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Program and Abstracts

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Third International Congress of Plant Molecular Biology

Molecular Biology of Plant Growth and Development

6-11 October 1991
Tucson Convention Center
Tucson, Arizona USA

Edited by
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MASTER

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Final Project Report

Project Title: Partial Support of the 3rd International Congress of Plant Molecular Biology

The Congress was held as scheduled October 6-11, 1991 in Tucson, Arizona with roughly 3000 scientists in attendance. Over 300 oral presentations were given and 1800 posters displayed. Twenty-five percent of the participants were female and about the same percentage attended from outside the United States.

Plant molecular biology is one of the most rapidly developing areas of the biological sciences. Recent advances in the ability to isolate genes, to study their expression and to create transgenic plants have had a major impact on our understanding of the many fundamental plant processes. Additionally, new approaches have been created to improve plants for agricultural purposes. The Congress provided an opportunity for all members of the society, as well as others supportive of this field, to learn of the most recent research developments and attend workshops on topical issues. Further, it provided the opportunity for senior and junior researchers, postdoctorals and graduate students to meet and share their most recent discoveries and furnished an opportunity to address the important funding and organizational issues that challenge this discipline at the international level.

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Exhibitors

The following will have products and information on display during the Poster Sessions in the Exhibition Hall.

American Society of Plant Physiologists: Featuring membership information, books and journals, and the opportunity to visit with representatives of ASPP's editorial staff.
Bio-Rad Laboratories: Displaying the biolistic particle delivery system and Chef Mapper post field system
Blackwell Scientific Publishers: Books and journals
Boehringer-Mannheim: biochemical reagents will be featured
Conviron: Specializing in plant growth chambers, incubators and tissue culture rooms.
DuPont: Instrumentation for DNA sequencing and biological separations will be exhibited. Look for poster also.
Elsevier Science Publishers B.V. Books and journals
Gauthier-Villars North America: Books and journals
Hoefer Scientific Instruments: Electrophoresis and electroporation instruments will be shown.
Kluwer Academic Publishers: Books and journals
LI-COR, Inc: Demonstrations of instruments for investigating the biochemistry of photosynthesis through gas exchange measurements; light meters and sensors and a field-portable spectroradiometer
Molecular Plant-Microbe Interactions: Books and journals
National Agricultural Library: Plant genome data and information and biotechnology information will be available.
New Brunswick Scientific: Biological shakers will be featured at this booth.
Phoenix Flow Systems: Presenting software for cell cycle and DNA analysis; flow cytometry
Springer-Verlag, New York: Books and journals
U.S. Biochemical Corporation: High quality biochemicals, molecular biology products and ultrapure reagents; also the SciScan 5000 digital imaging system
USDA Competitive Grants: Information on granting programs

Acknowledgements

The members of the Local Organizing Committee gratefully acknowledge the assistance of Jane Huff and Pam Larkins. We thank Lisa H. Thomas for the design of the Congress Logo.

Concurrent Symposia Schedule

Meeting Room	Monday 12:30-14:30	Tuesday 12:30-14:30	Wednesday 12:30-14:30	Thursday 10:30-12:30	Friday 12:30-14:30
Music Hall	1.2 Transcrip-tional regulation	1.1 RNA Processing	1.3 Manipulating gene expression	1.4 Post-transcriptional regulation	
Ballroom 1	6.1 Viruses, viroids	6.2 Necrotrophic bacterial pathogens	6.3 Biotrophic bacterial pathogens	6.4 Fungal/Plant Interactions	6.5 Plant/fungal interactions
Ballroom 2	2.1 Flowering	2.2 Fertilization	2.3 Meristems	2.4 Embryo-genesis	12.2 Mitochond. gene organization
Ballroom 3	3.1 Seed development		3.2 Fruit ripening	3.3 Lipid and carbohydrate biosynthesis	3.4 Storage proteins
Graham /Greenlee	4.1 Tissue culture I	4.2 Tissue culture II	12.1 Male sterility	4.3 Hormones I	4.4 Hormones II
Gila/Coconino	5.1 Intracellular protein transport	5.2 Solute transport	5.3 Cell wall	5.5 Synthesis of secondary products	5.4 Signal transduction
Holiday Inn East	9.1 Mapping QTLS	9.2 Transposable elements		9.3 Evolution of organelle genomes	9.4 Epigenetics and development
Holiday Inn West	8.1 Phytochrome	8.2 Tropisms, receptors	8.3 Water, desiccation and salt stresses	8.4 Heat, cold, heavy metal stresses	8.5 Wounding responses
Holiday Inn Central		7.1 Nitrogen fixation genes	7.2 Nodulation/ host range	7.3 Plant genes in nitrogen fixation	7.4 Regulation of bacterial nitrogen fixation
Maricopa	10.1 Carbon assimilation.	10.2 Photosystem I	10.3 Photosystem II	10.4 Light regulation	10.5 Electron transport
Mohave	11.1 Chloroplast gene expression	11.2 Chloroplast development	11.3 Genetics and transformation		11.4 Mol. genetics of cyanobacteria

Workshop/Poster Viewing Schedule

Meeting Room	Monday 15:00-17:00	Tuesday 15:00-17:00	Wednesday 15:00-17:00	Friday 15:00-17:00
Exhibition Hall	Poster Presentations: *Odd Numbered Posters**	Poster Presentations: *Even Numbered Posters**	Poster Presentations: *Odd Numbered Posters**	Poster Presentations: *Even Numbered Posters**
Music Hall	E. Communicating the Benefits of Ag Biotech Products	F. Future Research Priorities and their Funding	K. Viruses II--Plant Virus Interactions	A. Chromosome Walking and Genome Mapping
Ballroom 2		B. International Training and Research Opportunities	G. Agriculture in Third World Countries	J. Viruses I--Cross Protection
Ballroom 3		L. Organelle Inheritance	D. Managing Plant Genes and Genomes: Nomenclature and Informatics	M. Molecular Biology of Eukaryotic Algae
Graham/ Greenlee		I. Molecular Biology of the Cell Cycle	H. Application of Rapid-cycling Brassicas in Teaching	C. Interaction of Genes in Transgenic Plants

*Poster authors/presentors are scheduled to stand by their posters on the appointed days.

Poster Discussion Session Schedule

Meeting Room	Monday 17:00-18:30	Tuesday 17:00-18:30	Wednesday 17:00-18:30	Friday 17:00-18:30
Music Hall		20. Viruses, viroids	23. Fungal/plant interactions	24. Plant/fungal interactions
Ballroom 1	13. Hormones	31. Water, desiccation and salt stress	18. Signal transduction	32. Heat, cold and heavy metal stress
Ballroom 2	1. RNA Processing	2. Transcriptional regulation	3. Manipulating gene expression	4. Post-transcriptional regulation
Ballroom 3	15. Intracellular protein transport	16. Solute transport, ion pumps, plasmodesmata		19. Synthesis of secondary products
Graham /Greenlee	9. Seed development	10. Fruit ripening	11. Lipid and carbohydrate biosynthesis	12. Storage proteins
Gila/Coconino	43. Gene expression	35. Transposable elements	44. Development, genetics and transformation	14. Tissue culture
Holiday Inn East	5. Flowering	6. Fertilization	7. Meristems	8. Embryogenesis
Holiday Inn West	29. Phytochrome	30. Tropisms, receptors	22. Biotrophic bacterial pathogens	33. Wounding responses
Holiday Inn Central	34. Mapping QTLS	21. Necrotrophic bacterial pathogens	27. Plant genes involved in nitrogen fixation	36. Evolution of organelle genomes
Maricopa	38. Carbon assimilation	17. Cell wall	39. Photosystem I, Photosystem II	41. Light regulation
Mohave	25. Nitrogen fixation genes	47. Gene organization, male sterility	37. Epigenetics and development	46. Molecular genetics of cyanobacteria

Sunday October 6

Registration at Convention Center Ballroom Lobby (13:00-18:00)

Reception with Musical Entertainment and Refreshments in the Plaza outside the Music Hall (16:00-19:00)

Opening Session (Music Hall). B. Larkins, President ISPMB, presiding (19:00-20:00)

Official opening ceremony for the Congress. Conferees will be provided with last minute instructions regarding the format, schedule and procedures for the Congress.

Monday October 7

Plenary Session (Music Hall). J. Verbeke, Session Convenor (8:30-11:15)

8:30. Enrico Coen, John Innes Institute, Norwich, "The molecular genetics of floral homeotic mutations in Antirrhinum" (2)
9:15. Elliot Meyerowitz, California Institute of Technology, Pasadena, "Genetic and molecular mechanisms of flower development" (6)
10:00. Break
10:30. Adrienne Clarke, Melbourne University, Melbourne, "Cell-cell communication in pollen-pistil interactions" (9)

Monday Concurrent Symposia (12:30-14:30)

1.2 Advances in Gene Regulation: Transcriptional Regulation, Chair: E.S. Dennis (Music Hall)

12:30. Elizabeth S. Dennis, CSIRO, Canberra, "Transcriptional regulation of the alcohol dehydrogenase gene" (19)
13:00. Karam Singh, University of California, Los Angeles, "Ocs elements and bZIP transcription factors: the pathogen's choice for expressing genes in plants" (20)
13:30. Eric Lam, Rutgers University, Piscataway, "Hormonal modulation of the activity conferred by the binding sites of the nuclear factor ASF-1" (21)
14:00. Thomas W. Okita, Washington State University, Pullman, "In vivo and in vitro studies on the transcriptional regulation of a wheat gliadin gene" (22)

2.1 Differentiation of Tissues and Organs: Flowering, Chair: A. Lang (Ballroom 2)

12:30. Susan Singer, Carleton College, Northfield, "Induction and determination for flowering" (31)
13:00. Klaus Apel, ETH Institute for Plant Research, Zurich, "Changes in gene expression during the transition from vegetative to generative growth in the long-day plant Sinapis alba" (32)
13:30. Keith Roberts, John Innes Institute, Norwich, "Regulated expression of cell surface markers during floral development" (33)
14:00. Zsuzsanna Schwarz-Sommer, Max Planck Institute for Plant Breeding, Cologne, "The control of floral organogenesis by homeotic MADS-box genes in Antirrhinum majus" (34)

3.1 Seed and Fruit Development: Seed Development, Chair: J. Harada (Ballroom 3)

12:30. John Harada, University of California, Davis, "Gene regulation during the transition from embryogeny to postgermination" (47)

13:00. Rebecca S. Boston, North Carolina State University, Raleigh, "Overproduction of a molecular chaperone homolog in maize endosperm mutants" (48)

13:30. Donald R. McCarty, University of Florida, Gainesville, "The role of Vp1 in integration of hormonal and developmental signals in seed maturation" (49)

14:00. Terry Thomas, Texas A&M University, College Station, "Hierarchical control of seed protein gene expression" (50)

4.1 Hormonal Regulation: Tissue Culture I., Chair: R. Horsch (Graham/Greenlee)

12:30. Maud Hinchee, Monsanto Corporation, St. Louis, "Experimental gene transfer systems for plants" (63)

13:00. Marc De Block, Plant Genetic Systems, Gent, "Histochemical beta-glucuronidase assay with high cytological resolution" (64)

13:30. Jerzy Paszkowski, Swiss Federal Institute of Technology, Zurich, "Homologous recombination as a tool for precise modifications of plant genomes" (65)

14:00. Atsushi Komamine, Tohoku University, Sendai, "Gene expression during the cell cycle in synchronous cell cultures of Catharanthus roseus" (66)

5.1 Cell Biology and Metabolism: Intracellular Protein Transport, Chair: M. Chrispeels (Gila/Coconino)

12:30. Kenneth Keegstra, University of Wisconsin, Madison, "Transport of proteins into and across the envelope membrane of chloroplasts" (79)

13:00. Natasha Raikhel, Michigan State University, East Lansing, "Protein targeting to vacuoles and the nucleus of plant cells" (80)

13:30. Kenzo Nakamura, Nagoya University, Nagoya, "Characterization of the propeptide sequence of a precursor of sporamin that is required for vacuolar targeting" (81)

14:00. Christine Gietl, University of Munich, Munich, "Partitioning of malate dehydrogenase isoenzymes into glyoxysomes and mitochondria" (82)

6.1 Plant Pathogenesis: Viruses/viroids, Chair: R. Symons (Ballroom 1)

12:30. Robert Symons, Waite Agricultural Research Institute, Glen Osmond, "Variations in the hammerhead self-cleavage reaction of viroid RNA" (99)

13:00. Paul Ahlquist, University of Wisconsin, Madison, "Bromovirus replication and host adaptation" (100)

13:30. Anne Simon, University of Massachusetts, Amherst, "Resistance and susceptibility of Arabidopsis thaliana to turnip crinkle virus" (101)

14:00. Rosemarie Hammond, United States Department of Agriculture, Beltsville, "Investigations of the molecular interactions between a viroid and its host plant" (102)

8.1 Plant Responses to the Environment: Phytochrome, Chair: P. Quail (Holiday Inn West)

12:30. Peter Quail, USDA Plant Gene Expression Center, Albany, "Phytochrome and gene expression" (135)

13:00. Masaki Furuya, RIKEN Laboratory of Plant Biological Regulation, Saitama, "Type I and Type II phytochrome-dependent gene expression" (136)

13:30. Jane Silverthorne, University of California, Santa Cruz, "Structure and function of gymnosperm phytochrome genes" (137)
14:00. Lee H. Pratt, University of Georgia, Athens, "Differential expression of three phytochromes in Avena" (138)

9.1 Genome Organization, Mapping, Evolution and Stability: Mapping QTLs, Chair: T. Osborn (Holiday Inn East)

12:30. Thomas Osborn, University of Wisconsin, Madison, "Molecular marker analysis of low temperature responses in Brassica" (155)
13:00. Michael Murray, Agrigenetics, Madison, "Application of restriction fragment length polymorphism to maize breeding" (156)
13:30. John Doebley, University of Minnesota, St. Paul, "Genetic analysis of the morphological evolution of maize" (157)
14:00. Scott Tingey, DuPont, Wilmington, "Development and application of DNA-based diagnostics to problems in plant genetics" (158)

10.1 Photosynthesis: Carbon Assimilation., Chair: G. Lorimer (Maricopa)

12:30. George Lorimer, DuPont, Wilmington, "Folding Rubisco with chaperonins" (171)
13:00. Stefan Knight, Swedish University of Agricultural Sciences, Uppsala, "Enzymatic catalysis of carbon dioxide fixation - a structural view" (172)
13:30. Mark Stitt, Botanical Institute, Bayreuth, "The use of mutant and transgenic plants to study the regulation of carbon metabolism" (173)
14:00. Murray Badger, Australian National University, Canberra, "Molecular analysis of mutants of the CO_2-concentrating mechanism in Synechococcus PCC7942" (174)

11.1 Chloroplasts: Chloroplast Gene Expression, Chair: J. Gray (Mohave)

12:30. John C. Gray, Cambridge University, Cambridge, "Synthesis and assembly of photosynthetic complexes in the chloroplast thylakoid membrane" (191)
13:00. David Stern, Cornell University, Ithaca, "Regulation of mRNA processing and turnover in chloroplasts" (192)
13:30. Alice Barkan, University of Oregon, Eugene, "Molecular and genetic studies of chloroplast development in maize" (193)
14:00. Reinhold Herrmann, University of Munich, Munich, "Genes for thylakoid proteins: expression, modification, and interaction" (194)

Monday Workshop (15:00-18:00)

E. Communicating the Benefits of Ag Biotech Products, Chair: B. Miflin and R. Fraley, (Music Hall)

This workshop will involve introductory comments, followed by two presentations: Peter Sandman (Rutgers University) "Hazard versus Outrage; Responding to Public Concerns about Biotechnology"; Leonard Gianessi (Resources for the Future) "Alternative Pest Control--The Benefits of Biotech Products". This will be followed by a Roundtable Discussion with audience participation). Panelists will include Gianessi, Sandman, Martha Crouch, Heinz Saedler, Quentin Kubicek.

Monday Poster Discussion Sessions (17:00-18:30)

1. RNA Processing (215-247). Discussion Leader: A. Hunt (Ballroom 2)

Sanfacon, H "Dissection of the cauliflower mosaic virus polyadenylation signal" (223)
Barta, A "Processing of chimeric introns in plants: evidence for a close cooperation between 5' and 3' splice site" (240)
Egeland, DB "Molecular comparison of monocot and dicot U1 and U2 snRNAs" (237)

Thompson, AJ "Autocatalytic splicing of a group I intron from chloroplasts in vitro and in vivo" (219)
Miller, WA "Alternative tertiary structure attenuates self-cleavage of BYDV satellite RNA" (216)
Sanger, HL "Enzyme-catalyzed processing of potato spindle tuber viroid (PSTVd) and related viroids" (226)
Mulligen, RM "RNA editing intermediates of cox2 transcripts in maize mitochondria" (241)

5. Flowering (468-519). Discussion Leader: C. Gasser (Holiday Inn East)

Regan, SM "Expression of adenine phosphoribosyltransferase during pollen development in Arabidopsis" (481)
Ruezinsky, DM "Characterization of a floral specific C-MYB homolog from A. thaliana" (477)
Cornish, EC "Gene expression during petal senescence" (499)
Riggs, CD "Meiotin-1: a conserved, meiosis-specific protein which associates with nucleosome strings" (489)
Jansen, M "Novel stylar bioenergetics reveals a unique interaction between styles and pollen tubes" (469)
Harikrishna, K "A basic chitinase is expressed at high levels in the transmitting tissue of tomatoes" (511)
Roe, JL "FM-98:a gene required during early floral development in Arabidopsis" (488)
O'Neill, SD "Megasporogenesis: A pollination regulated development process" (505)
Schultz, E "The stud gene is requried to terminate stamen synthesis in flowers of A. thaliana" (504)

9. Seed development (622-676). Discussion Leader: A. Kriz (Graham/Greenlee)

Heck, GR "Characterization of a cDNA expressed during imbibition germination of barley seeds" (633)
Naito, S "Arabidopsis mutants that down regulate the expression of a soybean seed storage protein gene" (623)
Oishi, KK "Characterization of the maize Vp7 gene" (628)
Hutchings, CJ "Characterization of differentially-expressed gene families in seeds of tomato" (632)
Reiss, B "Identification of genes involved in the regulation of tobacco seed mutagenesis" (650)
Loer, D "Molecular and structural studies of soybean oil body assembly" (661)
Joudrier, P "Characterization of cDNAs encoding wheat CM proteins-heterologous expression in E. coli" (634)
Bass, HW "Characterization of b-32, a novel ribosome-inactivating protein of maize" (629)
Olsen, O-A "The rate of gene transcription increases tenfold during the syncytial stage of barley endosperm development" (667)
Tanaka, K "Construction of aleurone tissue specific cDNA library from a single rice grain using polymerase chain reaction and detection of aleurone specific gene expression" (638)

13. Hormones (784-869). Discussion Leader: A. Binns (Ballroom 1)

Binns, AN "Auxin sensitivity of cytokinin habituated tobacco cell lines" (784)
Bartling, D "Expression of a nitrilase from an Arabidopsis cDNA synthesizing the plant hormone indoleacetic acid" (855)
Sun, T-P "Cloning the A. thaliana ga-1 locus by genomic subtraction" (823)
Wu, Y "T-DNA insertional mutagenesis in Arabidopsis: the dwarf mutants" (860)
Su, W "Isolation and characterization of cytokinin response mutants in A. thaliana" (827)

Maurel, C "The ROLB gene of Agrobacterium rhizogenes allows auxin to potentiate auxin action in tobacco" (813)
Capone, I "Analysis of the regulation and function of the root inducing gene rolB" (837)
Upadhyaya, NM "Cytokinin production by Rhizobium strain IC3342 and possible role of Icr1 genes" (807)

15. Intracellular Protein transport (936-999). Discussion Leader: J. Carrington (Ballroom 3)

Varagona, MJ "Nuclear localization signal(s) required for nuclear targeting of the maize regulatory protein opaque 2" (970)
Carrington, JC "Regulation of nuclear transport of a plant viral protein" (963)
Nechushtai, R "pLHCP retention by chloroplast hsp70: unfolded conformation facilitates insertion into thylakoid membranes" (965)
Oblong, JE "Chromatographic characterization of the chloroplast soluble processing enzyme using as a substrate the LHCP precursor synthesized in E. coli" (942)
Kohorn, BD "Insertion of chlorophyll binding protein into a membrane lacking chlorophyll" (945)
Wrobel, R "Expression of maize b-70, an endoplasmic recticulum molecular chaperone homolog" (940)
Neuhaus, J-M "The vacuolar targeting signal of the basic chitinases of tobacco" (980)
Hofte, H "Targeting of an intrinsic membrane protein to the tonoplast in plant cells" (975)

25. Nitrogen Fixation Genes (1395-1416). Discussion Leader: C. Kennedy (Mohave)

Thiel, T "Characterization of genes for an alternate nitrogenase in the cyanobacterium A. variabilis" (1415)
Agron, PG "Mutational analysis of the R. meliloti nifA promoter" (1407)
Monson, EK "Heme-binding and kinase domains of the R. meliloti oxygen sensor fixl" (1408)
Holland, D "In-vivo localization of transcription of hetA: A gene involved in the differentiation of heterocysts" (1414)
Kosslak, RM "Do isoflavonoid pools change in soybean root exudates and extracts during nodulation" (1401)
Stokkermans, T "Rhizobitoxine is involved in overcoming the Rj4 genotype specific host restriction in soybean" (1402)
Ruan, X "Rhizobitoxine is differentially expressed in legume nodules in a genotype and species specific manner" (1401)
Spaink, H "NOD modulation of B. japonicum that induce a plant nodulation response" (1399)
Gresshoff, PM "Mapping and isolation of an important developmental locus of soybean (Glycine max) by molecular genetic means" (1412)
Boonjawat, J "Rice lectin: synthesis and induction by associative nitrogen-fixing bacteria" (1413)

29. Phytochrome (1462-1491). Discussion Leader: E. Schaefer (Holiday Inn West)

Wahleithner, JA "Characterization of requirements for chromophore attachment to E. coli expressed apophytochrome" (1475)
Edgerton, MD "Localization of a dimerization region within the phytochrome polypeptide " (1462)
Lopez, E "Detection of phyA and phyB-like gene products in two complementary phytochrome mutants" (1472)
Wagner, D "Rice and Arabidopsis phyB genes encode functional phytochromes as shown by overexpression in A. thaliana" (1464)

Nagatani, A "Phytochrome responses in transgenic tobacco plants which over-express rice phytochrome I" (1470)
Brusslan, J "The isolation of A. thaliana mutants defective in phytochrome signal transduction" (1479)
Pepper, AE "Genetic suppressors of the detl-I mutation of A. thaliana" (1477)
Weatherwax, S "Characterization of a light-regulated DNA-binding factor (LRF-1) in L. gibba and A. thaliana" (1481)
Dehesh, K "GT-2:a trans-acting factor that binds in a highly sequence specific manner to a GT motif in the rice PHYA gene promoter" (1490)

34. Mapping QTLs (1655-1727). Discussion Leader: A. Patterson (Holiday Inn Central)

Keim, P "RFLP analysis of soybean breeding populations: genetic mapping of markers and QTLs" (1717)
Landry, BS "Construction of a detailed RFLP-based genetic map and tagging of resistance genes to race 2 of clubroot disease in B. oleracea" (1692)
Weeden, NF "Evaluation and characterization of RAPD markers for genome mapping" (1721)
Carlson, JE "Genetic linkage maps for individual trees from haploid DNA using RAPD markers" (1667)
Williamson, VM "A high resolution linkage map of the nematode resistance gene region in tomato" (1710)
Ganal, M "Mapping and progress towards cloning of the Tm2a gene on chromosome 9 of tomato" (1724)
Matallana, E "Physical mapping of the Arabidopsis genome with anchored YACs" (1722)
Weber, G "Microdissection of chromosomes of B. napus at 4000 x magnification with a UV laser microbeam and stable transformation of higher plants" (1685)

38. Carbon assimilation (1823-1852). Discussion Leader: J. Preiss (Maricopa)

Hudson, GS "Reduction of Rubisco content by antisense RNA reduces photosynthesis in trangenic tobacco plants" (1829)
Flachmann, R "Site-directed mutagenesis of the Rubisco LSU to SSU interface" (1827)
Smith-White, B "Subunits of ADP-glucose pyrophosphorylase:spinach leaf subunit cDNAs and comparison to those from diverse sources" (1845)
Nakata, PA "Subunit structure of potato tuber ADP glucose pyrophosphorylase" (1837)
Muller-Rober, B "Approaches to influence starch quantity and quality in transgenic plants" (1834)
Stark, DM "Enhancement of starch content of plant cells by expression of a carbohydrate biosynthetic gene" (1846)

43. Gene expression (1913-1964). Discussion Leader: G. Link (Gila/Coconino)

Khanna, NC "Two distinct transcriptional activities of pea chloroplasts share immunologically related functional polypeptides" (1963)
Christopher, DA "Light-induced switch in promoter utilization for a chloroplast psbD-psbC operon" (1927)
Stevenson, JK "Intercistronic RNA processing of a chloroplast pre-mRNA coding for protein components of photosystem I and II reaction centers" (1926)
Copertino, D "Group III twintron in a chloroplast gene is excised by alternative splicing" (1922)
Chen, Q "Functional analysis of proteins that bind to the 3' inverted repeat region of spinach chloroplast petD mRNA" (1940)
Ye, L "Ribonucleoprotein family in tobacco chloroplasts " (1950)

Schuster, G "Chloroplast mRNA 3' end processing requires a nuclear-encoded RNA-binding protein" (1958)

Monod, C "Expression of Chlamydomonas reinhardtii chloroplast psbB gene depends on a nuclear gene product" (1935)

Tuesday October 8

Plenary Session (Music Hall). H. Bohnert, Session Convenor (8:30-11:15)

8:30. Steven D. Tanksley, Cornell University, Ithaca, "Molecular characterization of the tomato nuclear genome"

9:15. Clarence Ryan, Washington State University, Pullman, "Inducible signals for proteinase inhibitor genes" (4)

10:00. Break

10:30. Sharon Long, Stanford University, Stanford, "Cell and molecular biology of Rhizobium symbiosis" (1)

Tuesday Concurrent Symposia (12:30-14:30)

1.1 Advances in Gene Regulation: RNA Processing, Chair: W. Gerlach (Music Hall)

12:30. George Bruening, University of California, Davis, "Spontaneous formation of stable circles by oligoribonucleotides containing sequences of the satellite RNA of tobacco ringspot virus" (15)

13:00. John W. S. Brown, Scottish Crop Research Institute, Dundee, "Components of the plant pre-mRNA splicing machinery" (16)

13:30. Axel Brennicke, IGF, Berlin, "RNA editing and trans-splicing in Oenothera and Arabidopsis mitochondria" (18)

14:00. James Haseloff, Massachusetts General Hospital, Cambridge, "Ribozymes for targeted trans-splicing" (17)

2.2 Differentiation of Tissues and Organs: Fertilization, Chair: J. Nasrallah (Ballroom 2)

12:30. Mary K. Thorsness, Cornell University, Ithaca, "Expression of a Brassica S-gene promoter in transgenic plants" (35)

13:00. Martin Trick, John Innes Institute, Norwich, "Structure and expression of S-linked and S-related genes in Brassica" (36)

13:30. Richard Thompson, Max Planck Institute for Plant Breeding, Cologne, "The relationship of major pistil proteins of Solanum tuberosum to self-incompatibility" (37)

14:00. Sheila McCormick, USDA Plant Gene Expression Center, Albany, "Functional analysis of pollen-expressed genes" (38)

4.2 Hormonal Regulation: Tissue Culture II., Chair: E. Galun (Graham/Greenlee)

12:30. Indra K. Vasil, University of Florida, Gainesville, "Progress in the regeneration and genetic transformation of wheat" (67)

13:00. Albert Kausch, DeKalb Plant Genetics, Groton, "Transformation of maize" (68)

13:30. Ko Shimamoto, Plantech Research Institute, Yokohama, "Transgenic rice plants as tools for studies in regulation of monocot genes" (69)

14:00. Dennis McCabe, Agracetus, Middleton, "Particle gun transformation applied to cotton" (70)

5.2 Cell Biology and Metabolism: Solute Transport, Chair: A. Bennett (Gila/Coconino)

12:30. Norbert Sauer, University of Regensburg, Regensburg, "Sugar carriers of the plant plasma membrane" (83)

13:00. Julian Schroeder, University of California, San Diego, "Molecular mechanisms of abscisic acid-induced signal transduction in guard cells" (84)

13:30. Ramon Serrano, EMBL, Heidelberg, "Plasma membrane H+-ATPase: immunocytolocalization and heterologous expression in plants and yeast" (85)

14:00. Heven Sze, University of Maryland, College Park, "Molecular structure of the vacuolar H+-pumping ATPase" (86)

6.2 Plant Pathogenesis: Necrotrophic Bacterial Pathogens, Chair: B. Staskawicz (Ballroom 1)

12:30. Brian Staskawicz, University of California, Berkeley, "Genetic interactions specifying expression of bacterial disease resistance in Arabidopsis and tomato" (103)

13:00. Jan Leach, Kansas State University, Manhattan, "Genetics and physiology of incompatible interactions between Xanthomonas oryzae pv. oryzae and rice" (104)

13:30. Mike Daniels, John Innes Institute, Norwich, "Interaction of Xanthomonas with crucifers" (105)

14:00. Noel Keen, University of California, Riverside, "Towards understanding the function of pathogen avirulence genes and plant resistance genes" (106)

7.1 Nitrogen Fixation: Nitrogen Fixation Genes, Chair: A. Kondorosi (Holiday Inn Central)

12:30. Dennis Dean, Virginia Polytechnic and State University, Blacksburg, "Nitrogen fixation genes: structure and function" (119)

13:00. Alfred Puhler, University of Bielefeld, Beilefeld, "In vitro and site-directed mutageneiss to study the function of the R. meliloti nitrogen fixation genes FdxN and NifA" (120)

13:30. Rafael Palacios, Center for Investigation of Nitrogen Fixation, Cuernavaca, "Amplification of nodulation and nitrogen fixation genes in Rhizobium phaseoli" (121)

14:00. Hans-Martin Fischer, ETH Institute for Microbiology, Zurich, "Multiple control levels of nif and fix gene expression in Bradyrhizobium japonicum" (122)

8.2 Plant Responses to the Environment: Tropisms/Receptors, Chair: W. Briggs (Holiday Inn West)

12:30. Lon S. Kaufman, University of Illinois at Chicago, Chicago, "A blue-light activated GTP-binding protein in the plasma membrane of etiolated peas" (139)

13:00. Sarah M. Assmann, Harvard University, Cambridge, "Regulation of guard cell responses by blue light and second messengers" (140)

13:30. Masamitsu Wada, Tokyo Metropolitan University, Tokyo, "Phytochrome-mediated phototropism in fern protonemata" (141)

14:00. Timothy W. Short, Carnegie Institute of Washington, Stanford, "The photoreceptor for phototropism as a mediator of protein phosphorylation" (142)

9.2 Genome Organization, Mapping, Evolution and Stablity: Transposable Elements, Chair: P. Starlinger (Holiday Inn East)

12:30. Reinhard Kunze, University of Cologne, Cologne, "Functions of the ORFa protein, the putative transposase of maize transposable element AC" (159)

13:00. Jeffrey Bennetzen, Purdue University, West Lafayette, "Activities and evolution of the Mu and Ds mobile elements of Zea mays" (160)
13:30. Cathie Martin, John Innes Institute, Norwich, "Transposition and genome change in Antirrhinum majus" (161)
14:00. Heinz Saedler, Max Planck Institute for Plant Breeding, Cologne, "The En/Spm transposable element of Zea mays" (162)

10.2 Photosynthesis: Photosystem I, Chair: B. Lindberg-Møller (Maricopa)

12:30. Nathan Nelson, Roche Institute of Molecular Biology, Nutley, "Molecular biology of Photosystem I reaction center" (175)
13:00. Richard Malkin, University of California, Berkeley, "Subunit function and organization in Photosystem I complex" (176)
13:30. Henrik Vibe-Scheller, Royal Veterinary and Agricultural University, Frederiksberg, "Organization and development of Photosystem I in barley" (177)
14:00. John H. Golbeck, University of Nebraska, Lincoln, "Reconstitution of the Photosystem I reaction center with genetically modified psaC, psaD and psaE proteins expressed in Escherichia coli" (178)

11.2 Chloroplasts: Chloroplast Development, Chair: M. Edelman (Mohave)

12:30. Marvin Edelman, Weizman Institute, Rehovot, "The molecular basis for tentoxin resistance in chloroplasts of Nicotiana" (195)
13:00. Werner Kuhlbrandt, EMBL, Heidelberg, "Three-dimensional structure of light-harvesting chlorophyll a/b-protein complex" (196)
13:30. Gayle Lamppa, University of Chicago, Chicago, "Import and processing of the precursor for LHCP" (197)
14:00. Colin Robinson, Warwick University, Warwick, "Mechanism of protein transport across the thylakoid membrane" (198)

Tuesday Workshops (15:00-17:00)

B. International Training and Research Opportunities, Chair: I. Vasil, (Ballroom 2)

The purpose of this workshop is to highlight the value of education and training in plant molecular biology and biotechnology, encourage the participation of ISPMB members in such activities, and describe the opportunities available internationally in this rapidly advancing field of biology, particularly for the developing countries. Invited speakers include Denes Dudits (Hungary), Arturo Falaschi (ICGEB), Luis Herrera-Estrella (Mexico) and Jacques Weil (France).

F. Future Research Priorities and their Funding, Chair: M. Dilworth, (Music Hall)

This workshop will start with a one-hour informational session to present opportunities available for support of plant science research. The second hour will be a discussion session about what is wrong with the current system of plant science funding, what can be done about it, and how the community working with funding agencies can secure a bright future for plant science research. Speakers will include: Machi F. Dilworth (NSF), Bruce Umminger (NSF), Judith Greenberg (NIGMS), Robert Rabson (DOE), Anne Datko (USDA), Robert Goodman (NRC/NAS).

I. Molecular Biology of the Cell Cycle, Chairs: J. van t'Hof and J. Bryant, (Graham/Greenlee)

This workshop will consist of two parts. The first will concentrate on methods for the experimental analysis of the cell cycle in higher plants. The second will focus on molecular aspects underlying the operation of the cell cycle. Speakers and titles of presentations are as follows: Jack

9.3 Genome Organization, Mapping, Evolution and Stablity: Evolution of Organelle Genomes, Chair: J. Palmer (Holiday Inn East)

10:30. Jeffrey Palmer, Indiana University, Bloomington, "Evolution of chloroplast gene and intron content" (163)

11:00. Claude dePamphilis, Vanderbilt University, Nashville, "Plastid DNA evolution and function: the complete sequence of the plastid genome of a non-photosynthetic plant" (164)

11:30. Susan Douglas, National Research Council of Canada, Halifax, "Phylogenetic origin of the Cryptomonad plastid genome" (165)

12:00. Masahiro Sugiura, Nagoya University, Nagoya, "The chloroplast genomes from monocot, dicot and gymnosperm" (166)

10.4 Photosynthesis: Light Regulation, Chair: E. Tobin (Maricopa)

10:30. Elaine Tobin, University of California, Los Angeles, "Approaches to understanding phytochrome regulation of transcription" (183)

11:00. William Thompson, North Carolina State University, Raleigh, "Regulation of ferredoxin mRNA abundance" (184)

11:30. Eberhard Schäfer, University of Freiburg, Freiburg, "Analysis of photoregulation of chalcone synthase during plant development" (185)

12:00. Steve Kay, Rockefeller University, New York, "Circadian-regulated Cab gene transcription in Arabidopsis" (186)

Friday, October 11

Plenary Session (Music Hall). B. Larkins, Session Convenor (8:30-11:15)

8:30. Yasuyuki Yamada, Kyoto University, Kyoto, "Enzymology and molecular biology in alkaloid biosynthesis" (5)

9:15. Gerald E. Fink, Whitehead Institute, Cambridge, "Molecular genetics of hormone biosynthesis in Arabidopsis thaliana"

10:00. Break

10:30. Robert Goldberg, University of California, Los Angeles, "Developmental gene regulation: implications for agriculture"

Friday Concurrent Symposia (12:30-14:30)

3.4 Seed and Fruit Development: Storage Proteins, Chair: P. Shewry (Ballroom 3)

12:30. Peter Shewry, Bristol University, Bristol, "The structures and deposition of cereal seed storage proteins" (59)

13:00. Gad Galili, Weizman Institute, Rehovot, "Subcellular transport of wheat storage proteins and their deposition in protein bodies: signals and mechanisms" (60)

13:30. Ulrich Wobus, Institute for Plant Genetics and Tissue Culture, Gatersleben, "Legume seed proteins: gene expression and its regulation" (61)

14:00. Enno Krebbers, Plant Genetic Systems, Gent, "Expression of modified seed storage proteins in Brassicaceae" (62)

4.4 Hormonal Regulation: Hormones II., Chair: H. Kende (Graham/Greenlee)

12:30. Anthony Bleecker, University of Wisconsin, Madison, "Molecular analysis of an Arabidopsis gene involved in ethylene responses" (75)

13:00. Jan A.D. Zeevart, Michigan State University, East Lansing, "Gibberellin biosynthesis and its regulation" (76)

13:30. Dominique Van Der Straeten, State University, Gent, "Ethylene in higher plants: a molecular-genetic approach" (77)
14:00. Alan Jones, University of North Carolina, Chapel Hill, "Auxin-binding proteins and their possible roles in auxin-mediated plant cell growth" (78)

5.4 Cell Biology and Metabolism: Signal Transduction, Chair: D. Bowles (Gila/Coconino)

12:30. Edward E. Farmer, Washington State University, Pullman, "Airborne methyljasmonate activates wound-inducible proteinase inhibitor genes" (91)
13:00. Dianna J. Bowles, Leeds University, Leeds, "Local and systemic signaling in a plant defense response" (92)
13:30. Janet Braam, Rice University, Houston, "Touch, heat-shock, and calcium-induced expression of the calmodulin-related Tch genes of Arabidopsis" (93)
14:00. John Walker, University of Missouri, Columbia, "Receptor-like protein kinases of higher plants" (94)

6.5 Plant Pathogenesis: Plant/Fungal Interactions, Chair: R. Michelmore (Ballroom 1)

12:30. Richard Michelmore, University of California, Davis, "Specificity in lettuce downy mildew" (115)
13:00. Thomas J. Wolpert, Oregon State University, Corvallis, "Molecular interactions in Victoria blight of oats" (116)
13:30. Michael G. Hahn, University of Georgia, Athens, "Hepta-beta-glucoside elicitor binding protein(s) in soybean" (117)
14:00. V. Gianinazzi-Pearson, INRA, Dijon, "Endomycorrhiza: an emerging model for studying compatible plant-fungal interactions" (118)

7.4 Nitrogen Fixation: Regulation of Bacterial Nitrogen Fixation, Chair: R. Haselkorn (Holiday Inn Central)

12:30. William Buikema, University of Chicago, Chicago, "The hetR gene controls heterocyst development in Anabaena 7120" (131)
13:00. Paul Bishop, North Carolina State University, Raleigh, "Regulation of alternative nitrogenases in Azotobacter vinelandii" (132)
13:30. Claudine Elmerich, Pasteur Institute, Paris, "Free-living and symbiotic nitrogen fixation in Azorhizobium caulinodans" (133)
14:00. Werner Klipp, University of Bielefeld, Beilefeld, "Organization and regulation of genes encoding molybdenum and alternative nitrogenase in the phototrophic purple bacterium Rhodobacter capsulatus" (134)

8.5 Plant Responses to the Environment: Wounding Responses, Chair: C. Lamb (Holiday Inn West)

12:30. Chris Lamb, Salk Institute, La Jolla, "Integration of plant defense responses into developmental programs" (151)
13:00. Richard A. Dixon, Noble Foundation, Ardmore, "Expression and manipulation of genes encoding enzymes of phenylpropanoid biosynthesis in alfalfa" (152)
13:30. Ilya Raskin, Rutgers University, New Brunswick, "Salicylic acid is a systemic signal and an inducer of pathogenic-related proteins in virus-infected tobacco" (153)
14:00. John F. Bol, Leiden University, Leiden, "Induction of PR-genes by virus infection and wounding" (154)

9.4 Genome Organization, Mapping, Evolution and Stablity: Epigenetics and Development, Chair: R. Jorgensen (Holiday Inn East)

12:30. Nina Fedoroff, Carnegie Institution of Washington, Baltimore, "Epigenetic control of the maize suppressor-mutator transposable element" (167)

13:00. Marjorie Matzke, Austrian Academy of Sciences, Salzburg, "Gene interactions and epigenetic variation in transgenic plants" (168)

13:30. Fred Meins, Friederich Miescher Institute, Basel, "Reversible inactivation of chitinase gene expression in transgenic plants" (170)

14:00. Richard Jorgensen, University of California, Davis, "Programming of flower color patterns associated with co-suppression of CHS genes"

10.5 Photosynthesis: Electron Transport, Chair: W. Cramer (Maricopa)

12:30. William Cramer, Purdue University, West Lafayette, "Topography structure-function in the chloroplast cytochromes" (187)

13:00. Bruce Diner, DuPont, Wilmington, "Site-directed mutagenesis affecting electron transport on the donor side of Photosystem II" (188)

13:30. Paul Mathis, CNRS, Gif-Sur-Yvette, "Kinetics of QA to QB electron transfer in Rhodopseudomonas viridis" (189)

14:00. Mary Blackwell, Lawrence College, Appleton, "The plastoquinone connection between Photosystem II and the cytochrome b_6-f complex" (190)

11.4 Chloroplasts: Molecular Genetics of Cyanobacteria, Chair: L. Sherman (Mohave)

12:30. Louis Sherman, Purdue University, West Lafayette, "Plasticity in cyanobacterial oxygen evolution and chlorophyll-protein organization" (203)

13:00. Donald A. Bryant, Pennsylvania State University, University Park, "Mutagenesis of genes encoding phycobilisome components in Synechococcus Sp. PCC 7942" (204)

13:30. Arthur Grossman, Carnegie Institution, Stanford, "The acclimation of cyanobacteria to hard times" (205)

14:00. Nicole Tandeau de Marsac, Pasteur Institute, Paris, "Molecular mechanisms controlling adaptation of cyanobacteria to their environment" (206)

12.2 Mitochondria: Mitochondrial Gene Organization, Chair: M. Gray (Ballroom 2)

12:30. Michael W. Gray, Dalhousie University, Halifax, "RNA editing and gene migration in plant mitochondria" (211)

13:00. Jean-Michel Grienenberger, CNRS, Strasbourg, "RNA editing modulates gene expression in wheat mitochondria" (212)

13:30. Linda Bonen, University of Ottawa, Ottawa, "Wheat mitochondrial genes and their expression" (213)

14:00. Kanji Ohyama, Kyoto University, Kyoto, "Gene organization of liverwort Marchantia polymorpha mitochondrial DNA" (214)

Friday Workshops (15:00-17:00)

A. Chromosome Walking and Genome Mapping, Chair: H. Goodman, (Music Hall)

This workshop will consist of a number of short presentations followed by open discussion on the current status of the gene maps in Arabidopsis, maize, rice, soybean, and tomato. The topics will include both RFLP and physical maps, and integration of these with classical genetic maps. Emphasis will be placed on the resolution and practical utility of the maps for gene isolation and chromosome walking. Data will be presented on the correlation of genetic and physical distance in

various plant species. Another general topic of the workshop will be new technology such as the use of RAPD markers for RFLP mapping. Participants in this workshop include Howard Goodman (Chair), Martin Ganal, Tim Helentjaris, Pamela Ronald, and Scott Tingey. Attendees with relevant data are encouraged to contact the workshop Chair to discuss presenting their work at this session.

C. Interaction of Genes in Transgenic Plants, Chairs: J. Mol & R. Jorgensen, (Graham/Greenlee)

Recent studies indicate that duplicated gene sequences may undergo co-supression and that this may be a widespread phenomenon in plants. Better understanding of co-suppression may shed some light on the dynamic behavior in the nucleus. Moreover, this phenomenon may be of practical importance in the field of plant biotechnology. The aim of this workshop is to summarize all the data available today and to discuss possible models and implications. Participants are Rich Jorgenson (flower color genes), Joseph Mol, Don Grierson (polygalacturonase genes), Steven Rothstein (nopaline synthase genes), Majori Ann Matzke (T-DNA genes), Fred Meins (chitinase genes), Jerzy Paszkowski (hygromycin resistance genes), Jim English (streptomycin resistance genes), and Farhah Assaad (antibiotic resistance genes).

J. Viruses I--Cross Protection, Chair: N. Tumer, (Ballroom 2)

The workshop will concentrate on analysis of viral gene expression in transgenic plants and strategies for developing viral disease resistance. The topics will include virus resistance mediated via expression of viral coat protein genes, results of field evaluation of virus resistance plants, resistance mediated by expression of ribozymes, analysis of expression of viral replicase genes in plants and effects of coat protein mutations on virus replication and protection. The speakers will include Wayne Gerlach, "Ribozyme approach to virus resistance," John Bol, "Effect of alfalfa mosaic virus coat protein mutations on virus replication and cross-protection," Jerry Slightom, "Field tests of transgenic cucumber plants that express the cucumbr mosaic virus coat protein gene," Carl Braun, "Expression of potato virus X replicase in transgenic plants," Nilgun Tumer, "Development of potato plants resistant to potato leafroll virus," and Margit Laimer, "Coat protein-mediated protection against plum pox virus."

M. Molecular Biology of Eukaryotic Algae, Chair: W. Loefflehardt, (Ballroom 3)

There will be some emphasis on Chlamydomonas , because many problems that cannot be studied adequately with higher plants are approachable via transformation of Chlamydomonas chloroplasts. Two contributions deal with introns in nuclear and mitochondrial genes, respectively. Plasmids with transposon-like properties and plastid genes from chlorophyll b-less algae will also be discussed. Participants: Rose Ann Cattolico, John Davies, Hideya Fukuzawa, Michel Goldschmitt-Clermont, Mark Hildebrand, Ulrich Kuck, Umesh Muchhal, Marie Petracek.

Friday Poster Discussion Sessions (17:00-18:30)

4. Post-transcriptional regulation (429-467). Discussion Leader: J. Weil (Ballroom 2)

Luehrsen, KR "Mutants of maize introns affect both RNA accumulation and splicing efficiency" (446)

Newman, TC "Identification of an instability determinant derived from an auxin-inducible transcript" (449)

Danon, A "Light regulated RNA-binding proteins: Candidates for chloroplast translational activators" (436)

Vayda, ME "Translational control in potato tubers subjected to environmental stresses" (463)

Hensgens, LAM "Expression of chimeric GUS fusions is not proportional to the steady state levels of mRNA" (441)
Cloney, LP "Chaperonins and rubisco gene expression" (435)
Tacke, E "Expression strategies of the potato leafroll luteovirus" (460)
Schmidt-Puchta, W "Cellular proteins bind to the leader region of the cauliflower mosaic virus 35S RNA" (467)

8. Embryogenesis (589-621). Discussion Leader: L. Zimmerman (Holiday Inn East)

Dudits, D "Cell cycle control and induction of embryogenesis in hormone shocked somatic cells" (615)
Zhong, H "In vitro manipulation of corn morphogenesis for transformation" (591)
Berleth, T "Basal deletions in embryonic pattern mutants of Arabidopsis " (609)
Castle, LA "Molecular characterization of T-DNA tagged genes essential for Arabidopsis embryo development" (594)
Moore, KB "Characterization of maize HMG-CoA reductase" (601)
de Jong, AJ "Secreted proteins in plant embryogenesis" (598)
Brady, J "Isolation of an Arabidopsis WNT-1 homolog" (612)
LeVesque, CS "Embryo- and light-specific regulatory elements of a CAB gene of soybean" (616)
Sinha, NR "Isolation and characterization of three homeodomain containing genes involved in fruit development in tomatoes" (603)
Apuya, N "Differential gene expression in carrot somatic embryos is accomplished through translational control" (610)

12. Storage Proteins (730-783). Discussion Leader: R. Flavell (Graham/Greenlee)

Grimes, HD "The 94 kD vegetative storage protein in soybean leaves is a lipoxygenase " (783)
Ko, T-P "The three-dimensional structures of reserve proteins from the jack bean" (781)
Shani, N "Role of the amino and carboxy terminal regions in the structure and folding of wheat high molecular weight glutenin subunits" (773)
Fujiwara, T "Cis-acting transcriptional regulatory elements from soybean B-conglycinin genes" (745)
Motto, M "Regulatory genes affecting zein deposition in maize endosperm" (762)
Zheng, Z "Bean seed storage protein B-phaseolin is expressed in the endosperm of transgenic rice plants" (733)

14. Tissue Culture (870-935). Discussion Leader: E. Earle (Gila/Coconino)

Gallois, P "Transfer of a herbicide tolerance gene by direct gene transfer of Arabidopsis genomic DNA into tobacco protoplasts" (922)
Hirt, H "Isolation and characterization of a plant cyclin gene" (891)
Kausch, A "Cytodifferentiation of maize suspension cultures" (895)
Kikuchi, S "Cloning and characterization of the genes which specifically express in callus of rice" (872)
Li, Z "Field trials with transgenic rice plants: Molecular and genetic evaluation" (907)
MacRae, S "Use of Agrobacterium rhizogenes-mediated transformation to improve rooting of eucalypts" (903)
McElroy, D "Utilization of the rice actin 1 gene for foreign gene expression in transgenic rice" (879)
Radin, D "A tissue culture system for molecular analysis of root-knot nematode disease" (920)

19. Synthesis of secondary products (1091-1132). Discussion Leader: R. Last (Ballroom 3)

Jacobs, JJMR "Isolation and characterization of mutants of Tagetes erecta with altered thiophene contents" (1103)
Henstrand, JM "Preliminary characterization of the promoter for the first enzyme of the shikimate pathway in potato" (1113)
Fecker, L "Expression of a bacterial lysine decarboxylase gene in tobacco " (1092)
Rose, A "The gene defective in blue fluorescent Arabidopsis tryptophan mutants" (1115)
Goddijn, OJM "Regulation and overexpression of the tryptophan decarboxylase gene in C. roseus" (1128)
Bodeau, JP "Non-coordinate regulated expression of BZ1 and BZ2 promoters in maize protoplasts" (1130)
Hunt, MD "Cloning of plastid polyphenol oxidase (PPO) cDNAs from S. tuberosum" (1095)
Shirley, BW "Analysis of radiation-induced mutations at two flavonoid biosynthetic loci in Arabidopsis" (1110)

24. Plant/fungal interactions (1347-1394). Discussion Leader: J. Ebel (Music Hall)

Lotan, T "Chaperones as a candidate conduit for general plant pathogenesis response" (1374)
Alexander, D "Evidence for resistance to P. parasitica in transgenic tobacco expressing constitutively a newly-discovered pathogenesis-related cDNA" (1361)
Logemann, J "Expression of a ribosome inhibiting protein (RIP) leads to fungal resistance in transgenic tobacco plants" (1363)
Broglie, R "Transgenic plants with enhanced resistance to R. solani" (1364)
Somssich, IE "Function and transcriptional regulation of defense-related plant genes" (1372)
Daklin, K "Analysis of a bean chalcone synthase promoter in transgenic alfalfa" (1393)
Tsuji, J "Arabilexin, a novel phytoalexin from Arabidopsis thaliana" (1392)
Lerner, DR "A novel gene structure encodes a lectin from stinging nettle (U. dioica)" (1356)
Cammue, BPA "A new type of antifungal plant protein from M. jalapa L. seeds" (1380)
Mauch, F "Pathogen-induced genes in wheat " (1376)

32. Heat, cold and heavy metal stress (1563-1624). Discussion Leader: C. Guy (Ballroom 1)

Ow, D "Molecular cellular biology of heavy metal tolerance in Schizosaccharomyces pombe" (1619)
Vierling, E "Molecular and functional analysis of a plastid-localized heat shock protein" (1570)
Inze, D "Molecular study of oxidative stress in plants" (1572)
Christie, PJ "Low-temperature induction of maize genes associated with respiration, oxidative stress, and the phenylpropanoid pathway" (1613)
Sarhan, F "Identification of a gene family associated with freezing tolerance in wheat" (1588)

33. Wounding responses (1625-1654). Discussion Leader: R. Broglie (Holiday Inn West)

Draper, J "Gene expression at sites of wounding and pathogen invasion" (1641)
Rickey, T "Expression of HSP70 and ubiquitin gene families in potato" (1638)
Fritig, B "Isolation, catalytic activities and molecular cloning of tobacco lysozymes" (1642)
Froissart, D "Isolation and characterization of an auxin dependent tobacco gene activated upon infection with Pseudomonas solanacearum" (1635)
Schuch, W "Purification, characterization and cloning of tobacco cinnamyl alcohol dehydrogenase" (1653)

Kush, A "Hevelin, a lectin-like glycoprotein from Hevea brasiliensis (rubber tree) is involved in the coagulation of latex" (1651)
Hollick, JB "Wound-induced expression of poplar proteinase inhibitor-like genes" (1649)
Zhu, Q "Functional dissection of the rice basic chitinase RCH10 gene promoter " (1644)

36. Evolution of organelle genomes (1781-1805). Discussion Leader: R. Cattolico (Holiday Inn Central)

Igloi, G "DNA sequence analysis of the maize chloroplast genome: structure and expression of rpo genes" (1795)
Zhang, M "Structure of a maize mitochondrial RNA replicon" (1789)
Nugent, JM "RNA mediated transfer of the gene COXII from the mitochondrion to the nucleus" (1803)
Wolff, G "The chondriome of the alga Prototheca wickerhamii is closely related to plant mitochondria" (1797)
Kowallik, KV "Molecular evolution of chlorophyll a+c-containing plastids" (1786)
Reith, M "A 14 Kb region of the plastid genome of Porphyra umbilicalis encodes at least five genes not located on the plastid genome of green plants" (1792)
Löffelhardt, W "SecY, part of the bacterial protein export complex, from C. paradoxa cyanelles" (1799)
Bult, CJ "Organellar and nuclear ribosomal gene sequences within Onagraceae: phylogenetic and evolutionary considerations" (1793)
Herzog, M "Molecular phylogenetic analysis of Pyrrhophyta inferred from the sequence of the 24S rRNA conservative and divergent domains" (1794)
De Benedetto, C "Organization of coxII gene and its possible correlation with the evolution of angiosperms" (1790)

41. Light regulation (1882-1912). Discussion Leader: B. Green (Maricopa)

Green, BR "Identification of the chlorophyll a/b (CAB) polypeptides of photosystems I and II with their corresponding genes" (1903)
Spano, AJ "Protochlorophyllide reductase in pine" (1894)
Pilgrim, ML "Circadian regulation of gene expression in Arabidopsis" (1884)
Millar, AJ "Novel phenotypes based on luciferase expression in transgenic plants" (1895)
Tjaden, G "Characterization of trans-acting protein factors which interact with the light regulated GS2 promoter" (1888)
Armstrong, GA "Parsley DNA-binding proteins which recognize ACGT-containing promoter elements" (1882)

46. Molecular genetics of Cyanobacteria (2006-2029). Discussion Leader: S. Curtis (Mohave)

Smith, D "Protein targeting in cyanobacteria" (2006)
Martin, JA "Analysis of Anabaena PCC 7120 photosynthesis gene promoters" (2018)
Schwarz, R "The operon rbc and its genomic region are involved in the ability of cyanobacteria to grow under low-CO_2" (2010)
McLaughlin, S "Transcription of a cytochrome P-450 w-hydroxylase and other open reading frames within the Anabaena 7120 nifD excision element" (2023)
Heinhorst, S "Isolation and characterization of a DNA topoisomerase I from A. nidulans and preliminary characterization of its gene" (2019)
Heinhorst, S "A DNA polymerase from the cyanobacterium A. nidulans R2" (2020)
Robinson, SJ "Putative glycogen metabolism gene adjacent to NIFH in Anabaena 7120" (2022)
Potts, M "Components for high-affinity Fe uptake in cyanobacteria" (2015)

PLENARY SPEAKERS

1 GENETIC ANALYSIS OF *RHIZOBIUM MELILOTI* - ALFALFA NODULATION. Sharon R. Long, Robert F. Fisher, Joy Ogawa, Jean Swanson, Melanie Barnett, Julie Schwedock, Edward Atkinson and David Ehrhardt. Stanford University, Department of Biological Sciences, Stanford, California, 94305-5020

How do cells interact to carry out complex developmental events? In the *Rhizobium*-legume symbiosis, evidence from many research labs is pointing to the idea that the bacteria and plants coordinate action through exchange of molecular signals. We are studying the early interaction of *R. meliloti* and its hosts, such as alfalfa (*Medicago sativa*). We have found that the *nod* gene transcriptional activator NodD1 requires flavonoid signals such as luteolin from the host plant; another protein, NodD3, is able to activate expression of *nod* gene promoters without inducers and may be important later in symbiotic development. A gene with homology to *E. coli groEL* is necessary for NodD function; this may indicate that the folding of NodD protein is complex and requires the participation of a molecular chaperone. The function of the *nod* genes is proposed to be the synthesis of Nod-factors such as NodRm-1; this work will be described by several speakers during the Congress. We have found a number of *nod*-gene dependent factors similar to NodRm-1, and have shown that *nodPQ* genes encode enzymes that synthesize activated sulfate required for the transfer of sulfate to a Nod-factor. We have established that individual root hairs display a rapid response of membrane potential, when they contact purified Nod-factors. We hope this will lead to an understanding of plant signal transduction.

2 HOMEOTIC GENES CONTROLLING FLOWER DEVELOPMENT IN *ANTIRRHINUM*. Enrico Coen, Sandra Doyle, Robert Elliott, José Romero, Desmond Bradley, Sabine Hantke, Rüdiger Simon, Da Luo and Rosemary Carpenter. John Innes Institute, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

Three classes of homeotic floral mutations have been defined in *Antirrhinum*, depending on whether they affect (1) the overall fate of the floral meristem (2) the identity and sometimes also the number of whorls in the flower (3) the assymetry of the flower. We have carried out a molecular and genetic analysis of these mutants in order to understand how genes interact to control the fate of meristems. An early-acting class(1) gene, *floricaula* (*flo*), is required for the initiation of the floral programme: in the *flo* mutant, indeterminate shoots grow in place of flowers. The *flo* transcript is absent in vegetative apices and is detected 2 days following floral induction. The *flo* gene is transiently expressed in bract, sepal, petal and carpel primordia but no expression is detected in stamen primordia. Several class(2) genes have also been isolated from *Antirrhinum*. We will discuss models for how class(1) and class(2) genes interact based on studies of the timing and spatial patterns of gene expression together with phenotypic analysis of single and double mutants.

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3 COMMERCIALIZATION OF GENETICALLY MODIFIED PLANTS: PROGRESS TOWARDS THE MARKETPLACE. Robert T. Fraley, Monsanto Agricultural Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198 USA.

The development of new crops that are resistant to insect and viral diseases or are compatible with environmentally-friendly herbicides represent the first wave of genetically modified plant products which will be commercialized in the 1990's by Monsanto Company. Advances in expression of *Bt* genes in plants have allowed for exciting progress in the development of caterpillar resistant cotton and corn lines as well as potato varieties resistant to Colorado potato beetle. The emphasis on these projects has now shifted to commercial development activities which include registration, large-scale field testing and breeding. Similarly, the discovery of highly tolerant EPSPS enzymes that retain high substrate affinity and activity have enabled the production of canola and soybean lines that demonstrate commercial level tolerance to glyphosate herbicide. This technology will provide farmers with superior, cost-effective and environmentally sensitive weed control alternatives for the future.

In addition to pest control and other agronomic improvements, lies the attractive possibility of improving consumer and processor directed traits such as calorie reduction, fat reduction, taste improvement, longer shelf life, nutritional value, etc. Two advances--enhancement of starch levels in potatoes and the control of fruit ripening in tomato--will be discussed as examples of the enormous potential for opportunities in this area. Dramatic increases in the levels of tuber starch have been obtained by expressing a bacterial starch biosynthetic gene under control of a potatin promoter. Higher starch potatoes can potentially reduce processor costs as well as reduce oil adsorption during frying. Control of tomato fruit ripening has been obtained by expressing a gene encoding an enzyme that degrades the immediate precursor of ethylene, ACC. Expression of this gene results in greater than 90% reduction in ethylene production and delays in ripening of several weeks. These advances serve to illustrate that plant metabolic pathways can be regulated at both gene expression and enzyme activity level to regulate metabolic flux and hence influence plant growth and development.

4 POLYPEPTIDE SIGNALLING FOR WOUND-INDUCIBLE PROTEINASE INHIBITOR GENES IN PLANTS. Clarence A. Ryan, Gregory Pearce and Barry McGurl, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

Several signalling molecules have been identified that regulate the expression of genes coding for two proteinase inhibitor proteins that are synthesized in tomato and potato leaves in response to predator and/or pathogen attacks. Regulatory molecules include oligosaccharide fragments of plant and fungal cell walls, plant hormones such as abscisic acid and auxin, and methyl jasmonate. We have recently isolated an eighteen amino acid polypeptide from tomato leaves that powerfully activates the expression of proteinase inhibitor genes in tomato plants. This polypeptide, called systemin, is the most potent inducer of the two proteinase inhibitor genes yet found. The structure and properties of systemin and its cDNA and gene will be presented. Antisense constructs of the cDNA, under the regulation of the CaMV 35S promoter, when stably integrated into the tomato genome and expressed, result in plants that only weakly respond to wounding. The possible role of systemin as a systemic signal generated by wounding will be discussed. (Supported in part by Washington State College of Agriculture and Home Economics Project #1791 and grants from NSF and the McKnight Foundation.)

5 ENZYMOLOGY AND MOLECULAR BIOLOGY IN ALKALOID BIOSYNTHESIS. Yasuyuki Yamada, Takashi Hashimoto and Fumihiko Sato, Kyoto University, Kyoto 606, Japan

Fast-growing cultured cells and roots not only may lead to large-scale production systems for commercially important pharmaceuticals but also offer excellent experimental systems to study secondary metabolism. *In vitro* culture systems of medicinal plants have been established in our laboratory to study enzymology and molecular biology of two types of alkaloids. Cultured *Coptis japonica* cells that accumulate high amounts of berberine, an antibacterial drug, have been used to characterize two *O*-methyltransferases with distinct substrate specificity involved in berberine biosynthesis. Roots of several solanaceous plants produce anticholinergic tropane alkaloids, hyoscyamine and scopolamine. Hyoscyamine 6β-hydroxylase (H6H) catalyzes two consecutive oxidation reactions that epoxidize hyoscyamine to scopolamine by way of 6β-hydroxyhyoscyamine. Full-length cDNA clones and genomic clones encoding H6H have been isolated from *Hyoscyamus niger*. Northern blotting and immunohistochemical studies indicated that expression of H6H is highly tissue- and cell-specific. Abundant H6H mRNA was present only in the developmentally young root in which H6H protein was localized to the pericycle cells. For biotechnological application, we introduced and expressed constitutively the H6H gene under the control of CaMV 35S promoter in hyoscyamine-rich *Atropa belladonna* plants. The transgenic plants expressing *H. niger* H6H gene exclusively contained the commercially more valuable scopolamine in their leaves.

6 GENETIC AND MOLECULAR MECHANISMS OF FLOWER DEVELOPMENT. E.M. Meyerowitz, J.L. Bowman, L.L. Brockman, G.N. Drews, K. Goto, T. Jack, Z. Liu, M. Running, H. Sakai, L.E. Sieburth and D. Weigel. California Institute of Technology, Division of Biology, Pasadena, California 91125, USA.

We study a group of mutations that cause homeotic conversions of floral organs in *Arabidopsis*. The mutations fall into three predominant classes, each class affecting the organ types that appear in two adjacent whorls of the flower. By a series of genetic experiments, we have demonstrated that these three classes of genes specify the four types of floral organ in *Arabidopsis* in a combinatorial fashion: the three gene classes express in an overlapping pattern that defines four different domains of homeotic gene activity, and the genes expressed in each domain determine the nature of the organs that will differentiate there. Molecular cloning of homeotic genes from two of the three classes has been accomplished, and by *in situ* hybridization we have shown that the pattern of expression of RNA from these genes corresponds with the domain of gene activity. In other words, the spatial control of the expression of the homeotic genes is at the RNA level, and this spatial regulation defines organ type in developing flowers. We have also found two types of regulatory activity that determine the spatial pattern of homeotic gene RNA expression, and have identified genes whose function is to regulate the pattern of RNA expression of different of the homeotic genes. This work thus identifies both a pattern of homeotic gene expression that determines organ fate, and a prepattern of regulatory gene expression that specifies the pattern of homeotic gene expression.

PLENARY SPEAKERS

7 MANIPULATING THE CHLOROPLAST GENOME: PRESENT REALITIES AND FUTURE PROSPECTS. J.E. Boynton, N.W. Gillham, S.M. Newman, E.H. Harris, A.M. Johnson & B. L. Randolph-Anderson, Duke Univ., Depts. of Botany and Zoology, Durham NC

Biolistic transformation of the single chloroplast of Chlamydomonas reinhardtii occurs by homologous gene replacement at frequencies of 10^{-4} to 10^{-6}. Transformants are selected by complementation of defects in recipient strains carrying nonphotosynthetic chloroplast mutations or by introduction into wildtype strains of chloroplast rRNA or psbA genes carrying selectable antibiotic or herbicide resistance markers. Recombination events usually occur near vector : insert junctions, leading to integration of most of the donor fragment but no vector sequences. When targeted genes reside in the inverted repeat, donor DNA integrates into one element and spreads by copy correction to the opposite element. Genomes of initially heteroplasmic chloroplasts segregate rapidly to produce cells homoplasmic for introduced genes. Foreign genes have also been stably integrated downstream of a selectable atpB donor sequence. Chloroplasts of recipient cells can be co-transformed efficiently with mixtures of two different plasmids under conditions where selection is imposed for only one donor sequence, permitting development of a system for targeted disruption of chloroplast genes. This may allow identification of functions of certain unknown open reading frames as well as creation of null mutations in photosynthetic protein genes. Several laboratories are now using either cotransformation or complementation of null mutations to analyze in vivo effects of site directed changes in structural and regulatory chloroplast sequences. Technology for chloroplast transformation in Chlamydomonas has recently been adapted by Maliga and colleagues to transform tobacco chloroplasts, using selectable antibiotic resistance mutations in the 16S chloroplast rRNA gene. Thus, with sufficiently strong selection, the large number of chloroplasts in higher plant cells is not an insurmountable barrier for manipulation of their chloroplast genomes. Supported by NIH Grant GM-19427.

8 INTER-INTRACELLULAR TRANSIT OF NUCLEIC ACID-PROTEIN COMPLEXES-& ARABIDOPSIS FLOWERS. Patricia C. Zambryski, University of California, Department of Plant Biology, Berkeley, CA 94720.

How nucleic acids move into, within, and between cells is a fundamental question throughout biology. We are investigating this problem using two different biological systems: Agrobacterium mediated genetic transformation of plant cells, and plant virus spread through intercellular connections, the plasmodesmata. Evidence suggests that both systems transport single strand (ss) nucleic acid; furthermore, in both systems this nucleic acid is complexed with protein during its transit. The T-strand of Agrobacterium is complexed with VirD2 at its 5' end, and the VirE2 ss binding protein (SSB) along its length. Similarly, we have shown that at least two different (TMV and CaMV) plant virus movement proteins are SSBs. Interestingly, the physical dimensions (by electron microscopy) of the Agrobacterium ssDNA complex and the viral nucleic acid (RNA) movement protein complex are comparable. Thus, we propose that their movement is mediated by essentially similar mechanims, i.e., the formation of exceptionally thin protein nucleic acid complexes that facilitate transit through thin extracellular and intracellular channels. Our lab also is investigating developmental patterns during flower formation in Arabidopsis. We have several T-DNA tagged mutant lines (in collaboration with Dr. Kenneth Feldmann) that produce flowers with abnormal phenotypes. One of these, fm-98, produces flowers with alterations in all whorls, and all organs have a generally rumpled appearance. Scanning EM indicates that the fm-98 gene is required very early during initiation of floral development. Double mutant lines between fm-98 and existing floral mutants are being generated to test (i) possible epistatic relationships, and (ii) whether the phenotype controlled by fm-98 fits into current models for Arabidopsis flower development. Genomic as well as cDNA clones from the fm-98 regions have been obtained. Nucleotide sequence analysis of the fm-98 transcript as well as its expression pattern in developing flowers will be presented. In a separate study, Arabidopsis genes containing homeoboxes have been isolated using probes (in collaboration with Dr. Sarah Hake) homologous to the homeobox from the maize knotted gene.

9 RIBONUCLEASES IN SELF-INCOMPATIBILITY. Marilyn A. Anderson, Bruce A. McClure and Adrienne E. Clarke.

Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052 Australia.

Self-incompatibility (SI) in *Nicotiana alata* is controlled by a single gene. A number of cDNAs corresponding to the style *S*-allele products of a number of solanaceaous plants have been isolated. Analysis of these *S*-DNAs shows that their sequences contain both variable and conserved domains. Two of the conserved domains are also homologous to regions surrounding the proposed active sites of certain fungal ribonucleases. This finding led to the direct demonstration that the *N. alata S*-glycoproteins are ribonucleases.

Based on these results, we proposed a cytotoxic model for SI in solanaceous plants. In this model, pollen bearing the same *S*-allele as the style is selectively susceptible to the *S*-RNases. This could lead to an inhibition of translation and account for the characteristic inhibition of incompatible pollen tube growth. Labelling and RNA tracer experiments show that pollen rRNA is degraded in incompatible pollinations but not in compatible pollinations.

We are exploring the mechanism of action of the *S*-gene by a number of approaches. One approach is using transformation of both *N.alata* and *N. tabacum* plants with a genomic S_6-clone that contained 2.8 kb of sequence upstream from the start colour. The transgenic *N. alata* plants expressed the gene specifically in styles. However, although intact copies of the gene were present in the transgenic *N. tabacum* plants, no gene expression could be detected. A further approach is to establish details of the structure of the *S*-glycoproteins. We have isolated individual N-linked glycosyl chains from *S*-glycoproteins and established their structures.

SYMPOSIUM SPEAKERS

15 SPONTANEOUS FORMATION OF STABLE CIRCLES BY OLIORIBONUCLEOTIDES CONTAINING SEQUENCES OF THE SATELLITE RNA OF TOBACCO RINGSPOT VIRUS.

Paul A. Feldstein and George Bruening. University of California, Davis, California 95616. RNA that is complementary to the most abundant polarity of the satellite RNA of tobacco ringspot virus is designated sTobRV(-)RNA. sTobRV(-)RNA contains an endoribonucleolytic RNA chain segment: a ribozyme that recognizes and cleaves at a specific sequence. The self-cleavage reaction of multimeric sTobRV(-)RNA and its reverse, the ligation of linear sTobRV(-)RNA into circles, very likely are essential steps in the replication of sTobRV RNA. We demonstrate that the ribozyme sequence mediates ligation, both in trans and in cis. Based on these results, we designed an oligoribonucleotide of 75 residues that spontaneously, efficiently and reversibly circularized. Tests of molecules with various lengths of a loop connecting two helices revealed an aspect of the tertiary structure: that the two helices are not co-axial in reactive molecules. The local conformation of actively ligating sTobRV(-)RNA may resemble that of a bent paper clip.

16 COMPONENTS OF THE PLANT PRE-mRNA SPLICING MACHINERY.

John W. S. Brown, R. Waugh, G. G. Simpson, P. Vaux, D. J. Leader, C. G. Simpson and G. Clark. Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, U. K.

Splicing, the removal of introns from pre-messenger mRNA (pre-mRNA), is a fundamental process in gene expression. The mechanism of splicing and the components of the splicing machinery have been extensively studied in animal and yeast systems. Splicing is mediated by uridylate-rich small nuclear ribonucleoprotein particles (UsnRNPs) which consist of one or two UsnRNA molecules in association with a number of proteins. The UsnRNPs bind to pre-mRNAs and along with other proteins form a large ribonucleoprotein complex called the spliceosome where intron removal and exon ligation take place.

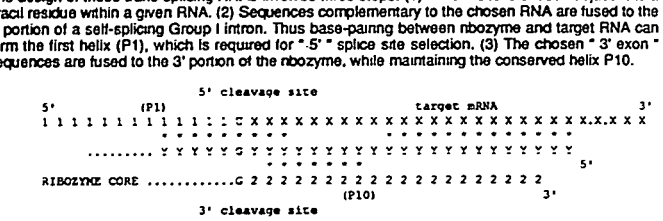
Genes coding for U1, U2 and U5snRNAs have been isolated from potato and maize. The coding regions show clear homology with their animal counterparts at the primary sequence and secondary structure levels, but plant UsnRNAs show far greater sequence variation than observed animal systems. Plant UsnRNAs also exhibit variation in sequences, known from animal systems, to determine the binding of specific UsnRNP proteins. The protein components of plant spliceosomal UsnRNPs are almost wholly uncharacterised. Using antibodies raised against yeast and mammalian UsnRNP proteins, plant UsnRNP proteins are being identified. The isolation of a full length cDNA for the U2snRNP-associated protein, U2B", represents the first plant pre-mRNA processing protein gene to be reported. The sequence exhibits extensive homology with the human U2B" protein. The characterisation of other pre-mRNA processing proteins, including U1snRNP-specific 70kDa protein, U5snRNP-specific PRP8 protein and RNA helicases is in progress.

17 DESIGN OF RIBOZYMES FOR TRANS-SPLICING.

Jim Haseloff and Howard Goodman, Department of Molecular Biology, Massachusetts General Hospital. Boston. MA 02114. U.S.A.

Ribozymes are potential tools for genetic manipulation. We describe the design of new ribozymes capable of trans-splicing which are based upon the catalytic core of a Group I intron. Using this design, it has been possible to construct ribozymes capable of efficiently splicing a new 3' exon sequence into a chosen target RNA sequence in a highly precise manner.

The design of these trans-splicing RNAs involves three steps. (1) A " 5' " site is chosen adjacent to a uracil residue within a given RNA. (2) Sequences complementary to the chosen RNA are fused to the 5' portion of a self-splicing Group I intron. Thus base-pairing between ribozyme and target RNA can form the first helix (P1), which is required for " 5' " splice site selection. (3) The chosen " 3' exon " sequences are fused to the 3' portion of the ribozyme, while maintaining the conserved helix P10.



Using this design, ribozyme sequences have been constructed. (1) to fuse the lacZ a-peptide coding sequence (in frame) downstream of the CAT mRNA translation start site, and (2) to fuse the coding sequence of the A chain of diphtheria toxin downstream of the GAL4 mRNA start codon. Mis-folded "pro-ribozyme" forms of these catalytic RNAs were produced by alteration of the ribozyme core sequences. Using such trans-splicing ribozymes, the presence of an arbitrary but specific target RNA within a cell may be used as a key for the introduction of a new gene activity.

18 RNA PROCESSING BY TRANS SPLICING AND EDITING IN PLANT MITOCHONDRIA.

Wolfgang Schuster, Bernd Wissinger, Stefan Binder, Anita Marchfelder, Volker Knoop, Rainer Ternes, Rudolf Hiesel and Axel Brennicke. Institut für Genbiologische Forschung, Ihnestraße 63, D-W1000 Berlin 33, FRG

Plant mitochondrial transcripts are processed by excision of introns and modification of the primary sequence by RNA editing. Several intron sequences are interrupted and are encoded together with the adjacent exons in distant genomic locations. The mature mRNAs for these genes must be assembled from several precursor transcripts by trans-splicing. RNA editing is observed in open reading frames and also in intron-sequences. Comparative analyses of spliced and unspliced transcripts show editing to occur both before and after splicing. Several RNA editing events in intron sequences appear to be required for proper folding of the intron structure. The interconnection between cis- and trans-splicing and RNA editing in plant mitochondria is discussed.

19 TRANSCRIPTIONAL REGULATION OF ALCOHOL DEHYDROGENASE GENES.

E.S. Dennis, M.R. Olive, R. Dolferus and W.J. Peacock. CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601

Anaerobic induction of the maize alcohol dehydrogenase (*Adh1*) gene occurs at the transcriptional level. The sequences necessary for this anaerobic regulation lie between -140 and -100 relative to the start of transcription. We have called this region the Anaerobic Responsive Element (ARE). A detailed analysis of this region has identified bases critical for transcriptional activity using a transient assay of DNA electroporated into maize protoplasts. There are two regions of importance and bases between these regions are not critical for activity. We have identified a protein factor which binds specifically to multimerized repeats of the ARE. This factor resembles the animal transcription factor *Sp1* in the sequence of its DNA binding site and it does bind purified *Sp1*. Binding of this factor is only one component of anaerobically induced transcription.

Regulation of the Arabidopsis *Adh* gene has also been studied. As well as showing induction by anaerobic conditions, Arabidopsis *Adh* is induced by the auxin 2,4D, by cold, wilting and 10^{-4} M ABA. Deletion analysis of the promoter will determine whether all these inducing agents work through the same cis element on the promoter and share a common signal transduction pathway or whether each stimulus works through a separate promoter element.

20 OCS ELEMENTS AND bZIP TRANSCRIPTION FACTORS: THE PATHOGENS CHOICE FOR EXPRESSING GENES IN PLANTS.

Karam Singh*, Danny Llewellyn#, Jeff Ellis#, Carol Grossman#, Liz Dennis# and Jim Peacock#. *UCLA, Department of Biology, Los Angeles, CA 90024 and #CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, Australia.

We have identified a family of related 20 bp enhancer sequences, the ocs-elements, which are a functional component of the promoters of several genes introduced into the plant by Agrobacterium transformation or infection by DNA viruses. Although the cellular functions of the ocs-elements are unknown, their ability to function in both monocot and dicot plants and their exploitation by a diverse range of plant pathogens suggests an essential role(s) in plant metabolism. We have cloned the genes for a number of maize ocs-element binding proteins (OCSBFs) suggesting that the interaction between ocs-element sequences and the plant encoded transcription factors is complex. The OCSBFs, which contain the bZIP motif, have characteristics of the leucine zipper family of transcription factors and appear to have overlapping but distinct DNA binding and RNA expression patterns. One of the clones, OCSBF-3, appears to correspond to OCSTF, a maize transcription factor that is the major binding activity detectable in extracts from maize suspension cells. OCSBF-3 also shares significant homology with the wheat HBP-1B and tobacco TGA1a proteins including regions that lie outside the bZIP motif.

SYMPOSIUM SPEAKERS

21 HORMONAL MODULATION OF THE ACTIVITY CONFERRED BY THE BINDING SITES OF THE NUCLEAR FACTOR ASF-1. Eric Lam, Olga Pensic and Xiangjun Liu. AgBiotech Center, Box 759, Waksman Institute, Rutgers State University, Piscataway, NJ 08854.

Auxin is a class of plant hormones that has been well-characterized in its effects on growth and differentiation processes. Alterations in auxin concentrations by exogenous applications of the hormone have been shown to induce cell division, cell elongation and lateral root initiation in plants. However, the mechanisms by which auxins cause these changes remain unknown. Recently, we found that two related DNA sequences of 21 base-pairs act as auxin-responsive elements in transgenic tobacco. These sequences were originally found in the promoter region of the nopaline synthase gene from the T-DNA of *Agrobacterium tumafaciens* and the 35S promoter from cauliflower mosaic virus. Both sequences are binding sites for the tobacco transcription factor ASF-1. Northern analysis of ASF-1 expression and pre-treatment with cycloheximide suggest that auxin activation of these promoter elements does not involve *de novo* synthesis of ASF-1. We propose that transcriptional activation by ASF-1 can be modulated by auxin through modification of pre-existing factors. These results suggest a role for ASF-1 in mediating the effects of auxin *in vivo*. At present, we are investigating the effects of other plant hormones on the activity of ASF-1 binding sites. These studies may reveal evidence for "cross-talk" between different regulatory pathways.

22 *IN VIVO* AND *IN VITRO* STUDIES ON THE TRANSCRIPTIONAL REGULATION OF A WHEAT GLIADIN GENE. Thomas W. Okita, Robert L. Vellanoweth, Arun P Aryan, Mary J. Laughlin, and Mark W. Roberts. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

The gliadins form one of the principal storage protein classes which are synthesized and accumulated in wheat endosperm. In order to identify the regulatory elements responsible for tissue specific and temporal expression of this multigene family we have analyzed the promoter region of an α/β-type gliadin gene by both *in vivo* and *in vitro* approaches. Based on transient expression analysis in tobacco protoplasts of a series of deletions of the gliadin promoter fused upstream to a functional and inactivated nopaline synthase (*nos*) promoter fragment, a region, -218 bp to -142 bp, from the transcriptional initiation site was observed to be important in either enhancing or restoring the activity of the *nos* promoter. The role of this promoter region in gliadin transcription is also supported by gel retardation studies using nuclear extracts isolated from developing wheat endosperm. Within this 77 bp region, at least six different nuclear protein-DNA complexes are observed. One of the binding proteins binds to a CA-rich element, conserved in many wheat and barley storage protein genes, while a second binding activity maps to an adjacent downstream AT-rich palindromic region. Present efforts are directed at the purification of these nuclear proteins by biochemical approaches, and the development and employment of an *in vitro* transcription assay to determine the role of these factors in gliadin gene expression.

23 ANALYZING CARBOHYDRATE METABOLISM VIA CHANGING GENE EXPRESSION IN TRANSGENIC PLANTS. L. Willmitzer, J. Koßmann, B. Muller-Rober and U. Sonnewald. Institut fur Genbiologische Forschung Berlin GmbH, Ihnestr. 63, 1000 Berlin 33, Germany

Carbohydrate metabolism has been widely studied on the physiological and biochemical level. However, despite the fact that this metabolism is of central importance to plant development, no such studies have yet been described trying to approach this problem on the molecular level and more specifically using the tools supplied by molecular genetics.

Here we describe a concept and first results of a molecular approach. Based on the (partly) known biochemistry of carbohydrate metabolism and on mainly assumptions with respect to factors which might be important for partitioning of carbohydrates between sink-source tissue, we have tried to identify key steps controlling carbohydrate metabolism and partitioning. Subsequently by using tools of reversed genetics we have tried to modulate these key steps by either inhibiting the expression of endogenous genes using anti-sense strategies or by ectopic expression of either plant endogenous or alien enzymes in transgenic plants. These plants are finally analysed for a variety of biochemical and physiological parameters. Results obtained using this approach will be described.

24 PROGRESS IN ENGINEERING GENES FOR FERTILITY CONTROL. Jan Leemans, Arlette Reynaerts, Marc De Block, Marc De Beuckeleer, Patrick Rüdelsheim, Robert Goldberg and Celestina Mariani. Plant Genetic Systems N.V., Gent, Belgium. UCLA, Dept. of Biology, Los Angeles, CA 90024-1606.

We have reported previously the isolation of tapetal-specific genes from tobacco (1). The 5' region of such gene has been used to target expression of a ribonuclease specifically to the tapetum. Expression of the RNase Barnase leads to tapetum ablation and results in transgenic plants which are male sterile (2). The approach has been applied successfully to various crops, including oilseed rape, cabbage, chicory and cotton. Field trials have shown the stability of the male sterile phenotype and the normal agronomic performance of male sterile lines.

Linkage of the male sterility to a herbicide tolerance gene provides a general way to multiply the male sterile plants by using the fertile parental line as a maintainer in backcrosses. Pollinator lines capable of producing fully fertile hybrid seed on the male sterile plants have been obtained by expressing Barstar, the specific inhibitor of Barnase. Hybrid progeny express both Barnase and Barstar in the tapetum. The two proteins form an inactive complex resulting in the full restoration of fertility. The utility of this fertility control system for the production of hybrid seed will be discussed.

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2 Mariani, C. et al, Induction of male sterility in plants by a chimaeric ribonuclease gene, Nature, vol 347, nr. 6295, pp 737-741, 1990

25 OPAQUE-2 AND ZEIN GENE TRANSCRIPTION. Robert J. Schmidt. Dept. of Biology, and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093.

The *opaque-2* locus (*o2*) plays a central role in regulating the expression of certain members of the α-zein gene family. The protein encoded by *o2* is a member of the bZIP family of eukaryotic transcription factors. Expression of *o2* is restricted to maize endosperms, and the Opaque-2 protein (O2) levels during endosperm development appear to be regulated by a posttranscriptional mechanism. DNA binding analyses indicates that O2 recognizes a target site that is present in promoters of zein genes encoding proteins of the 22-kD size class but not in members of the 19-kD size class. This provides a molecular explanation for the differential effect that *o2* has on the expression of 22 and 19-kD zein genes. When a multimer of the O2 binding site was placed upstream of a minimal promoter for *lac Z* and introduced into yeast, β-galactosidase activity increased 500 fold in response to O2 protein expression. This demonstrates that the O2 protein is capable of activating gene expression in the absence of other maize proteins and provides a convenient system for analyzing the effect of alternative target sites on the ability of O2 to activate gene expression. In 22-kD zein gene promoters the O2 target site is situated approximately 20 nucleotides downstream from the conserved *cis*-acting element known as the prolamine box. By site-directed mutagenesis of promoter elements we are analyzing the effect of various mutation on the function of the zein gene promoter in cultured endosperm cells and developing endosperm tissue.

26 DISSECTION OF THE CIS-REGULATORY ELEMENTS OF THE CLASS-I PATATIN GENE OF POTATO CONTROLLING "TUBER-SPECIFIC" AND SUCROSE-INDUCIBLE EXPRESSION. William Park and Jie Du, Texas A&M University, Dept. of Biochemistry and Biophysics, College Station, TX 77843

The Class-I genes of potato are expressed at high levels in tubers and can be induced to express at high levels in stems and leaves by exposure to 0.2 to 0.3M sucrose. This specificity appears to result from the interaction of two separate regulatory domains. While the sequences between -40 and -460 have "tuber-specific" enhancer activity when attached to a -90 truncated CaMV 35S promoter, we found that this "tuber-specificity" is critically dependent on the presence of the sequences between -87 and -40. When the patatin sequences between -460 and -88 are attached to a -90 truncated 35S promoter, they still are expressed in tubers, but are expressed at even higher levels in the vascular tissue of leaves. They are not normally expressed in leaf mesophyll cells, but like intact Class-I genes, they can be induced to do so by high levels of sucrose. The importance of the sequences between -87 to -40 is shown even more strikingly when they are deleted from similar constructs truncated at -595. Rather than being "tuber-specific", these constructs are expressed at very high levels both in tubers and in essentially all of the cell types of normal leaves and stems even without adding additional sucrose. That the sequences between -87 and -40 should play a critical role in "tuber-specific" expression is very surprising since they are shared with the Class-II patatin genes which are expressed at only low levels in tubers and unlike the Class-I genes are also normally expressed in root tips.

SYMPOSIUM SPEAKERS

28 CONTROL OF mRNA STABILITY IN HIGHER PLANTS. Pamela J. Green, Thomas C. Newman, Crispin B. Taylor, Masaru Ohme-Takagi, Yang Yen, Christie Howard, Pedro Gil, Yang Liu, and Wan-Ling Chiu. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824-1312. As a first step towards elucidating the fundamental principles that govern mRNA stability in higher plants, we have begun to identify mRNA sequences and cellular factors that facilitate general and selective mRNA decay. We have developed a system to measure directly mRNA decay rates in stably transformed tobacco cells and have used this system to measure the stability of a variety of endogenous and genetically engineered transcripts. These studies have shown that the GUS and CAT transcripts each decay with a half-life of about 80 min. Moreover, we have identified an AU-rich motif and a short sequence from an auxin-inducible transcript that can markedly destabilize the GUS transcript (relative to an unmodified CAT transcript) in tobacco cells. Preliminary data suggests that these sequences have a similar affect in transgenic plants. Among the cellular factors that participate in mRNA degradation, ribonucleases (RNases) undoubtedly play a major role. Using a substrate-based gel assay, we have identified 16 RNase activities in crude extracts from *Arabidopsis*, some of which accumulate differentially in stems vs leaves. We have characterized these RNases according to their biochemical properties and have begun to screen for mutants that lack any of these activities. Our long-term goal is to use RNase mutants to determine which activities are important for mRNA decay. In addition, we have identified three putative RNase genes from *Arabidopsis* whose structure and expression characteristics resemble those of the S-gene RNases associated with self-incompatibility. The expression of these genes in *Arabidopsis*, which is self-compatible, suggests that these RNases have a more general function in RNA metabolism.

29 MOLECULAR ANALYSIS OF THE UBIQUITIN CONJUGATION PATHWAY IN HIGHER PLANTS. Richard D. Vierstra, Judy Callis, Tami Carpenter, Pierre-Alain Girod, Peggy Hatfield, Michael Sullivan, and Steve van Nocker. Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706

Ubiquitin is a highly conserved, 76-amino acid eukaryotic protein that has a variety of important regulatory roles as a result of its covalent attachment to other cellular proteins. Best characterized of these roles is as a reusable recognition signal for proteolysis. In an effort to determine the specificity and functions of the system, we have initiated a molecular characterization of the ubiquitin conjugation pathway from both wheat and *Arabidopsis*. Ubiquitin is encoded by a novel multi-gene family that synthesizes ubiquitin as protein fusions. At least eleven distinct ubiquitin genes have been identified in *Arabidopsis*. Conjugation requires ATP and is accomplished by a multienzyme pathway involving at least three families of enzymes: ubiquitin activating enzyme (E1), ubiquitin carrier protein (E2) and ubiquitin protein ligase (E3). Several different E1 genes have been cloned, all encoding ~120-kDa proteins. By site-directed mutagenesis of E1 genes expressed in *E. coli*, the cysteine residue required for catalysis has been identified. Over 12 different E2 proteins have been isolated by "ubiquitin affinity" chromatography and DEAE-HPLC and the corresponding DNA clones have been obtained for 5 of them. One E2 appears responsible for making the "multi-ubiquitin" chains necessary for targeting proteins to catabolism while another is structurally and functionally similar to the yeast DNA repair enzyme *RAD6*. Amino acid sequence comparisons of E2s have located conserved domains that may be important for function. Several of these domains have been confirmed as necessary by *in vitro* mutagenesis including a sequence of amino acids at the N-terminus, the active-site cysteine, and a domain at the C-terminus. The C-terminal domain appears to be required for target protein specificity such that transfer of this domain to heterologous E2s also transfers the ability to conjugate ubiquitin to specific substrate proteins. Work is now in progress to isolate E3s, which in combination with E2s are responsible for substrate specificity. Preliminary studies indicate that multiple forms of this enzyme exist in plants as well.

30 INTRON RECOGNITION IN MAIZE. Virginia Walbot, Kenneth Luehrsen and Juli Nash. Stanford University, Department of Biological Sciences, Stanford, California 94305-5020

Introns play a dual role in post-transcriptional regulation. Their positive role, intron enhancement, results in higher RNA levels from intron-containing compared to intronless genes (Callis *et al.* 1987 Genes & Development 1: 1187; Luehrsen & Walbot 1991 Mol. Gen. Genetics 225: 81). We speculate that a spliceable intron increases the efficiency of other post-transcriptional steps such as capping and poly(A) addition. Low splicing efficiency can decrease expression. Compared to fungi and animals, plants accumulate considerable unspliced transcript (up to two-thirds of *Bronze-2* transcript is unspliced (Nash *et al.* 1990 The Plant Cell 2: 1039)) suggesting that particular introns could exert either positive or negative regulation depending on cellular conditions. Using substitution, deletion and insertion mutants we are defining the nucleotide requirements for splicing and asking whether enhancement depends on splicing. We propose that splicing in maize is preceded by a novel step — intron recognition. Recognition is mediated by internal intron sequence as well as the exon:intron junctions. We propose that U-rich motifs found every 50-100 bases in the maize introns examined serve to demarcate the intron. We speculate that the splice junctions are defined after the recognition step at nearby 5' GT and 3' AG sites. This model could explain the absence of highly conserved motifs at the intron junctions and the ability of transposons to so readily alter splice site choice in maize.

31 INDUCTION AND DETERMINATION FOR FLOWERING. Susan R. Singer. Carleton College, Department of Biology, Northfield, Minnesota 55057

The production of a flower results from a sequence of developmental processes which can be regulated at unique control points in different angiosperms. Identifying these control points spatially and temporally is prerequisite to addressing mechanistic questions regarding these processes. In well studied systems, the transition of an apex from a vegetative to reproductive state begins when a leaf signal(s) induces a competent apex resulting in determination for flowering. A spatial and temporal investigation of acquisition of this developmental state in tobacco and pea indicates that determination can occur several nodes before flowers are produced. In tobacco, the terminal bud becomes florally determined; then, the terminal meristem initiates about three nodes before it is consumed in the production of a terminal flower. In pea, the terminal meristem never produces a flower. However, determination for inflorescence development occurs at the level of the meristem about eight nodes before the first floral node is initiated. Thus, this vegetative meristem becomes a stably determined inflorescence meristem, but never becomes determined to directly produce a flower. Mutations, including *floricaula* in snapdragon and *veg* in pea, appear to block the transition from inflorescence to floral meristems in axillary buds (Coen, E.S. et al. 1990 Cell 63:1311; Reid, J.B., Murfet, I.C. 1984 Ann. Bot. 53:369). As determination for inflorescence development is a stable developmental state in pea, mutants which block the transition from vegetative to inflorescence meristem should be obtainable. (Supported by USDA grants 87-1-2554 and 90-37261-5516.)

32 CHANGES IN GENE EXPRESSION DURING THE TRANSITION FROM VEGETATIVE TO GENERATIVE GROWTH IN THE LONG DAY PLANT Sinapis alba.

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Changes in gene expression during flower formation were studied in the long-day plant Sinapis alba. The day length dependence was exploited to synchronize flower formation in a large population of mustard plants. After an inductive light treatment leaves and apices were harvested after different lengths of time and changes in gene expression were analyzed.
Two different aspects of flower induction have been studied.
I. Changes of gene expression in leaves of induced plants have been measured that may be related to the release of the floral stimulus.
II. In apices two major groups of genes were identified whose expression was affected during flower formation. Transcripts of the first group began to accumulate strongly and transiently after the end of the inductive light period. Transcripts of the second group of genes could be detected first at the time when floral organs began to form. They disappeared again before the flower reached maturity.

33 REGULATED EXPRESSION OF CELL SURFACE MARKERS DURING FLORAL DEVELOPMENT. Keith Roberts, Luise Jannicke and Roger I. Pennell. John Innes Institute, Department of Cell Biology, Norwich NR4 7UH, U.K.

We have raised a family of monoclonal antibodies to oligosaccharide epitopes that are expressed on the outer face of plant plasma membrane. Some of these epitopes are virtually ubiquitous in their distribution, while other related ones show unusual and very precise developmental regulation in their pattern of expression. Three features of the patterns observed are 1. that expression is non-graded, being either on or off in a cell, 2. that it appears to be a function of cell position rather than of a present or future cell or tissue type, and 3. that it is highly labile. These features will be illustrated using JIM8, an antibody that recognizes an arabinogalactan protein whose expression is spatially regulated during root development in carrot and *Arabidopsis*. In *Brassica* floral development the same cell surface epitope appears and disappears in a complex manner in the male and female compartments of the flower. The epitope is present on both the sperm cell and the egg cell as well as on the endothecium, nucellar epidermis, synergids and embryo sac. The young embryo proper then loses the epitope, which is retained only in the suspensor. The significance of such complex regulation of cell surface carbohydrate markers will be discussed.

SYMPOSIUM SPEAKERS

34 THE CONTROL OF FLORAL ORGANOGENESIS BY HOMEOTIC MADS-BOX GENES IN *Antirrhinum majus* Zs. Schwarz-Sommer, P. Huijser, W. Nacken, H. Saedler and H. Sommer
Max-Planck-Institut für Züchtungsforschung, 5 Köln 30

Floral morphogenesis in *Antirrhinum majus* seems to be controlled by a set of homeotic genes[1]. Three of these genes (*deficiens*, *globosa*, *squamosa*) have been isolated and their structure and expression pattern determined. Mutations in *deficiens* and *globosa* lead to homeotic transformation of petals to sepals and stamens to carpels. These two genes are expressed at elevated levels in wildtype petals and stamens during the whole period of organogenesis. Mutational inactivation of *squamosa* causes the production of "shoots" instead of flowers in the axils of the bracts. *Squamosa* is transcribed prior to expression of *deficiens* and *globosa* and its expression is maintained in all floral organs except stamens.
The proteins encoded by the three genes are putative transcription factors: they have a conserved domain at the N-terminus in common that displays extensive homology to two known transcription factors, SRF of mammals and MCM1 of yeast. The conserved putative DNA-binding domain (the so-called MADS-box[1]) was found to be present in twelve additional genes, the majority of which is expressed only in floral organs, in a specific manner. We speculate that specific combinations of these MADS-box genes, expressed in the respective organs, specify organ identity in the process of organogenesis.
[1]Schwarz-Sommer et al. (1990) Science 250,931-936

35 EXPRESSION OF A BRASSICA S-LOCUS GENE PROMOTER IN TRANSGENIC PLANTS. M. K. Thorsness, K. Toriyama, T. Sato, M. K. Kandasamy, M. E. Nasrallah and J. B. Nasrallah. Cornell University, Section of Plant Biology, Ithaca, NY 14853.

The self-incompatibility response is a genetic barrier to self-fertilization in flowering plants. In many species this response is controlled by a single genetic locus, the S-locus, such that when pistil and pollen express identical S-alleles fertilization is blocked. Reporter gene fusions to a *Brassica* S-locus gene promoter were used to analyze the sites of S-locus gene expression in transgenic plants. A fusion of the S-locus gene promoter to the β-glucuronidase gene was introduced into *Brassica* and into the heterologous species *Nicotiana tabacum* and *Arabidopsis thaliana*. This gene fusion was expressed in specific tissues of the pistil and anther in all species, however the precise pattern and timing of expression differed between the three hosts. In addition, a toxic gene fusion was constructed by linking the S-locus gene promoter to the Diptheria toxin A gene. This fusion was used to genetically target cell killing to those floral tissues that express the S-locus gene promoter. The consequences of toxic gene expression were dramatic, and led to alterations in floral structure, development and fertility.

36 STRUCTURE AND EXPRESSION OF *S*-LINKED AND S-RELATED GENES IN *BRASSICA*. Martin Trick. Cambridge Laboratory. John Innes Centre, Norwich NR4 7UJ, UK

The self-incompatibility system of *Brassica* species is sporophytically controlled by a single, multi-allelic locus *S*. It is thought that *S* locus glycoproteins (SLGs), secreted by the stigma papillae cells, mediate the female part of the pollen-stigma interaction underlying the incompatibility response. The SLG gene is part of a multigene family of sequences, at least three of whose members (SLG, SLR1 and SLR2) are co-expressed in the stigma. The SLG is linked to *S* while SLR1 and SLR2 form a separate linkage unit. cDNA clones for an SLG and an SLR1 were isolated from a homozygous S_{29} plant and sequenced. These cDNA probes have been used to obtain genomic clones of each type from an S_{63} genotype. In addition, the sequence conservation of SLR1 alleles (as opposed to the extraordinary divergence of SLGs) has allowed their amplification from genomic DNA by inverse PCR. The evolution of SLG and *S*-related sequences will be discussed. SLR1 expression has been obtained in pollen and stigma of transgenic tobacco (like the SLG expression reported), indicating some specific recognition of the *Brassica* promoter in the heterologous host. The SLG-63 and SLR1-63 promoters are being compared to define these responsive sequences. Suitable SLG constructs are also being delivered into *Brassica* in order to test their proposed implication in the determination of incompatibility type.

37 THE RELATIONSHIP OF MAJOR PISTIL PROTEINS OF SOLANUM TUBEROSUM TO SELF-INCOMPATIBILITY. Helgard Kaufmann, Hans-Hubert Kirch, Thomas Wemmer and Richard Thompson. MPI fur Zuchtungsforschung, Carl-von-Linné-Weg 10, W-5000 Koln 30, FRG

A group of abundant basic polypeptides has been purified from pistils of diploid potato lines. Genetic analysis and N-terminal sequencing demonstrated that these included S-linked glycoproteins (SLGs) related in sequence to those described for other Solanaceous species. The corresponding genes have been isolated, and experiments are underway to re-introduce these into potato and tobacco plants via Ti-mediated transformation. No transcript homologous to SLG sequences could be detected in RNA preparation from developing potato anthers, mature or germinated pollen, either by Northern blot analysis or PCR. A self-compatible potato mutant has been analysed molecularly, and found to be capable of distinguishing in its action pollen carrying the S1-allele from other pollen grains. This locus is, however, not homologous to the S1-SLG. The results suggest that the S-locus-encoded pollen gene product may differ in sequence from the SLGs. A non-S-linked abundant pistil protein has been identified as a pistil-specific endochitinase, located in the transmitting tract tissue.

38 **FUNCTIONAL ANALYSIS OF POLLEN-EXPRESSED GENES.** Sheila McCormick, Judy Yamaguchi, Guy Vancanneyt and Keith Hamby. Plant Gene Expression Center, USDA-ARS, Albany,CA 94710

We want to understand the regulatory circuits that control differential gene expression in pollen, and we want to determine the roles that pollen proteins play during pollen maturation and germination. Towards this end, we have characterized several pollen-expressed genes (LAT52, LAT56 and LAT59) from tomato. We used both transient and stable expression assays to define cis -acting sequences of these promoters that are important for pollen expression. The LAT56 and LAT52 promoters share a common sequence element, as do the LAT56 and LAT59 promoters; these elements are important for quantitative promoter activity. We are examining protein interactions of these cis elements via gel retardation, in vivo DNA footprinting and southwestern assays. The LAT56 and LAT59 proteins show extensive sequence similarity to the ragweed allergen Amb a I and to a tomato pistil-expressed protein, and some similarity to pectate lyases, while the LAT52 protein shows some sequence similarity to proteinase inhibitors and to a maize pollen-expressed gene. Immunoblot analyses indicate that these LAT proteins are heat stable and probably located in the pollen wall. We are sequencing analogs of these genes from several other plant species, in order to further determine conserved features of the promoters and coding regions.

39 MOLECULAR ANATOMY OF THE SHOOT APICAL MERISTEM. Andrew Fleming, George Owttrim and Criş Kuhlemeier
Institute of Plant Physiology, University of Berne, Altenbergrain 21, CH-3013 Berne, Switzerland

The shoot apical meristem is a highly organized tissue that in a precisely determined spatial and temporal order (i) perpetuates itself and (ii) produces leaf primordia. Over the past two centuries numerous theories have attempted to describe and explain the phenomena of apical growth, differentiation and leaf pattern formation. We would like to understand the underlying molecular-genetic mechanisms. In order to define different cell types within the shoot apex we use gene probes as specific molecular markers. We carefully dissected 200 tomato apical meristems and from the resulting tissue (approx. 2 mg) constructed a cDNA library. Reasoning that many genes with interesting expression patterns and functions might not be expressed exclusively in the apex, we tested random clones from the library by Northern blotting and in situ hybridization. The first gene analyzed in this way, HC13, is highly expressed in the adaxial cells of leaf primordia and may be a good marker for primordia development. Additionally, we are determining the expression pattern of genes coding for proteins with known biochemical function. A gene for a lipid transfer protein is constitutively expressed in the meristem, but in primordia and young leaves, expression gradually becomes restricted to the epidermis. Two divergent genes for translation initiation factor eIF-4A are highly expressed in the apex; possible cell-specific expression within the apex is under study.

SYMPOSIUM SPEAKERS

THE FLORAL TRANSITION

40 D.Ry Meeks-Wagner, Mark Bonnlander, Carolyn Jacobs, Alan Kelly, Genevieve Reid, Susan Shannon and Michelle Zagotta.

University of Oregon, Institute of Molecular Biology, Eugene, OR 97405

We are using molecular and genetic approaches to understand shoot apical meristem function during the transition to flowering. The floral transition represents a distinct signal/response situation which influences the developmental potential of the apical meristem. One of the earliest responses of the meristem is to alter patterns of gene expression. These changes in gene expression are likely to facilitate the cessation of vegetative growth and the initiation of inflorescence and flower development. We are studying the changes in meristem specific gene expression in tobacco and in *Lolium temulentum* that accompany the floral transition in order to identify important genetic functions controlling meristem developmental potential, and to elucidate the molecular mechanisms which regulate the levels of meristem-specific gene transcripts. We are also analyzing mutants of *Arabidopsis* that display altered floral initiation. Recent results with these projects will be discussed in terms of meristem function and organization during the floral transition.

41 FATE MAPPING THE SHOOT APICAL MERISTEM IN ARABIDOPSIS. Vivian F. Irish[1] and Ian M. Sussex[2].
1. Yale University, Department of Biology, New Haven, CT 06511.
2. University of California, Department of Plant Biology, Berkeley, CA 94720.

We have assessed the relative contributions of cells in the dry seed shoot apical meristem to the differentiated tissues of the mature Arabidopsis plant. Individual meristematic cells were marked by mutation, and the resulting sectors were scored. These sectors represent the lineage derived from a single marked meristematic cell. The frequency and size of mutant sectors can be used in combination with histological analysis of the meristem to construct a fate map of the dry seed shoot apical meristem. This fate map reflects the probability with which a cell in the shoot apical meristem will give rise to a particular organ or tissue in the plant.

42 MOLECULAR CONTROLS OF QUIESCENT CENTER ACTIVITY IN THE ROOT MERISTEM OF CORN. Peter W. Barlow, Jesus Vicente and Paolo A. Sabelli. University of Bristol, Long Ashton Research Station, Bristol, BS18 9AF, UK.

The quiescent centre (QC) has at least four properties that define it as a stem-cell zone of roots: it is self-renewing, its rate of cell division is lov due to a prolonged G_0/G_1 phase, its cells are morphologically undifferentiated, and at least some of its cells can be recruited to the proliferative pool when surrounding meristematic cells are depleted. We are probing the molecular controls of these processes and comparing hov the molecular species of the QC differ from those of neighbouring meristematic and differentiating cells. QCs have been isolated from corn roots. The unfractionated RNA extracted from them has then been transcribed into cDNA and amplified by PCR. Probes to the QC cells cycle in either its quiescent or proliferative state include oligonucleotides partially homologous to yeast genes *cdc2*, *cdc25* and *wee1* and also the animal cyclin genes. We have also explored the molecular nature of the niche occupied by the QC. This enables us to say whether the QC is an actively-induced property through inhibition, or whether, more simply, it is a neutral poise due to lack of some essential inducer of the $G_0/G_1/S$ transitions. This latter approach defines, in molecular terms, a position dependent-morphogen important for root development.

43 PROPERTIES OF TWO LEA PROTEINS IN RELATION TO THEIR FUNCTIONS IN STRESS TOLERANCE. Leon Dure III, Department of Biochemistry, University of Georgia, Athens, GA 30602

Lea proteins are Late Embryogenesis Abundant. A subset of these proteins also becomes abundant in tissues undergoing stresses that involve the loss of water (dehydration, chilling, salt). Thus a function for protecting cells during periods of water loss can be entertained for this subset. Other Lea proteins may be involved in the acquisition of desiccation tolerance (the extreme form of water stress), in preventing vivipary or in other aspects of the late stages of seed formation. Some Lea proteins can be induced into abundancy by exogenous ABA, even in non-seed tissues not experiencing stresses.

Two Lea proteins from cotton have been singled out for study based on the fact that their amino acid sequences predict structures that, if verifiable, may predict their function. Further, the two proteins are foils in that one (D-7) can be induced in non-seed tissue by ABA or water stress, whereas the other (D-113) is not. D-7 most likely exists as an amphiphilic α helix, whereas D-133 most likely is largely a random coil. For purification purposes, both proteins are being produced in *E. coli* from full length cDNAs in pET-3 plasmids. Polyclonal antibodies to each protein have been used to examine cell type specificity or abundancy of the two proteins in embryos. These antibodies are also being used to measure absolute amounts of the protein in staged embryos and in wilting leaves via rocket electrophoresis. Biophysical data indicates that D-7 exists as a dimer, which suggests that it forms a coiled coil by means of its apolar helical stripe. The D-7 helical regions are composed of a tandemly repeating 11-mer amino acid sequence that is also found in D-7 homologs of other species, found in other Lea proteins in less stringent form and in a desiccation-induced protein from the "resurrection plant." The random coil nature of D-113, on the other hand, suggests a "reverse chaperone" role for this protein in highly desiccated cells.

Other structure/function aspects of these proteins will be presented.

44 GENETIC AND MOLECULAR CHARACTERIZATION OF EMBRYONIC MUTANTS OF ARABIDOPSIS THALIANA. David W. Meinke, Linda A. Castle, Deena Errampalli, Joe Shellhammer, and Linda H. Franzmann. Oklahoma State University, Department of Botany, Stillwater, OK 74078. Embryonic mutants of Arabidopsis thaliana represent a valuable tool in the genetic dissection of plant embryogenesis. More than 180 recessive mutants with a wide range of embryonic defects have been isolated and characterized in our laboratory. Some of these mutants are likely to be defective in housekeeping functions not directly related to the regulation of embryogenesis. One such mutant, a biotin auxotroph that becomes lethal during embryo development, has provided valuable information on the pathway of biotin synthesis in plants. Our current model is that bio1 is defective in conversion of 7-keto-8-amino-pelargonic acid to 7,8-diaminopelargonic acid. Several pattern mutants with phenotypes indicative of defects in regulatory genes have also been analyzed and mapped in preparation for gene isolation through chromosome walking. We have already mapped over 20 embryonic mutants relative to visible markers and have constructed tester lines to facilitate mapping a large number of genes with essential functions during plant embryogenesis. We have also isolated and characterized over 75 embryonic mutants of Arabidopsis generated by Ken Feldmann at Du Pont following Agrobacterium-mediated seed transformation. Many of these families contain multiple T-DNA inserts and rearrangements that have complicated genetic analysis and molecular recovery of tagged genes. In addition, a significant number of these mutations do not appear to be caused by T-DNA insertion. A major focus of current research in our laboratory is therefore to eliminate extraneous inserts in these families, identify mutants that are tagged with T-DNA, and use plasmid rescue and inverse PCR to clone flanking plant sequences. Recent advances in this area will be discussed.

45 GENETIC ANALYSIS OF PATTERN FORMATION IN THE *ARABIDOPSIS* EMBRYO. G. Jürgens, U. Mayer, R.A. Torres Ruiz, T. Berleth & S. Misera. Universität München, Institut für Genetik, D-8000 München 19, Fed.Rep.Germany

Little is known about the mechanisms that generate the basic body pattern in plant embryogenesis. As a first step towards the analysis of pattern formation, we have isolated 250 putative pattern mutants in the flowering plant, *Arabidopsis thaliana*. A large-scale screen for morphologically abnormal seedling mutants yielded about 250 lines for further study, and genetic evidence suggests saturation of the genome for this kind of mutation. The phenotypes of putative pattern mutants fall into distinct categories, classes and groups, which may reflect specific aspects of embryonic pattern formation. Mutant seedling phenotypes result from abnormal development in the early embryo. The implications of our findings are discussed.

SYMPOSIUM SPEAKERS

46 PATTERNS OF mRNA ACCUMULATION IN DEVELOPING *BRASSICA NAPUS* EMBRYOS. Donna E. Fernandez[1] and Martha L. Crouch[2]. [1]Dept. of Botany, University of Wisconsin, Madison, WI 53706. [2]Dept. of Biology, Indiana University, Bloomington, IN 47405.

Information about early events in embryogeny has been gained by studying patterns of mRNA accumulation in developing rapeseed embryos. Using *in situ* hybridization techniques and probes derived from cDNA clones of napin and cruciferin, the major storage proteins of *Brassica napus*, we found that these mRNAs accumulate in a variety of cell types, but at distinctly different times in different regions of the embryo. Napin mRNAs begin to accumulate in the cortex of the axis during heart stage, in the outer faces of the cotyledons during torpedo stage, in the inner faces of the cotyledons during cotyledon stage and in the shoot apex during maturation stage. Cruciferin mRNAs accumulate in a similar pattern but approximately 5 days later. Cells in the apical regions where root and shoot meristems develop do not accumulate storage protein messages during early stages of embryogeny. In the upper axis, the boundary between these apical cells and immediately adjacent cells that accumulate napin and cruciferin mRNAs is distinctly linear. Our analysis indicates that this boundary is not related to differences in tissue or cell type, but appears instead to be coincident with the site of a particular set of very early cell divisions. This pattern suggests that cells at the apex are set aside at an extremely early stage and function as a distinct entity, at least in terms of accumulation of storage protein mRNAs, until the shoot apical meristem is fully organized. In order to look at this process in greater detail, molecular probes for gene activity during very early stages of embryogeny will be necessary. As a first step, we are currently isolating mRNAs from globular and transition stage zygotic embryos and will use them to generate a cDNA library.

47 GENE REGULATION DURING THE TRANSITION FROM EMBRYOGENY TO POSTGERMINATION. John J. Harada[1], Robert A. Dietrich[1], James Z. Zhang[1], Debbie Laudencia-Chingcuanco[1], Kelly L. Matsudaira[1], Lucio Comai[1], and Sharon E. Radke[2], [1]Department of Botany, University of California, Davis and [2]Calgene Inc., Davis, CA 95616.

To obtain information about the processes that operate during the transition from embryogeny to postgerminative growth, we are studying the regulation of genes from *Brassica napus* L that encode the storage proteins cruciferin and napin, a late embryogenesis-abundant protein, the glyoxylate cycle enzymes isocitrate lyase and malate synthase, and an mRNA that accumulates during the early stages of cortical cell differentiation in roots. Several of these genes are expressed in both embryos and seedlings, suggesting that the gene products may be involved in similar processes at both stages of development. We will describe studies that provide information about the transcriptional regulation of these genes and discuss the implications of our results to the regulatory processes that operate during plant development. This work is supported by a grant from the National Science Foundation.

48 OVERPRODUCTION OF A MOLECULAR CHAPERONE HOMOLOG IN MAIZE ENDOSPERM MUTANTS. Rebecca S. Boston, Elizabeth P. B. Fontes, Brian B. Shank, Russell L. Wrobel and Fan Zhang. North Carolina State University, Department of Botany, Raleigh, North Carolina 27695

The maize b-70 protein is a homolog of the mammalian immunoglobin-binding protein, BiP. Levels of b-70 are increased in an endosperm-specific manner in the zein regulatory mutants *floury-2*, *Defective endosperm-B30*, and *Mucronate*. In cell cultures, b-70 is induced by treatment with the glycosylation inhibitor, tunicamycin. Purified b-70 migrates as two 75kD polypeptides of pI 5.3 and 5.4. The pI 5.3 form of b-70 is post-translationally modified by phosphorylation and ADP-ribosylation. The increases in b-70 are due in part to increases in the level of b-70 mRNA. In the three endosperm mutants that have altered b-70 levels, protein bodies are abnormal. Immuno-localization of zeins demonstrates an altered arrangement of zeins within these protein bodies. The physical similarity of b-70 to known molecular chaperone proteins and its association with abnormal protein accumulation in endosperm mutants may reflect a biological function to mediate protein folding and assembly in maize endosperm.

49 THE ROLE OF VP1 IN INTEGRATION OF HORMONAL AND DEVELOPMENTAL SIGNALS IN SEED MATURATION. Donald R. McCarty, Vimla Vasil, Tsukaho Hattori, Leonard Rosenkrans, Christian B. Carson and Indra K. Vasil. Vegetable Crops Dept. University of Florida, Gainesville, FL. 32611.

Mutations in the *viviparous-1* (*vp1*) gene specifically block maturation and anthocyanin biosynthesis in the developing maize seed. These effects are attributed to a seed specific insensitivity to abscisic acid. We have shown that the protein encoded by *Vp1* is a novel transcription factor. To address the role of VP1 in transcriptional regulation and its interaction with ABA, we have used transient over-expression of VP1 in protoplasts to study activation of two downstream target genes, the Em LEA gene of wheat and the *C1* regulatory gene of the anthocyanin pathway. The Em and *C1* promoters exhibit markedly different responses to VP1 and ABA alone and in combination. Furthermore, mutations in *vp1* can differentially effect regulation of these genes. For example, the product of the *vp1-Mc* mutant is an activator of Em transcription, but a weak inhibitor of *C1*. This is consistent with the colorless/dormant phenotype of the *vp1-Mc* mutant. The apparent functional complexity of VP1 is discussed in terms of a model for its role in integrating hormonal and developmental signals in the seed.

50 HIERARCHICAL CONTROL OF SEED PROTEIN GENE EXPRESSION Molly A. Bogue, Jeevalatha Vivekananda and Terry L. Thomas
Texas A&M University, College Station, Texas 77843

A 2.4 kb upstream region of a sunflower gene encoding the seed protein helianthinin confers rigorous developmental expression to a β-glucuronidase (GUS) reporter in transgenic tobacco seeds with no detectable GUS activity in non-embryonic tissues. Discrete elements of the helianthinin upstream regulatory ensemble (URE) confer novel regulatory patterns when analyzed outside the context of the complete sunflower regulatory complex; these include *cis* regulatory elements that confer ABA-responsive gene expression in vegetative tissues. In the full-length helianthinin URE, these elements only respond to ABA in the developing seed suggesting that the helianthinin gene contains additional regulatory elements that ensure hierarchical control in the developing seed. In contrast, a 1.5 kb 5' upstream region of the carrot *lea*-class gene *Dc*3 drives GUS expression in developing transgenic tobacco seeds and also is expressed in vegetative tissues in response to ABA and desiccation. The global organization of the sunflower helianthinin genes and the *Dc*3 gene appear quite similar. Current data suggests that embryonic specification is maintained by promoter proximal regions of the *Dc*3 and helianthinin genes and that more distal regions have a modulatory function, including response to fluctuations in ABA concentrations. We speculate that the structure of the helianthinin and *Dc*3 UREs may reflect a general pattern in highly expressed genes of domesticated plants.

51 CONTROLLING ETHYLENE SYNTHESIS AND RIPENING WITH ANTISENSE GENES. Don Grierson, AFRC Research Group in Plant Gene Regulation, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, U.K.

Ripening involves alterations in colour, flavour, texture and aroma and in climacteric fruits these changes are stimulated by ethylene. A number of genes expressed during tomato ripening have now been identified, including those encoding polygalacturonase, pectinesterase, phytoene synthase, ACC synthase, ethylene forming enzyme (EFE), a putative membrane channel and a heat-shock-related protein. The development of antisense techniques has played an important part in the assessment of gene function during ripening and also made it possible to produce directed mutations for the improvement of fruit quality. A cDNA for tomato EFE has been identified by antisense techniques and the identification confirmed by expression in yeast. Transgenic tomatoes homozygous for EFE antisense genes produce very little ethylene. When picked mature green these fruit slowly turn yellow but ripening is attenuated. The normal rate of ripening is restored by adding ethylene externally. The effect of reduced ethylene production on the expression of other ripening genes will be discussed.

SYMPOSIUM SPEAKERS

52 ROLE OF CELL WALL HYDROLASES IN FRUIT RIPENING, Alan B. Bennett, Coralie C. Lashbrook, Thomas Moore, Jamie Stotz and Robert L. Fischer, Department of Vegetable Crops, University of California, Davis, CA 95616 and Plant Biology Department, University of California, Berkeley, CA 94720.

Degradation of cell wall polymers plays an important role in fruit ripening. However, the complexity of the cell wall hinders precise predictions as to which polymers contribute significantly to the changing rheological properties that accompany fruit ripening. To dissect the processes that modify cell wall structure in ripening fruit we have cloned cell wall hydrolase genes and modified their expression in transgenic fruit. These experiments have provided the basis for critically assessing the role of polygalacturonase in pectin degradation and have directed our attention to a non-catalytic subunit of polygalacturonase isozyme I as a potentially critical component of the physiologically relevant form of polygalacturonase *in vivo*. These experiments have also suggested that cell wall hydrolases, other than polygalacturonase, contribute significantly to fruit softening. Likely candidates in this regard are the endo-β-1,4-glucanases, commonly referred to as cellulases, that may degrade hemicelluloses *in vivo*. Multiple cDNA clones encoding endo-β-1,4-glucanases have been isolated from tomato and their expression analyzed in relation to fruit development and in relation to other plant development processes. Analysis of tomato fruit expressing an antisense gene corresponding to one endo-β-1,4-glucanase will be presented.

53 REVERSIBLE INHIBITION OF TOMATO FRUIT RIPENING BY ANTISENSE 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE RNA.[1,2,3,4] Paul W. Oeller, William H. Rottmann, Lu Min Wong, Gary F. Peter, Loverine P. Taylor, Julie A. Keller, Nancy F. Shen, Brian P. Nagy, Deborah A. Pike, Alan D. Campbell & Athanasios Theologis. Plant Gene Expression Ctr, USDA-ARS, 800 Buchanan St., Albany, CA 94710 & Dept. of Plant Biol., UC Berkeley, 94720

The key regulatory enzyme in the biosynthetic pathway of the plant hormone ethylene is 1-aminocyclopropane-1-carboxylic acid (ACC) synthase. It catalyzes the conversion of AdoMet to ACC, the precursor of ethylene. We isolated complementary DNA sequences, ptACC2 and ptACC4, for two distinct and differentially regulated ACC synthase mRNAs expressed in ripe tomato fruit. The authenticity of the clones has been confirmed by expression experiments in *E. coli*. The predicted size of the encoded polypeptides (54,690 and 53,519 Da) is similar to that of the primary *in vitro* translation products and to the proteins found *in vivo*. Expression of antisense ptACC2 RNA inhibits tomato fruit ripening. The inhibition is reversible by exogenous ethylene or propylene. The sequence of the gene encoding one mRNA, LE-ACC2, has been determined, and its transcription initiation site defined. Four additional genes, LE-ACC1A, LE-ACC1B, LE-ACC3 and LE-ACC4, have been identified and the sequence of their coding regions determined. The LE-ACC1A and LE-ACC1B genes are adjacent to each other and are convergently transcribed. Their encoded polypeptides are 96% identical; the identity of the other polypeptides to each other varies between 50-70%. The proteins predicted to be encoded by the ACC synthase genes so far cloned from tomato and zucchini contain eleven of the twelve conserved amino acid residues found in various aminotransferases involved in the binding of the substrate and the cofactor pyridoxal-5'-phosphate. The data indicate that ACC synthase is encoded by a divergent multigene family in tomato that encodes proteins related to aminotransferases. Futhermore ethylene is indeed the hormone that controls fruit ripening and not the biproduct of ripening.

(1) Sato, T. & Theologis, A. (1989) *PNAS* 86, 6621-6625.
(2) Sato, T., Oeller, P.W. & Theologis, A. (1991) *J. Biol. Chem.* 266, 3752-3759.
(3) Huang, P.L., Parks, J.E., Rottmann, W.H. & Theologis, A. (1991) *PNAS* in press.
(4) Rottmann, W. H., et. al. (1991) *J. Mol. Biol.* submitted.

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54 TOMATO FRUIT DEVELOPMENT AND EXPRESSION OF HYDROXYMETHYL GLUTARYL COA REDUCTASE. Wilhelm Gruissem, Jon Narita, Susan Dean and Glenda Gillaspy. Department of Plant Biology, University of California, Berkeley, CA 94720

A large production of mevalonic acid (MVA) by the enzyme hydroxymethyl glutaryl CoA reductase (HMGR) is required at two phases of fruit development, early during fruit growth and in ripening fruit for the synthesis of lycopene. We have identified and cloned four different genes for HMGR in tomato, which are differentially expressed during fruit development. *Hmg1* gene activity is required early in development, indicating that HMGR1 is central to the sterol biosynthesis pathway. The promoter of the *hmg1* gene has two conserved sterol regulatory elements, which bind proteins in nuclear extracts from young fruit. The activation of *hmg2* coincides with the accumulation of lycopene in chromoplasts, suggesting that the subcellular location of HMGR2 differs from HMGR1. We have demonstrated that mevinolin, which blocks HMGR activity, interferes with both fruit growth and lycopene accumulation, thus establishing the requirement of MVA synthesis for fruit development. Arachadonic acid, which in animal cells is regulated by receptor-stimulated lipid hydrolysis, has a significant effect on *hmg* expression in young fruit, but not mature fruit. The levels of *hmg1* mRNA decreases to non-detectable levels, while both *hmg2* and *hmg3* mRNAs accumulate. We are currently testing the effects of over- and anti-sense expression of *hmg* on tomato fruit development. Experiments are underway to determine the spatial distribution of the individual *hmg* mRNAs, as well as the subcellular location of HMGR1 and HMGR2 in tomato fruit.

55 GENE-TECHNOLOGICAL MANIPULATION OF FATTY-ACID UNSATURATION: DESATURASE AND ACYLTRANSFERASE. Norio Murata. National Institute for Basic Biology, Okazaki 444, Japan.

Quality of plant lipids, including plant oils, depends on the level of unsaturation and the length of carbon chain of the constituting fatty acids. Desaturase and glycerol-3-phosphate acyltransferase determine the level of fatty-acid unsaturation. The fatty-acid desaturation of plants and cyanobacteria is unique in that fatty acids are desaturated while they are bound to glycerolipids. This type of desaturation is termed "acyl-lipid desaturation" or "plant-type desaturation". We have cloned, from the cyanobacterium, Synechocystis PCC6803, a gene (desA) for acyl-lipid desaturation at the delta-12 position of fatty acids. With use of this gene, we have cloned several genes for fatty-acid desaturation from cyanobacteria and higher plants. Transformation with the desaturation genes has enabled us to produce cyanobacterial mutants having different levels of fatty acid unsaturation. It has been demonstrated that the low-temperature sensitivity can be modified with this transformation. Glycerol-3-phosphate acyltransferase in chloroplasts determines the level of saturated molecular species of phosphatidylglycerol, which, according to our hypothesis, is related to the low-temperature sensitivity of higher plants. We have transformed tobacco plants with cDNAs for this enzyme from Arabidopsis and squash. The level of saturated molecular species of phosphatidylglycerol in the transgenic tobacco has been changed with the introduction of cDNA from the different plants.

56 MANIPULATION OF THE STARCH COMPOSITION IN ROOT AND TUBER CROPS. Richard G.F. Visser, Elise Flipse, Anja G.J. Kuipers, Shah N.I.M. Salehuzzaman and Evert Jacobsen. Agricultural University Wageningen, Department of Plant Breeding, PO Box 386, 6700 AJ Wageningen, The Netherlands.

Starch is the major storage carbohydrate in plants, it is formed in amyloplasts and consists of amylose and amylopectin. In maize and potato a large number of enzymes involved in starch synthesis have been identified and a number of them have been cloned. Also in cassava, like potato a vegetatively propageted polyploid, these enzymes are present. Despite the fact that identical enzymes and organels (amyloplasts) are present the properties of starches from different sources vary considerably and the chemical and physical characteristics are typical to the biological origin. The differences in starch characteristics might be due to the differently regulated genes or isoenzymes of the enzymes involved in starch biosynthesis, as well as to the organ in which starch is deposited (tuber in potato, tuberous root in cassava). In order to be able to manipulate the starch composition and/or content in these plants the regulation of the different enzymes and genes needs to be known. We have analyzed expression patterns in cassava and potato of two genes involved in the final step of the starch synthesizing process; granule-bound starch synthase (GBSS) and branching enzyme (BE). GBSS is responsible for the formation of amylose, BE for the formation of amylopectin. The expression of these genes was not only studied in the organs where starch is deposited, but also in leaves and stems. We have furthermore been able, in potato, to interfere with the amylose/amylopectin content using antisense genes of GBSS and BE.

57 REGULATION OF STARCH SYNTHETIC GENES OF MAIZE. L. C. Hannah, J. Bae, J. Baier, M. Clancy, M. Giroux, J. Lee, L. Ingham, J. Shaw and C. Boyer*, University of Florida, Vegetable Crops Department, Gainesville, FL 32611 and *Penn State University, Department of Horticulture, University Park, PA 16802

The synthesis of starch in the endosperm of maize is of interest for several reasons. Approximately 70% of the weight of the maize seed is starch, and there exists a wealth of genetic variation in the genes expressed in this pathway. In addition, the genes expressed have been shown to exhibit a high degree of tissue specificity. At least eight genes in this path have been cloned. ADP-glucose pyrophosphorylase, an allosteric enzyme which is possibly rate limiting, is encoded by the two unlinked genes *shrunken-2* (*Sh2*) and *brittle-2* (*Bt2*). The enzyme is a tetramer composed of subunits encoded by these two genes whereas in *E coli*, studies from other laboratories have shown that there is only one structural gene. Both maize genes show sequence similarity to the *E coli* gene suggesting that the complementary genes *Sh2* and *Bt2* arose via a gene duplication. Analysis of *sh2* and *bt2* mutations reveals that alternative RNA splicing or alternative transcription termination giving rise to multiple transcripts is a common type of mutation. Expression of *Sh1*, *Wx*, *Sh2* and *Bt2* is maximal early in endosperm development (12 to 14 days post-pollination under Florida conditions) as judged by steady-state transcript levels per total RNA. Mutation at one starch synthetic gene changes this developmental profile such that transcript levels decrease at a much slower rate during endosperm development. This points to the existence of a regulatory network that is important in the expression of these genes. Sequences important in the control of these genes, some of which lie within the gene, will be also discussed.

SYMPOSIUM SPEAKERS

58 ELUCIDATION OF *IN VIVO* REGULATION OF PLANT FATTY ACID BIOSYNTHESIS BY EXAMINING THE POOLS OF ACETYL-CoA, MALONYL-CoA AND THE ACYL-ACP INTERMEDIATES.

D. Post-Beittenmiller[1], J.G. Jaworski[2], P.G. Roughan[3], and J.B. Ohlrogge[1]. 1. Michigan State University, Department of Botany and Plant Pathology, E. Lansing, MI 48824-1312; 2. Miami University, Chemistry Department, Oxford, OH 45056; 3. Mt. Albert Research Center, Division of Horticulture and Processing, DSIR, Auckland, New Zealand.

In order to elucidate the *in vivo* regulatory steps of plant fatty acid biosynthesis, we examined changes in the chloroplast pools of acetyl-, malonyl- and free CoA and in the *in vivo* pools of acyl-acyl carrier protein (ACP) intermediates in spinach leaves. When light-grown spinach leaves are harvested, all of the saturated acyl-ACP intermediates are present. The level of malonyl-ACPs is lower than the levels of short and medium chain acyl-ACPs. During the shift from light to dark growing conditions, there is a rapid 5-fold increase in the level of acetyl-ACPs, which corresponds to a 6-fold decrease in the rate of fatty acid biosynthesis. In addition to the changes observed in acetyl- and malonyl-ACP levels, a previously unidentified acyl-ACP was detected in dark leaf. The unknown acyl-ACP is not detected in leaf samples harvested shortly after the lights were turned on again. The significance of this unknown acyl-ACP will be discussed. The TCA-soluble CoA pool in freshly isolated chloroplasts is low (27-30 μM), with virtually all of the CoA esterified to acetate. In chloroplasts incubated with acetate, the level of acetyl-CoA decreases transiently during a shift from light to dark, and returns to normal (light) levels after 10 minutes in the dark. Chloroplasts incubated in the light with Triton X-100 have increased rates of fatty acid biosynthesis, and under these conditions the levels of both malonyl-CoA and malonyl-ACP increase. These data together with previous work [J. Biol. Chem. 266: 1858-1865 (1991)] provide *in vivo* evidence that acetyl-CoA carboxylase is a regulatory step of plant fatty acid biosynthesis.

59 THE STRUCTURE AND DEPOSITION OF CEREAL SEED STORAGE PROTEINS. Peter R. Shewry. University of Bristol, AFRC Institute of Arable Crops Research, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, UK.

The prolamins of barley, wheat and rye have immense economic importance as they determine the nutritional and processing properties of the grain. They are complex polymorphic families of proteins that are characterized by the presence of highly repetitive sequences. A range of physico-chemical studies, including X-ray scattering and scanning tunnelling microscopy, have been used to demonstrate that these sequences may form an unusual spiral supersecondary structure, based on repetitive β-turns. These may in turn confer an extended rod-like structure to the whole molecule. Many prolamins are assembled into complex high M_r ($1x10^6$ to $1x10^7$) polymers, stabilized by interchain disulphide bonds. The control of disulphide bond formation in monomeric and polymeric prolamins is being studied by expression of wild type and mutant genes in heterologous hosts: yeast, cultured insect cells and the developing seeds of transgenic tobacco plants. Finally, the synthesis of prolamins and their deposition into protein bodies in the developing cereal grain is being studied by a combination of *in situ* hybridization and immunogold labelling.

60 SUBCELLULAR TRANSPORT OF WHEAT STORAGE PROTEINS AND THEIR DEPOSITION IN PROTEIN BODIES: SIGNALS AND MECHANISMS. G. Galili, Y. Altschuler, N. Rosenberg, M. Volokita*, R. Rubin, S. Giorini, R. Simon and H. Levanony, Department of Plant Genetics and Department of Biochemistry*, The Weizmann Institute of Science, Rehovot 76 100, Israel.

Wheat storage proteins are synthesized on membrane bound polysomes and are subsequently sequestered into the rough endoplasmic reticulum (RER). From the RER, these proteins continue a subcellular route ending by their deposition in dense protein bodies that are localized within storage vacuoles. Our laboratory is studying the subcellular route of wheat storage proteins by their analysis in developing wheat grains as well as by expression of individual storage protein genes in the surrogate systems of *Xenopus laevis* oocytes as well as transgenic yeast and plant cells. We find that individual storage proteins are transported by two different routes. One is the general route from the RER via the Golgi to storage vacuoles and the second is a direct aggregation of the storage proteins within the RER to form protein bodies that are then discharged into the cytoplasm and transported to storage vacuoles, by-passing the Golgi. The preferred route appears to be the direct aggregation within the RER while transport via the Golgi may occur only when a specific factor within the RER, that probably binds to newly synthesized storage proteins, is saturated. Site-directed mutagenesis analyses have shown that the signal for aggregation within the RER is found in the N-terminal repetitive domain of the storage proteins, while the signal for transport via the Golgi is encountered in the structure and specific folding of the C-terminal unique domain. The possible relationship between the function of wheat storage proteins in the protein body deposition process and their evolution will be discussed.

61 LEGUME SEED PROTEINS: GENE EXPRESSION AND ITS REGULATION. Ulrich Wobus, Istvan Nagy, Reinhard Panitz, Roland Schubert, Winfriede Weschke and Helmut Bäumlein. *Institute of Genetics and Crop Plant Research*, O-4325 Gatersleben, Germany.

Legumin-like and vicilin-like globulins provide the major nitrogen reserves for the dicot seedling but are also found in the seeds of some monocot grasses. They are encoded by multigene families and expressed coordinately during seed development. We have investigated four seed protein genes from faba bean and oat in transgenic tobacco plants. Final protein levels of legumin LeB4 depend on 5'flanking as well as on 5'untranslated sequences of the gene. *Cis* elements have been defined by deletion analysis which regulate the level and seed specifity of expression. The CATGCATG core element of the legumin box around position -100 is of central importance since its destruction alone reduces expression levels more then fifty fold and induces weak expression in leaves. Quasi constitutive expression is also induced by deletions upstream of the legumin box suggesting a complex mechanism which suppresses LeB4 promoter activity in non-seed tissues. An oat legumin-like gene, Asglo5, is also transcribed at relatively high levels in tobacco seeds but no protein is found. The distinct expression patterns of the four genes and their modes of regulation will be presented and discussed.

62 EXPRESSION OF MODIFIED SEED STORAGE PROTEINS IN *BRASSICACEAE*. Enno Krebbers, Patrick Rudelsheim, and Joel Vandekerkhove*, Plant Genetic Systems, Plateaustraat 22 and *Rijksuniversiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium

Plant seeds contain abundant quantities of storage proteins which appear to have no enzymatic function but serve as a source of nutrition for the geminating seed. Examination of sequences and other characteristics of one class of such proteins, the 2S albumins, suggested that it might be possible to modify the region between the sixth and seventh cysteine residues without disrupting the overall structure. Two classes of modifications have been made. In the first, sequences encoding biologically active peptides flanked by protease cleavage sites have been substituted into the 2S albumin. When they are separated from the other seed proteins, a simple step due to their solubility and size characteristics, the peptide can be recovered after cleavage with the appropriate protease. The yields obtained at laboratory scale using both *Brassica napus* and *Arabidopsis* suggest that hundreds of grams of peptide (depending on the size of the peptide) could be recovered per hectare of transformed canola plants. In the second class of modifications, additional methionine or lysine residues were inserted into the 2S albumin. The goal is to increase the content of these essential amino acids in Canola seed cake, making it more useful for livestock feed. For such applications the field and agronomic performance of such transgenic plants must be examined. Canola plants expressing related chimeric storage protein genes have now undergone field trials at various sites. Data has been collected concerning their field performance, yield, and the nutritional and industrial quality of the seed products and will be presented. Overall the results are encouraging, suggesting that expression of such genes has no undesired effects.

63 EXPERIMENTAL GENE TRANSFER SYSTEMS FOR PLANTS. Maud A.W. Hinchee, Joyce E. Fry, Patrica R. Sanders, Chuck L. Armstrong, William L. Petersen, David D. Songstad, Nancy L. Mathis, Yvonne M. Muskopf, Arlene R. Barnason, Jeanne G. Layton, David R. Corbin, Dannette V. Connor-Ward, Robert B. Horsch. Monsanto, 700 Chesterfield Village Parkway, St. Louis, MO. 63198.

Plant transformation is a complex process which involves many steps for gene transfer and plant regeneration. Improvements in the transformation of dicotyledonous and monocotyleonous crops are possible by addressing early transformation efficiency, selection efficiency and plant regeneration capacity. The Agrobacterium transformation of dicotyledonous crops, such as potato and canola, has been significantly improved by enhancing *Agrobacterium* transformation at low bacterial inoculums and by improved explant treatment methods. It has been possible to increase the absolute number of transgenic plants as well as decrease the number of "escape" non-transgenics by selectable marker modifications as well as regeneration response improvements. Improvements in the transformation of monocots have relied on improved gene transfer techniques, selectable markers and regeneration technology. The production of transgenic corn plants has been achieved by DNA bombardment technology of embryogenic cultures. Improved transformation efficiency assays, based on anthocyanin gene expression, have allowed for testing many different bombardment conditions for the optimization of corn transformation. Several different selection systems have proven useful for the production of transgenic corn plants containing the *Bacillus thuringenesis* insecticidal protein.

SYMPOSIUM SPEAKERS

64 HISTOCHEMICAL β-GLUCURONIDASE ASSAY WITH HIGH CYTOLOGICAL RESOLUTION.
Marc De Block and Dirk De Brouwer.
Plant Genetic Systems, Jozef Plateaustraat 22, 9000 Gent, Belgium.

The tissue and cell specific expression of plant genes can be studied by fusing their promoter to the GUS structural gene. The GUS gene codes for the enzyme β-glucuronidase, which can be demonstrated histochemically by using X-Gluc as substrate. X-gluc renders after hydrolysis by β-glucuronidase an insoluble, intense indigo-blue chromophore. However, the histochemical methods to demonstrate β-glucuronidase activity have up to now a poor resolution and are susceptible to artefacts.
We developed an enzyme histochemical method with a high cytological resolution and without loss of enzymatic activity. The principles of the technique are : no fixation, pretreatment with spermidine, partial dehydration with acetone and embedding in a water soluble glycol-methacrylate. The enzymatic assays are done on 3 to 10 µm sections. This technique was used to follow in transgenic rapeseed the 'cell'-specific regulation of the GUS gene under the control of a tapetum specific promoter. Succinate dehydrogenase activity was used as a standard for the metabolic activity of the cells. The presence of catalases and peroxidases in the plant cells, and an inefficient indigo-blue chromophore formation was studied in relation to the GUS assay.

65 HOMOLOGOUS RECOMBINATION AS A TOOL FOR PRECISE MODIFICATIONS OF PLANT GENOMES

Jerzy Paszkowski

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Integration of foreign genes into plant genomes has become routine. However, foreign DNA is integrated predominantly in random chromosome locations by the pathway of illegitimate recombination. The use of homologous recombination for foreign DNA integration, as well as homologous recombination within chromosomal DNA as a means of precise modification of plant nuclear genes, will be discussed.

66 GENE EXPRESSION DURING THE CELL CYCLE IN THE SYNCHRONOUS CULTURES OF CATHARANTHUS ROSEUS CELLS. Atsushi Komamine, Masaki Ito, Hiroaki Kodama and Naoto Ohnishi, Biological Institute, Faculty of Science, Tohoku University, Sendai 980, Japan

Several cell-cycle specific cDNAs were isolated in the two different synchronous cell division systems of *Catharanthus roseus* cells. In one system, synchronized cell division is induced by double phosphate-starvation method and in the other it is induced by auxin starvation and subsequent addition of auxin. One of the genes isolated from synchronous culture induced by double phosphate starvation method, which we refer as *cyc07*, was characterized in detail. *cyc07* mRNA was detected specifically in cells of S phase in both synchronous cell division systems. The induction and accumulation of mRNA in the S phase were suppressed when DNA synthesis was inhibited by aphidicolin. In the intact plant, *cyc07* mRNA was expressed in root tips that contained meristematic tissue. The nucleotide sequence of this cDNA indicated that the protein encoded by *cyc07* consisted of 245 amino acids. The amino acid sequence predicted from the nucleotide sequence is highly basic as are mammalian histones. It was revealed that the homologous gene to *cyc07* was present in the yeast genome. The expression and structure of this homologous gene is also reported. Another cDNA clone isolated from synchronous culture induced by auxin starvation, named as *cyc19*, was also characterized. The pattern of expression of *cyc19* was quite similar to that of *cyc07*. *cyc19* was expressed also in the S phase in the synchronous cultures. The nucleotide sequence had a similarity to that of heat shock protein 90 (hsp90). The function of this gene has not been identified clearly. These findings led us to hypothesize that hsp90 played an important role in the cell proliferation in higher plant cells.

67 PROGRESS IN REGENERATION AND GENETIC TRANSFORMATION OF WHEAT. Indra K. Vasil and Vimla Vasil. Laboratory of Plant Cell and Molecular Biology, Department of Vegetable Crops, University of Florida, Gainesville, FL 32611-0514, USA

Plants were regenerated by somatic embryogenesis from cultured immature embryos, cell suspension cultures and protoplasts of hexaploid bread wheat. Identification and maintenance of a rare type of embryogenic callus was found to be critical for the establishment of long-term regenerable callus as well as suspension cultures, the latter proving to be an ideal source for the isolation of regenerable protoplasts. Plated suspension cultures were bombarded with high velocity microprojectiles coated with plasmid DNA containing the NPTII, GUS and EPSP synthase genes. Putative transformed calli were selected on kanamycin and further identified by histochemical staining for GUS activity. Each of the three introduced genes were shown to be integrated into the genome of the transformed calli by Southern analysis. In addition, expression of each of the three genes was demonstrated by enzyme assays. In other experiments, embryogenic calli were bombarded with plasmid DNA containing the BAR and GUS genes. PAT activity was demonstrated in putative calli selected on the basis of GUS expression and growth on basta. Information on regeneration of plants and further analyses will be presented. These results once again highlight the significance of embryogenic cultures in cereal regeneration and genetic transformation. Supported by Monsanto Co. (St. Louis, MO), which also provided each of the plasmids used in the study.

68 TRANSFORMATION OF MAIZE. Discovery Research, DEKALB Plant Genetics, Eastern Point Road, Groton, CT 06340.

Fertile transgenic maize plants have been recovered using embryogenic suspension cultures as recipient cells for direct gene transfer by microprojectile bombardment (Gordon-Kamm et al.,1990,The Plant Cell 2:603). Cells were transformed with the bacterial gene bar and selected using the herbicide bialaphos. Southern blot analyses indicated that transformation occured as a single cell event. Recently, we have observed that particle penetration is limited to surface cell layers in embryogenic suspension cell clusters. Various bombardment parameters have been optimized for transient expression and recovery of stable transformants. We have studied patterns of cytodifferentiation in two transformable embryogenic suspension cultures, namely SC82 and SC716, to address several unaswered questions regarding recipient cells in maize suspension cultures: what are the cells that have allowed transformation and recovery of fertile transgenic maize plants by microprojectile bombardment; what role does developmental fate play during selection of stable transformants that retain morphogenetic competency; and, why has plant regeneration and recovery of fertile transformants from protoplast based systems remained such an unreliable process for maize?

69 TRANSGENIC RICE PLANTS AS A TOOL FOR STUDIES IN REGULATION OF MONOCOT GENES. Ko Shimamoto, Junko Kyozuka, Rie Terada and Akemi Ono. Plantech Research Institute, 1000 Kamoshida,Midori-ku, Yokohama 227 Japan

Transgenic plants are an important tool for studies in regulation of plant genes. Because some monocot genes are not expressed at all or expressed but not in a correct mannerin transgenic dicots it is desirable to establish a model monocot system to study expression of modified promoters. Rice is one such candidate since routine production of fertiletransgenic plants is possible. To examine this possibility we analyzed expression of three monocot promoters in transgenic rice plants and their progeny.
1. Maize Adh1: A close examination of the Adh1 promoter expression in transgenic rice showed that it is constitutively expressed in seed, root, flower and pollen. A strong anaerobic induction is found in seedling roots. These results closely parallel the known expression patterns of Adh1 in maize.
2. Wheat histone H3(in collaboration with Dr.M.Iwabuchi): Histone genes are expressed in S-phase specific manner in cell cycle. Rice plants transgenic for H3-gus showed that it is expressed in shoot and root meristems and that its leaf expression is strongly correlated with leaf age.
3. Rice rab16A(in collaboration with Dr.N.-H.Chua): The rab genes are induced by ABA and stresses. Transgenic rice plants carrying rab16A-gus showed that the rab promoter is induced by ABA, desiccation and salt in root, leaf and flower tissues.
These studies clearly indicated that transgenic rice plants are useful for the analysis of expression of promoters derived from rice and othermonocots We are currently analyzing in vivo functions of various upstream regulatory elements present in these promoters using transgenic rice plants.

SYMPOSIUM SPEAKERS

70 PARTICLE GUN TRANSFORMATION APPLIED TO COTTON. Dennis E. McCabe and Brian Martinell. Agracetus, Inc., 8520 University Green, Middleton, Wisconsin 53562

The use of high velocity particles to carry DNA into living cells and tissues allows access to a new range of explants for genetic transformation of plants. Among these are the meristems of germinated cotton seeds, which can be bombarded directly while still retaining their ability to develop into normal shoots. When leaf material from such shoots is analyzed for expression of transferred DNA, such as that encoding the marker enzyme, β-glucuronidase (GUS), we find GUS enzymatic activity in approximately 0.5% of the shoots. In most of these transformants, the entire upper portion of the plant expresses the new gene. Analysis of the inserted DNA and GUS enzymatic activity in tissues of progeny plants have confirmed the apparent integration into nuclear DNA and inheritance of the DNA as a dominant Mendelian trait. We have now used this procedure with equal success to insert new genes into a number of commercially important cotton genotypes, including Sea Island, Pima S-6, Delta Pine 90, and Delta Pine 50.

71 EXPRESSION PATTERNS AND ANALYSIS OF AN AUXIN-INDUCIBLE PROMOTER. Ron T. Nagao, Robert E. Wyatt, W. Michael Ainley, Joscelyn W. Hill, and Joe L. Key. Department of Botany, University of Georgia, Athens, GA. 30602. Two genes from Arabidopsis thaliana corresponding to the auxin-inducible genes Aux28 and Aux22 of soybean (Ainley et al., J. Biol. Chem. 263: 10658-10666, 1988) have been isolated (Conner et al., Plant Mol. Biol. 15: 623-632, 1990). These genes belong to a small multi-gene family and are similar to the soybean Aux gene family in deduced amino acid sequence of the proteins, intron/exon locations, and auxin-enhanced expression of their transcripts. Comparison of the promoter sequences of the soybean and Arabidopsis genes yielded no extensive sequence conservation: however, three regions of near sequence identity (Q, R, S) are present between the two Arabidopsis Aux genes. The regulatory properties of the promoter region of one of these genes, AtAux2-11, are being analyzed in transgenic Arabidopsis plants using deletion constructs containing fragments beginning at positions of approximately -3.0, -1.5, -0.6, -0.5, and -0.4 kb upstream of the transcriptional start site and all ending at position +109. In another set of experiments 70 base pair linker scan deletions and fragment replacement mutations from approximately -600 to -46 were also constructed and transformed into Arabidopsis. All of these promoter constructs were fused to the β-galactosidase reporter gene (lacZ) and assayed for control of expression. The five promoter deletions tested can confer tissue-specific expression patterns that are correlated with processes associated with auxin action. The promoter deletions were active at several developmental stages of S_2 transgenic plants, from seedling to flowering. β-Galactosidase assays performed on auxin-sprayed transgenic seedlings indicated that the five promoter deletions remained auxin-inducible, but basal activity and degree of induction vary between constructs. Little difference was observed between spatial expression patterns of auxin-sprayed and control plants.

72 REGULATION OF GENE EXPRESSION BY AUXIN. G.Hagen, T.Guilfoyle, Y.Li, M.Gee* and G. Martin. Departments of Biochemistry and *Biology, University of Missouri, Columbia, MO 65211

Auxin application has been shown to cause a rapid increase in the expression of specific sets of genes. We have used several approaches (RNA blots, in situ and tissue print hybridization and transgenic tobacco plants) to study the expression of several auxin-responsive soybean genes. Our results show that while these genes are all rapidly induced by auxin application, there are distinct differences among the sequences in the organ and tissue-localized expression in response to auxin. Differences are also observed in the endogenous level and localization of expression throughout the plant and during growth and development. Despite these differences, our results indicate that most, if not all, organs and tissues respond rapidly to auxin by expressing one or more of these genes. Previously, our studies showed that some of the auxin-responsive genes (SAURs) are responsive to gravity, and that during gravitropism, the transcripts turn-over rapidly. Using transgenic plants containing the SAUR promoter fused to the β-glucuronidase gene, we have shown that the gravity-responsive (and auxin-responsive) element is localized to the promoter. Other chimeric constructs have been used to show that the sequence(s) involved in the rapid turn-over of SAUR is contained within the coding region of SAUR. We have also used transgenic plants to study the effect of the expression of hormone biosynthetic genes under the control of auxin-responsive promoters. To date, the transgenic plants containing these fusion constructs show a variety of changes in phenotype.

73 REGULATION OF GENE EXPRESSION BY ETHYLENE DURING TOMATO (*Lycopersicon esculentum*) FRUIT RIPENING. Jill Deikman, Julie Montgomery, Linda Margossian, Lola Peñarrubia and Robert Fischer. Department of Plant Biology, University of California, Berkeley, CA 94720.

The plant hormone ethylene plays an important role in controlling fruit ripening. The onset of ripening of many fruits is initiated by increased levels of ethylene that, in turn, activates gene expression. To study this process we have cloned mRNAs that accumulate during fruit ripening and in unripe fruit exposed to exogenous ethylene. Genes that encode two of these mRNAs (E4 and E8) have been studied in detail and are rapidly transcriptionally activated by ethylene. We are currently studying E4 and E8 gene function by analyzing fruit from transgenic tomato plants bearing their respective antisense genes. To begin to understand how ethylene regulates E4 and E8 gene transcription we have mutagenized their 5'-flanking regions and have analyzed expression of these mutated genes in both stably transformed plants and in a micro-projectile transient assay system. These experiments indicate that short, discrete DNA sequences are responsible for transmitting the ethylene signal. We are currently analyzing nuclear proteins that interact with these regulatory DNA sequences.

74 CIS- ELEMENTS AND TRANS- ACTING FACTORS INVOLVED IN THE ABSCISIC ACID REGULATION OF EM GENE EXPRESSION. Ralph S Quatrano, Mark J. Guiltinan, William R. Marcotte, Jr. Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

The Em protein and its mRNA normally accumulate in mature wheat embryos. They can be induced to precociously accumulate in immature embryos by exposure to abscisic acid (ABA) in culture. Expression of Em is not confined to the embryo, since increases in the levels of ABA in seedlings or in suspension cultures results in the accumulation of Em mRNA. Fusions of the intact and deleted 5' promoter regions of the Em gene with the glucuronidase (GUS) reporter gene have been tested for ABA-dependent expression in transient assays using rice protoplasts and in transgenic tobacco plants. Results from both assays indicate that a 646bp region (-554 to +92) from the 5' untranslated region of the Em gene contains all the information needed for ABA regulation and embryo-specific expression. A detailed analysis of the sequences in the ABA response element [ABRE] involved in ABA-modulated regulation of chimeric genes in a transient assay reveals a 20bp sequence that can confer ABA-responsiveness on a non-responsive CaMV viral promoter. Nuclear extracts from embryonic cells contain protein factors which specifically bind to the ABRE, as evidenced by gel-shift and footprinting experiments. A cDNA clone (GC19) was isolated by screening a lambda gt11 expression library. The protein product of GC19 is a DNA binding protein (EmBP-1) of the bZIP class that interacts specifically with an 8bp sequence (CACGTGGC) in the ABRE. A 2bp mutation in this sequence prevents binding of EmBP-1 and reduces the ability of the ABRE to respond to ABA in the transient assay. The 8bp EmBP-1 target sequence is conserved in several other ABA-responsive promoters and in promoters from plant genes that respond to signals other than ABA, e.g. light. The deduced amino acid sequence of EmBP-1 contains conserved basic and leucine zipper domains found in other plant transcription factors of the bZIP class. Progress on the analysis of the role of EmBp-1 in the ABA response pathway of higher plants will be discussed.

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75 MOLECULAR ANALYSIS OF AN ARABIDOPSIS GENE INVOLVED IN ETHYLENE RESPONSES. Caren Chang, Anthony B. Bleecker, Shing F. Kwok and Elliot M. Meyerowitz.

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California Institute of Technology, Biology Division, Pasadena, California 91125

The plant hormone ethylene regulates a wide range of physiological processes during growth and development in higher plants. In order to begin to understand how ethylene is perceived and how the signal is transduced into the observed responses, we are focusing on cloning the ETR gene in Arabidopsis. The ETR locus is defined by a dominant mutation that causes insensitivity to ethylene, as evidenced by the absence of a variety of wild type ethylene responses. Ethylene binding studies on the mutant and wild type suggest that the ETR gene product may encode a receptor for ethylene. Our strategy for cloning the ETR gene has been to isolate genomic DNA clones from the dominant mutant by chromosome walking, and then to test the clones' ability to confer the mutant phenotype to wild type Arabidopsis when introduced by transformation. We have cloned over 100 kb of contiguous DNA that is genetically linked to the ETR locus. Wild type plants transformed with either of two overlapping cosmids from the walk display partial insensitivity to ethylene. From additional transformations, we have delimited the source of this phenotype to a 10 kb DNA fragment contained on both cosmids. Molecular analysis of this 10 kb DNA region has revealed a gene whose DNA sequence strongly suggests that it encodes a zinc finger of the type found in a number of transcription factors. Further analyses are in progress to determine whether this gene is indeed the ETR gene.

SYMPOSIUM SPEAKERS

76 GIBBERELLIN BIOSYNTHESIS AND ITS REGULATION. Jan A.D. Zeevaart and Manuel Talon. Michigan State University, DOE Plant Research Laboratory, East Lansing, MI 48824

Higher plants contain a large number of gibberellins (GAs) which can be arranged in parallel biosynthetic pathways. In these pathways, only 3ß-hydroxy C_{19}-GAs appear to be biologically active *per se*, while the others are either precursors or inactivated GAs (2ß-hydroxylated). Dwarf mutants that respond to GA by restoring normal phenotypic growth have played an important role in establishing GA biosynthetic pathways and in identifying the active GAs in these pathways. For example, the semidwarf *ga4* mutant of *Arabidopsis* is partially blocked in 3ß-hydroxylation. This mutant has reduced levels of the 3ß-hydroxy-GAs, GA_1 and GA_4, and accumulates the non-3-hydroxy-GAs, GA_9 and GA_{20}. Although applied GA_4 is more active than GA_1 in promoting stem growth in *Arabidopsis* seedlings, GA_4 is converted to GA_1.

In the long-day rosette plant spinach, GA_{19} accumulates under short-day (SD) conditions, whereas long days (LD) enhance its conversion to GA_{20} and GA_1. Results of experiments with feeds of radioactive GAs to plants in light or darkness as well as with cell-free systems indicate that the activities of GA_{53}-oxidase and GA_{19}-oxidase are lost quickly when spinach plants are transferred from light to darkness. Thus, the biochemical basis for photoperiodic control of stem growth in spinach when plants are exposed to LD is the increase in enzyme activities in the later part of the GA biosynthetic pathway, resulting in increased synthesis of bioactive GA_1.

77 ETHYLENE IN HIGHER PLANTS: A MOLECULAR-GENETIC APPROACH Dominique Van Der Straeten, Renato Alberto Rodrigues-Pousada, Jan Smalle, Howard M. Goodman*, and Marc Van Montagu. Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium); *Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

In the past years, there has been an increasing interest in understanding the molecular basis of plant hormone biosynthesis and signal transduction, a key to unravel fundamental aspects of plant development. The Laboratory of Genetics (Gent, Belgium) is interested in molecular physiology of ethylene, the smallest molecule with plant growth regulatory properties. One can address two basic biological questions concerning ethylene biology: (i) Which are the molecular mechanisms governing ethylene formation in plants? (ii) How can the plant perceive this 2-carbon-atom molecule and transfer the signal to result in the well-characterized responses? We have been trying to bring some insight to these questions. A first step in the molecular analysis of ethylene biosynthesis, was the cloning of genes encoding 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase), the key regulatory enzyme in ethylene formation. ACC synthase promoter/ß-glucuronidase fusions will allow to assess different induction conditions, and give information about aspects of temporal and spatial regulation of ACC synthase genes. The molecular studies of ethylene signal transduction involve the analysis of an ACC and ethylene-insensitive mutant in *Arabidopsis thaliana*, designated *ain1*. *Ain1* is being characterized physiologically and genetically. The long-term goal is cloning the *ain* gene using the combination of RFLP markers, with overlapping YAC and cosmid libraries.

78 AUXIN-BINDING PROTEINS AND THEIR POSSIBLE ROLES IN AUXIN-MEDIATED PLANT CELL GROWTH. Alan Jones and Paruchuri V. Prasad, Dept. of Biology, Univ. of North Carolina, Chapel Hill, NC 27599.

Like several other classes of hormones, the auxin class exert myriad effects on cell development. While auxins are most noted for inducing cell elongation, they also are involved in cell differentiation, cell and organ polarity, and wound responsiveness. Consistent with this pleiotropy, is the recent identification of several putative auxin receptors that, in theory, could represent the primary elements of more than one auxin signal pathway leading to distinct responses or leading in parallel to a single response. Our current working hypothesis is that some auxin-mediated responses may be mediated by multiple receptors. What is known about each of the new putative receptors is summarized and a hypothesis on the role of these receptors in cell elongation is presented. Specifically for the case of cell elongation, we proposed that two rapid events, specfic gene transcription and cell wall acidification, are separately mediated by at least two receptors, acting in the nucleus and at the plasma membrane, respectively.

79 TRANSPORT OF PROTEINS INTO AND ACROSS THE ENVELOPE MEMBRANES OF CHLOROPLASTS. Kenneth Keegstra, Barry Bruce, Yasuko Kaneko, Hsou-min Li, Jerry Marshall, Jennifer Ostrom and Sharyn Perry. University of Wisconsin, Department of Botany, Madison, Wisconsin 53706.

Proteins destined for several different chloroplastic compartments are synthesized in the cytoplasm before being transported to their proper location. Some cytoplasmically synthesized proteins are directed to the envelope membranes. One protein destined for the outer membrane (OM14) is synthesized as a precursor that is the same size as the mature form. It is inserted into the outer envelope membrane via an energy independent process that is distinct in several respects from the transport of other imported proteins. A protein destined for the inner envelope membrane (IM30) is synthesized as a higher molecular weight precursor and its transport shares several similarities with the transport of other imported proteins. The transport of these envelope proteins will be compared with that of stromal and thylakoid proteins, which must cross both envelope membranes. Results of our recent efforts to understand the mechanism of protein translocation across the two envelope membranes will be presented.

80 MECHANISM OF PROTEIN SORTING TO VACUOLES AND THE NUCLEUS OF PLANT CELLS. Natasha V. Raikhel, Sebastian Y. Bednarek, Martin R. Shroeder, Jim E. Dombrowski, Marguerite J. Varagona and Mark W. Shieh. MSU-DOE Plant Research Laboratory, East Lansing, Michigan 48824-1312.

Maintence of separate subcellular compartments in eukaryotic cells depends on the correct sorting and targeting of newly-synthesized proteins. In our laboratory we are investigating the molecular mechanisms of protein targeting to the vacuoles and the nucleus.

Proteins that enter the secretory pathway are either secreted or localized within several distinct subcellular compartments. Recently we have demonstrated that the carboxyl-terminal propeptide (CTPP) of probarley lectin is necessary for efficient sorting of this protein to the vacuoles of transgenic tobacco. To test whether the CTPP is sufficient to redirect a secreted reporter protein to the vacuoles we have prepared chimeric genes containing the CTPP of probarley lectin fused to reporter proteins and have transformed the constructs into plants. Analysis of the transgenic plants indicates that CTPP is sufficient to redirect a reporter protein to vacuoles. To analyze the functional elements of the CTPP which are necessary for proper sorting and processing, several mutants of the CTPP were prepared and have been analyzed in transgenic plants.

Proteins that function in the nucleus are synthesized in the cytosol and then are either transported or diffused into the nucleus through the nuclear pores. We are using a transgenic approach to examine the transport of two maize trans-acting factors: the opaque-2 (O2) and the R proteins. We have prepared chimeric genes containing the reporter gene encoding β-glucoronidase fused with the O2 and the R cDNA clones. Analyses of transiently expressed fusion proteins indicate that both trans-acting factors are capable of redirecting a reporter protein to the nucleus. In addition further characterization of in frame deletions of these proteins have determined putative nuclear localization sequences.

81 CHARACTERIZATION OF THE PROPEPTIDE OF A PRECURSOR TO SPORAMIN THAT IS REQUIRED FOR VACUOLAR TARGETING Kenzo Nakamura and Ken Matsuoka, Lab. of Biochem., Sch. of Agric., Nagoya University, Chikusa, Nagoya 464-01, Japan

Sporamin, a vacuolar protein in tuberous roots of sweet potato, is synthesized on rER as a non-glycosylated prepro-precursor with an N-terminal extra sequence. The propeptide that follows N-terminal signal peptide is composed of 16 amino acid residues. In transformed tobacco cells, the precursor to sporamin was sequentially processed from the N-terminus and correctly targeted to the vacuole (1). The propeptide of the precursor to sporamin is required for this targeting since deletion of the propeptide from the precursor results in almost complete secretion of sporamin into the medium (2). This N-terminal propeptide does not show homologies to the C-terminal propeptide of barley lectin that is required for targeting of this protein to the vacuole (3). However, sequences homologous to a part of the propeptide of sporamin are present in precursors to several other plant vacuolar proteins. Some of the amino acid-substitution and -deletion mutations in the propeptide of sporamin resulted in alteration of the site of cotranslational cleavage of the signal peptide. The site of cleavage in these mutant precursors jumped to a cryptic site located downstream of the propeptide and resulted in secretion of processed polypeptides. Another mutation in the C-terminal part of the propeptide produced a mutant precursor with uncleavable signal peptide that is removed only posttranslationally together with the propeptide. These results indicate that the propeptide of sporamin is important in defining the site of cotranlsational cleavage of the signal peptide, in addition to target sporamin to the vacuole.

(1) Matsuoka et al. J. Biol. Chem., 265: 19750-19757 (1990). (2) Matsuoka and Nakamura, Proc. Natl. Acad Sci. USA 88: 834-838 (1991). (3) Bednarek et al. Plant Cell 2: 1145-1155 (1990)

SYMPOSIUM SPEAKERS

82 PARTITIONING OF MALATE DEHYDROGENASE ISOENZYMES INTO GLYOXYSOMES AND MITOCHONDRIA. Christine Gietl. Lehrstuhl fur Botanik. Technische Universität München, Germany, and Department of Physiology, Carlsberg Laboratory, Copenhagen Valby, Denmark.

Glyoxysomal as well as mitochondrial malate dehydrogenase in watermelon cotyledons is synthesized as a higher molecular weight precursor on cytoplasmic ribosomes. This special feature is exploited to study the targeting of the two isoenzymes into their respective organelle.

Full-length cDNA clones for glyoxysomal and mitochondrial malate dehydrogenases have been isolated. The nucleotide sequences revealed the following features: Glyoxysomal malate dehydrogenase has homomeric subunits with a 320 amino acid long polypeptide chain (Mol.weight 33.400) while the mitochondrial isoenzyme subunit is one amino acid shorter (319 residues; Mol.weight 33.200). The primary structures of the two isoenzymes have 60% amino acid homology with strict conservation of the amino acids in the catalytic pocket and at NAD cofactor binding sites.

Glyoxysomal malate dehydrogenase is synthesized with a 37 amino acid long presequence which is cleaved off during import into the organelle. The mitochondrial counterpart has a 27 amino acid long presequence with no homology to the glyoxysomal transit peptide. A microbody tripeptide targeting signal PTS is absent from the mature glyoxysomal malate dehydrogenase but the related Ala-His-Leu sequence is present in the transit peptide. Comparison with the 26 amino acid long transit peptide of rat peroxisomal 3-ketoacyl thiolase identifies a glutamate and a cluster of serine residues at equal distance from the cleavage site as a putative microbody target signal. A PTS tripeptide (Gly-His-Leu) is found at a homologous position in the thiolase transit peptide.

Hybrid genes giving malate dehydrogenases which combine the mitochondrial transit peptide with the mature glyoxysomal polypeptide and the reciprocal combination have been constructed with the procedure of nucleotide splicing by overlap extension. These as well as in vitro synthesized peptides of the presequences and of discriminating domains of the mature subunit are used to determine organelle targeting signals in the two isoenzymes.

83 SUGAR CARRIERS OF THE PLANT PLASMA MEMBRANE. Norbert Sauer and Kerstin Friedlander. University of Regensburg, Universitatsstrasse 31, Department of Cell Biology and Plant Physiology, D-8400 Regensburg, Germany.

It has frequently been discussed to which extent specific carrier proteins of the plant plasma membrane could be involved in processes like the distribution of photosynthetically fixed carbon in higher plants. The fact that individual cells of a higher plant are separated by a large apoplastic space and connected by plasmodesmata, structures allowing the diffusion of a wide variety of substances from one cell to the other, has been used as argument against an important role of transport proteins. Using the unicellular green alga *Chlorella kessleri* as model organism and the classical method of differential screening we were able to isolate cDNA and genomic clones of the inducible monosaccharide carrier from *Chlorella*. Heterologous screening of an *Arabidopsis thaliana* genomic library with the *Chlorella* probe resulted in the identification of a family of four highly homologous genes encoding putative sugar transport proteins (STP1-STP4). The derived amino acid sequences are 20-30% identical to human or procaryotic sugar transporters and suggest 12 transmembrane helices for each of these proteins with the N- and C-termini on the cytoplasmic side of the plasmamembrane. Full length cDNA clones of some of these carriers have been further investigated using heterologous expression of their respective cDNAs in the fission yeast *Schizosaccharomyces pombe*. One of the clones could be characterized as an active H^+/monosaccharide cotransporter. This expression system had also been used for the identification of the *Chlorella* monosaccharide transporter. In this case the kinetic parameters determined for the protein expressed in *S. pombe* were undistinguishable from those in *Chlorella*

84 MOLECULAR MECHANISMS OF ABSCISIC ACID-INDUCED SIGNAL TRANSDUCTION IN GUARD CELLS. Julian I. Schroeder. Department of Biology, University of California San Diego, La Jolla, CA 92093-0116.

Hormonal and environmental stimuli regulate the gas exchange in plants by controlling guard cell movements. The molecular mechanisms by which abscisic acid (ABA) triggers stomatal closing in response to drought stress were investigated by patch clamp studies and recordings of changes in the cytosolic Ca^{2+} concentration. Abscisic acid was found to stimulate opening of Ca^{2+}-permeable channels. ABA-induced Ca^{2+} channel opening is controlled by cellular coupling mechanisms. Ca^{2+} channel activation leads to rises in the cytosolic Ca^{2+} concentration which in turn have previously been shown to activate voltage-dependent anion channels (Schroeder, J.I. and Hagiwara, S., Nature 338, 427-430, 1989). These anion channels have been suggested to play a central role in depolarizing guard cells, thereby initiating stomatal closing. New results show that two classes of anion channels exist. The different modes of second messenger and voltage-dependent regulation of these two types of anion channels suggest that they may be important during different phases of stomatal closing. These findings of primary events in signal transduction in guard cells give rise to a novel understanding of the molecular mechanisms involved in ABA-stimulated stomatal closing.

85 PLASMA MEMBRANE H^+-ATPase: IMMUNOCYTOLOCALIZATION AND HETEROLOGOUS EXPRESSION IN PLANTS AND YEAST
R. Serrano, A.Parets-Soler, J.M. Villalba, M.G. Palmgren, G.E. Berberian. EMBL, Meyerhofstr.1, 6900 Heidelberg, FRG

The plant plasma membrane H^+-ATPasedrives the active transport of nutrients and ions. Immunocytolocalization studies with specific polyclonal and monoclonal antibodies indicate that the enzyme is concentrated at the phloem, stomata guard cells, root epidermis and root endodermis and perycicle. This support the role of the enzyme in phloem loading with sucrose, stomatal opening by potassium accumulation, ion absorption by the root epidermis and active xylem loading at the root central tissues. In order to probe the physiological role of the enzyme we have attempted to generate transgenic plants with alterations on proton pumping. One approach is the expression of an hyperactive mutant of yeast plasma membrane H^+-ATPase under the control of the 35S viral promoter. Although good levels of yeast ATPase mRNA are produced in the transgenic plants, the level of protein is very low and is not located at the plasma membrane. The reciprocal experiment. expression of plant ATPase in yeast, resulted in high levels of expression of active enzyme. It could pump protons after reconstitution in liposomes and it could make a phosphorylated intermediate both from ATP and from Pi. However it was not localized in the yeast plasma membrane suggesting again mistargeting. Fusions between yeast and plant ATPases have also failed to be targerted to the yeast plasma membrane.

86 MOLECULAR STRUCTURE OF THE VACUOLAR H^+-PUMPING ATPASE. Heven Sze, John Ward and Shoupeng Lai. University of Maryland, Department of Botany, College Park, MD 20742.

The vacuolar H^+-pumping ATPase generates a proton electrochemical gradient that acidifies the vacuolar lumen and drives active transport of numerous solutes across the tonoplast. The H^+-ATPase from oat roots is a multi-subunit complex (650 kDa) that represents a major component of the tonoplast. It is composed of a large peripheral sector (540 kDa) that includes the 70 kDa catalytic subunit, the 60 kDa "regulatory" subunit, plus polypeptides of 44, 42, 36 and 29 kDa whose functions have yet to be determined. The peripheral part of the ATPase appear as knobs that occur in patches on the surface of tonoplast vesicles. The Triton solubilized and purified enzyme demonstrated electrogenic H^+ pumping activity in reconstituted proteoliposomes. Thus the purified and transport-competent ATPase consisted of 10 different polypeptides of 70, 60, 44, 42, 36, 32, 29, 16, 13 and 12 kDa. A major subunit of the membraneous sector is the N,N'-dicyclohexylcarbodiimide-binding 16 kDa. Six copies of the 16 kDa proteolipid per ATPase is thought to form the H^+ conductance pathway. The amino acid sequence of a 16 kDa proteolipid has been deduced from the nucleotide sequence of a cDNA from oat. It has four membrane-spanning domains in which region IV contained a glutamate residue, the putative DCCD-binding residue. Interestingly, the 16 kDa proteolipid in oat is encoded by a multigene family. The presence of a multigene family suggest the possibility of isoforms of the V-ATPase. Their expression, regulation and distribution will be crucial to understanding the role of vacuolar H^+-ATPases in the growth and development of plant cells.

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87 MOLECULAR BIOLOGY OF HIGHER PLANT HRGPs. Allan M. Showalter. Ohio University, Department of Botany, Molecular and Cellular Biology Program, Athens, Ohio 45701, USA

There are at least four classes of hydroxyproline-rich glycoproteins (HRGPs) in the cell walls of higher plants: 1) extensins, 2) certain lectins from the Solanaceae family, 3) arabinogalactan proteins, and 4) repetitive proline-rich proteins. In addition, another class of plant cell wall proteins, the glycine-rich proteins (GRPs), exists and may be related to the extensins. All of these proteins are thought to function as structural cell wall proteins and as such may also have important roles in development, wound healing, and disease resistance. This presentation focuses on the molecular cloning and characterization of the extensin and GRP gene families in tomato. To date, my lab has isolated and characterized two distinct classes of extensin cDNA clones and two distinct classes of GRP cDNA clones (see Plant Molecular Biology 16: 547-565, 1991). These classes are distinguished from one another based on their encoded DNA sequences and their respective RNA gel blot hybridization patterns obtained from unwounded and wounded tomato plant tissues. Using such cloned extensin sequences as probes, my lab has also isolated and characterized two genomic clones for extensin which correspond to the two extensin cDNA classes. One genomic clone, Tom L4, encodes an extensin containing multiple $SP_4SPSP_4TY_{1-3}S$ and $SP_{2-5}TPSYEHPKTP$ repeat units. The other genomic clone, Tom J10, encodes an extensin containing multiple $SP_4SPKYVYK$ and $SP_4SPSP_4Y_3K$ repeats. These cloned extensin and GRP sequences were used as probes in RNA gel blot hybridization experiments to monitor changes in extensin and GRP mRNA levels in response to various stress conditions, including wounding, pathogen infection, and drought stress. The observed stress-induced changes will be discussed in the context of the proposed functions for the extensins and GRPs.

SYMPOSIUM SPEAKERS

88 ptGRP1 IS BOTH A SPECIFIC PROMERISTEMATIC AND GENER AL CELL PROTEIN. Carol M. Condit, Depts. of Plant Science and Biochemistry, University of Nevada, Reno, Reno, NV. 89557

Immunocytochemical localization of ptGRP1 in the apical bud show that this protein is highly expressed in the medial portion of the apical meristem. It is not expressed in the ground or flank meristem.In addition ptGRP1 is highly expressed in all promeristematic cells of undifferentiated lateral buds. Once the lateral bud has differentiated to the extent that leaf primordia can be discerned, expression of ptGRP1 resembles that of the apical meristem. Although ptGRP1 is, as stated above, highly expressed in the medial portion of the apical meristem and all promeristematic cells of undifferentiated lateral buds, expression of ptGRP1 is at its highest in the second layer of cells of these tissues. Further, ptGRP1 is also very highly expressed in one or two cells of the second layer of cells at the junction point of the apical meristem and its adjacent leaf primordia. These highly expressing ptGRP1 cells are located at the site at which lateral bud formation will occur and likely indicate than these cells are progenitors of this structure.

The pattern of expression of ptGRP1 in the developing plant, however, is more complex than indicated above. Excluding lateral buds, ptGRP1 expression is turned on and off twice during the development of the plant. As indicated above this protein is first expressed in the medial portion of the apical meristem. Its expression then appears to be turned off and no evidence of the presence of this protein can be found in cells for the next five to ten cell generations. ptGRP1 expression then occurs first in the procambium and is followed by all other cell types of the stem and leaf (except the protoxylem). Expression of ptGRP1 is seen until the organ the cell resides in appears to be completely expanded and then dissappears. Thus ptGRP1 expression correlates with the promeristematic state and also with cell division and expansion of "relatively" differentiated and differentiated cell types.

89 SOME NEW WAYS OF LOOKING AT CELL WALL PECTINS Andrew J. Mort, Niels Maness, Michael Freitas, Jinhua An and Xiaoyang Qi. Oklahoma State University, Department of Biochemistry, Stillwater, Oklahoma 74078 USA. A quantitatively major component of the cell walls in the vegetative parts of plants is pectin. Pectin is thought to be involved in several important plant processes such as adhesion between cells, regulating the elasticity of cell walls, regulating the permeability and electrical charge of cell walls and acting as a source of endogenous signals to be released from the wall upon appropriate stimulation such as invasion by pathogens or physical damage. To understand these roles of pectin it is helpful to understand its structure. A dominant feature of pectin is its negative charge carried on the abundant galacturonic acid (GalA) residues. The plant can modulate the charge on pectin by varying the degree of methyl esterification of these residues. We will describe a sensitive method for chemically determining the degree of methyl esterification of pectin and application of the method to showing variation of methyl esterification. In celery petioles we found that the pectin of parenchymal tissue is 60% methyl esterified whereas that of epidermal, vascular and collenchymal tissue is 47, 37 and 34% esterified, respectively. Using selective methods for cleaving pectins (endopolygalacturonase for cleaving GalA-rich regions, cold liquid HF for cleaving rhamnosyl and other neutral sugar linkages), we can solubilize fragments of pectin. Characterization of these fragments by NMR and mass spectrometry allows us to deduce the structural arrangement of pectins. In cotton suspension culture cell walls pectins consist of regions: ~45% are homogalacturonans of very low degree of methyl esterification, ~ 40% are long stretches rich in rhamnose-galacturonic acid repeating disaccharides interspersed with highly methyl esterified oligomers of GalA, and ~15% are short complex regions. (Supported in part by DOE grant DE-FGO5-86ER13496)

90 MAIZE HRGP GENE EXPRESSION IN CELL DIVISION AND WOUNDING. Luis Ruiz-Avila, Denis Tagu, Regina Raz, Carme Caelles, Matilde José, Shirley Burgess, M. Dolors Ludevid,José-Antonio Martínez Izquierdo and Pere Puigdomènech. Dept. de Genética Molecular. CID-CSIC. Jordi Girona, 18 08034 Barcelona. Spain.

One of the main extractable components of maize cell wall is a hydroxyproline-rich glycoprotein (HRPG) having some similarities with the extensins from dicotyledonous species. This protein is coded in maize and related species (teosinte, sorghum, rice) by either a low-copy number or a single gene. It has a very repetitive sequence formed by well-conserved elements. the accumulation of the HRGP mRNA is correlated with that of histone H4 both by northern blot and in situ hybridization analysis except in a number of situations : induction by wounding or ethylene and tissue-specifity in defined parts of the embryo. Accumulation of mRNA in provascular cells is observed by in situ hybridisation. In scutellum both HRGP and its mRNA cannot be detected but another proline-rich protein mRNA can be observed. HRGP mRNA accumulates in embryo in cells with a small size and thin walls indicating that HRPG gene expression may be an early event in maize cell wall formation occuring prior to complete cellulose deposition. The 5'-flanking region of the HRGP gene contains a 500 bp sequence having a number of boxes conserved between the species studied and it is able to direct the expression of the gus and cat genes in maize protoplasts and calli.

91 Local and systemic signalling events in the defence response of a plant D.J. Bowles Centre for Plant Biochemistry and Biotechnology, Department of Biochemistry and Molecular Biology, The University of Leeds, LS2 9JT, U.K.

Wounding, or attack by pathogens and pests, induces changes in gene activation at the local site of the stimulus and at distant regions of the plant. In contrast to the characterization of local events, systemic changes are much less studied, although they are known to be implicated in acquired resistance. We have studied local and systemic signalling in tomato and potato plants both in response to wounding and invasion by an important root pathogen : parasitic nematodes. We have been interested in particular to determine the mechanism(s) underlying long-distance signalling in a defence response : the nature of the systemic signal or indeed, the need if any, for a mobile signal to achieve long-range effects.

92 AIRBORNE METHYL JASMONATE ACTIVATES WOUND-INDUCIBLE PROTEINASE INHIBITOR GENES. Edward E. Farmer and Clarence A Ryan, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Several signaling molecules that regulate the expression of genes coding for two wound-inducible proteinase inhibitor proteins have been identified in this laboratory. The signal-active molecules include oligosaccharide fragments of plant and pathogen cell walls, volatile methyl jasmonate, and a recently discovered novel polypeptide called Systemin Methyl jasmonate and jasmonic acid powerfully activate proteinase inhibitor genes in tomato, potato, tobacco and the legume alfalfa and are thought to be interacting with the intracellular signal transduction pathways close to the gene level. Additionally, mRNA coding for proSystemin was found to accumulate in response to methyl jasmonate. Recent research on the effects of methyl jasmonate on wound-inducible gene expression as well as the potential role of methyl jasmonate to act as an airborne, interplant defense signal will be discussed. A current model for the possible roles of methyl jasmonate and jasmonic acid in wound-inducible signal transduction will be presented. (Supported in part by the Washington State University College of Agriculture and Home Economics, the McKnight Foundation and grants from the National Science Foundation).

93 TOUCH, HEAT SHOCK AND CALCIUM-INDUCED EXPRESSION OF THE CALMODULIN-RELATED TCH GENES OF *ARABIDOPSIS* Janet Braam. Rice University, Department of Biochemistry and Cell Biology, Houston, TX 77251

Plants are exquisitely sensitive to the environment and have evolved the ability to adapt to many environmental stresses by altering development. Our long-term goal is to determine the mechanisms by which environmental stimuli are sensed, signals are transduced in the responding cells and the cellular responses are evoked. We have discovered an early response to a variety of environmental stimuli in *Arabidopsis*, that is, the strong induction of expression of five genes--termed the TCH genes. The inductive stimuli include touch, wind, rain, wounding, darkness and heat shock. Three of the TCH genes encode proteins related to calmodulin, the protein thought to mediate physiological responses to changes in the concentration of the second messenger calcium. Calcium ion and calmodulin may therefore be involved in the signal transduction pathways used in response to stimuli as diverse as touch, wounding and heat shock. Using cultured *Arabidopsis* cells that are relatively homogeneous and easily manipulated, we are investigating aspects of the regulation of TCH gene expression. We will describe experiments that demonstrate that the TCH genes are regulated by a variety of stimuli in both intact plants and cultured cells and that calcium itself may be sufficient to induce TCH gene expression.

SYMPOSIUM SPEAKERS

94 RECEPTOR-LIKE PROTEIN KINASES OF HIGHER PLANTS. John C. Walker, Veronica P. Counihan, Thomas Moran and Ren Zhang, Biological Sciences. University of Missouri, Columbia, MO 65211.

Extracellular signals are important regulators of physiological and developmental processes in all cells. In many cases perception of these signals is mediated by cell surface receptors which couple extracellular ques to intracelluar responses. Yet, despite the importance of these processes, the understanding of both the structure and function of cell surface receptors in higher plants is very limited. We have recently identified a gene that encodes a protein that is a good candidate for a plant cell receptor. The architecture of this novel protein is reminiscent of the receptor tyrosine kinases (e.g. epidermal growth factor receptor) of invertebrates and vertebrates. The maize gene that we have isolated (*ZmPK1*) consists of an cytoplasmic domain that is homologous to the serine/threonine-specific family of protein kinases which is linked, via a membrane spanning domain, to an extracellular domain that is related to S-locus (self-incompatibility) glycoproteins (SLGs) of *Brassica*. By analogy to the receptor tyrosine kinases in vertebrates we postulate that the gene product encoded by *ZmPK1* acts as a plant cell receptor. Consistent with this hypothesis we have shown that the ZmPK1 protein is localized to the plasma membrane of young maize seedling. Immunolocalization experiments are in progress to further characterize the cellular and subcellular localization of ZmPK1.

To gain a broader insight into the distribution and function of the receptor-like protein kinases in higher plants we have initiated screen to identify these genes in *Arabidopsis thaliana*. Using the protein kinase domain of *ZmPK1* as a hybridization probe we have isolated five different genes that appear to be related to the receptor protein kinases. One of these genes, like *ZmPK1*, contains an extracellular domain that is similar to the SLG's of *Brassica*. We are currently analyzing the expression of this gene in transgenic *Arabidopsis* plants.

95 INDOLE AND BENZOPHENANTHRIDINE ALKALOID BIOSYNTHESIS: NEW SYSTEMS FOR THE STUDY OF ENZYME MECHANISMS AND PLANT GENE EXPRESSION.

Toni M. Kutchan. *Lehrstuhl fur pharmazeutische Biologie, Universität München, Karlstraße 29. D-8000 München 2, Germany*

Many important pharmaceutical agents, from chemotherapeutics to anti-hypertensives, are derived from members of the indole class of alkaloids. All of these alkaloids, over 1800 different chemical structures, are formed from the first alkaloid in the biosynthetic pathway, strictosidine. The formation of certain of these alkaloids appears to be highly regulated in the plant, leading to low levels of accumulation, and , therefore, to insufficient quantities of drug material. Given unlimited quantities of strictosidine, the possibility exists, through chemical modification or enzymatic transformation, to synthetically prepare existing or new pharmaceuticals. A cDNA for strictosidine synthase from *Rauvolfia serpentina*, the enzyme which stereospecifically condenses tryptamine with secologanin to form strictosidine, has been isolated and the enzyme heterologously expressed. Virtually unlimited quantities of the indole alkaloid precursor, strictosidine, and potentially strictosidine analogs formed with a modified enzyme, can be produced in a microbial system.

Alkaloids also serve as part of the plant chemical defense system against pathogenic attack. The model currently under study if that of the biosynthesis and accumulation of benzophenanthridine alkaloids in the plant, *Eschscholtzia californica*, in response to elicitor treatment. A key enzyme in the formation of benzophenanthridine alkaloids, the berberine bridge enzyme (BBE), which catalyzes the formation of the berberine bridge in the conversion of (S)-reticuline to (S)-scoulerine, has been purified to homogeneity, a cDNA for this enzyme has been isolated, and the enzyme has been heterologously expressed. The induction kinetics of the mRNA for BBE, the enzyme activity and the accumulation of benzophenanthridine alkaloids has been determined for elicited cell suspension cultures of *E. californica*.

96 ACTIVATION OF PHENYLPROPANOID SYNTHESIS IN PLANT DEFENSE. Dierk Scheel, Gregory Armstrong, Christiane Colling, Oswaldo da Costa e Silva, Harald Keller, Annette Renelt, Wendy R. Sacks, Bernd Weißhaar and Klaus Hahlbrock. Max-Planck-Institut für Züchtungsforschung, Abteilung Biochemie, Carl-von-Linné-Weg 10, D-5000 Köln 30, FRG.

Plants express specific defense responses towards different forms of environmental stress, often involving the accumulation of secondary products derived from general phenylpropanoid metabolism. Parsley has been used extensively as a model system to study reactions to UV light and pathogen attack. Both stimuli activate the transcription of genes encoding enzymes of partly overlapping phenylpropanoid biosynthetic pathways. Irradiation induces vacuolar accumulation of flavonoids, whereas elicitor treatment stimulates the synthesis of secreted furanocoumarins and predominantly wall-bound cinnamic acid derivatives. Regulatory elements were identified in the promoters of genes encoding phenylalanine ammonia-lyase and chalcone synthase and cDNAs have been isolated encoding putative transcription factors that bind to these motifs. Elicitor treatment of irradiated parsley cells completely blocks the light-induced accumulation of flavonoids by repressing the transcription of the chalcone synthase gene. While the mechanisms of perception and signal transduction involved in light-induced flavonoid synthesis are largely unknown, a purified glycoprotein elicitor appears to act in pathogen defense by specific binding to a target site in the parsley plasma membrane. This leads to transient changes in membrane permeability to Ca^{2+}, Cl^-, H^+ and K^+. Increased cytosolic Ca^{2+} levels are involved in elicitor-mediated protein phosphorylation and activation of genes encoding phenylpropanoid biosynthetic enzymes and may, therefore, represent part of the elicitor signal transduction chain.

97 AROMATIC SECONDARY PRODUCTS REGULATE CARBON FLOW INTO THE SHIKIMATE PATHWAY. Klaus M. Herrmann, Avtar K. Handa, and John M. Henstrand, Purdue University, Departments of Biochemistry and Horticulture, West Lafayette, Indiana 47907

The first step in the shikimate pathway is catalyzed by the enzyme 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase. The endproduct of the shikimate pathway, chorismate, is the precursor for phenylalanine, tyrosine, and tryptophan, from which plants synthesize a number of aromatic secondary metabolites required for defense and repair. Demands for secondary metabolites cause not only changes in enzyme levels of the controlling catalysts for the respective secondary pathways but also in the level of the enzyme that controls carbon flow into the primary common aromatic or shikimate pathway, the DAHP synthase. The mechanism of this enzyme activity modulation is - at least in part - transcriptional. Wounding that causes increased lignin biosynthesis induces DAHP synthase rapidly. The increase in enzyme activity is associated with an increase in the DAHP synthase polypeptide as determined by immunoblots and an increase in DAHP synthase specific mRNA as probed with a cDNA encoding DAHP synthase. Within the same plant tissue that shows wound-induction of DAHP synthase, developmental control signals can cause a down regulation of the enzyme. Again, the decrease in enzyme activity is correlated with a loss of DAHP synthase polypeptide and a decline in the DAHP synthase specific mRNA levels. Our data demonstrate for the first time that environmental and developmental signals can either up- or down-regulate DAHP synthase within the same tissue. It remains to be seen whether or not induction and repression of DAHP synthase under different conditions reflect the synthesis or degradation of different isoenzymes.

98 INHIBITION OF FLAVONOID BIOSYNTHESIS IN ANTHERS OF PETUNIA HYBRIDA BY AN ANTISENSE APPROACH: A NOVEL WAY TO ENGINEER NUCLEAR MALE STERILITY. Joseph Mol, Ingrid van der Meer, Toon Stuitje and Arjen van Tunen. Free University, Dept. of Genetics, Amsterdam, The Netherlands.

Pigmentation of flower corollas and anthers is caused by the accumulation of flavonoids and anthocyanins. Apparently, the function of these pigments is to attract pollinators. In recent years it has become clear that flavonoids have multiple functions in the plant kingdom: in some plant species they act as phytoalexins, they can protect the plant against UV-light damage, they are nodulation signals in legumes. Furthermore they have been implicated in insect resistance and hormone transport. Here we report on experiments using antisense technology that point to a new function in gametophyte development.

Previous experiments from this laboratory (Nature 333, 1988, 866-869) showed for the first time that an antisense chalcone synthase (chs) gene could effectively down-regulate flower pigmentation in Petunia when driven by a CaMV 35S promoter. Pigmentation of the anthers, however, was never affected possibly caused by an incorrect temporal or spatial expression of the antisense *chs* gene. In an attempt to alter the expression pattern of the 35S promoter an 18 bp synthetic sequence of the chs promoter, previously called "antherbox" (Plant Mol. Biol. 12, 1989, 539-551), was inserted and the new promoter used to drive a ß-glucuronidase (gus) reporter gene. Transgenic plants containing this construct showed a novel specificity: expression in tapetum cells surrounding and nourishing the pollen grains. The flavonoids attached to the pollen originate from the tapetum. Inhibition of flavonoid gene expression in tapetum cells should therefore lead to pollen grains devoid of these pigments. The antherbox-containing CaMV 35S promoter was fused to an antisense chs gene and the construct transferred to Petunia plants. Four types of phenotypic effects were found in transformants: coloured corolla, coloured anthers; coloured corolla, white anthers; white corolla, coloured anthers and white corolla, white anthers. The transformants carrying white anthers did not produce fertile pollen. Pollen development was arrested at the early microspore stage. This finding strongly argues for a key function of flavonoids in microsporogenesis and suggests a novel way to engineer nuclear male sterility.

99 VARIATIONS IN THE HAMMERHEAD SELF-CLEAVAGE REACTION OF VIROID RNA

Candice C Sheldon, Chris Davies & Robert H Symons. Department of Plant Science, Waite Institute, Glen Osmond, SA 5064, Australia

The plus and minus RNAs of the 247 nt avocado sunblotch viroid (ASBV) and the RNAs of four encapsidated, viroid-like, satellite RNAs or virusoids undergo site-specific self-cleavage *in vivo*, (a reaction) considered to play an essential role in their *in vivo* replication (1-5). We have characterized the single hammerhead self-cleavage structure common to the virusoid RNAs and also shown that *dimeric plus* ASBV RNA cleaved via a double hammerhead structure both during transcripuon but via a single hammerhead structure after purification.

By contrast, *dimeric minus* ASBV cleaved via a double-hammerhead structure during transcripuon but via a single hammerhead structure after purification. This result illustrated the importance of the folding pathway of the RNA in determining whether an active single- or double-hammerhead structure was formed.

Results with *monomeric plus* and *minus* ASBV RNAs were more complex since no self-cleavage occurred during transcripuon while, with purified RNAs, the presence of vector sequences at both 5'- and 3'-ends of the transcripts influenced whether self-cleavage occurred at all or via a single- or double-hammerhead structure.

The stability of stem III of the hammerhead structure is an important factor in determining single- or double-hammerhead cleavage in all ASBV RNAs, a conclusion supported by mutating this stem in *plus monomeric* RNA to a more stable structure which cleaved by a single-hammerhead. Further, sequences outside the hammerhead structure play a role in enhancing its stability.

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SYMPOSIUM SPEAKERS

100 BROMOVIRUS RNA REPLICATION AND HOST ADAPTATION. Paul Ahlquist, Walter DeJong, Radiya Pacha, and Patricia Traynor. Institute for Molecular Virology, University of Wisconsin, Madison, Wisconsin 53706.

Brome mosaic virus (BMV) and cowpea chlorotic mottle virus (CCMV) are related bromoviruses that systemically infect certain monocots and dicots, respectively. Each virus has a genome divided among ss, messenger-sense RNAs 1, 2 and 3 (3.2, 2.9, 2.1 kb). RNA1 and RNA2 encode proteins 1a and 2a, respectively, which are involved in viral RNA replication. 1a (109 kD) contains an N-terminal domain implicated in RNA capping and a C-terminal helicase-like domain, while 2a (94 kD) contains a central polymerase-like region. Intervirral exchanges show that RNA replication requires mutual adaptation of 1a and 2a, implying direct or indirect interaction between the two proteins. The 200 amino acid (aa) N-terminal domain of 2a flanking the polymerase-like core is required for RNA replication and appears to influence 2a interaction with 1a. The 125 aa C-terminal domain of 2a following the polymerase-like region is dispensable for RNA replicanon; however, BMV mutants lacking this region or having it replaced with the corresponding segment of the CCMV 2a gene are deficient in systemic spread of infection, suggesting either that 2a carries out function(s) beyond its recognized role in RNA replicanon, or that the export of progeny genomes is somehow linked to their synthesis. RNA3 encodes the coat protein and the 3a noncapsid protein (30 kD), which are both dispensable for RNA replicanon but required for systemic infection. BMV-CCMV gene exchanges show that bromovirus host specificity is a multigenic property involving at least the 3a gene, some RNA3 regulatory sequences, and determinants on RNA1 and/or RNA2. Continuing studies of bromovirus regulatory sequences have identified separate 125-base and 145-base noncoding segments in CCMV RNA3 that are fully dispensable for systemic infection, but whose loss makes the virus unable to compete with wild type CCMV in mixed infections and, in one case, dramancally exacerbates infecnon symptoms.

101 RESISTANCE AND SUSCEPTIBILITY OF ARABIDOPSIS THALIANA TO TURNIP CRINKLE VIRUS. Anne E. Simon, Mary Polacco, Xiao Hua Li and Clifford D. Carpenter. Department of Biochemistry, of Massachusetts, Amherst, MA 01003

The complexity of the plant response in both resistant and susceptible interactions with pathogens complicates studies of the molecular events involved. We are developing a model system for the study of plant-virus interactions using *Arabidopsis thaliana* and turnip crinkle virus (TCV). TCV is one of the smallest and simplest RNA viruses and has been extensively characterized at the molecular and structural levels. Most ecotypes of *A. thaliana* are killed by one isolate of the virus within 22 days of inoculation. We have screened 26 different ecotypes and over 6000 EMS treated M2 plants and found that one ecotype, Dijon, was resistant to TCV infection at all stages of growth tested. Genetic analyses revealed that the resistance trait best fits a single copy, partially dominant gene. Viral genomic RNA accumulates to high levels in the susceptible Col-0 ecotype three days post-inoculation, while TCV is first detected in Dijon after an additional eight days. Nearly all Dijon plants remain symptomless at 16-20 days of infection, by which time the susceptible ecotypes are all dead or dying. Differences in proteins accumulating in response to viral infection between Dijon and col-0 ecotypes are apparent within a few days post-inoculation. To characterize the susceptible response, a cDNA library was constructed of Col-0 mRNA four days after inoculation with TCV. Results produced from differential screening of the library will be presented.

102 INVESTIGATIONS OF THE MOLECULAR INTERACTIONS BETWEEN A VIROID AND ITS HOST PLANT. Rosemarie W. Hammond. U. S. Department of Agriculture, Agricultural Research Service, Microbiology and Plant Pathology Laboratory, Beltsville, Maryland 20705

Viroid pathogenicity in infected plants is a complex process that is influenced by both the viroid and host plant genomes. Manipulation of the viroid RNA genome by mutagenesis combined with analysis of infectivity, structure, and tissue localization suggests functional roles for regions, and in some cases, specific nucleotides in replication, symptom induction, and cell-to-cell movement. Surprisingly, potato spindle tuber viroid (PSTVd) may be modified at a number of positions without losing its ability to replicate and move systemically in its tomato host, yet other modifications render the molecule nonviable. A series of modifications were introduced into the virulence modulating region of PSTVd. Not all of the mutant PSTVd molecules were infectious, and those that were had an altered phenotype from that of the parent molecule. The results obtained from these and other experiments challenge previous models of structure/function relationships in viroid pathogenicity and point to the need to determine the structure of the molecule as it occurs in vivo. Investigation of host components involved in the viroid infection process are also being investigated and progress along these two lines will be discussed.

103 Genetic Interactions Specifying Expression of Bacterial Disease Resistance in *Arabidopsis* and Tomato. S. J. Barker, A. F. Bent, F. M. Carland, D. Dahlbeck, R. Innes, B. Kunkel, J. M. Salmeron, and B. J. Staskawicz. Department of Plant Pathology, University of California, Berkeley, CA, USA 94720.

We are currently developing *Arabidopsis thaliana* and tomato as model host plants to study genes from both the the host and the pathogen that specify disease resistance. Our goal is to clone genes from the two interacting partners that determine the molecular basis of disease resistance. We have successfully cloned and characterized avirulence genes from *Pseudomonas syringae* that specify resistance on specific ecotypes of *Arabidopsis thaliana*. The avirulence genes *avrRpt2* and *avrB* are recognized by the *Arabidopsis* ecotype Col-0. When these genes are conjugated into a normally virulent strain of *Pseudomonas syringae* pv. *tomato* (DC3000), the transconjugants are able to induce a hypersensitive reaction on Col-0. We have also identified other ecotypes of *Arabidopsis* that do not recognize these avirulence genes and are currently genetically analyzing crosses between these ecotypes to determine the genetic control of resistance. In addition, we are have isolated two putative susceptible mutants of the ecotype Col-0 from a M2 population of diepoxybutane (DEB) mutagenized seed that do not recognize the avirulence gene *avrRpt2*. One of these mutants still is able to recognize *avrB* suggesting that the mutation is specific for resistance caused by the *avrRpt2* gene. Genetic characterization of these mutants is currently under investigation. Efforts are also underway to clone the tomato resistance gene *Pto*. We are currently employing two methods to construct mutations at this locus that will assist in the cloning of this gene. Our first approach is to employ the Maize transposon *Ac* to insertionally inactivate the *Pto* locus. In addition, we are attempting to construct deletion mutations at the *Pto* locus by employing the mutagen DEB. Our progress in the isolation of *Pto* mutations will be presented.

104 GENETICS AND PHYSIOLOGY OF INCOMPATIBLE INTERACTIONS BETWEEN *XANTHOMONAS ORYZAE* PV. *ORYZAE* AND RICE. Jan E. Leach and Frank F. White. Kansas State University, Department of Plant Pathology, Manhattan, Kansas 66506-5502.

Races of *Xanthomonas oryzae* pv. *oryzae* the bacterial blight pathogen of rice, are defined by their interactions with rice cultivars that contain specific genes for resistance. Resistance responses in cultivars carrying the *Xa-10* bacterial blight resistance gene are characterized by an accelerated (16-24 h) increase in extracellular cationic peroxidase (pI 8.5) activity, lignin deposition (24-48 h), host cell death (48 h), and a decrease in the rate of bacterial multiplication. Late (48-72 h) in the susceptible interactions, the cationic peroxidase activity increases, but at reduced levels compared to resistant interactions, and bacterial multiplication is not inhibited. Purification and characterization of the peroxidase and identification of cloned peroxidase sequences are in progress. The potential role of the peroxidase in resistance will be assessed. Avirulence genes which specify the resistant phenotype on rice cultivars have been cloned from *X. o.* pv. *oryzae*. A cosmid clone from a *X. o.* pv. *oryzae* genomic library was identified which contains a functional avirulence gene corresponding to the *Xa-10* rice gene for resistance. The clone hybridizes with *avrBs3*, a gene from *X. campestris* pv. *vesicatoria* that specifies a resistant phenotype to pepper cultivars containing the *Bs3* resistance gene.

105 INTERACTION OF XANTHOMONAS WITH CRUCIFERS. M.J. Daniels, J.M. Dow, J. Conrads-Strauch, C.E. Barber and J.E. Parker. The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH,UK.

Pathogenesis of Xanthomonas campestris pathovar campestris (Xcc) to crucifers requires a large number of genes which are not essential for growth in vitro. Extracellular enzymes and polysaccharide contribute to symptom development. Production of these factors is subject not only to individual but also to complex global regulation by at least three independent systems. One of these functions as a positive regulator of transcription, one regulates negatively and one resembles catabolic activation factor of enteric bacteria. hrp genes, which are required both for pathogenesis and for induction of resistance responses in plants, do not regulate enzyme and polysaccharide synthesis, but their expression depends on the metabolic state of the bacteria. Plants respond to infection by major increases in expression of about 20 genes, the products of some being "pathogenesis-related" proteins. Although the pattern of gene expression depends on whether the plant is resistant or susceptible to the bacterium, the role of these genes in influencing the course of the infection is not understood. A more general approach to analysing plant resistance and response exploits the fact that Arabidopsis is susceptible to Xcc. Recent results on natural and induced variation for susceptibility of Arabidopsis, and the interaction of resistance genes and bacterial avirulence genes will be discussed.

SYMPOSIUM SPEAKERS

106 TOWARD UNDERSTANDING THE FUNCTION OF PATHOGEN AVIRULENCE GENES AND PLANT DISEASE RESISTANCE GENES. Noel T. Keen. University of California, Department of Plant Pathology, Riverside, CA 92521

Several avirulence genes lead to elaboration by a pathogen of specific chemical signals, called elicitors. These are recognized by host plants carrying the complementary disease resistance gene and thereby invoke active plant defense reactions. Pathogens, however, have in some cases evolved mechanisms to alter avirulence gene function in order to escape plant surveillance. We have studied several alleles of avrD, present in certain pathovars of Pseudomonas syringae, which interact with the Rpg4 resistance gene in soybean. Although all of the studied pathovars carry complete genes encoding highly homologous AvrD proteins with 311 amino acids, the avrD allele from P.s. pv. glycinea does not function as an avirulence gene. This appears to result from a small number of amino acid substitutions at the carboxyl end of the protein. Bacteria expressing functional alleles of avrD produce significant quantities of the avrD elicitor, but only trace amounts are directed by the allele of avrD from P.s. pv. glycinea. The elicitor, identified as a novel lipid, is only active in soybeans carrying the Rpg4 resistance gene. We are also interested in cloning Rpg4 and other disease resistance genes from soybean and are experimenting with approaches that may permit the identification of clones by complementation methods.

107 EARLY EVENTS IN PLANT CELL TRANSFORMATION BY *AGROBACTERIUM*. Eugene W. Nester, Milton Gordon, University of Washington, Departments of Microbiology and Biochemistry, Seattle, Washington, 98195

Agrobacterium induces tumors by inserting a fragment (T-DNA) of its tumor inducing (Ti) plasmid into the plant chromosome. Some understanding of the functioning of genes, the virulence or *vir* genes required for this process has been elucidated. The *vir* genes are activated by two classes of plant signal molecules, plant phenolic compounds and a variety of sugars, which are components of the plant cell wall. These molecules interact with the VirA protein which then activates the VirG protein, which in turn transcriptionally activates all of the *vir* genes following binding to their promoter regions. The VirA and VirG proteins, members of a two component regulatory system undergo phosphorylation and phosphate transfer demonstrated by other members of the group. The *virD* operon is concerned with early stages in the processing of T-DNA. The VirD1 protein has topoisomerase activity for the Ti plasmid. The *virD2* locus codes for a site specific endonuclease which nicks at the right and left borders of the T-DNA. It also codes for a nuclear targeting sequence. The VirC1 protein interacts with the overdrive sequence and in conjunction with the VirD1 and VirD2 proteins promotes T-strand formation and the efficiency of transfer of the T-DNA. The *virE* operon codes for a single stranded DNA binding protein which associates with the T-DNA. The *virB* operon has now been sequenced and many of its protein products are associated with the cytoplasmic membrane. Presumably these protein components provide the pore through the bacterial envelope for the exit of the T-DNA. The *virB11* gene product has ATPase activity and autophosphorylates. Its DNA sequence is similar to that of a gene in *B. subtilis* required for development of competence. The VirB10 protein is oligomeric and anchored in the inner membrane. Open reading frames 9, 10, and 11 all code for gene products which are essential for tumor formation.

The components of the *vir* regulon function to process and transfer the T-DNA from the bacteria into the plant nucleus by a process which bears a resemblance to bacterial conjugation.

108 Agrobacterium Ti PLASMID GENE EXPRESSION BEFORE AND AFTER PLANT TUMORIGENESIS. Stephen C. Winans. Cornell University, Section of Microbiology, Ithaca, New York 14853.

A. tumefaciens uses a transmembrane protein kinase (VirA) and a transcriptional activator (VirG) to induce a set of pathogenesis genes in response to compounds released from plant wounds. VirA contains one periplasmic domain and three cytoplasmic domains. We have made site-directed deletions of the virA gene which remove the entire periplasmic domain of the protein, or the carboxyl terminal cytoplasmic domain, or both domains. All three alleles of virA are able to activate VirG in response to phenolic compounds. We have also isolated a virG point mutant whose product no longer requires activation by VirA. Like plant wounds, crown gall tumors also release compounds (including opines) which induce the transcription of specific bacterial genes. We have identified a gene (occR) which, in response to the opine octopine, activates transcription of the octopine catabolism operon. OccR is a member of the LysR family, and negatively regulates its own gene as well as activating the catabolic operon. OccR protects from DNase digestion a site between these two promoters, and the region protected is altered by the addition of octopine. We have isolated 30 strains containing gus fusions which are induced by octopine, only 5 of which disrupt the occ region. We are currently mapping these fusions and determining whether they are regulated by occR.

109 EARLY STEPS IN THE AGROBACTERIUM-PLANT INTERACTION. Paul J. J. Hooykaas, Alice Beyersbergen, Leo Melchers, Teresa Mozo, Kees Rodenburg and Stefan Turk Leiden University, Department of Plant Molecular Biology, 2333 AL, Leiden, Netherlands

The bacterium Agrobacterium tumefaciens induces crown gall (tumorous overgrowths) on dicotyledonous plants. During tumorigenesis agrobacteria transfer a segment of their tumor inducing (Ti) plasmid to plant cells at the infection site. Expression of onc-genes located in the T (transferred)-DNA results in tumor formation. Data will be presented showing that T-DNA transfer to plants is based on a system which is comparable to bacterial conjugation. The transfer apparatus is largely encoded by virulence (vir-) genes that are located on the Ti plasmid in a region called the virulence region. As an early step in crown gall tumorigenesis the genes in this region are induced by certain specific plant phenolic compounds. Regulation is mediated by the VirA (chemoreceptor) and VirG (activator) proteins, whereby signal transduction occurs via fosforylation. Recent results concerning the characterization of virA and virG mutants will be discussed.

110 GENETIC INTERRELATIONSHIPS AMONG *AGROBACTERIUM* INFECTION PROCESSES. Stanton B. Gelvin, Todd Steck, M. Krishnan and Lillian L. Habeck. Purdue University, Department of Biological Sciences, West Lafayette, Indiana 47907. We have previously shown that, using acetosyringone as an inducer, we can hyperinduce the *vir* genes of the *Agrobacterium* Ti-plasmid using octopine. This hyperinduction requires only *virA*, *virG*, and genes on the chromosome. We now show that this potentiation of *vir* gene induction does not involve the periplasmic domain of VirA. In addition, the extent of hyperinduction using tobacco protoplasts depends upon the amount of exogenous octopine added to the medium. Low concentrations of octopine (less than 0.2 mM) hyperinduce the *vir* genes, but higher concentrations repress this induction. *Agrobacterium* strains mutant in the octopine synthase gene are less virulent than are wild-type bacteria. The *vir* genes of these mutant bacteria cannot be induced to the same extent by normal tobacco protoplasts as can the *vir* genes of the corresponding wild-type bacteria. However, when transgenic tobacco protoplasts expressing the octopine synthase gene are used for induction, the *vir* genes of both bacterial strains are induced to the same extent. We speculate that *Agrobacterium* senses the amount of opines in the wound environment and uses this information to determine the state of growth (induction of *vir* genes or vegetative growth). Because opines can affect the induction of *vir* genes in a complex way, we tested the possibility that *vir* genes may influence a process induced by some opines, conjugal Ti-plasmid transfer. We found that, under limiting opine induction conditions, conjugal transfer can be diminished by up to four orders of magnitude by mutations in certain *vir* genes. We shall also present data suggesting that the single-stranded form of the T-DNA is the form that is transferred to plant cells.

111 DELETION FORMATION AND GENETIC VARIATION IN THE RICE BLAST FUNGUS. Barbara Valent, Marc J. Orbach, James A. Sweigard, Leonard Farrall, Anne Walter and Forrest C. Chumley. The Du Pont Co., Cen. Res. & Dev., Experimental Station, Wilmington, DE. 19880-0402.

Understanding the basis for genetic variation in the rice blast fungus, Magnaporthe grisea, will aid in the design of strategies for controlling rice blast disease. Many rice pathogen field isolates exhibit frequent mutation in the melanin biosynthesis gene, BUF1, which is critical for infection. That is, 0.5% of the conidia produced by these strains during vegetative growth are Buf$^-$. Hybridization analysis using the cloned BUF1 gene has determined that this high rate of mutation is due to frequent deletion of the gene, most likely by homologous recombination between repeated sequences that flank the gene. The abundance of repeated DNA sequences in the fungus suggests such deletions may account for other examples of genetic instability. We have identified two unlinked, highly-mutable avirulence genes, Avr2-YAMO and Avr1-TSUY, which are specific for rice cultivars Yashiro-mochi and Tsuyuake respectively. Mapping has shown that each gene is telomere-linked. Spontaneous mutants derived from several strains with the Avr2-YAMO gene have now been characterized. Several of these mutants, which have specifically gained virulence on rice cultivar Yashiro-mochi, contain deletions of various sizes within one kb of the telomere to which Avr2-YAMO is linked. Progress toward cloning the unstable avirulence genes as well as other stable ones will be described.

SYMPOSIUM SPEAKERS

112 MATING-TYPE LOCI AND THE CONTROL OF PATHOGENIC DEVELOPMENT IN USTILAGO MAYDIS. Regine Kahmann, Jörg Bergemann, Michael Bölker, Marlis Dahl, Bernd Gillissen, Claus Sandmann, Florian Schauwecker, Ramona Schlesinger, Birgit Schroeer. Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, W-1000 Berlin 33, FRG

The basidiomycete fungus Ustilago maydis ist the causative agent for corn smut disease. The dikaryon generated by fusion of two compatible haploid lines represents the infectious form. Mating and sexual development is controlled by the two mating-type loci a and b which exist in two and multiple (> 25) alleles, respectively. A successful mating reaction requires that both partners carry different alleles at both loci. We have cloned and analyzed the two alleles of the a locus and present evidence that a1 and a2 encode mating-peromone precursors. The b locus on the other hand has been shown to encode two regulatory proteins with no apparent homology except for a homeodomain related motif. Both genes exist in many alleles. We propose that the two proteins encoded by the b locus interact with each other. A heterodimeric protein complex active in turning on the developmental program consists of two polypeptides originating from different alleles of the b locus. The interaction of the two gene products from the same allele, on the other hand, produces a complex that is inactive for turning on development.

115 SPECIFICITY IN LETTUCE DOWNY MILDEW. Richard W. Michelmore, Richard V. Kesseli, Howard Judelson, David M. Francis and Ilan Paran. Department of Vegetable Crops, University of California, Davis, CA 95616.

The molecular genetic basis of specificity in the gene-for-gene interaction between lettuce (*Lactuca sativa*) and its obligate fungal pathogen, *Bremia lactucae*, is being determined in both host and parasite. Parallel studies minimize the assumptions that have to be made about genes being expressed in each organism. Classical genetics has defined the numbers and linkage of the genes determining specificity. Thirteen dominant resistance (*Dm*) genes have been mapped to four linkage groups in the host. Thirteen matching dominant avirulence genes in the pathogen are not tightly linked. Extensive genetic maps are being developed predominantly using RFLP and RAPD markers. Additional crosses are being analyzed to map genes for resistance to diverse pathogens. Target regions containing *Dm* genes are being saturated with markers using near-isogenic lines and analysis of bulks of individuals from segregating populations. Sequence and genetic complexity of these regions is being characterized. RAPD fragments have been cloned, sequenced and used to identify primers for Sequence Characterized Amplified Regions (SCARs). A long-range restriction map of the major cluster of resistance genes is being made and correlated with the genetic map. Resistance and avirulence genes will be cloned by chromosome walking from linked molecular markers using a variety of libraries. Partial YAC libraries have been constructed for both organisms. Progress of walks will be analyzed using segregation analyses of terminal sequences. Ultimately, sequences that absolutely cosegregate with resistance or avirulence will be tested for function in transgenics. Transformation of lettuce mediated by *Agrobacterium rumefaciens* is routine; transformation of *B. lactucae* is under development.

113 MOLECULAR CLONING OF AVIRULENCE AND PATHOGENICITY GENES OF THE TOMATO PATHOGEN CLADOSPORIUM FULVUM. Pierre J.G.M. de Wit, Guido F.J.M. van den Ackerveken, Jan A.L. van Kan, Matthieu H.A.J. Joosten, Nadia Danhash and Jos P. Wubben. Agricultural University, Department of Phytopathology, Wageningen, the Netherlands.

The avirulence gene avr9 and two potential pathogenicity genes of Cladosporium fulvum were isolated and characterized. All genes are specifically induced in planta. The avr9 gene encodes a race-specific elicitor inducing a hypersensitive response (HR) in tomato cultivars carrying the corresponding resistance gene Cf9. Races of C. fulvum which are virulent (pathogenic) on Cf9 cultivars become avirulent (non-pathogenic) when they are transformed with the avr9 gene. This indicates that transformants become non-pathogenic by their gained ability to produce the HR-inducing race-specific elicitor. Experiments are underway to transform Cf9 cultivars with avr9 gene constructs under control of promotors that are only locally induced by various pathogens giving rise to HR. The level of resistance of these transgenic plants to their native pathogens will be checked. The biological function of the two cloned pathogenicity genes is not known yet. Their role in pathogenicity will be studied by disruption of the wild type genes which occur as single copy in the genome of C. fulvum. Regulation of the genes in vitro as well as in vivo will be discussed.

116 MOLECULAR EVALUATION OF VICTORIA BLIGHT OF OATS. Thomas J. Wolpert. Department of Botany and Plant Pathology, Oregon State University, Corvallis OR 97331-2902.

The interaction of several plant-pathogenic fungi with their hosts is mediated by the ability of these fungi to produce phytotoxic compounds called host-selective toxins (HST). A HST is toxic only to a specific genotype of a plant species, and toxin sensitivity is typically mediated by a single host gene. Because of the apparent role of these compounds in conferring the pathogens' ability to cause disease and the simple genetics of the host response to the toxin and thus, the pathogen, HSTs present unique opportunities for the study of host-parasite interactions. We have been investigating a disease called victoria blight of oats caused by the fungus *Cochliobolus victoriae*. The fungus is pathogenic due to its production of the host-specific toxin, victorin. Toxin sensitivity and susceptibility to the pathogen, is controlled by a single dominant gene (*Vb*). Furthermore, the *Vb* gene is either closely linked or identical to the *Pc-2* gene which confers resistance to crown rust. The hypothetical product of the *Vb* gene is a toxin receptor. Thus, identification of the toxin receptor could simultaneously identify the product of a disease susceptibility gene, and a disease resistance gene. Toward this identification we have: A) chemically characterized victorin; B) determined the molecular features of the toxin essential to its activity; C) prepared labelled, biologically-active derivatives of the toxin; D) identified a 100 kd protein from oat plants that binds the toxin in a genotype-specific and ligand-specific manner; E) purified the 100 kd victorin-binding protein (VBP) and developed anti-VBP antibodies; and F) isolated a full-length cDNA clone of the VBP and expressed it in *E. coli*.

114 MOLECULAR SPECIFITY IN THE COCHLIOBOLUS CARBONUM RACE 1-MAIZE INTERACTION. Jonathan D. Walton, DOE-Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824

Specificity in this host/pathogen system is controlled by a single dominant gene (Hm1) for resistance in maize and a single gene (Tox2) in the fungus. Tox2 controls production of the host-selective compound HC-toxin, a cyclic tetrapeptide containing L-2-amino-9,10-epoxy-8-oxo-decanoic acid (AEO). We have isolated the gene encoding an enzyme, HTS-1, involved in the production of HC-toxin. This gene along with 22 kb of contiguous flanking DNA is present as two copies in all isolates of C. carbonum that make HC-toxin and is absent in all isolates that do not. Presence of the 22-kb region genetically segregates with Tox2. We propose that Tox2 is a gene cluster encoding the enzymes necessary to make HC-toxin, and that a major DNA deletion or insertion event has occurred in the evolution of host-range in C. carbonum.

Major resistance in maize to HC-toxin (and hence to C. carbonum race 1) is controlled by the Hm1 locus. Extracts of resistant (Hm1/hm1 or Hm1/Hm1) maize contain an enzyme, "HC-toxin reductase", that utilizes NADPH to reduce the 8-keto group of AEO to the non-toxic 8-alcohol derivative. Although excised leaves of both susceptible and resistant maize reduce HC-toxin when it is delivered through the transpiration stream, the enzyme activity is undetectable in cell-free extracts from near-isogenic sensitive plants. Several maize lines that were mutated from resistance to susceptibility due to the insertion of transposable elements at Hm1 were obtained from Steve Briggs, Pioneer Hi-Bred. These transposon-induced mutants lack in vitro HC-toxin reductase activity. Experiments to date indicate that this enzyme is the biochemical basis of specific resistance to C. carbonum race 1. The Hm1 gene either encodes or controls expression of HC-toxin reductase.

117 HEPTA-β-GLUCOSIDE ELICITOR BINDING PROTEIN(S) IN SOYBEAN. M. G. Hahn, J.-J. Cheong, F. Côté and R. M. Alba, Univ. of Georgia, Complex Carbohydr. Res. Ctr. & Depts. of Botany and Biochemistry, Athens, GA 30602, USA

The accumulation of phytoalexins in response to fungal infection is an important plant defense mechanism. In soybean tissues, phytoalexin accumulation is also induced by oligoglucoside fragments (elicitors) that originate from fungal cell wall polysaccharides. A branched hepta-β-glucoside, originally purified from acid-hydrolyzates of mycelial walls of *Phytophthora megasperma* f. sp. *glycinea*, encompasses a structural motif required for a specific recognition event in soybean tissues that leads to phytoalexin accumulation. Structure-activity studies, utilizing a family of synthetic oligoglucosides structurally related to the elicitor-active hepta-β-glucoside, have identified specific structural elements in the hepta-β-glucoside that are required for maximum elicitor activity. These structural elements include the branched trisaccharide at the non-reducing end of the hepta-β-glucoside and a specific distribution of terminal glucosyl side chains along the backbone. The putative receptor(s) in soybean that are responsible for recognizing elicitor-active oligoglucosides are being identified and characterized. The binding site(s) co-sediment with markers for plant plasma membranes and are protease sensitive. The results of ligand binding studies have demonstrated that a single class of specific, reversible, high-affinity ($K_d \approx 1$ nM) binding sites for a radiolabeled hepta-β-glucoside exist in soybean microsomal membranes. Competitive inhibition of the binding of radiolabeled hepta-β-glucoside elicitor to the binding protein(s) by structurally related, synthetic oligoglucosides demonstrated a direct correlation between the binding affinities and the abilities of the oligoglucosides to induce phytoalexin accumulation. These results suggest that the binding protein(s) are physiological receptors. The hepta-β-glucoside binding protein(s) were solubilized from soybean root microsomal membranes with detergents, and retain their affinity and binding specificities. The elicitor binding protein(s) are being identified and purified using techniques such as photo-affinity labeling and affinity chromatography. Purification and characterization of the hepta-β-glucoside elicitor binding protein(s) is a prerequisite for defining their role in the signal cascade that ultimately leads to phytoalexin accumulation. (Supported by NSF grant DCB-8904574; and in part by the USDA/DOE/NSF Plant Science Centers Program through funding by DOE grant DE-FG09-87ER13810)

SYMPOSIUM SPEAKERS

118 ENDOMYCORRHIZA : AN EMERGING MODEL FOR STUDYING COMPATIBLE PLANT-FUNGAL MOLECULAR INTERACTIONS.

Vivienne Gianinazzi-Pearson and Silvio Gianinazzi.
Laboratoire de Phytoparasitologie. INRA-CNRS, Station de Génetique et d'Amelioration des Plantes, INRA, BV 1540, 21034 Dijon, France.

The fact that the large majority of land plants have co-evolved symbiotic associations with vesicular-arbuscular endomycorrhizal fungi (Glomales) implies that the compatibility systems which are reciprocally activated during symbiont interactions must be based on widely occurring molecular mechanisms. These and the resulting morphofunctional integration between host and fungus must involve coordinated changes in gene expression in both partners. Plant defence reactions and associated molecules are only weakly induced during host-fungus interactions in vesicular-arbuscular endomycorrhiza[1], and protein analyses indicate that the symbiotic state in mycorrhizal tissues is characterised by modifications in host gene expression which differ from those occurring in the presence of pathogens[2]. Furthermore, studies of isogenic plant mutants, deficient for the endomycorrhizal phenotype, suggest that the symbiosis is under multiple, dominant host gene control and that the lack of endomycorrhiza formation is related to localised activation of host defence reactions[3]. The possible rôle of symbiosis-specific genes in controlling the expression of plant defence genes during the establishment of compatible endomycorrhiza associations will be discussed.

1 S. Gianinazzi (1991). Agriculture, Ecosystems & Environment, 35, 105-119.
2. E. Dumas, A. Tahiri-Alaoui, S. Gianinazzi, V. Gianinazzi-Pearson (1990). Endocytobiology IV. INRA Press, Paris, 153-157
3 V. Gianinazzi-Pearson, S. Gianinazzi, J.P. Guillemin, A. Trouvelot, G. Duc (1991). Advances in Molecular Genetics of Plant-Microbe Interactions 1, 336-342

119 NITROGEN FIXATION GENES: STRUCTURE AND FUNCTION. Dennis R. Dean and William E. Newton. Virginia Polytechnic Institute and State University, Departments of Anaerobic Microbiology and Biochemistry, Blacksburg, Virginia, 24060.

Biological nitrogen fixation is catalyzed by nitrogenase, a complex metalloenzyme comprised of two component proteins, the Fe protein and the MoFe protein. Substrate reduction occurs at or near a metal center called FeMo-cofactor which is located within the MoFe protein. We have been probing the contribution of FeMo-cofactor's polypeptide environment to substrate binding and reduction by placing amino acid substitutions within regions assigned as FeMo-cofactor-binding domains. Results of these experiments have led to the assignment of the cysteinyl-275 residue and the histidinyl-195 residue as providing coordinating ligands to the FeMo-cofactor. Perturbation of FeMo-cofactor's polypeptide environment at or near either of these positions effects the catalytic and biophysical properties of the nitrogenase MoFe protein. We have also been analyzing mutant strains which produce altered forms of the FeMo-cofactor. In particular, our recent results indicate that the nifW and the nifZ gene products are directly involved in FeMo-cofactor biosynthesis.

120 IN VITRO AND SITE DIRECTED MUTAGENESIS TO STUDY THE FUNCTION OF THE RHIZOBIUM MELILOTI NITROGEN FIXATION GENES fdxN AND nifA. Alfred Pühler, Werner Klipp, Reiner Krey, Michael Kutsche, Bernd Masepohl and Kai-Uwe Riedel. Universität Bielefeld, Lehrstuhl für Genetik, D-4800 Bielefeld 1, Deutschland

The Rhizobium meliloti nitrogen fixation genes nifA, nifB and fdxN form an operon. The fdxN gene which is absolutely required for symbiotic nitrogen fixation encodes a small protein of 68 amino acid and a molecular mass of 6,830. The fdxN protein contains two cysteine motifs, typical for bacterial ferredoxins. By site directed mutagenesis the role of the cysteine motifs was analyzed. The biochemical function of FdxN is still under investigation. It is proposed that FdxN takes part in the electron supply of the nitrogenase or is involved in the biosynthesis of the FeMoCo.

The R.meliloti NifA protein activates specific nif promoters in front of nif and fix operons. The NifA protein was found to be oxygen sensitive. In contrast to the Klebsiella NifA the R.meliloti NifA contains an additional interdomain linker which is thought to be involved in the oxygen sensitivity. By mutational analysis using the in vitro hydroxylamine technique oxygen tolerant nifA mutants were isolated. By sequence analysis we were able to demonstrate that the mutation sites were not located in the interdomain linker region but in the central domain close to the nucleotide binding site.

121 AMPLIFICATION OF NODULATION AND NITROGEN FIXATION GENES IN Rhizobium phaseoli. Rafael Palacios, Susana Brom, Guillermo Dávila, Margarita Flores, Ma. de Lourdes Girard and David Romero. Centro de Investigación sobre Fijación de Nitrógeno, Universidad Nacional Autónoma de México. Apdo. postal 565-A, Cuernavaca, Mor. MEXICO

The genome of Rhizobium phaseoli; the symbiont of the bean plant Phaseolus vulgaris, is a complex structure containing a large amount of reiterated DNA sequences and presenting frequent rearrangements. A genetic element (GDYNI) which allows the positive selection of different types of rearrangements was constructed and inserted in different regions of the genome of R. phaseoli strain CFN-42. We found that tandem amplification of DNA sequences occurs at high frequency in different regions of the genome, including various large plasmids and the chromosome. Such amplifications of genetic material are unstable structures that revert to the basal state at high frequency. This dynamic state of the genome might be involved in the adaptation of Rhizobium strains to different environmental conditions or to the interaction with different plants. In the symbiotic plasmid, homologous recombination between reiterated DNA sequences resulted in amplification of DNA regions containing nodulation and nitrogen fixation genes. The physiological effects of amplification of symbiotic genes in R. phaseoli is now under study.

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122 MULTIPLE CONTROL LEVELS OF *NIF* AND *FIX* GENE EXPRESSION IN *BRADYRHIZOBIUM JAPONICUM*. Hans-Martin Fischer, Denise Anthamatten, Enrique Morett, Ines Kullik and Hauke Hennecke. Mikrobiologisches Institut, Eidgenössische Technische Hochschule, Schmelzbergstrasse 7, CH-8092 Zürich, Switzerland.

Expression of genes involved in the process of nitrogen fixation by the soybean symbiont *Bradyrhizobium japonicum* is strictly controlled by oxygen. Low oxygen tensions prevailing in soybean root nodules or in microaerobic cultures of free-living bacteria result in the coordinated activation of numerous *nif* and *fix* genes. We are investigating at the genetic and molecular levels the regulatory elements that are involved in this type of control. The transcriptional activator protein NifA is regulated by oxygen at the level of (i) its activity, (ii) its synthesis and (iii) its stability. *In vivo* footprinting experiments demonstrated that binding of NifA to its target sequences located around 100 bp upstream of –24/–12-type *nif* consensus promoters and NifA-dependent formation of an open promoter-RNA polymerase-σ^{54} complex are oxygen sensitive. Under microaerobic conditions, expression of *nifA* is increased at least fivefold by a mechanism that requires NifA itself. Aerobic *nifA* expression depends on a yet-to-be-identified activator protein that probably binds to an upstream DNA sequence located around position –66 of the *fixRnifA* operon. Furthermore, it was found that in aerobically (but not microaerobically) growing *Escherichia coli* cells the NifA protein was degraded rapidly. As a second oxygen regulatory system, the *fixLJ*-like genes were identified in *B.japonicum* and shown to be essential for a fully effective symbiosis. Potential targets for *fixLJ*-mediated regulation are a *fixK*-like gene and a region with homology to *Rhizobium meliloti fixN*. Finally, we found in *B.japonicum* two highly homologous genes ($rpoN_1$, $rpoN_2$) encoding σ^{54} which enables RNA polymerase core enzyme to recognize and activate –24/–12-type promoters. In contrast to $rpoN_2$, expression of $rpoN_1$ is oxygen regulated in a *fixLJ*-dependent manner. At least one *rpoN* gene product is required for symbiotic nitrogen fixation, assimilation of nitrate and motility of *B.japonicum*.

123 MOLECULES INVOLVED IN THE DETERMINATION OF HOST-SPECIFICITY IN THE SYMBIOTIC INTERACTION OF RHIZOBIUM LEGUMINOSARUM BV VICIAE AND ITS HOST PLANTS. Herman P. Spaink, Anton A.N. van Brussel, Jan W. Kijne, Clara Díaz and Ben J.J. Lugtenberg. Leiden University, Department of Plant Molecular Biology, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands

Nodulation of leguminous plants by Rhizobium bacteria is a host-specific process. When R.leguminosarum bv. viciae is compared with R.leguminosarum bv. trifolii, the genes nodABCIJ and L are common whereas nodFE determine host-specificity. The mentioned genes are responsible for synthesis and secretion of a series of low molecular weight components, designated as Nod-metabolites, which are basically lipo-oligosaccharides, similar to NodRm1 secreted by R.meliloti. Several Nod-metabolites have been purified and their structures have been elucidated. The use of various nod clones has enabled us to relate certain nod genes to certain moieties of the Nod-metabolites, including those involved in host specificity. Purified Nod-metabolites were related to biological processes by testing their abilities to induce root hair deformation (Had), thick and short roots (Tsr), meristems and increased nod-gene inducing activity (Ini). An intriguing question is which pathways are used by the Nod-metabolites to activate these biological processes. Since the bacterial nodFE genes and the plant's lectin are both major determinants of host-specificity and since both are involved in the initiation of infection thread formation, it is tempting to speculate that Nod-metabolites and plant lectin interact in a host-specific way.

124 REGULATION OF *RHIZOBIUM MELILOTI* NODULATION GENES. Eva Kondorosi[1], Michael Schultze[1], Michèle Pierre[1], Michèle Cren[1], Beate Hoffmann[1], Michel Buiré[1] and Adam Kondorosi[1,2].
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[2]Biological Research Center, Szeged, Hungary

Induction of nodules on leguminous plant roots by rhizobia is governed by signal exchanges between the symbiotic partners that result in the activation of genes in the two partners. Flavonoids produced by the host plant are required to induce the nodulation (*nod* and *nol*) genes in rhizobia in conjunction with the bacterial activator NodD protein. The *nod* gene products direct the synthesis of the *Rhizobium* Nod factors which activate plant genes involved in nodule initiation. In *Rhizobium meliloti* the expression of *nod* genes is under both positive and negative control. The *nodD* gene is present in three allelic forms (*nodD1*, *nodD2*, *nodD3*) which have distinct flavonoid specificies. A structurally and functionally related gene, *syrM*, when provided in several copies, directs flavonoid-independent *nod* gene activation *via* the NodD proteins, resulting in host range extension to certain non-host plants, such as *Macroptilium*. Negative regulation is mediated primarily by the 13.3 kDa NolR repressor protein which binds in dimeric form to the operator site of the overlapping *nodD* - inducible *nod* gene promoters. Combined nitrogen *via nodD3* also negatively affects *nod* gene expression. Moreover, at least two additional chromosomal loci contribute to the regulatory circuitry. The structure of several Nod-Rm factors has been determined which exhibited striking differences in the concentrations optimal for biological activity tested on host and non-host plants. We suggest that fine-tuning of *nod* gene regulation is required for the production of Nod factors in amounts optimal for the host plants.

SYMPOSIUM SPEAKERS

125 *RHIZOBIUM MELILOTI* NODULATION SIGNALS

G. Truchet[1], P. Roche[2], F. Debellé[1], P. Lerouge[2], C. Rosenberg[1], M.Y. Ardourel[1], N. Demont[2], J. Vasse[1], J.C. Promé[2] and J. Dénarié[1]. [1]Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA, BP27, 31326 Castanet-Tolosan Cedex; [2]Centre de Recherches de Biochimie et de Génétique Cellulaire, CNRS-UPS, 118, route de Narbonne, 31062 Toulouse Cedex, France.

The *R. meliloti nod* genes which control host specificity, infection and nodulation are classified into common *nod* genes present in all rhizobia studied so far and species-specific *nod* genes which control the host-range.

The common *nodABC* genes are required for the production of extracellular Nod factors which are sulphated lipo-oligosaccharides. They are tetra- or pentasaccharides of N-acetyl-D-glucosamine, N-acylated and O-acetylated on the terminal non-reducing sugar residue and sulphated on the reducing sugar (1, 2). On alfalfa, these purified factors elicit root hair deformation at nanomolar concentrations and cortical cell divisions and nodule formation in the micromolar-nanomolar range (1, 3). Structure-function relationship studies have shown that the sulphate group, the reducing function of the terminal sugar and the double bonds of the acyl chain are all important in eliciting nodule organogenesis on alfalfa (3).

The host-range genes *nodH* and *nodPQ* determine the sulphation of the Nod factors which is responsible for the alfalfa specificity. The role of other *nod* genes in the synthesis of Nod factors will be described.

(1) Lerouge et al. (1990) Nature, 344, 781.
(2) Roche et al. (1991) J. Biol. Chem. in press.
(3) Truchet et al. (1991) Nature, 351, 670.

126 NODULIN GENE INDUCTION: ROLE OF CIS-ACTING ELEMENTS, TRANS-ACTING FACTORS AND CYTOKININ. Frans J. de Bruijn[1,2*], Sylvie Bianchi[3], Christoph Dehio[2], Anurag Goel[1,2], Britta Grunenberg[2], Pascal Ratet[2,3], Laszlo Szabados[2,4], Krzysztoph Szczyglowski[1], and Peter Welters[2]. MSU-DOE Plant Research Laboratory and Department of Microbiology, Michigan State University, E. Lansing, MI, 48824, USA[1]; Max Planck Institut für Züchtungsforschung, 5000 Köln 30, FRG[2]; Present Address: ISV-CNRS, Gif sur Yvette, France[3]; Present Address: BRC, Szeged, Hungary[4].

Plant genes activated during nodule ontogeny and in mature nitrogen-fixing nodules (nodulin genes) are not only symbiotically induced in a developmentally controlled fashion, but are also expressed in a cell-specific pattern. We are investigating the cis-acting elements and trans-acting factors involved in controlling the expression of the *Sesbania rostrata* early nodulin Enod2 and late nodulin leghemoglobin (lb) genes. By examining the activity of chimeric Enod2 - and lb-uidA (glucuronidase) gene fusions in transgenic Lotus plants, we have delimited promoter elements responsible for symbiotically induced, cell-specific expression. We have also identified trans-acting, DNA-binding proteins of both plant and rhizobial origin which interact specifically with the lb promoter. In addition, we will demonstrate that the *S. rostrata* Enod2 gene is specifically and rapidly activated by physiologically significant concentrations of cytokinin.

127 MECHANISMS INVOLVED IN EARLY NODULIN GENE EXPRESSION. Beatrix Horvath, Henk Franssen, Renze Heidstra, Igor Kardailsky, Miklos Lados, Tetsuo Meshi, Marja Moerman, Panagiota Mylona, Karel Novak, Irma Vijn, Wei-Cai Yang and Ton Bisseling. Agricultural University, Dept. of Molecular Biology, Wageningen 6700 ET, The Netherlands.

The development of nitrogen fixing nodules on the roots of leguminous plants is induced by soil bacteria of the genus (*Brady*) *Rhizobium*. During early steps of nodulation, root hair curling, infection and nodule primordium formation are induced. These processes accompanied by the expression of sets of early nodulin genes. The bacterial nodulation genes (*nod*) play a pivotal role in the induction of these three developmental processes. The *nod* genes are involved in the production of the Nod factors, which serve as signal molecules triggering root hair curling and cell division. Moreover, the Nod factors secreted by *R. leguminosarum* elicit the expression of two early nodulin genes in pea root hairs. These two early nodulin genes (ENOD5 & ENOD12) are both involved in the infection process but are induced in a spatially different manner[1,2,3]. In this report we will present the inducing activity of different closely related Nod factors on early nodulin gene expression. Based on the correlation between chemical structure and induction of early nodulin gene expression, we suggest a model for host-specific signal perception and transduction.

128 NATURE OF THE MEMBRANE COMPARTMENT HOUSING THE BACTEROID AND TARGETING OF PBM NODULINS. D. P. S. Verma, Department of Molecular Genetics and Biotechnology Center, The Ohio State University, 1060 Carmack Rd, Columbus, OH 43210.

The release of *Rhizobium* inside an infected cell of nodule necessitates formation of a new subcellular compartment. The formation of this compartment and the release of *Rhizobium* can be disassociated from nodule morphogenesis, suggesting that nodule development and infection proceed independently. Although the nature of this compartment is not fully understood, it is becoming apparent that it has features common with the vacuole. This is consistent with the presence of several hydrolytic enzymes and the presence of nodulin-26 which is found to have homology with a vacuolar protein, TIP. A nodule-specific sequence encoding protease inhibitor has recently been isolated. This protein generally localized in vacuoles. Nodulin-26 is induced prior to the formation of the PBM compartment and is synthesized on ER without a cleavable signal sequence. This membrane protein is co-translationally inserted in PBM with both amino and carboxy ends facing the cytoplasm and forms an ion channel. It is phosphorylated at its carboxy end with a membrane kinase which may control the activity of this channel. Nodulin-24, on the other hand, is a surface protein which is synthesized as a precursor and the signal is removed co-translationally. It does not have any trans-membrane domain and seems to anchor with PBM by covalent attachment with a molecule that increases its size by 12 kD. The function of PBM nodulins is being deciphered by creating antisense mutations in transgenic *Lotus* and *Vigna* plants for which PBM-specific clones have been isolated using immunological screening of the recombinant libraries.

129 CHARACTERIZING THE SYMBIOSIS (SYM) GENES OF PISUM SATIVUM. Thomas A. LaRue. Boyce Thompson Institute for Plant Research, Ithaca, NY 14853-1801

The pea cv. 'Sparkle' has proven easy to mutate, and we have obtained mutants defective in nodule formation or symbiotic nitrogen fixation. We are determining the inheritance of the symbiosis (sym) genes defined by these mutants, and the loci are being mapped. The collection of mutants in a near-isogenic background provides a means for studying the plant role in nodule development. We will describe three phenotypically different mutants blocked at the stage of rhizobial infection (sym 8), at the stage of nodule primordium initiation (sym 5), and at the stage of nitrogen fixation (sym 13).

130 PLANT GENE EXPRESSION DURING NODULE INITIATION AND DEVELOPMENT IN SOYBEANS. C. Sengupta-Gopalan, New Mexico State Univ., Agron & Hort Dept/Mol. Bio. Program/Plant Gen. Eng. Labs., Las Cruces, NM 88003.

Among the many functional roles plant flavonoids play, the relevant roles in context of Legume/*Rhizobium* symbiosis are: i. induction of the nod genes; ii. plant growth hormones and iii. Host defense. Expression of genes encoding for two key enzymes in flavonoid biosynthesis, phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) were monitored to trace flavonoid synthesis during nodule initiation and nodule development in soybeans. We have identified and characterized gene members whose expression is induced 2 to 4 days following infection and appears to be associated with cortical cell proliferation. The expression of the host defense related PAL and CHS gene members was also investigated in context to release of bacteria from infection thread, disintegration of the peribacteroid membrane and failure to fix N_2. Work is in progress to determine the molecular signals involved.

To understand the role and regulation of soybean genes in nodule genesis we have focused on a highly expressed late nodulin gene cluster. The gene members have been characterized, cis-elements have been identified and trans-activating factors have been characterized. Our discussion will include the complex interaction between the nuclear factors and the cis elements and the implications of those interactions on gene expression. Glutamine synthetase gene expression in nodules has been investigated in order to determine if it is regulated by N_2 fixation.

SYMPOSIUM SPEAKERS

131 THE *hetR* GENE CONTROLS HETEROCYST DEVELOPMENT IN *ANABAENA* 7120. William J. Buikema and Robert Haselkorn, Dept. of Molecular Genetics & Cell Biology, Univ. of Chicago, Chicago, IL 60637

Following mutagenesis with diethyl sulfate, approximately 140 mutants of Anabaena 7120 were selected based on an inability to grow aerobically without a source of fixed nitrogen. Seven mutants representing several morphologically abnormal heterocyst phenotypes were complemented by conjugation with a cosmid library of wild-type Anabaena 7120 DNA. One mutant, 216, fails to differentiate heterocysts under all growth conditions. Subcloning identified a 2.4 kb partial Sau3A fragment able to complement mutant 216, and DNA sequencing of this fragment revealed a single open reading frame, *hetR*, capable of encoding a 299 amino acid protein. *hetR* is transcribed at a low level under nitrogen replete conditions but at a much higher level within six hours of transfer to N^- conditions. Conjugation of deletion subclones of this region into strain 216 showed that the *hetR* ORF is both necessary and sufficient for complementation of the Het^- phenotype. The mutation in 216 is located at position 535 in *hetR*, and converts a serine at position 179 in the expected wild-type protein to an asparagine in the mutant. Five independent revertants of 216 all show a return to the wild-type DNA residue at position 535. A null mutation of *hetR* recombined into wild-type cells displays an identical phenotype to 216. Both 216 and wild-type cells containing the intact *hetR* gene on a plasmid display increased frequency of heterocysts, even on media containing fixed nitrogen. Many of these heterocysts appear in pairs and occasionally in higher multiples. Infrequently, pairs of heterocysts occur that appear to have been formed from incompletely divided vegetative cells. These results suggest that *hetR* encodes a product that is not only essential for but also controls heterocyst development.

132 REGULATION OF ALTERNATIVE NITROGENASES IN AZOTOBACTER VINELANDII Paul E. Bishop. North Carolina State University, Department of Microbiology, and United States Department of Agriculture, Agricultural Research Service, Raleigh, North Carolina 27695

The diazotrophic bacterium A. vinelandii is able to express three genetically distinct nitrogenases. Nitrogenase-1 (encoded by nifHDK) contains Mo and is expressed only in the presence of this metal. In Mo-deficient growth medium containing V, nitrogenase-2 (a V-containing enzyme complex) is synthesized and nitrogenase-3 is made in the absence of Mo and V. The genes encoding nitrogenase-2 are split into two independently regulated operons; vnfHorfFd and vnfDGK while the subunits for nitrogenase-3 are encoded by a single operon, anfHDGK. Each of these operons is preceded by a potential promoter site that conforms to a consensus sequence that is recognized by RNA polymerase-sigma^{54}. All three nitrogenases are repressed by NH_4^+. Ammonium repression of nitrogenases-2 and -3 occurs at the level of transcription whereas repression of nitrogenase-2 appears to be post-transcriptional. Synthesis of nitrogenase-2 and -3 requires vnfA and anfA, respectively. These genes presumably encode activator proteins which are similar to NifA, a protein required for transcription of all nif operons. In addition, dinitrogenase reductase-2 (which is encoded by vnfH and is a component of nitrogenase-2) is required for transcription of anfHDGK. All vnf and anf operons are repressed by Mo. The anfHDGK operon is also repressed by V. This repression requires vnfDGK and nifB (a gene involved in nitrogenase cofactor synthesis). Finally it should be mentioned that study of the regulation of alternative Mo-independent nitrogenases is in its infancy and our knowledge of the components that are involved in this regulation remains incomplete.

133 FREE-LIVING AND SYMBIOTIC NITROGEN FIXATION IN *AZORHIZOBIUM CAULINODANS*. P.A. Kaminski, F. Arigoni, K. Mandon, N. Desnoues, N. Domigan and C. Elmerich.

Unité de Physiologie Cellulaire, Département des Biotechnologies, Institut Pasteur, 75724 Paris Cedex 15, France.

Azorhizobium caulinodans ORS571, isolated from stem nodules of the tropical legume *Sesbania rostrata*, can grow at the expense of dinitrogen as the sole nitrogen source and fixes nitrogen symbiotically in root and stem nodules of the host plant. Genes corresponding to *nifA* and *ntrC* have been found in *A. caulinodans*. Although *nifA* mutants were strictly Nif^- Fix^-, *ntrC* mutants displayed residual nitrogenase activity in pure culture and a delayed Fix phenotype in symbiosis. This suggested the existence of another regulatory circuit. Homologues of *fixL*, *fixJ* and *fixK* have been characterized. Kanamycin cartridges were inserted into the cloned genes and recombined into the host genome. The resulting mutants were Nif^- Fix^-, suggesting that the three genes were required for symbiotic nitrogen fixation and for nitrogen fixation in the free-living state. However, the mutants displayed 10 % residual nitrogenase activity in the free-living state, but a double *ntrC-fixL* mutant was completely devoid of nitrogenase activity. Using a *pfixK-lacZ* fusion, the FixLJ products were shown to control expression of *fixK*. Using a *pnifA-lacZ* fusion, the FixK product was shown to regulate positively the transcription of *nifA* in bacteria grown in the free-living state. A model of regulation, which differs from that established in *Rhizobium meliloti* is presented and accounts for the unusual property of *A. caulinodans* to fix nitrogen both under symbiotic conditions and in the free-living state.

134 ORGANIZATION AND REGULATION OF GENES ENCODING MOLYBDENUM AND ALTERNATIVE NITROGENASES IN THE PHOTOSYNTHETIC PURPLEBACTERIUM RHODOBACTER CAPSULATUS. Werner Klipp, Silke Hennecke, Kerstin Schüddekopf and Ge Wang. University of Bielefeld, Department of Genetics, D-4800 Bielefeld, Germany

Rhodobacter capsulatus is able to fix nitrogen in the absence of molybdenum (<0.05ppb) using a molybdenum-independent nitrogenase. The alternative nitrogenase of R.capsulatus, which could be demonstrated in wild-type cells as well as in cells carrying a complete deletion of the structural genes for molybdenum nitrogenase (nifHDK), is not a vanadium enzyme but a heterometal-free iron nitrogenase. Expression of the alternative system is repressed by molybdenum. The gene region responsible for the molybdenum repression was identified. Double mutants carrying deletions in nifHDK and in the new gene region are able to fix nitrogen via the alternative nitrogenase even in the presence of molybdenum. Two operons of nif region A are essential for both nitrogenase systems. One of these operons encodes two ferredoxin-like proteins, one plant-type ferredoxin and one bacterial-type ferredoxin. The other transcription unit contains genes coding for high molecular weight iron sulfur proteins. These results indicate that both nitrogenases use the same electron donor system. Transcription of genes encoding the conventional nitrogenase as well as genes encoding the molybdenum-free nitrogenase is dependent on the nifR4 gene product, an alternative RNA polymerase sigma factor. In contrast, the transcriptional activator, which is encoded by two functional copies of nifA in R.capsulatus, is only necessary for the expression of the molybdenum nitrogenase.

135 PHYTOCHROME AND GENE EXPRESSION. Peter H. Quail, Plant Gene Expression Center, UC Berkeley/USDA, 800 Buchanan St., Albany, CA 94710

To understand the molecular mechanism by which phytochrome regulates gene expression, we are pursuing a threefold general strategy: (a) examining the phytochrome molecule for clues to its mechanism of action; (b) studying genes under its control for DNA elements and regulatory factors involved in induction or repression of expression; and (c) characterizing potential mutants in transduction pathway components between the photoreceptor and the genes it regulates. Analysis of the *Arabidopsis hy3* mutant shows that it has wild-type levels of phytochrome A but is deficient in phytochrome B, consistent with a discrete physiological role for the *phyB* gene product. Overexpression of either *phyA* or *phyB* sequences in transgenic *Arabidopsis* results in short hypocotyls in the light. We are exploiting this phenotype to probe for functionally important regions in these phytochromes by site-directed mutagenesis. In parallel, the mutated polypeptides are being overexpressed in *E. coli* and examined for *in vitro* chromophore attachment and dimerization. We have isolated, mapped and molecularly characterized recessive *Arabidopsis* mutants at a locus designated *cop1*, whose seedlings grow in darkness like the wild type in light. The phenotype suggests a mutation potentially in an early transduction pathway component that normally acts in darkness to repress genes involved in photomorphogenic development. Progress toward cloning this locus will be reported. Using microprojectile-mediated gene transfer we have identified *cis*-elements that are functionally important to the autoregulated expression of rice and oat *phyA* genes. This information has enabled us to clone DNA-binding proteins that interact with some of these functionally defined elements and are potential transcription factors.

136 TYPE I AND TYPE II PHYTOCHROME-DEPENDENT GENE EXPRESSION. Masaki Furuya, Frontier Research Program, RIKEN Institute, Wako City, Saitama, Japan 351-01

Evidence has accumulated to indicate that there are at least two physiologically and spectrophotometrically distinct, "labile" and "stable", pools of phytochrome in plants (see the review by Furuya, 1989, Adv. Biophys. 25: 133). Two different molecular species of phytochrome apoprotein, type I and type II, in terms of immunochemical reactions and amino-acid sequences are found in pea (Abe et al. 1989), and their gene sequences correspond to phyA and phyB in Arabidopsis (Sharrock & Quail 1989). A stable phytochrome pool regulates the expression of phyA in both dark- and light-grown pea seedlings, but it was unknown as to which of the phytochrome species results in the stable Pfr pool (Furuya et al. 1991).

The useful tools to determine the functions of each phytochrome species are provided by phytochrome-deficient mutants like cucumber lh or Arabidopsis hy-3, which has no PHYB (type II), and transgenic plants expressing variously gene-engineered phy genes. It is proposed that (i) phyA gene expression is regulated by type II phytochrome; (ii) Cab and rbcS genes are under the control of type I phytochrome, and (iii) phyB gene expression seems to be constitutive.

SYMPOSIUM SPEAKERS

137 STRUCTURE AND FUNCTION OF GYMNOSPERM PHYTOCHROME GENES. Jane Silverthorne, Ellen Chinn and Wendy Peer. University of California at Santa Cruz, Department of Biology, Santa Cruz, California 95064.

Gymnosperms have a variable dependence on light for chlorophyll biosynthesis and chloroplast development. *Ginkgo biloba*, like angiosperms, requires light for both of these processes. In the absence of light, *Ginkgo* seedlings show a classical etiolated morphology and are white. By contrast, seedlings of the pine *Pinus palustris* can synthesize chlorophyll and carry out steps in chloroplast development in complete darkness. We are cloning and characterizing the phytochrome genes from these two gymnosperms as part of our effort to elucidate the role of light in their developmental processes.

138 DIFFERENTIAL EXPRESSION OF THREE PHYTOCHROMES IN *AVENA*. Lee H. Pratt[1], Marie-Michèle Cordonnier-Pratt[1], Yu-Chie Wang[1] and Sandy J. Stewart[2]. [1]University of Georgia, Department of Botany, Athens, Georgia 30602, and [2]CIBA-GEIGY Biotechnology, Research Triangle Park, North Carolina 27709

Phytochrome that predominates in green plant tissues is different from that which is abundant in etiolated seedlings. With phytochrome from green *Avena* leaves as immunogen, seven monoclonal antibodies (MAbs) directed to this immunochemically distinct phytochrome have been obtained. These seven MAbs recognize at least four epitopes. Only one MAb cross reacts with the phytochrome that is most abundant in etiolated shoots. Results obtained with these seven MAbs indicate that the immunochemically distinct phytochrome isolated from green oat leaves is itself of at least two types. Together with the abundant phytochrome in etiolated shoots, we therefore detect a total of three phytochromes. Including MAbs produced previously, we have probes highly specific to each of these three phytochromes. These MAbs, the apparent monomer sizes of the phytochrome detected by each, and the tissue in which each phytochrome predominates are: Oat-22, 124 kDal, etiolated shoots; GO-4, 125 kDal, green leaves; and GO-7, 123-kDal, green leaves. Microsequencing data indicate that 125-kDal phytochrome is encoded by a gene other than that which produces 124-kDal phytochrome. It is as yet uncertain whether 123-kDal phytochrome derives from a third gene. Immunoblot analyses indicate that 123- and 125-kDal phytochromes can form heterodimers with 124-kDal phytochrome. While each of the three phytochromes has been detected in unimbibed embryos, etiolated shoots and green leaves, it is nevertheless evident that they are expressed differentially. The existence of at least three independently expressed phytochromes not only complicates the analysis of phytochrome mutants and transgenic plants containing introduced phytochrome genes, but also indicates the present futility of attempts to correlate phytochrome abundance and photostationary state with photomorphogenic responses.

139 A BLUE-LIGHT ACTIVATED GTP BINDING PROTEIN IN THE PLASMA MEMBRANES OF ETIOLATED PEAS. L.S. Kaufman*, K.M.F. Warpeha*, K. Bhattacharya*, H.E. Hamm+, and M.M. Rasenick+. *Dept of Biol Sci, and +Dept of Physiol and Biophys, Univ of Il at Chicago, Chicago, Il 60680.

We have established the presence of a heterotrimeric GTP-binding protein associated with the plasma membranes of the apical bud of etiolated peas. The GTPase activity is induced by low fluences of blue-light administered to plasma membrane encriched fractions. This activity is not responsive to red light irradiation and is specific for GTP. The threshold for the excitation of the GTPase activity in vitro is below 10^{-1} μmol m^{-2} of blue light consistent with participation in the blue low-fluence system identified in the same tissue. A 40 kD polypeptide is recognized by polyclonal antisera directed against the alpha subunit of the G protein transducin. The polypeptide also serves as a substrate for ADP-ribosylation by both cholera and pertusis toxins. The ability of the 40 kD polypeptide to serve as substrate for the toxin-induced ADP-ribosylation is mediated by blue-light irradiation, implying that the 40 kD polypeptide is the alpha subunit of a blue-light stimulated G protein. The 40 kD polypeptide binds a non-hydrolyzable photoaffinity labeling analog of GTP only after irradiation with blue light. The protein we have described may function as an α-subunit of a G-protein active in the process of light-mediated development in higher plants. We are currently attempting to purify the 40 kD polypeptide responsible for these blue-light regulated activities, as well as obtain cDNA clones for this polypeptide.

140 REGULATION OF GUARD CELL RESPONSES BY BLUE LIGHT AND SECOND MESSENGERS. Sarah M. Assmann, Harvard Univ., The Biological Laboratories, Cambridge, MA 02138

Light-stimulated stomatal opening is mediated by chlorophyll and a blue-light photoreceptor. Research conducted on the specific blue light response at both the single cell and the whole leaf level will be described. At the single cell level, patch clamp techniques have allowed us to detect blue-light activation of a H^+ pump at the guard cell plasmalemma. Recent experiments demonstrate that lipids (e.g., diacylglycerols) also activate the H^+ pump, and thus may comprise part of the signal transduction pathway for stomatal opening. Activation of H^+ extrusion creates an electrical gradient which presumably drives the K^+ uptake associated with stomatal opening. K^+ influx through K^+ selective ion channels is also regulated by signal transduction pathways: experiments indicating G-protein regulation of inward K^+ current will be described. At the level of the intact leaf, recent findings from gas exchange experiments include the discoveries that: 1) the magnitude of the blue light response is increased in plants of *Commelina communis* grown under high light conditions; 2) the blue light response is observed in the facultative CAM species, *Portulacaria afra*. These results do not support previous hypotheses that the blue light response is most relevant to low light (shade) conditions.

141 PHYTOCHROME-MEDIATED PHOTOTROPISM IN FERN PROTONEMATA Masamitsu Wada Biology Department, Faculty of Science, Tokyo Metropolitan University, Hachioji, Tokyo 192-03

Photo- and polarotropic response in fern protonemata is mediated by phytochrome and blue light-absorbing pigment. In Adiantum, the phytochrome which mediates the phototropism is localized in the ectoplasm of the apical and subapical portions of the protonemata, showing dichroic orientation. Pr is parallel and Pfr is perpendicular to the plasma membrane. The direction of the light may be detected by a Pfr gradient in the subapical region. When tropic response is induced, circumferentially-arranged cortical microtubule (Mt-band) and microfilament bands (Mf-band) around the subapical part of a protonema start to change their orientation from perpendicular to oblique to the initial growing axis, preceding the morphological change of cell shape. Mf-band changes its orientation much earlier than Mt-band does. When protonemata bend with large degree, the Mf- and Mt-bands disappear thereafter. In phototropic responses induced by local irradiation of a side of the subapical part of a protonema with a red microbeam, the Mt-band disappears or becomes faint, but neither disappearance or a change of orientaiton of Mts occur on the non-irradiated side. The innermost layer of microfibrils in the subapical part of the protonema also changes its orientation from perpendicular to oblique to the growing axis, corresponding to the changes in the orientaion of the Mt-band. Based on these observations, the mechanism of the phototropic response in a protonema will be discussed.

142 THE PHOTORECEPTOR FOR PHOTOTROPISM AS A MEDIATOR OF PROTEIN PHOSPHORYLATION? Timothy W. Short, Markus Porst, and Winslow R. Briggs. Carnegie Institution, 290 Panama St., Stanford, CA 94305.

When plasma membranes from etiololated pea stem segments are incubated with [γ-^{32}P]ATP, autoradiographs of subsequent sodium dodecylsulfate polyacrylamide gels show a protein with an apparent molecular weight of 120,000 to be strongly phosphorylated. However, if the tissue is exposed to a brief pulse of blue light prior to membrane isolation, subsequent analysis shows the *in vitro* phosphorylation of the 120 kilodalton protein to be greatly reduced. Characterization of the response suggests that protein phosphorylation may be an early step in the signal transduction pathway for phototropism. When the tissue is incubated with labelled phosphate and allowed to produce endogenous [γ-^{32}P]ATP, blue light exposure leads to rapid *in vivo* phosphorylation of the protein. Membranes isolated from etiolated tissue also show light sensitivity, with blue light causing an increase in subsequent *in vitro* phosphorylation of the protein. The light-induced phosphorylation increase remains active even in detergent-solubilized plasma membranes. This fact has allowed us to partially characterize the photoreceptor, kinase, and substrate activities involved in the reaction. At this time it is not known whether the active moieties are part of a single polypeptide or as distinct subunits. A putative substrate has been identified on protein gels, and this protein is being analyzed for kinase activity and association with likely chromophores. Antibodies generated against this protein and microsequencing of proteolytic fragments are being used to improve our understanding of the mechanism at work and to aid in attempts to isolate the blue light photoreceptor involved. Similar light-mediated phosphorylation changes in other dicots and in monocots have also been detected and are being investigated further.

SYMPOSIUM SPEAKERS

143 REGULATION OF GENES BY ABSCISIC ACID AND JASMONIC ACID DURING PLANT WATER DEFICIT. John E. Mullet, Robert Creelman, Hugh Mason, Erin Bell, Jennifer Jones, Daryll DeWald and Mark Whitsitt, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128

Plant response to water deficit is complex and depends on the severity and duration of water deficit, the stage of plant development, and plant species. Mild water deficit often reduces cell turgor, inhibits growth and results in accumulation of ABA. Elevated ABA levels in turn can differentially inhibit shoot vs root growth, aid stomatal closure and induce changes in gene expression. The search for genes involved in ABA synthesis has led us to identify several genes which are rapidly induced in response to decreased cell turgor. The turgor inducible genes encode a putative membrane channel forming protein, a thiol protease, an aldehyde reductase and lipoxygenase. In addition to the "turgor responsive" genes we have also identified several genes which exhibit altered expression in the hypocotyl growing zone of water stressed soybean seedlings. Genes showing decreased expression include the genes encoding ß-tubulin, actin, and glycine rich proteins. In contrast other genes are up-regulated in the water deficient seedlings. These results may provide insight into the differential inhibition of hypocotyl vs root growth in water deficient soybean seedlings. We have also reported that the genes encoding the soybean vegetative storage proteins show increased expression in water limited soybean plants. These genes are co-regulated by jasmonic acid and carbon. The *cis*-elements which control the expression of the vegetative storage protein genes are presently under investigation. In addition, a quantitative assay for jasmonic acid has been developed and used to investigate jasmonate levels in water stressed plants.

144 REGULATION OF ABA AND WATER STRESS RESPONSIVE GENES IN MAIZE Montserrat Pagès, Maria Pla, Josep Vilardell, Miguel Angel Freire and Adela Godav. Departamento de Genética Molecular Centro de Investigacion y Desarrollo. C.S.I.C. Barcelona. Spain.

Earlier studies on the regulation of gene expression during embryogenesis in Zea mays L. have shown that a group of stage specific mRNAs and proteins which accumulate in late embryogenesis are induced precociously in immature embryos by abscisic acid (ABA) treatment(1). Expression of maize RAB genes, coding for these proteins, can be induced in tissues other than those of the embryo, and at other stages besides embryo development by ABA or water stress (2,3). However, the mechanism by which embryo and vegetative organs share responses to ABA and water stress remains unclear. Analysis of the several viviparous mutants of maize that are deficient in embryo ABA or insensitive to exogenous ABA, failed to show major differences in the developmental induction of RAB genes during embryogenesis. However, there are differences in RAB gene expression in the vegetative tissues of the ABA-deficient viviparous mutants, particularly the absence of detectable RAB transcripts in water-stressed tissues (4). Functional analyses using transient systems and transgenic plants have been performed to identify ABA and water stress-responsive DNA elements in the promoters of different maize RAB genes. Our data suggest that more than one mechanism determines the level of expression of RAB genes and that control at the transcriptional and post-transcriptional level is involved.

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145 DESICCATION-INDUCED GENE PRODUCTS: FUNCTIONAL ANALYSIS AND REGULATION OF EXPRESSION. Dorothea Bartels, Detlef Piatkowski, Kathrin Schneider, Roland Alexander, Gabriel Iturriaga, Renza Roncarati, Christiane Hanke, Ralph Elster and Francesco Salamini. MPI for Plant Breeding Research, W-5000 Köln 30, Germany

The developing barley embryo and C. plantagineum as a representative of the poikilohydrous or resurrection plants were chosen as experimental systems to analyse molecular mechanisms of desiccation tolerance. Common to both systems is the unique feature of desiccation tolerance at the protoplastic level. In the barley embryo the tolerance to desiccation is developmentally regulated and restricted to particular stages of seed development whereas the resurrection plant exhibits desiccation tolerance in all tissues throughout the whole life cycle of the plant. By differential hybridization several cDNA clones were isolated which encode desiccation-related transcripts. Three cDNA clones isolated from the resurrection plant display homologies to desiccation-related genes expressed in many higher plants during late embryogenesis; a fourth cDNA clone shares tracts of sequences with a light-regulated transcript and three cDNA clones are unique to the resurrection plant. These studies showed that at least in part the gene products involved in desiccation pathways are common to maturing seeds and resurrection plants. From the barley embryo an ABA and GA modulated gene has been analysed which encodes an aldose reductase related protein. Data about the subcellular localization will be presented and the developmental and tissue specific expression will be discussed for these different desiccation-related gene products.

146 DEVELOPMENTAL, HORMONAL AND ENVIRONMENTAL REGULATION OF THE OSMOTIN GENE. R.A. Bressan, D.E. Nelson, K.G. Raghothama, P.C. LaRosa, A. Kononowicz, Z. Chen, A. Casas, M.Paino D'urzo, I. Ilahi, D. Liu N.K. Singh, and P. M. Hasegawa. Dept. of Horticulture, Purdue University, West Lafayette, Indiana 47907.

The osmotin gene is regulated by osmotic stress and several other factors including abscisic acid (ABA), ethylene, wounding, virus infection and spatial and temporal development of the plant. It has sequence similarity to several other genes which, along with its pattern of regulation, has led to its classification as a family -5 pathogenesis related (PR) gene. Nuclear run on experiments with ABA induction indicate transcriptional regulation. A number of regulatory conditions result in increased accumulation of osmotin mRNA but not protein suggesting that post-transcriptional regulation also occurs. Osmotin/GUS promoter fusion studies indicate a very tissue-specific pattern of promoter control including an interestingly high level of expression in mature pollen. Promoter-deletion/gel retardation studies indicate the presence of more than one cis-acting element that binds nuclear factors. Transgenic plants with chimaeric osmotin genes that over- and under-produce osmotin were made and are being used to test the possible function of this gene in osmotic stress tolerance. Two osmotin cDNA clones were isolated from an *Atriplex nummularia* cDNA library. One of these halophytic genes is highly induced by NaCl, the other is mostly constitutive. They also have distinctly different patterns of tissue expression.

147 CLONING, EXPRESSION AND FUNCTIONAL ANALYSIS OF THREE PLANT HEAT STRESS TRANSCRIPTION FACTOR GENES.
K.-D. Scharf[1], E. Treuter[1], S. Rose[1], W. Zott[1], F. Schoffl[2] and L. Nover[1]. Institute of Plant Biochemistry[1], O-4050 Halle; Department of Genetics[2], University, W-7400 Tubingen, FR Germany

Induced binding of the tomato heat stress transcription factor(s) (HSF) is observed after treatment with heat or chemical stressors. Using differential screening of a λ gt11 expression library with a functional HSE oligonucleotide and a mutant HSE respectively (Southwestern screening), we isolated 7 classes of clones. Three of them, coding for tomato HSFs, are characterized by a highly conserved 93 amino acid HSE-binding domain at the N-terminus, which is also found in HSFs of yeast, Drosophila and mammals. The C-terminal parts of the three factors are not conserved. Surprisingly, two of the HSF are heat stress-induced themselves, suggesting a cascade of HSFs, which may influence quantitative and qualitative aspects of the ns response (1, 2). Functional analysis of the HSF clones was done (i) with the proteins over-expressed in E. coli and (ii) by transient expression in tobacco mesophyll protoplasts using ns promoter x gus reporter constructs. Depending on the position of inserted HSEs activator and repressor functions of different HSF constructs can be monitored.

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148 ISOLATION AND CHARATERIZATION OF THE GENES CODING FOR HEAT-SHOCK PROTEINS IN ARABIDOPSIS THALIANA. Y. Komeda, T. Takanashi and S. Naito. University of Tokyo, Japan.

A family of proteins called heat-shock proteins (HSPs) are induced after the exposure of living organisms to elevated temperatures. We have begun an analysis of the HSP genes in Arabidopsis thaliana in order to study the genetic control of the synthesis of HSPs in plants. We have isolated the cDNAs for both two low-molecular-mass HSPs and two high-molecular-mass HSPs and their corresponding genes. The analysis of genes for small HSPs, HSP17.4 and HSP18.2 has been published. The analysis of those for large HSPs, HSP81-1 and HSP81-2 will be presented. The expression of these genes for HSPs was shown to be induced heavily by heat shock at 35C and as well induced by cadmium and arsenite. 5'-upstream region of these HSP genes were highly homologous to one another and had homologous regions with animal heat-shock elements. Gel-shift assay using nuclear extracts showed the existence of a factor that binds to the homologous region. To examine the function of the upstream regions of HSP18.2 as a heat-shock promoter, the HSP18.2-GUS chimeric gene was introduced into Arabidopsis and the induction of GUS activity occurred in a heat regulated manner. The transgenic plants were further used to isolate regulatory mutants of the heat-shock response. Analysis of such mutants will be presented and should help us to understand the molecular mechanisms that regulate the synthesis of HSPs.

SYMPOSIUM SPEAKERS

149 COLD ACCLIMATION IN ARABIDOPSIS THALIANA. M Thomashow[1,2], S Gilmour[1], R Hajela[1], D Horvath[2], C Lin[1] and W Guo[1]. Department of Crop and Soil Sciences1, Program in Genetics2, Michigan State University, East Lansing, MI 48824, USA.

We have established that Arabidopsis, like many other plants, increases in freezing tolerance in response to low temperature and that changes in gene expression occur during the cold acclimation process. Our long term objectives are to determine both the functions of the cor (cold-regulated) genes and the mechanism(s) responsible for their thermoregulation. Studies have centered on four genes designated cor160, cor47, cor15 and cor6.6. The transcript levels of these genes increase at least 10 fold in response to low temperature (4°C). Nuclear run-on transcription assays indicate that cor15 is regulated largely at the transcriptional level while cor160, cor47, and cor6.6 are regulated primarily at the posttranscriptional level. All four genes are ABA responsive. A number of COR polypeptides from both Arabidopsis and wheat share the unusual property of remaining soluble upon boiling. One of these, encoded by cor15, is a 15 kDa hydrophilic polypeptide that appears to be located in the chloroplasts; the polypeptide has potent cryoprotective activity in an in vitro assay. DNA sequence analysis of cor47 and cor6.6 indicates that the former encodes a polypeptide related to Group II LEA (late-embryogenesis-abundant) proteins, and the latter, a polypeptide almost identical to KIN1, a cold-regulated Arabidopsis polypeptide having sequence similarities with certain fish antifreeze proteins [Kurkela and Frank (1990) Plant Mol. Biol. 15:137]. The possible relationships between COR, LEA and antifreeze proteins and their potential roles in cold acclimation will be discussed.

150 SOYBEAN HEAT SHOCK GENE Gmhsp17.5E IS REGULATED BY HSE's AND AT-RICH PROMOTER ELEMENTS. E. Czarnecka, D. Barros, and W. B. Gurley. Microbiology & Cell Science, Univ. of Florida, Gainesville, FL 32611. Proteins in nuclear extracts of soybean plumules were characterized with respect to *in vitro* binding to AT-rich DNA sequences located in the *Gmhsp17.5E* promoter by gel mobility retardation assays and by DNase I footprinting. Molecular weights were established by UV crosslinking analysis. The two sets of protein:DNA complexes present in crude nuclear extracts comprised two distinct classes of proteins. The nuclear proteins were purified by ammonium sulfate precipitation, phosphocellulose, heparin Sepharose and AT-composite Sepharose affinity chromatography. The nuclear proteins of soybean plumules were shown to bind all of the AT-rich probes from a variety of inducible plant promoters in addition to oligonucleotide probes homologous to homeobox protein binding sites. Binding activity specific for AT-rich sequences seemed to be regulated by phosphorylation, and showed a wide distribution among the various plant organs tested in soybean and other plant species. The 8 bp 5'-TAATATTA-3' consensus sequence for nuclear proteins binding was derived *via* application of point-mutated oligomers as specific competitors in gel shift assays. The bases within the consensus were not of equal importance when tested by protein:DNA complex formation. The AT-rich sequences were shown to act as enhancer-like elements by their stimulation of the heterologous promoter for maize *Adh1* when assayed in anaerobically stressed sunflower tumors. A 33 bp double-stranded oligonucleotide homologous to two AT-rich sequences located upstream (-907 to -889 and -843 to -826) to the start of transcription of heat shock gene *Gmhsp17.5E* stimulated transcription when placed 5' to a truncated (-140) maize *Adh1* promoter.

151 INTEGRATION OF PLANT DEFENSE RESPONSES INTO DEVELOPMENTAL PROGRAMS. Christopher J. Lamb. Plant Biology Laboratory, Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800

Induction of phytoalexins, lytic enzymes and wall reinforcement involves transcriptional activation of defense genes, in some cases within 2-3 minutes of an elicitation signal. Rapid response genes include those encoding chitinase and phenylpropanoid biosynthetic enzymes for production of phytoalexins and lignin monomers. We have recently observed the apparent disappearance of two specific cell wall structural proteins in response to fungal elicitor or glutathione. This response, which reflects the insolubilization of these major wall proteins by H2O2-mediated oxidative cross-linking, is initiated within 1 to 2 minutes of elicitor addition, and complete within 5 minutes. We propose that this protein cross-linking, which presumably toughens the cell wall, is a novel, ultra-rapid defense mechanism.

152 EXPRESSION AND MANIPULATION OF GENES ENCODING ENZYMES OF PHENYLPROPANOID BIOSYNTHESIS IN ALFALFA. Richard Dixon, Karen Dalkin, Theodor Fahrendorf, Ganesan Gowri, Maria Harrison, Gary Loake, Carl Maxwell, Weiting Ni, Nancy Paiva and Lloyd Yu. Plant Biology Division, The Noble Foundation, P. O. Box 2180, Ardmore, Oklahoma 73402, USA.

The phenylpropanoid pathway is regulated by developmental and environmental (wounding, light, pathogen infection) signals. These result in activation of genes encoding enzymes involved in specific branch pathways leading to the formation of a range of functionally distinct end-products including defense related phytoalexins, structural polymers such as lignin, and inducers of bacterial nodulation genes. We have recently purified and/or cloned a number of enzymes involved in general and specific pathways of phenylpropanoid metabolism in alfalfa (Medicago sativa L.). These include L-phenylalanine ammonia-lyase, chalcone synthase (CHS), chalcone 2'-O-methyltransferase, caffeic acid O-methyl transferase (COMT), isoflavone O-methyltransferase and isoflavone reductase (IFR). We discuss the genomic organization and environmental and developmental expression patterns of several of these phenylpropanoid biosynthetic genes. Studies are now in progress aimed at modifying the expression of the lignin and phytoalexin pathways in transgenic plants by manipulation of COMT and IFR respectively. Transient gene expression assays and stable transformation studies have helped define a number of cis-acting elements involved in determining quantitative and qualitative expression patterns of a bean CHS promoter in alfalfa cells. Our current understanding of the transcription factors which interact with these elements will be reviewed.

153 SALICYLIC ACID IS A SYSTEMIC SIGNAL AND AN INDUCER OF PATHOGENESIS-RELATED PROTEINS IN VIRUS-INFECTED TOBACCO. Ilya Raskin, Nasser Yalpani and Paul Silverman. AgBiotech Center, Cook College, Rutgers University, P.O. Box 231, New Brunswick, New Jersey 08903-0231

As reported earlier, systemic acquired resistance (SAR) to tobacco mosaic virus (TMV) in tobacco and the associated induction of pathogenesis-related (PR) proteins may be caused by a minimum 10-fold systemic increase in endogenous levels of salicylic acid (SA). This rise in SA parallels PR protein induction and occurs in TMV-resistant Xanthi-nc tobacco carrying the 'N' gene but not in TMV- susceptible 'nn' tobacco. By feeding SA to the excised leaves of Xanthi-nc (NN) tobacco we have shown that the increase in SA levels observed during SAR is sufficient for the systemic induction of PR proteins. TMV infection became systemic and Xanthi-nc plants failed to accumulate PRI proteins at 32 °C. This loss of HR at high temperature was associated with inability to accumulate SA. However, spraying leaves with SA induced PRI proteins at both 24 °C and 32 °C. SA is most likely exported from the primary site of infection to the uninfected tissues. A computer model predicts that SA should move rapidly in phloem. When leaves of Xanthi-nc tobacco were excised 24 hr after inoculation and exudates from the cut petioles collected, the increase in endogenous SA in TMV- inoculated leaves paralleled SA levels in exudates Exudation and leaf accumulation of SA were proportional to TMV concentration and were higher in light than in darkness. Different components of TMV were compared for their ability to induce SA accumulation and exudation: three different aggregates of coat protein failed to induce SA, but unencapsidated viral RNA elicited SA accumulation in leaves and phloem. These results further support the hypothesis that SA acts as an endogenous signal, which activates some components of resistance and systemic induction of PR proteins.

154 INDUCTION OF PR-GENES BY VIRUS INFECTION AND WOUNDING. John F. Bol, Frans Th. Brederode and Huub J.M. Linthorst. Leiden University, Gorlaeus Laboratories, Einsteinweg 5, 2333 CC Leiden, The Netherlands.

After infection with tobacco mosaic virus (TMV) Samsun NN tobacco plants accumulate at least five different groups of pathogenesis-related proteins and the plants acquire a systemic resistance to further infection. The function of proteins from PR groups 1, 4 and 5 is not known; proteins from groups 2 and 3 have beta-1,3-glucanase and chitinase activity, respectively. All five groups contain acidic extracellular proteins. In addition, groups 1, 2, 3 and 5 contain basic vacuolar isoforms. Cloned cDNAs were used as probes to follow the local and systemic induction of PR genes by various treatments. All PR genes were induced in TMV-inoculated leaves but systemic induction was observed mainly for genes encoding acidic isoforms. Incission-wounding of tobacco leaves strongly induced PR genes encoding basic isoforms but did not induce genes encoding acidic isoforms. Because wounding did not induce an antiviral response of the plant, the basic isoforms may not be involved in this defense. Irradiation of tobacco plants with UV light resulted in the induction of both acidic and basic PR proteins and the plants became resistant to TMV infection. These data favor a role of acidic PR proteins in the defense of the plant against virus infection. Some data on newly characterized PR proteins will be presented.

SYMPOSIUM SPEAKERS

155 MOLECULAR MARKER ANALYSIS OF LOW TEMPERATURE RESPONSES IN *BRASSICA*. Rita A. Teutonico[1], Philippe Moreau[1], Jiwan P Palta[2], and Thomas C. Osborn[1] University of Wisconsin. Departments of Agronomy[1] and Horticulture[2]. Madison, Wisconsin. 53706 Plants within the genus *Brassica* have several important responses to low temperature Many genotypes require exposure to low temperature (vernalization) to induce flowering and responses to freezing temperatures are important for winter survival. There is substantial genetic variation for these responses, however, the genetic control of this variation is not well understood In order to study genetic control of low temperature responses, we are using molecular markers to analyze a segregating population of turnip rape (*B rapa* syn. *campestris*) for genes controlling responses to low temperature. The parents of this population differ for vernalization requirement, freezing tolerance, acclimation ability and winter survival Molecular markers are being used to identify genes controlling these traits and to determine if the same sets of genes control different responses to low temperature. In addition, molecular and biochemical changes induced by low temperature are being analyzed to determine if any of the same genes also control these responses to low temperature.

156 APPLICATION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM TO MAIZE BREEDING. Michael G. Murray, Yan-San Chyi, Jane Harris Cramer, Jeanne Romero-Severson, David P. West, Dave Zaitlin. Agrigenetics Company, Madison, Wisconsin 53716

Restriction Fragment Length Polymorphism (RFLP) technology has made the concept of linked-marker breeding a practical reality in maize. Today's well-saturated maize maps make it possible to tag desired chromosomal segments and follow their assortment through the recombination process. The resources heretofore devoted to large progeny populations and extensive replication can thus be used more efficiently. RFLP technology is particularly effective in accelerating the rate at which relatively simple traits can be introgressed into elite germplasm.

RFLP technology is also proving effective in elucidating the number and nature of gene effects which result in quantitatively inherited traits. However, the predictive value of RFLP Marker Linkages to genes is only as good as the accuracy of the phenotypic data upon which the linkage was calculated. Our experience suggests that complex trait mapping efforts are generally limited more by inadequate replication, imprecise scoring, and small mapping population sizes than by the sheer number of molecular markers available for use. The time needed to prepare appropriate recombinant inbred mapping populations is well worth the effort.

Optimum exploitation of linked marker breeding requires close integration between lab, greenhouse, and field operations. Practical examples of the mapping and manipulation of complex traits will be discussed.

157 GENETIC ANALYSIS OF THE MORPHOLOGICAL EVOLUTION OF MAIZE. John Doebley. University of Minnesota, Department of Plant Biology, St. Paul, MN 55108

Understanding the genetic basis of morphological change is a fundamental concern of both geneticists and evolutionary biologists The combined use of molecular markers and quantitative genetic models provide a powerful means for dissecting the genetic basis of morphological evolution. Specifically, molecular marker loci can be used as tags to locate morphological trait loci and investigate their effects on the phenotype This approach was used to investigate the genetic basis of the dramatic morphological differences between maize (Zea mays ssp. mays) and its presumed progenitor, teosinte (Z mays ssp. parviglumis) Results indicate that the key traits differentiating maize and teosinte are each under multigenic control, although for several traits, such as number of ranks of cupules, the data are consistent with a mode of inheritance that would involve a single major locus plus several modifiers For other traits, such as presence/absence of the pedicellate spikelet, the data indicate multigenic inheritance with no single locus having a dramatically larger effect than the others Results also indicate that the tunicate locus (Tu) had major no role in the origin of maize, despite previous opinion that it was involved. Rather, our data suggest that an undescribed gene (or genes) in 4S control the switch from hard to soft glumes in the evolution of the maize ear. Most of the variation for the dramatic differences in inflorescence morphology between maize and teosinte is explained by five restricted regions of the genome. One of these regions encompasses a previously described gene, tb1 (teosinte branched), and the effects of this region on inflorescence architecture are similar to the known effects of tb1 The data suggest that the differences between teosinte and maize involve, in part, developmental modifications that enable (1) secondary inflorescences, which are programmed to develop into tassels (male) in teosinte, to become ears (female) in maize, and (2) the expression of male secondary sex traits on a female background in maize. Similar changes were likely involved in the origin of maize.

158 DEVELOPMENT AND APPLICATION OF DNA BASED DIAGNOSTICS TO PROBLEMS IN PLANT GENETICS. Scott V. Tingey, J. Antoni Rafalski, and John G.K. Williams. E.I. du Pont de Nemours & Co., P.O. Box 80402, Wilmington, DE 19880-0402.

The use of DNA markers in genetic mapping, genetic diagnostics, molecular taxonomy, and evolutionary studies has been well established. The most commonly used DNA marker is the restriction fragment length polymorphism (RFLP). Detection of RFLPs by Southern blot hybridization is laborious and incompatible with applications requiring high analytical throughput. Genetic tests based on the polymerase chain reaction are simple to perform, but require target DNA sequence information in order to design specific primers.

We have recently described (Williams et al., 1990, NAR 18:6531) a novel class of genetic marker which is based on DNA amplification, but requires no knowledge of target DNA sequence. These markers, called RAPD markers (for Random Amplified Polymorphic DNA), are generated by the amplification of random target DNA segments with single primers of arbitrary nucleotide sequence. RAPD markers can be used for genetic mapping applications, as well as for genetic diagnostics. The non-radioactive assay requires nanogram quantities of DNA and is applicable to a broad range of species. Here we present experimental protocols, and discuss RAPD applications, with emphasis on plant genetics.

159 FUNCTIONS OF THE ORFa PROTEIN, THE PUTATIVE TRANSPOSASE OF MAIZE TRANSPOSABLE ELEMENT *Ac* Siegfried Feldmar, Detlef Becker, Ralf Lutticke, Reinhard Kunze and Peter Starlinger University of Cologne, Institute of Genetics, Weyertal 121, 5000 Koln 41, FRG

The 807 amino acids long *Ac* ORFa protein is a DNA-binding protein that recognizes subterminal AAACGG-motifs within the *Ac* element It has a basic DNA-binding domain with essential basic residues' located between amino acids 191 and 200, as was shown by DNA-binding assays with deletion and substitution derivatives The N-terminal 102 amino acids of the ORFa protein are neither involved in DNA-binding nor required for the transposing activity As their deletion even increases the transposition frequency in a transient assay, we suspect that the N-terminus rather has a reducing function. Between amino acids 109 and 128 a tenfold direct repetition of the dipeptide PQ or PE is localized A deletion of this segment does not prevent DNA-binding, but strongly reduces or even abolishes the transposition function At least 537 amino acids may be removed from the C-terminus of the ORFa protein without disturbing its DNA-binding domain However, the 220 C-terminal amino acids seem to be essential, because their deletion inactivates the transposition function Within this region a good homology to the C-terminus of the open reading frame of the *Antirrhinum* transposon *Tam3* is found The DNA-binding domain of the *Ac* protein, however, has no homology to *Tam3* Accordingly, the *Ac* ORFa protein does not bind to any *Tam3* sequences

160 ACTIVITIES AND EVOLUTION OF THE Mu AND Bs MOBILE ELEMENTS OF ZEA MAYS. Jeffrey L. Bennetzen, April D. Cresse, Young-Kwan Jin, Willis E. Brown, David Horvath and Patricia S. Springer. Purdue University, Department of Biological Sciences, West Lafayette, Indiana 47907.

Amongst all known mobile DNA systems, the Mutator transposable elements of maize are unique in their structural diversity and exceptional for their high rate and promiscuity of mutant induction. We have characterized the activities, regulation, specificity, diversity, and dispersion of subfamilies within the Mu system. We have found that different subfamilies transpose at different frequencies in the same populations. Fifteen insertions sites for the Mu1/Mu1.7 subfamily were analyzed in detail; insertions were detected primarily in low copy number DNA but no specific sequence requirements were detected at the target sites. The Mu1.7 element did not show any significant bias for transposition to linked chromosomal locations. We have also found and studied flanking deletions and excision legacies associated with Mu element activity. The degree to which different Mu element subfamilies are dispersed was found to vary in accord with different points of origin.

In striking contrast to the Mutator system, the Bs mobile elements of maize were found to be notable for their low level of mobility and their lack of element diversity. Our sequence of Bs1 indicated that it has the structure of a "non-autonomous" retrotransposon. We have also found that Bs1 has a region of sequence homology with the plasma membrane proton ATPases of plants. We have used this homology to clone a putative plasma membrane H+ ATPase gene from maize. The sequence of this gene confirmed that Bs1 has acquired part of six contiguous exons from this ATPase gene. Contrary to the published report, we have found many different Bs element types, at least one of which has the structure of a potential "autonomous" Bs element.

SYMPOSIUM SPEAKERS

161 TRANSPOSITION AND GENOME CHANGE IN Antirrhinum majus. Cathie Martin, Clare Lister, Steve Mackay, Rachel Burton, John Innes Institute, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

Transposons cause mutations by insertion into genes and by leaving footprints when they excise. They can also cause large-scale rearrangements at a high frequency which appear to result from aberrant transpositions. A study of such rearrangements therefore sheds light on the mechanism of transposition in relation to chromosome structures. Some rearrangements generated by Tam3 give rise to dominant or trans-acting mutations. These appear to be associated with the production of inverted duplications. Inverted duplications also appear to be very unstable and in combination with transposons give rise to further rearrangements of DNA. Transposons can therefore rapidly generate particularly unstable regions of the genome which may become hot spots for genome reorganization through mechanisms linked to transposition.

162 A MODEL FOR TRANSPOSITION OF En/Spm. Alfons Gierl, Monika Frey, Julio Reinecke, Guilermo H. Cardon Stefan Trentmann, Peter A. Peterson and Heinz Saedler. Max-Planck-Institut für Züchtungsforschung, Köln, FRG.

An assay system for En/Spm transposition was established in transgenic tobacco. A 2.2 kb reporter dSpm element was inserted infront of the GUS coding region. Excision of the dSpm element leads to restauration of GUS gene activity. Thus, somatic excision is displayed by a variegated phenotype upon staining tobacco seedlings with X-Glu, very similar to the situation with spotted kernels in maize. In this model system two En/Spm encoded proteins, TNPA and TNPD, are required for excision of the dSpm reporter element. The sequence alterations (footprints) generated during excision are similar to the ones observed in maize. The dSpm element in this system is also able to transpose to new locations in the tobacco genome, generating the characteristic 3 bp target site duplication. Therefore, the mechanism of excision and integration in the presence of TNPA and TNPD seems to be also identical to the one present in maize.

Based on these results and on DNA-binding studies with TNPA protein, a model for En/Spm transposition is proposed: Binding of TNPA to the subterminal regions of En/Spm induces complex formation between both ends by a "zipper" like mechanism, where the bound TNPA molecules represent the teeth of the zipper. This leads to correct alignement of the terminal inverted repeats (TIRs) of En/Spm. Accordingly, binding of TNPD to the TIRs leads to endonucleolytic cleavage and release of En/Spm, accompanied by healing of the chromosome breaks.

163 EVOLUTION OF CHLOROPLAST GENE AND INTRON CONTENT. Jeffrey D. Palmer. Department of Biology, Indiana University, Bloomington, Indiana 47405

I will discuss the evolution of chloroplast gene content from three perspectives. First, recent data will be reviewed indicating that chloroplasts of highly divergent lineages (land plants, Cyanophora, Euglena) contain substantially different sets of genes. Second, I will outline the multiplicity of pathways whereby genes for chloroplast proteins originate, are lost, or are transferred between compartments. Finally, ongoing studies from our laboratory will be presented dealing with cases of genes that have been transferred to the nucleus in relatively recent evolutionary time. The origin, diversification, and loss of chloroplast introns will also be discussed from three perspectives. First, it will be shown that most chloroplast introns are of relatively late, post-endosymbiotic origin. In contrast, two specific chloroplast introns, one a group I intron and the other a group II intron, will be shown to be ancient introns, present not only in the cyanobacterial ancestors of chloroplasts but, most likely, in the progenote ancestor of all extant life. Finally, data will be presented bearing on the tempo and pattern of intron gain and loss within land plant evolution.

164 PLASTID DNA EVOLUTION AND FUNCTION: THE COMPLETE SEQUENCE OF THE PLASTID GENOME OF A NONPHOTOSYNTHETIC PLANT. Claude W. dePamphilis[1,2], Clifford W. Morden[2], Kenneth H. Wolfe[2], Stephen R. Downie[2], and Jeffrey D. Palmer[2]. [1]Department of Biology, Vanderbilt University, Nashville, TN 37235, and [2]Department of Biology, Indiana University, Bloomington, IN 47405.

What happens to a plastid genome when it is no longer constrained for photosynthetic function? The obligate root parasite, Epifagus virginiana, while completely lacking photosynthetic ability, retains a much reduced plastid DNA (ptDNA) only 70 kb in size, the result of many small and large deletions from both single copy and inverted repeat regions of the genome. The complete sequence of this ptDNA shows that photosynthetic and ndh genes, and most open reading frames (ORFs) found in the ptDNAs of photosynthetic plants are deleted from Epifagus. A small portion of a few of these genes (rbcL, psbA, atpA, ndhB) remain. In addition, all four RNA polymerase genes, five ribosomal protein genes, and 11 tRNA geneshave been lost from the Epifagus ptDNA, and at least two of the remaining tRNA sequences are evidently pseudogenes. Despite the absence of these components of plastid gene expression, most plastid genes for the translational apparatus appear to be intact as do a small number of ORFs. The genome is apparently functional at a transcriptional level, with transcripts detected in Epifagus of plastid rRNA and ribosomal protein genes as well as two of the remaining ORFs. These results suggest that the ndh genes and most of the ORFs without known function in green plants will be found to be involved in photosynthetic or closely related functions, but that the ORFs remaining in Epifagus have distinct, nonphotosynthetic functions. Transcription of Epifagus ptDNA and translation of plastid mRNAs must involve RNA polymerase(s) and tRNAs imported from the cytoplasm.

165 PHYLOGENETIC ORIGIN OF THE CRYPTOMONAD PLASTID GENOME. Susan E. Douglas, Institute for Marine Biosciences, National Research Council, 1411 Oxford Street, Halifax, Nova Scotia, CANADA B3H 3C1

Two groups of algae, the cryptophytes and chromophytes, possess plastids surrounded by four membranes rather than the two found in most other plants. It has been proposed that these algal groups arose by two distinct endosymbiotic events. The first involved a photosynthetic prokaryote and a phagotrophic eukaryotic host and resulted in those organisms (rhodophytes, chlorophytes and land plants) whose plastids are surrounded by two membranes. The second endosymbiotic event between a primitive eukaryotic alga and a different phagotrophic eukaryotic host gave rise to those algae whose plastids are surrounded by more than two membranes. Cryptophyte algae are unique in retaining the nucleus of the first endosymbiont (the nucleomorph) in the space between the inner and outer plastid membrane pairs. Comparisons of small subunit rRNA sequences from the nucleomorph and nucleus of this alga show that two distinct endosymbiotic events involving phylogenetically distinct eukaryotes indeed contributed to the formation of the cryptomonad cell. Evidence from gene mapping studies and sequencing of 16S rRNA indicates that the plastid genome of the cryptophyte alga *Cryptomonas* φ, most closely resembles those of red algae.

166 THE CHLOROPLAST GENOMES FROM MONOCOT, DICOT AND GYMNOSPERM. Masahiro Sugiura. Nagoya University, Center for Gene Research, Nagoya 464-01, Japan

The complete nucleotide sequences of rice, monocot, and tobacco, dicot, chloroplast genomes were determined to be 155,844 bp and 134,525 bp, respectively. Four different rRNA genes, 30 different tRNA genes and 62 different polypeptide genes (including putative genes) have so far been located in both genomes. One-third of black pine, gymnosperm, chloroplast genome (ca.120 kbp) has so far been sequenced and 36 genes have been mapped.

The rice genome is 14% smaller than the tobacco genome, which is mainly due to lacking several long ORFs found in tobacco. The rice IR has expanded outward and its LSC has undergone a series of inversions, which has probably produce pseudo tRNA genes at the inversion points.

The pine genome apparently lacks IRs. It also lacks rps16 but contains ORF510 at the corresponding position, similar to the liverwort genome. We also compared the three genomes with that of liverwort. Based on the comparison, unidentified ORFs can be grouped such that it is possible to speculate about the functions of products which may be produced by these ORFs.

SYMPOSIUM SPEAKERS

167 PROGRAMMING OF FLOWER COLOR PATTERNS ASSOCIATED WITH CO-SUPPRESSION OF CHS GENES. Richard Jorgensen, Department of Environmental Horticulture, University of California, Davis, CA 95616

The introduction of a chalcone synthase (CHS) transgene to an ectopic position in the petunia genome can result in the coordinated and reversible suppression of the transgene and both alleles of the homologous, endogenous CHS gene. Co-suppression of CHS genes in petunia also results in striking flower pigmentation patterns which are highly regular and non-clonal. These patterns are subject to stochastic reprogramming events which occur in meristems. In these events, all cells of the meristem appear to be reprogrammed cooperatively, resulting in the expression of a new pattern in all subsequent flowers of the reprogrammed shoot. In addition, the new pattern program is often heritable. Lastly, transgene alleles determining different pattern programs interact in the same nucleus resulting in the alteration of one allele in a manner that persists after the alleles have segregated away from each other, as in paramutation in maize.

168 EPIGENETIC CONTROL OF THE MAIZE Spm TRANSPOSABLE ELEMENT. N. Fedoroff, J. Banks, P. Masson, V. Kolosha, D. Cook, and M. Schlaeppi. Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland, 21210.

The maize *Suppressor-mutator* (*Spm*) element is subject to epigenetic inactivation. Genetic analysis of the inactivate elements has permitted the dissection of the underlying mechanism into two components. One component determines the element's phase of activity (active or inactive), while the second component controls the stability of the phase setting in development, its heritability, and its phase in the next generation. The former is termed the *phase setting* and the latter, the *phase program*. The results of molecular studies reveal that differences in the *Spm*'s phase setting are correlated with differences in methylation of the sequence immediately upstream from its transcription start site, while differences in its phase program are correlated with differences in methylation of a GC-rich sequence immediately downstream from its transcription start site. There is both genetic and molecular evidence that active elements can reactivate inactive, methylated elements, as well as promote heritable changes in the phase program. Efforts are underway to characterize the responsible element-encoded gene products and the element sequences with which they interact, using *Spm* promoter-reporter gene fusions in a transient assay system.

170 GENE INTERACTIONS AND EPIGENETIC VARIATION IN TRANSGENIC PLANTS. M.A. Matzke, H. Oberkofler, F. Neuhuber, G. Lirk and A.J.M. Matzke. Institute of Molecular Biology, Academy of Sciences, Salzburg, Austria.

To study the phenomenon of trans-inactivation of gene expression, we have analyzed the interactions which can occur between model selection and screening marker genes in transformed tobacco plants. Such trans-inactivation, which appears to be dependent both on regions of DNA sequence homology between the interacting genes as well as their relative locations in the genome, has been observed both in doubly transformed plants obtained from sequential transformation steps, and in progeny of crosses between individual plants containing previously identified suppressor and suppressed loci, respectively. Information on the genetics of these interactions will be presented, as will data to support the following conclusions: 1) Suppression and the accompanying promoter methylation of an affected gene can be either partial or complete; 2) Methylation in the promoter of the suppressed gene is lost only gradually after suppressor loci are crossed out; 3) Within a suppressed locus, all genes having sequence homology with the suppressor locus can be affected.

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171 FOLDING RUBISCO WITH CHAPERONINS. George H. Lorimer, Paul V Viitanen, Anthony A. Gatenby and Saskia M. van der Vies. Central R&D Dept., DuPont Co. Exp. Sta., Wilmington DE 19880-0402

Chaperonins (cpn) are ubiqitous and abundant proteins which facilitate the folding of other proteins *in vivo* The non-native states of many proteins, including Rubisco, tend to aggregate irreversibly. (In the recombinant cell this leads to the formation of inclusion bodies.) Cpn60 prevents this aggregation by forming stable binary complexes with these non-native states. These complexes are sufficiently stable to be isolated by gel filtration chromatography. Cpn60 does not interact with the native states of these proteins. However, formation of a binary complex also inhibits the transition to the native states. The structural elements recognized by cpn60 remain to be defined. One or two mols of non-native, target protein are bound per mol of $[cpn60]_{14}$.Conversion of the binary complex of cpn60-Rubisco to the active Rubisco dimer requires the presence of a second protein, cpn10, and is associated with the K^+-dependent hydrolysis of ATP. In chloroplasts, cpn60, previously known as the Rubisco subunit binding protein, and a protein with functional properties similar to the bacterial cpn10, have been detected. The folding intermediate (F1) of Rubisco is sufficiently stable at 4^o to examine some of its physical properties. Its CD spectrum reveals considerable secondary structure, the trp fluorescence is more like the native state than the unfolded state but ANS fluorescence indicates a greater exposure of hydrophobic surface than in the native protein. *In vitro* complementation experiments show that Rubisco F1 is monomeric. It is much more sensitive to proteolysis than the native state. It is rapidly formed ($t_{1/2}$<1 sec at 4^o) upon diluting unfolded Rubisco, but reverts to the native state at 4^o with a $t_{1/2}$ of about 8h. At concentrations Rubisco F1> 150nM it forms to aggregates whereas the native state remains dimeric even at 5mM. It is not yet known if the transition state for folding o Rubisco occurs while in association with cpn60 or after release.

172 ENZYMATIC CATALYSIS OF CARBON DIOXIDE FIXATION - A STRUCTURAL VIEW. Stefan Knight, Inger Andersson, Carl-Ivar Branden, Ylva Lindqvist, Tomas Lundqvist and Gunter Schneider. Swedish University of Agricultural Sciences, Uppsala Bionedical Centre, Department of Molecular Biology, P.O. Box 590, S-751 24 Uppsala, Sweden.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyses the first steps of the two opposing metabolic pathways of photosynthetic carbon reduction and photorespiratory carbon oxidation. Both catalytic activities take place at a common active site and require the prior activation of the enzyme by formation of a catalytically functional ternary complex enzyme - CO_2 - Me^{2+}. Structures of both the non activated and activated enzyme and a number of complexes have been obtained by X-ray crystallographic methods. In higher plants the enzyme is a hexadecamer of two types of subunit (L_8S_8). The large subunit (L) harbors the active site which is located at the C-terminal side of an eight stranded parallel alpha/beta barrel. The binding site for substrate RuBP as well as for the reaction intermediate analogue CABP stretches across the opening of the barrel with two distinct phosphate binding sites. The active site magnesium ion is coordinated to three residues in loop 2 of the alpha/beta barrel, D203, E204 and carbamylated K201 and is apropriatly situated to polarize the O2-C2 bond in the initial enolization step of the reaction mechanism. During catalysis the active site is shielded from solution by loop 6 which blocks off the entrance to the active site. This loop is flexible when the substrate binding site is unoccupied. Here, the series of chemical events during the carboxylation reaction and the structure of the enzyme will be described and correlated to biochemical and genetic data.

173 THE USE OF TRANSGENIC PLANTS TO STUDY THE REGULATION OF CARBON METABOLISM. M. Stitt, Botanisches Institut, Im Neuenheimer Feld 360, Heidelberg, FRG

In transgenic plants the expression of a chosen enzyme can be decreased by using 'antisense' technology, or a heterologous protein can be expressed to disturb a particular process. I will discuss how genetically manipulated plants can be used to study the regulation of metabolism using as examples:

(1) The impact of decreased expression of Rubisco on photosynthesis, storage, allocation and growth.

(2) The effect of decreasing the activity of sucrose- and starch-synthesizing enzymes on partitioning and growth.

(3) Inhibition of sucrose export, by expressing invertase from yeast in the cell wall and vacuole of tobacco.

This work was carried out in collaboration with S. Rodermel and L. Bogorad (Harvard) and U. Sonnewald and L. Willmitzer (Berlin) and was supported by the Deutsche Forschungsgemeinschaft.

SYMPOSIUM SPEAKERS

174 MOLECULAR ANALYSIS OF MUTANTS OF THE CO_2 CONCENTRATING MECHANISM IN *SYNECHOCOCCUS* PCC7942. Murray R.Badger, G.Dean Price and Jian-Wei Yu. Research School of Biological Sciences, Australian National University, Canberra, Australia.

Cyanobacteria possess a CO_2 concentrating mechanism (CCM) which results in a high photosynthetic affinity for inorganic carbon (Ci) and improved photosynthetic efficiency. Mutants which are defective in various components of the CCM have been selected by screening for the ability to grow at high-CO_2 but not at air. In *Synechococcus* PCC7942 the most frequent type of mutant recovered under these conditions are those which are able to accumulate internal Ci but are not able to use it effectively for photosynthesis. Physiological and molecular analysis of some of these mutants indicates that these lesions affect the assembly and functioning of the carboxysome, the polyhedral protein body which contains most, if not all, of the cells Rubisco. A number of ORFs have been identified in the region 1 to 5 kb upstream of the 5' end of the *rbcL* gene. Inactivation of some of these ORFs either individually or together creates high-CO_2-requiring phenotypes with altered carboxysome morphology or the complete absence of carboxysomes. Another class of mutants appears to have altered carboxysome carbonic anhydrase (CA) activity. These mutants are complemented by a 3.5 kb BamH1 fragment which has some homology to the spinach chloroplast CA gene and may be located some 20 kb downstream of the *rbcS* gene. The analysis of these mutants and other data strongly support the notion that the carboxysome may be the localised site for CO_2 elevation within the cells and that this is achieved through a specific association of Rubisco and CA in these bodies, together with a mechanism to reduce CO_2 leakage. More recently, we have isolated a mutant which is able to grow at air but not at 20 ppm CO_2. This mutant is defective in the ability to induce high-affinity HCO_3^- transport activity. Analysis of these types of mutants should provide information about HCO_3^- transport activity and the ability to adapt to low-Ci conditions.

175 MOLECULAR BIOLOGY OF PHOTOSYSTEM I REACTION CENTER. Parag R. Chitnis, Dian-Lin Xie, Michael Büttner, Günter Hauska and Nathan Nelson. Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110

Photosystem I catalyzes the photoreduction of ferredoxin using plastocyanin as an electron donor. The complex contains two large molecular weight subunits (approximately 80 kDa) which bind all the pigments of the complex and catalyze the primary photochemical reaction. There are also several lower molecular weight subunits (8 to 22 kDa), whose functions are only now being identified. We have embarked on a program of cloning and characterizing genes encoding these subunits from the cyanobacterium *Synechocystis* sp. PCC 6803 and then generating subunit-specific mutants. We recently cloned and sequenced the genes (*psaD*, *psaE*, *psaF* and *psaB*) encoding four of the subunits of PS I. We have generated mutants for these genes by interrupting, deleting or substituting the wild type genes from the genome. The phenotypic characterization of these mutants with reference to the structure-function relationship in photosystem I will be described. We are also studying biogenesis of photosystem I. The in vitro synthesized, radiolabelled subunits II and IV were used to study their integration into thylakoid membranes and into photosystem I. Some *cis* and *trans* requirements for this process will be described. Recently we obtained several types of crystals of photosystem I. Our approach is to eliminate the lower molecular weight subunits and to crystallize the core complex. The gene encoding the reaction center protein of chlorobium was cloned and sequenced. This is the first bacterial gene encoding photosystem I-like reaction center that was cloned. The evolutionary implications revealed by the chlorobium gene will be discussed.

176 SUBUNIT FUNCTION AND ORGANIZATION IN THE CHLOROPLAST PSI COMPLEX. Richard Malkin and April Zilber. University of California, Dept. of Plant Biology, Berkeley, CA 94720.

The chloroplast Photosystem I complex contains at least 15 protein subunits, many of which still have no known function. We have been investigating the topology of these subunits with respect to their orientation relative to the membrane surface using proteolysis in conjunction with amino acid sequencing of proteolytic fragments. Trypsin treatment of intact membranes removes a small portion of the 22 kDa subunit (the psaD gene product) with only a marginal effect on the ability of the treated membranes to reduce NADP in the light. This result indicates that a portion of the psaD subunit is exposed at the stromal surface and that this segment of the protein is not required for interaction with ferredoxin. Under similar conditions, pronase treatment of intact membranes leads to the conversion of the high molecular mass psaA and psaB subunits to lower molecular mass forms of approximately 45 kDa. These digested subunits still bind Fx, the first iron-sulfur center-containing electron acceptor in the complex. Under conditions where the psaA and psaB subunits are digested by pronase, the psaK subunit of approximately 5 kDa is also degraded. These results and other experiments will be used to discuss models for the subunit organization in the Photosystem I complex.

177 ORGANIZATION AND DEVELOPMENT OF PHOTOSYSTEM I IN BARLEY. Henrik Vibe Scheller, Birgitte Andersen, Jens Sigurd Okkels, Vibeke Skovgaard Nielsen, Bodil Kjær, and Birger Lindberg Møller. Plant Biochemistry Laboratory, Department of Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark.

Photosystem I (PS I) from barley has been shown to contain ten different polypeptide subunits. Four subunits are encoded by the chloroplast and six by the nuclear genome. cDNA clones and clones for the chloroplast genes have been obtained. Based on functional and structural studies, a model of PS I has been proposed which emphasizes a pseudosymmetrical structure of the complex and the similarity to other photosynthetic reaction centers. Different methods have been developed for isolating PS I. Using a selection of detergents, we have been able to isolate PS I with a stoichiometric relationship between all subunits. PS I can be isolated together with or without the peripheral antenna complexes. The improved preparations are used in attempts to crystallize the PS I complex. The assembly of PS I has been studied in greening experiments. Immunoblotting shows that all subunits accumulate at the same rate. Northern blots have shown that all subunit genes are expressed in the dark. Therefore, the PS I subunits are under strong translational and posttranslational control. In the barley PS I mutant *viridis-zb*^{63}, mRNAs for all the analyzed PS I subunits are present, and very low amounts of some of the gene products can be detected. The rate of synthesis and degradation of the PS I subunits in dark-grown plants and in mutants is presently being investigated in pulse-chase experiments.

178 RECONSTITUTION OF THE PHOTOSYSTEM I REACTION CENTER WITH GENETICALLY MODIFIED PsaC AND PsaD POLYPEPTIDES EXPRESSED IN *ESCHERICHIA COLI*. John H. Golbeck[1], Ning Li[1], Jindong Zhao[2] and Don Bryant[2]. [1]University of Nebraska, Department of Biochemistry, Lincoln, NE 68583 and [2]The Pennsylvania State University, University Park, PA 16802.

The *psaC* gene product from *Synechococcus* sp. PCC 7002 and the *psaD* gene product from *Nostoc* sp. PCC 8009 were synthesized in *Escherichia coli* and purified to homogeneity. Incubation of the PsaC apoprotein with the *Synechococcus* sp. PCC 6301 Photosystem I core protein in the presence of $FeCl_3$, Na_2S and ß-mercaptoethanol resulted in a time-dependent transition in the flash-induced absorption change from a 1.2-ms, $P700^+ F_X^-$ backreaction to a long-lived, $P700^+ [F_A/F_B]^-$ backreaction. ESR studies showed that F_B and F_A were photoreduced about equally at 19 K, and while the resonances were shifted upfield, they remained as broad as in the free PsaC holoprotein. When the reconstituted complex was purified in a sucrose gradient containing 0.1% Triton X-100, most of the optical absorption transient reverted to that characteristic of the $P700^+ F_X^-$ backreaction. Addition of purified PsaD to the incubation mixture led to a greater extent of recovery of electron flow to F_A/F_B for any given concentration of PsaC. ESR studies showed that F_A, rather than F_B, became the preferred electron acceptor at 19 K; moreover, the resonances moved upfield and sharpened to become nearly identical to those of a control Photosystem I complex. When the sample was purified in a sucrose gradient containing 0.1% Triton X-100, the long-lived $P700^+ [F_A/F_B]^-$ optical transient remained stable. Analysis by denaturing polyacryalamide gel electrophoresis showed that the PsaC and PsaD proteins had rebound to the Photosystem I core. The data indicate that although PsaC can bind loosely, the presence of PsaD leads to a stable, isolable Photosystem I complex which is spectroscopically indistinguishable from the native complex. Site-directed mutants of the PsaC protein have been generated. The biochemical, optical kinetic and ESR properties of the modified Photosystem I complexes will be discussed.

179 MECHANISMS OF PHOTOINACTIVATION OF PHOTOSYSTEM II AS STUDIED WITH SITE-DIRECTED MUTANTS. Wim Vermaas and Frank van der Bolt, Department of Botany, and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe AZ 85287-1601, USA

Illumination at high light intensity inhibits electron transport in Photosystem II (PS II). The reason for this photoinactivation of PS II is as yet unknown. Several mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 have been made carrying site-directed changes in the D2 protein and featuring increased rates of photoinactivation. These mutants, E69Q, P161L, H197Q, and G215W (the mutant name indicating a sequence of the wild-type D2 residue, the number, and the residue in the mutant), all exhibit a half-time of PS II electron transport of several minutes or less at high light intensity. The mutants resemble wild type in all photoinactivation properties, except that the photoinactivation kinetics are much faster in the mutants. Thus, the mutants provide a convenient system to study photoinactivation *in vivo*. E69Q and P161L presumably are altered in the interaction of D2 with the water-splitting system, while H197Q and G215W are changed in residues close to P680 and Q_A, respectively. The rate of photoinactivation increases at increasing light intensity, even after saturation of electron transport has been achieved. The rate of photoinactivation is increased after inactivation of the water-splitting system, and artificial electron donors to Z (which is a redox intermediate in electron transfer from water to P680) protect from photoinactivation. Photoinactivation in all mutants is inhibited by diuron, which blocks PS II electron transfer. We conclude that *in vivo* photoinactivation is triggered by events (possibly the accumulation of oxidizing radicals) on the donor side of PS II. Even in the case of G215W we do not have evidence for the involvement of acceptor-side electron transport intermediates in the primary events leading to photoinactivation; however, photoinactivation triggers inhibition of PS II reaction center activity and turnover of PS II protein components. This work is supported by NSF90-58279.

SYMPOSIUM SPEAKERS

180 THE MOLECULAR BIOLOGY OF PHOTOSYSTEM II IN CHLAMYDOMONAS. Jeanne M. Erickson, Abe Domian and Julian Whitelegge. Department of Biology, UCLA. Los Angeles, CA 90024

The eukaryotic green unicellular alga, Chlamydomonas reinhardtii, provides an excellent system for investigating the molecular processes which regulate the expression of nuclear and chloroplast genes encoding polypeptides required for photosynthesis, and for dissecting the function of these polypeptides in the photosynthetic process. Mutants unable to photosynthesize can be maintained on medium containing acetate, and the specific blocks affecting regulatory processes at the transcriptional, post-transcriptional, translational and post-translational levels can be studied. We have used the DuPont biolistic transformation system to introduce mutant copies of psbA, coding for the D1 polypeptide of PSII, into the chloroplast genome of living algal cells. Co-transformation with DNA containing drug-resistant marker genes, selection for drug resistance, and subsequent screening for psbA mutants allows us to isolate transformants with specific point mutations in psbA which alter single amino acid residues of the D1 polypeptide. Oligonucleotides for in vitro mutagenesis are designed such that each mutation results in a change in restriction enzyme recognition site, so all transformants can be screened by rapid RFLP mapping. We report here the correlation between genotype and phenotype for transformants with specific lesions in psbA, and the effect of single amino acid substitutions in D1 on PSII phosphorylation and PSII electron transfer.

181 REGULATION OF LIGHT-RESPONSIVE PHOTOSYSTEM II GENES IN THE CYANOBACTERIUM *SYNECHOCOCCUS* SP. STRAIN PCC 7942. Susan S. Golden, Silvia A. Bustos, Resham D. Kulkarni, Ulrich W Mueller, A. Pratap Kumar, Michael R. Schaefer, and Rixin Li. Texas A&M University, Department of Biology, College Station, TX 77843-3258

The two proteins that comprise the photosystem II reaction center, termed D1 and D2, are encoded by small multigene families in cyanobacteria. *Synechococcus* sp. strain PCC 7942 has three *psbA* genes that encode two forms of the D1 protein, and two *psbD* genes that encode D2. Measurements of *psbA* and *psbD* gene expression by northern analysis of messenger RNA levels and through reporter gene activity have indicated that the genes respond differently to a change in light intensity. Three of the genes, *psbAII*, *psbAIII*, and *psbDII*, are expressed at relatively low levels when cells are grown at or below a light intensity of 125 $\mu E \cdot m^{-2} \cdot sec^{-1}$, but sharply increase expression if the light intensity is raised to at least 360 $\mu E \cdot m^{-2}$ sec^{-1}. The expression of *psbDI* is relatively constant during the light shift, and the level of *psbAI* message drops dramatically. A major transcriptional component has been demonstrated for the increased expression from *psbAII*, *psbAIII*, and *psbDII*, whereas accelerated degradation may account for the decrease in the *psbAI* message. DNA mobility shift assays and footprint analyses have indicated that proteins bind upstream of the open reading frames of each of the *psbA* genes and *psbDII*. For the genes whose binding sites have been mapped, binding has been shown to occur between the transcription start site and the translation initiation codon rather than upstream of the promoter. Small DNA fragments containing the promoters, transcription start sites, and protein binding sites for *psbAII* and *psbAIII* have been shown to drive light-responsive expression of a promoterless *lacZ* reporter gene in *Synechococcus*.

182 LOW-MOLECULAR-MASS PROTEINS IN PHOTOSYSTEM II. Masahiko Ikeuchi. Solar Energy Research Group, The Institute of Physical and Chemical Research, Wako, Saitama 351-01, Japan

There are many low-molecular-mass proteins in the PSII complexes. These can be detected by direct sequencing of protein bands of high resolution SDS-urea-PAGE. Of these, seven subunits (PSII-E, PSII-F, PSII-H, PSII-I, PSII-K, PSII-L and 4.1 kDa protein) are conserved between cyanobacteria and higher plants, suggesting that they play some significant role in PSII. Common feature of these proteins is the presence of a hydrophobic region, which may span the thylakoid membrane. Cytochrome b_{559} (PSII-E and PSII-F) and PSII-I are tightly bound to D1/D2 complex, while the others are less tightly bound to PSII. Proteins except for cytochrome b_{559} do not seem to bear any prosthetic group. We deleted the *psbK* gene in *Synechocystis* PCC6803 and observed only a slight decrease in PSII activity*. Thus, the role of the low-molecular-mass proteins has not yet been understood. Possible role of the low-molecular-mass proteins in PSII will be discussed on the basis of following approaches. (1) Developmental behavior of the low-molecular-mass proteins is studied during greening process from etioplasts to chloroplasts. Cytochrome b_{559} accumulates in etioplasts in the dark while the level of PSII-H is much lower than cytochrome b_{559}. (2) Interaction of the low-molecular-mass proteins with the PSII core proteins is studied using various PSII mutants of *Synechocystis* PCC6803. The absence of D2 and CP43 does not affect the level of PSII-K. (3) Topology of the low-molecular-mass proteins in thylakoid membrane is studied with regard to protease accessibility.

* Ikeuchi M, Eggers B, Shen G, Webber A, Yu J, Hirano A, Inoue Y, Vermaas W (1991) *J Biol Chem* 266: in press.

183 APPROACHES TO UNDERSTANDING PHYTOCHROME REGULATION OF TRANSCRIPTION. E.M. Tobin, J.A. Brusslan, G.A. Karlin-Neumann, D M Kehoe, P.A. Okubara, L.Sun, and S.C. Weatherwax, Department of Biology, University of California, Los Angeles, CA 90024, U.S.A.

In *Lemna gibba*, both the *rbcS* and *cab* gene families are regulated by phytochrome action We have also isolated three genes, designated NPR 1, 2 and 3, that are negatively regulated by phytochrome action [Okubara and Tobin (1991) Plant Physiol., in press]. We have developed a transient assay system in intact *Lemna* fronds to analyze the promoters of these genes *in vivo*. We have used the system to localize sequence elements important for phytochrome regulation in a deletion analysis of an rbcS promoter [Rolfe and Tobin (1991) Proc. Natl. Acad. Sci. U.S.A. 88:2683-2686]. Similar experiments are being done with promoters from the *cab* and NPR genes. Concurrently, nuclear factors that interact with these promoter regions and may be involved in the phytochrome regulation are being characterized A light-regulated factor (LRF-1) interacts with a specific sequence upstream of a *Lemna rbcS* promoter [Buzby, Yamada, and Tobin (1990) Plant Cell 2: 805-814], and deletion of this region resulted in loss of phytochrome regulation in the transient assay described above. A similar activity can be demonstrated to be present in nuclear extracts of *Arabidopsis thaliana*.

Two different strategies are being used to isolate *Arabidopsis thaliana* mutants that have altered transcriptional responses to phytochrome action. In the first of these, EMS mutagenized M-2 seeds have been screened for failure to germinate in response to red light The second approach is a directed one utilizing a "suicide" selection scheme in which we can screen for mutant survivors of treatment with a toxic substrate [Karlin-Neumann, Brusslan, and Tobin (1991) Plant Cell in press] Plant lines selected under both strategies are being tested for phytochrome regulation of the endogenous *cab* genes, and those showing an altered transcriptional response of these genes will be characterized further.

184 REGULATION OF FERREDOXIN mRNA ABUNDANCE. William F. Thompson, Lynn F. Dickey, Maria Gallo-Meagher, George C. Allen, Dolores Sowinski, and Deborah Marin Thompson. North Carolina State University, Departments of Botany and Genetics, Raleigh, North Carolina 27695, USA.

Ferredoxin I is encoded in pea (*Pisum sativum* L., cv. Alaska) by a single copy gene, lacking introns, whose mRNA abundance is strongly light responsive in both etiolated seedlings and green leaves. We have compared *Fed-1* light responses in pea and transgenic tobacco seedlings. White light induction is very similar in both species, but tobacco seedlings are markedly less responsive to red light pulses.

We have also shown that the abundance of *Fed-1* mRNA is modulated by an unusual mechanism involving *cis*-acting elements located within the transcribed portion of the gene rather than in 5' promoter sequences. The *Fed-1* promoter is active in transgenic plants and confers leaf-specific expression, but its activity is not strongly influenced by light. In addition, light treatment of transgenic plants containing chimaeric *Fed-1* constructs driven by the CaMV 35S promoter results in large changes in mRNA abundance with no change in transcriptional activity as measured in isolated nuclei. We conclude that transcriptional initiation does not limit *Fed-1* mRNA accumulation, and are currently attempting to distinguish between transcriptional elongation models and post-transcriptional mechanisms such as changes in mRNA stability.

Results with a variety of additional chimaeric gene constructs indicate that *Fed-1* 3' untranslated sequences are not required for light responsiveness, and that the active element(s) are contained in the 5' half of the mRNA. Element(s) in the both the 5' leader and the first third of the coding region are essential for light responses, but neither of these regions is sufficient by itself. These observations are consistent with models postulating cooperative interactions between two or more elements, perhaps as part of a complex mRNA secondary structure.

185 ANALYSIS OF PHOTOREGULATION OF CHALCONE-SYNTHASE DURING PLANT DEVELOPMENT. Eberhard Schäfer, University of Freiburg, D-7800 Freiburg, Germany

Chalcone synthase mRNA accumulation in parsley cell suspension cultures is under control of UV-B-, blue light photoreceptor and phytochrome. In transient gene expression studies using protoplasts from parsley cells unit 1 of the CHS promotor fused to a GUS reporter gene can transfer photoregulation by UV- and blue light. The same results were obtained using the promotor of the CHS 1 gene from mustard. In etiolated mustard and parsley seedlings CHS mRNA accumulation is only under control of phytochrome. In contrast, plants grown in white light need after-reetiolation in darkness or red light UV- and/or blue light for reaccumulation of high levels of CHS mRNA.

SYMPOSIUM SPEAKERS

186 CIRCADIAN-REGULATED CAB GENE TRANSCRIPTION IN *ARABIDOPSIS*. Steve A. Kay and Andrew J. Millar.
Rockefeller University, New York, NY 10021.

An intriguing property of many organisms is their ability to exhibit rhythmic cellular events that continue independently of environmental stimuli. These rhythmic processes are generated by an endogenous mechanism known as the biological clock. We have chosen *Arabidopsis thaliana* as a model plant system for a molecular genetic dissection of the circadian clock. To this end, we have investigated the expression of *Arabidopsis cab* genes throughout the circadian cycle. Steady-state mRNA levels of the *cab2* and *cab3* genes show a dramatic circadian cycling in plants shifted from light/dark cycles to constant darkness, whereas the *cab1* mRNA level exhibits little or no cycling under the same conditions. Analysis of *cab* promoter fusions in transgenic tobacco reveals that both the *cab1* and *cab2* 5' upstream regions confer circadian regulated expression on a *cat* reporter gene. In vitro nuclear run-on transcription assays also indicate that the transcription of both the *cab1* and *cab2* genes is circadian regulated in *Arabidopsis*. Taken together, these data suggest that a post-transcriptional mechanism influences *cab1* mRNA levels in *Arabidopsis*. We are currently analyzing in more detail the *cis*-elements and *trans*-factors that are responsible for clock-regulated transcription of the *cab2* gene. In addition we are also developing a screen for clock mutants in *Arabidopsis* by using *cab*-luciferase fusions to create a non-invasive marker for clock-regulated gene expression.

187 TOPOGRAPHY-STRUCTURE-FUNCTION IN THE CHLOROPLAST CYTOCHROMES. William A. Cramer, Paul N. Furbacher, Deru Huang, Sergio Martinez, Janet L. Smith, Andrzej Szczepaniak, and Guo-Sik Tae.
Purdue University, Department of Biological Sciences, West Lafayette, Indiana 47907.

1. Structure-Assembly: The thylakoid membrane contains three oligomeric hydrophobic *trans*-membrane electron transport complexes: (i) PSII containing cyt *b*-559, (ii) the cyt *b6f* complex, and (iii) PSI. The cyt *b6f* complex contains 4 polypeptides with MW≥15,000, cyt *f*, cyt b_6, the Rieske FeS protein, and "subunit IV," that span the membrane bilayer 9-10 times. The 214 residue cytochrome b_6 polypeptide is folded in the membrane as 4 *trans*-membrane α-helices, I-IV, with NH_2- and COOH-termini on the stromal side of the membrane. The 2 hemes form an intermolecular bridge of helices II and IV by coordination to His residues on each end of these helices. The hemes span the bilayer to create a *trans*-membrane electron transport pathway. The greater degree of homogeneity of the spectral and redox properties of the cyt b_6 hemes, compared to those of the cyt *b* of the mitochondrial and chromatophore bc_1 complexes, may arise from the unique Arg-86 near the heme ligand His-85 or the extra residue between the two His in helix IV. Cytochrome b_{559} in the PSII reaction center complex is organized by intermolecular bridging by heme near the stromal surface, of a His residue on the 9.2 kDa α and one on the 4.3 kDa β polypeptide, forming an $(\alpha\beta)_2$ heterodimer, although an $(\alpha)_2(\beta)_2$ organization of two hemes in PSII cannot be excluded. A role of the peripheral charged peptide domains in the assembly of the *b6f* and b_{559}-PSII complexes is implied by (i) sequential disassembly of the polypeptides of the *b6f* complex at alkaline pH (>10), and (ii) decreased probability of correct assembly of PSII in the cyanobacterium, *Synechocystis* sp. PCC 6803 upon truncation of the lumen-side COOH-terminal domain of the cyt b_{559} α-subunit.

2. Crystallization Studies: Crystals of the 250 residue lumen-side domain of cyt *f* from turnip chloroplasts have been obtained, with space group $P2_12_12_1$, a=81.9Å, b=79.2Å, c=46.3Å, that diffract to 2.2Å.

3. Function. Trans-membrane functions of the cyt *b6f* complex are: (i) the PSI cyclic electron transport pathway, using oxidant-induced reduction to reduce the lumen-side heme, as proposed for the Q-cycle mechanism, and/or (ii) a Q cycle. The energetic requirement for either pathway in C-3 photosynthesis is not compelling under steady-state and unstressed conditions.

The function of cyt b_{559} also appears to be linked to stress response. The stromal-side location of at least one, and probably both, b_{559} hemes allows reduction by electron donors, NADPH or ascorbate, in the stroma. The b_{559} can then act as a slow reductant to the auxiliary Chl or P_{680} on the opposite side of the membrane, and/or to poise the PQ pool for PSI cyclic phosphorylation under stress conditions when electron donation from H_2O and PSII is impaired. [Supported by NIH GM-38323.]

188 SITE-DIRECTED MUTAGENESIS AFFECTING ELECTRON TRANSFER ON THE DONOR-SIDE OF PHOTOSYSTEM II. Peter J. Nixon and Bruce A. Diner - CR&D, Experimental Station, E.I. duPont de Nemours & Co., Wilmington, DE 19880-0173

Tyrosine-161 of the D1 polypeptide is the redox active component Yz that connects the oxygen-evolving complex (OEC) to the primary electron donor P680. Site directed mutations at his190 slow the oxidation of Yz 200-fold compared to WT and shift the free energy difference between the Yz^+/Yz and $P680^+/P680$ redox couples. These observations are consistent with a close interaction between D1-his190 and D1-tyr161 as recently proposed by Svensson et al. (EMBO J. 9:2051).

D1-asp170 is crucial to the function of the Mn cluster responsible for light-driven water oxidation. Site-directed mutations at asp170 show the following ranking of relative light-saturated rates of oxygen-evolution: asp (100%)>glu (>60%)>his, cys (20%)>tyr, arg, met (10%)>trp (5%)>asn (1%)>ser, ala, phe (0%). Analysis of core complexes isolated from these mutants leads to the conclusion that this residue is part of a high affinity Mn binding site probably involved in the first step in the assembly of the oxygen-evolving complex.

Carboxy-terminal proteolytic processing of precursor D1 is known to be required for proper assembly of the OEC. This observation is now understandable in light of our identification of residues D1-his332, asp342 and ala344 at the mature carboxy-terminus that are involved in later steps in the assembly of the oxygen-evolving complex and are probable ligands to the assembled Mn cluster.

189 KINETICS OF QA TO QB ELECTRON TRANSFER IN RHODOPSEUDOMONAS VIRIDIS. Paul Mathis, Irmgard Sinning and Hartmut Michel. C.E. Saclay, Section of Bioenergetics, 91191 Gif sur Yvette Cedex, France, and Max-Planck-Institut für Biophysik,Heinrich-Hoffmann Str. 7, 6000 Frankfurt, Germany.

The reaction center of the purple bacterium Rps. viridis is now well known from a structural point of view. In particular the structure of the QB site is well defined in WT and in several mutants which are locally modified so as to be resistant to triazine inhibitors. The functional properties of QB however, have not yet been studied in detail in this bacterium. In an earlier step, we have studied ubiquinone binding and competition with herbicides, with the surprising finding that one mutant had striking similarities with PS-2 (Sinning et al. Biochemistry (1989) 28, 5544-5553 ; FEBS Lett (1989) 256, 192-194). We have now measured the kinetics of electron transfer from QA to QB by flash absorption techniques, in isolated reaction centers, relying on differential absorption shifts around 830 nm due to QA^- or to QB^-, and in chromatophores, using a double-flash technique and measuring the absorption due to $P-960^+$ around 1300 nm. We found similar kinetics in reaction centers and in chromatophores, with little pH dependance between pH 6 and 9 ($t_{1/2} \approx 20$ μs). One terbutryn-resistant mutant (mutant T_4, Tyr L222 → Phe) behaves nearly as the WT but another one (mutant T_1, Ser L223 → Ala and Arg L217 → His) shows an accelerated rate of transfer ($t_{1/2} \approx 6$ μs). These results are discussed in the context of the compared X-ray structures and of other functional data (QB binding, rates of charge recombination between $P-960^+$ and QA^- or QB^-). They will also be compared to similar data obtained in other purple bacteria and in PS-2 where the QA to QB transfer is always much slower.

190 THE PLASTOQUINONE CONNECTION BETWEEN PHOTOSYSTEM II AND THE CYTOCHROME BF COMPLEX. Mary F. Blackwell.
Department of Chemistry, Lawrence University, Appleton WI 54911.

Plastoquinone (PQ) is a membrane-bound carrier of reducing equivalents between the Q_B binding site on the D1 polypeptide in the photosystem II reaction center and a PQH_2 oxidation site on the cytochrome bf complex. The possible requirement of a significant role for long-range diffusion by PQ has been raised by observations that photosystem II is confined largely to chloroplast grana and that a significant proportion of cytochrome bf complexes may be present in stromal regions. Long-range diffusion of PQH_2 from granal PS II to stromal cytochrome bf may require travel over distances (r) of the order of 500-1000 nm in 2-20 ms (t). According to the Einstein relationship ($r=2(Dt)^{1/2}$), these time and distance constraints would require the diffusion coefficient to be of order 10^{-6} to 10^{-8} cm^2sec^{-1}. PQ and PQH_2 diffusion coefficients can be estimated by the technique of pyrene fluorescence quenching and have been found to be in the region of 1-3 x 10^{-7} cm^2sec^{-1} in model membranes containing only lipid. Integral membrane proteins were found to have a profound effect on PQ mobility: the rate of PQ diffusion decreased by more than an order of magnitude in model membranes containing both lipid and integral membrane proteins when the protein content exceeded 30-50 weight % protein. Pyrene fluorescence quenching techniques have also been used to explore the diffusional mobility of PQ in spinach chloroplasts and subchloroplasts. The diffusion coefficient of PQ was estimated to be less than O(10^{-9} cm^2sec^{-1}) in thylakoid membranes and stromal subthylakoids. This raises questions about PQ's ability to serve as a carrier of reducing equivalents from granal PS II to stromal cytochrome bf and thus about whether or not stromal cytochrome bf complex is functional in linear electron transport.

191 SYNTHESIS AND ASSEMBLY OF PHOTOSYNTHETIC COMPLEXES IN THE CHLOROPLAST THYLAKOID MEMBRANE. J.C. Gray, P. Dupree, J.S. Knight, F. Madueño, J.A Napier and A.H. Salter.
Botany School, University of Cambridge, Cambridge CB2 3EA, UK.

The mechanisms responsible for regulating the assembly of multisubunit photosynthetic complexes in the chloroplast thylakoid membrane are being investigated using transgenic plants with altered levels of expression of the genes for nuclear-encoded subunits.

The Rieske FeS protein is the only nuclear-encoded polypeptide of the cytochrome *bf* complex; the genes for the other four polypeptides, cytochrome *f*, cytochrome *b*-563 and subunits IV and V, are located in the chloroplast genome. Measurements of the amounts of transcripts and polypeptides for these components during the greening of etiolated pea shoots indicates that the synthesis of the Rieske FeS protein is the limiting factor in the assembly of a functional cytochrome *bf* complex. The effect of manipulation of the expression of the genes for the Rieske FeS protein on the assembly of the cytochrome *bf* complex is being examined in transgenic tobacco plants containing sense and antisense constructs. cDNA clones for the pea and tobacco Rieske FeS proteins and genomic clones for the tobacco and *Arabidopsis* proteins have been isolated and characterised, and are being used to examine chloroplast import, processing and assembly of the protein *in vitro*.

Two forms of ferredoxin-$NADP^+$ reductase (FNR) are found in chloroplasts; one is located in the stroma and the other is bound to the thylakoid membrane. To examine the molecular basis for these forms, cDNA and genomic clones encoding pea FNR have been expressed in transgenic tobacco plants. Active pea FNR was located in both the stromal and thylakoid membrane fractions of chloroplasts isolated from transgenic tobacco containing a single pea FNR gene, indicating that both forms of FNR are the product of a single gene. The total amount of FNR activity associated with the thylakoid membrane was the same in transgenic and wild-type tobacco, indicating that pea FNR was able to compete with tobacco FNR for binding to a limited number of binding sites.

SYMPOSIUM SPEAKERS

192 REGULATION OF mRNA PROCESSING AND TURNOVER IN CHLOROPLASTS. David Stern[1], Hsu-Ching Chen[1], Wataru Sakamoto[1], Xuemei Chen[1], Qiuyun Chen[1], Cynthia Adams[1] and Karen Kindle[2]. [1]Boyce Thompson Institute and [2]Plant Science Center, Cornell University, Ithaca, NY 14853

Gene expression in chloroplasts is light- and developmentally-regulated in a complex manner. We are using two model systems, spinach and *Chlamydomonas reinhardtii*, to study the molecular mechanisms of post-transcriptional regulation in chloroplasts, in particular translational control and the control of mRNA stability.

Based primarily on *in vitro* biochemical studies, our working model is that plastid mRNA maturation and degradation are regulated by 3' end hairpin structures and RNA-binding proteins. We are purifying several RNA-binding proteins that associate with the hairpin and flanking regions. These may regulate mRNA stability, processing, and/or transcription termination. We are also analyzing mRNA:protein complexes isolated from chloroplasts, to complement our biochemical approaches.

We are also studying post-transcriptional regulation of plastid gene expression in *Chlamydomonas reinhardtii*, a eukaryotic green alga. Using ballistic transformation of chloroplasts ("particle gun" technology), we are introducing defined mutations, into chloroplast genes. These mutations include deletions in the 3' untranslated region of the *atpB* gene, and site-directed mutations in the coding region, and deletions in the 5' untranslated region of the *petD* gene. Our strategy for *petD* is to select loss-of-function mutations by resistance to metronidazole, and then to isolate second-site suppressor genes by selecting for cells that have regained electron transport. In this way, we are studying *cis*-elements and *trans*-acting factors that control plastid gene expression in *Chlamydomonas*.

193 MOLECULAR AND GENETIC STUDIES OF CHLOROPLAST DEVELOPMENT IN MAIZE. Alice Barkan. Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

We are interested in understanding mechanisms that dictate the timing and cell-type specificity of chloroplast development. Towards this end, we are applying a combination of molecular and genetic approaches, using maize as a model organism. Its excellent genetics, well-characterized transposons, large seed, and mode of leaf development together make maize uniquely suited for this purpose.

Previously, we observed that the ratio of unspliced to spliced mRNAs from several plastid genes is approximately ten-fold higher in roots and leaf primordia than in leaves. This suggested that RNA splicing might be a regulatory step during the proplastid to chloroplast transition. To gain a more complete view of RNA metabolism in non-leaf plastids, we have now extended this analysis to other tissues, and to rRNA and tRNA processing. Nuclear mutants with lesions that block chloroplast translation initiation are being used to study other post-transcriptional aspects of chloroplast gene expression. These include the effects of translation on plastid mRNA stability and on plastid mRNA splicing, the coupling of ribosome assembly with rRNA processing, the involvement of chloroplast translation in greening, and the relationship between chloroplast translation rates and protein accumulation.

194 GENES FOR THYLAKOID PROTEINS: EXPRESSION, MODIFICATION AND INTERACTION. R. G. Herrmann, J. Steppuhn, J. Bichler, S. Clausmeyer and N. Wedel. Botanisches Institut der Ludwig-Maximilians-Universitat, Menzinger Str. 67, 8000 Munchen 19, FRG

Thylakoid membranes are composed of ca. 55 major polypeptide species, most of which are found in four supramolecular membrane-spanning multisubunit complexes. Genes for ca. 50 components, approximately half of plastic and half of nuclear origin, have been characterized from spinach. These include those for the nuclear-coded subunit CFo-II (atpG) and 22 kDa polypeptide (psbS) which are phylogenetically and structurally intriguing components of the ATP synthase and photosystem II assembly, respectively. - The unique feature of the biogenesis of the photosynthetic membranes is its dependence on two different sources of protein synthesis. This implies a coordinated delivery of the components of cytosolic and organelle origin and, consequently, requires regulation in space, time and stoichiometry. An eukaryote-type of transcription factor that can be imported into the organelle and bind to the organelle DNA represents a potential signal for the intercompartimental interaction. Appropriate gene domains (e. g., segments of several promotors and a cassette system for transit peptides) have been used to pin-point light-responsive, tissue-specific and developmentally active *cis*-elements, to isolate recombinant DNAs for *trans*-acting factors, to check the classification and to determine functional epitopes of chloroplast-targetting transit sequences, as well as to cure plastome mutants with defined lesions in photosynthesis by compartment-alien transformation. - Supported by the Deutsche Forschungsgemeinschaft (SFB 184)

195 MOLECULAR BASIS FOR TENTOXIN RESISTANCE IN PLASTIDS. A. Avni, J.D. Anderson, J.-D. Rochaix[1] and M. Edelman. Dept. Plant Genetics, Weizmann Institute of Science, Rehovot, Israel; [1]Dept. Mol. Biol., University of Geneva, Geneva, Switzerland.

Tentoxin, a cyclic tetrapeptide produced by the fungus *Alternaria*, causes a number of diverse effects in a variety of plants, suggesting multiple modes and sites of action. The effect of tentoxin most frequently studied is the prevention of chlorophyll accumulation ('chlorosis') in germinating seedlings of some, but not all, angiosperms. Based on a number of findings, chlorosis was suggested, several years ago, to result from inhibition of photophosphorylation due to a specific receptor site for tentoxin on the coupling factor (CF_1) of the plastid ATPase in sensitive species. We became interested in defining the molecular-genetic basis for the chlorosis response to tentoxin after successfully using this trait as a plastid marker in interspecific plastid fusions in *Nicotiana*. The identity of the CF1 subunit interacting with tentoxin was determined by recourse to LiCl-extracted *Rhodospirillum rubrum* chromatophores, which we show require both β subunit and trace amounts of α subunit for reconstituting ATPase activity. Reconstituted ATPase activity was shown to be sensitive to tentoxin when β subunit from lettuce (a chlorosis-sensitive specie) is used, but resistant to 100 fold higher concentration of tentoxin when β subunit from tobacco (a chlorosis-resistant specie) is used, irrespective of the source of the added α subunit. The receptor site for tentoxin on the β subunit was pinpointed by DNA sequence analysis of the *atp*B gene from six closely-related chlorosis-sensitive and -resistant *Nicotiana* species. Only a single amino acid difference was consistent between sensitive and resistant species. This site was verified by direct, particle-gun transformation experiments in *Chlamydomonas reinhardtii*. Upon site-directed plastid mutagenesis, *Chlamydomonas*, normally tentoxin resistant, was rendered tentoxin sensitive at the cellular and ATPase level.

196 THREE-DIMENSIONAL STRUCTURE OF THE LIGHT-HARVESTING CHLOROPHYLL a/b-PROTEIN COMPLEX.

Werner Kühlbrandt and Da Neng Wang. European Molecular Biology Laboratory, 6900 Heidelberg, F.R. Germany.

The three-dimensional structure of the light-harvesting chlorophyll a/b-protein complex has been determined at 6 Å resolution by electron crystallography. The complex is a trimer, consisting of three identical or nearly identical monomers. Within the monomer, three membrane-spanning α-helices and fifteen chlorophyll molecules are resolved. Two of the α-helices are tilted by about 30° relative to the membrane normal and measure 46 Å and 49 Å in length, respectively. The upper halves of these long helices are related by non-crystallographic 2-fold symmetry which is probably due to an internal homology in the polypeptide sequence. The third helix is shorter (30 Å) and runs roughly perpendicular to the membrane plane. The chlorophyll porphyrin head groups are approximately perpendicular to the membrane plane and seem to be arranged on two levels within the hydrophobic core of the membrane. The spacing of the chlorophylls suggests energy transfer by delocalized exciton coupling within the monomers and by the Förster mechanism between monomers and trimers.

197 IMPORT AND PROCESSING OF THE PRECURSOR FOR LHCP. Gayle Lamppa, Mark Abad, Steven Clark, John Oblong, and Li-Ming Yang. University of Chicago, Molec. Genetics and Cell Biol., Chicago, IL 60637

The precursor of the major light-harvesting chlorophyll a/b binding protein (LHCP type I of PSII), encoded by a wheat gene, was synthesized in E. coli, recovered from inclusion bodies in 6 M urea, and found to be competent for import. Two peptides of ~26 and 25 kD are produced by processing at two sites, referred to as primary (1°) and secondary (2°). Addition of a reticulocyte lysate to the reaction produces a major increase in the amount of the two mature peptides, suggesting that the lysate contains a factor that stimulates a step in the import pathway. In an organelle-free processing reaction the LHCP precursor (preLHCP) is cleaved at only one site, yielding a 25 kD peptide. N-terminal sequence analysis of the 25 kD peptide indicates that cleavage occurs between residues 40 and 41, removing the transit peptide and a basic hexapeptide domain (RKTAAK) from "mature" LHCP. To determine if the soluble enzyme that cleaves at the 2° site is specific for preLHCP, it was partially purified and preS, prePC, preRA, preHsp21 and preACP were tested as substrates. These precursors were all cleaved by the same chromatographic peak of activity that processes preLHCP in the organelle-free reaction. Mutational analysis of preLHCP made from wheat transcripts has demonstrated that cleavages at the 1° and 2° sites occur independently. In addition, the determinants for each cleavage are distinct. Using preLHCP from both pea and wheat, it was found that 1° site processing depends on a basic residue at position -4. On the other hand, neither preS or preRA require an amino-proximal basic residue for removal of the transit peptide. Cleavage at the 2° site of preLHCP requires the residues AK-A at the bond cleaved (denoted by the hyphen). Our results indicate that two different forms of LHCP can be produced with distinct N-termini by selective cleavage of a single precursor polypeptide.

SYMPOSIUM SPEAKERS

198 MECHANISM OF PROTEIN TRANSPORT ACROSS THE THYLAKOID MEMBRANE. Colin Robinson, Ruth M Mould and Jamie B Shackleton. Cytosolically-synthesised thylakoid lumen proteins, such as the extrinsic polypeptides of the photosystem II oxygen-evolving complex (OEC) are imported by a two-step pathway via stromal intermediate forms. The transport of these proteins across the thylakoid membrane has been analysed using an *in vitro* thylakoid import assay. Isolated pea thylakoids are capable of importing the 33 kDa and 23 kDa OEC proteins by a light-dependent mechanism. The 33 kDa protein is probably imported only as the intermediate-size form, whereas both the full precursor and the intermediate forms of the 23 kDa proteins are imported. The effects of electron transport inhibitors and uncouplers have demonstrated that, in both isolated thylakoids and intact chloroplasts, the transport of these proteins across the thylakoid membrane is driven by a proton gradient. Processing of these proteins to the mature sizes is carried out by a thylakoidal peptidase which is similar in some respects to "signal" peptidases, but which is more stringent in its recognition of cleavage sites.

199 CHLOROPLAST TRANSFORMATION IN CHLAMYDOMONAS: IDENTIFICATION OF A SMALL TRANS-ACTING RNA INVOLVED IN TRANS-SPLICING AND DIRECTED CHLOROPLAST GENE DISRUPTION. J.-D. Rochaix, M. Goldschmidt-Clermont, Y. Choquet†, Y. Takahashi, J. Girard-Bascou+ University of Geneva, Departments of Molecular Biology and Plant Biology, Geneva, Switzerland †Institut de Biologie Physico-Chimique, France

Many nuclear genes are involved in the expression of chloroplast photosynthetic genes. At least 14 nuclear loci are required for the maturation of the mRNA of the chloroplast psaA gene consisting of three exons each of which is transcribed independently. We have studied mainly the first trans-splicing reaction between exons 1 and 2 which requires the participation of at least 7 nuclear genes and one chloroplast locus tscA. Using particle gun mediated chloroplast transformation we have identified the product of pscA as a small 470 nt RNA which appears to interact with the 5' and 3' ends of the split intron 1 of psaA thereby completing the catalytic core structure of this group II intron. Amongst several artificial insertions in tscA only one, located within the core, blocks splicing completely.

We have disrupted the psaC gene encoding the iron-sulfur protein of photosystem I with an aadA gene cassette designed to express spectinomycin-streptomycin resistance in the chloroplast by particle gun transformation. Analysis of the DNA of several transformants reveals that all wild-type psaC genes have been replaced through homologous recombination. None of the PSI subunits accumulate stably in the thylakoid membranes of the transformants. Chloroplast reverse genetics is therefore feasible in C. reinhardtii.

200 PLASTID TRANSFORMATION IN HIGHER PLANTS. Pal Maliga, Jeffrey Staub, Helaine Carrer, Peter Hajdukiewicz, Renee Malone and Zora Svab. Waksman Institute of Molecular Genetics, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854

Plastid transformation was reported in *N. tabacum* by selection for the 16S rDNA-encoded spectinomycin resistance marker. A linked, unselected RFLP marker was efficiently introduced with, whereas a second unselected streptomycin resistance marker was often separated from the spectinomycin resistance marker (PNAS 87:8526, 1990). A study with five RFLP markers in a 6 kbp region of the inverted repeat confirmed good linkage with spectinomycin resistance. Benavior of the streptomycin resistance marker observed in the first study therefore is specific to the mutation. Introduction of chimeric genes into the inverted repeat by linkage to the spectinomycin resistance marker are in progress. Transformation in the unique region will be by selection for inserted chimeric antibiotic resistance genes. Transformation in the inverted repeat, and in the unique region of the plastid genome will be discussed.

201 GENETIC AND MOLECULAR ANALYSIS OF LEAF AND CHLOROPLAST DEVELOPMENT IN *ARABIDOPSIS*. Joanne Chory. Plant Biology Laboratory The Salk Institute, P.O. Box 85800, San Diego, 92186-5800.

Dicotyledonous seedling development is dramatically affected by light. The transition from heterotrophic to photoautrophic growth, with the associated development of leaves and chloroplasts is dependent on the presence of light and developmental signals. Several regulatory photoreceptors are involved in the perception of light signals, but little is known of the transduction pathways that mediate light-regulated development. We are taking a combined genetic and molecular biological approach to identify potential components of the light-signal transduction pathways. We have identified a class of *Arabidopsis thaliana* mutants that show many characteristics of light-grown plants even when grown in complete darkness. The mutants define at least 3 complementation groups, designated *det1*, *det2*, and *det3* (*de-etiolated*). Because these mutations are both pleiotropic and recessive, we have hypothesized that *DET* genes play a negative regulatory role in leaf and chloroplast development in *Arabidopsis*. In a separate set of experiments, work in our lab and by Dr M Koornneef (Wageningen) has identified a class of mutants (*hy*) that have long hypocotyls in the light. There are at least 7 loci which give the *hy* phenotype; 4 of these are defective in some aspect of light perception by phytochrome. We have constructed double mutants between the phytochrome-deficient *hy* mutants and the *det* mutants. Based on analysis of the phenotypes of the double mutants, we have constructed pathways of light-regulated seedling development in *Arabidopsis*. Our results are consistent with a model where formation of the active form of phytochrome results in a decrease in activity of DET, which in turn leads to the de-etiolation response. We are currently in the process of cloning the *DET1* and *DET2* loci by chromosome walking and rescue of the mutant phenotypes. Clones of the *DET* genes will lead to information on the mechanisms of the switch between light and dark growth modes in plants.

202 NUCLEAR GENES THAT REGULATE CHLOROPLAST DEVELOPMENT. William C. Taylor, CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia

Two nuclear genes in maize that play important roles in the differentiation of proplastids into chloroplasts have been identified by Coe et al. (1989, Amer. J Botany). Mutant alleles of *iojap* and *chloroplast mutator* cause white leaf stripes in which plastids are blocked at early stages of differentiation. The conditioned plastids are maternally inherited, demonstrating that the defect in a nuclear gene permanently blocks plastid differentiation. The pattern of striping indicates that the timing of *chloroplast mutator* gene expression is during embryogenesis and the initiation of leaf primordia, while the *iojap* gene is continually expressed during leaf development. I have generated new transposon insertion alleles of both genes to facilitate gene isolation by molecular cloning. Eight new *iojap* alleles with *Mu* insertions have been isolated, one of which is dominant. Thirteen new *chloroplast mutator* alleles with *Mu* insertions and six with -like insertions have been identified. Progress in the cloning and analysis of both genes will be discussed.

203 PLASTICITY IN CYANOBACTERIAL O_2-EVOLUTION AND CHLOROPHYLL-PROTEIN ORGANIZATION. Rob Burnap, Tracy Troyan and Louis A. Sherman. Purdue University, Dept. of Biological Sciences, West Lafayette, IN 47907.

The unicellular, transformable cyanobacteria have proven to be of great utility for the analysis of photosynthesis. The cyanobacterial photosynthetic mechanism is virtually identical to that of plants and algae; in addition, the cyanobacterial mechanism is highly plastic and can be altered substantially to fit the environmental conditions. We report here on genetic and environmental perturbations that still allow for oxygenic photosynthesis despite significant changes in the photosynthetic mechanism.

We have deleted the *psbO* gene (encoding for the manganese-stabilizing protein) in *Synechocystis* sp. PCC6803. The resulting deletion strain (Δ*psbO*) was capable of photoautotrophic growth and O_2-evolution at approximately 1/2 the normal rate, had significantly lower levels of fluorescence at 696nm and was much more sensitive to inactivation by hydroxylamine. We have also constructed site-directed mutants which grow and evolve oxygen at a lowered rate and which demonstrate some or all of these perturbed characteristics. We conclude that *psbO* is not directly involved in the binding of Mn, but is primarily involved in the regulation of O_2-evolution by effecting the release time of O_2. The growth of the cyanobacterium *Synechococcus* sp. PCC7942 in iron-deficient media results in significant changes in the photosynthetic mechanism. Among these changes, a new chlorophyll-protein complex (CP43') is synthesized and incorporated into membranes from iron-deficient cells. We have now cloned the gene coding for CP43' (*isiA*) so that we can study the nature of this alternative chlorophyll-binding protein. Using techniques similar to those above, we have produced a deletion mutant of this gene (Δ*isiA*). The deletion strain is now unable to grow under completely iron-deficient conditions, but can grow in 1/1000 the normal level of iron. We will discuss the impact of the pigment changes associated with iron-deficient cells in the presence or absence of CP43'.

SYMPOSIUM SPEAKERS

204 MUTAGENESIS OF GENES ENCODING PHYCOBILISOME COMPONENTS IN *SYNECHOCOCCUS* SP. PCC 7002.. Donald A. Bryant, Jianhua Zhou, and Veronica Strewalt. Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802

We are characterizing genes encoding phycobilisome (PBS) components in the marine unicellular cyanobacterium Synechococcus sp. PCC 7002 by analyses of mutants generated by interposon and site-directed mutagenesis. Our goal is to understand factors which contribute to energy transfer pathway and efficiency in these structures. All eleven structural genes encoding PBS components in this organism were cloned, mutated and transformed back to their chromosomal loci. Mutants were analyzed in terms of their ability to produce mature PBS and their efficiency of light-harvesting and excitation energy transfer. From these studies we found that the *apcC*, *apcD* and *apcF* gene products (encoding L^{8}_{C}, α^{APB}, and β^{18}, respectively) are not absolutely required for the formation of functional PBS. The *cpcG* gene product (L^{29}_{RC}) is absolutely required for the attachment of rods to the core subassembly. The *apcE* gene product (L^{99}_{CM}) is essential for the formation of the core subassembly and also acts as the major terminal energy-acceptor of the core which delivers excitation energy from PBS to chl *a* in PS II. We have also identified two genes (*cpcE* and *cpcF*) in the *cpc* operon, which are involved in posttranslational modification of the *cpcA* gene product, the α^{PC} subunit. At steady state both *cpcE* and *cpcF* mutant strains accumulate less phycocyanin than their wild-type counterpart. Further characterization showed that these mutants failed to accumulate chromophorylated α^{PC} subunit although the α^{PC} apoprotein was synthesized at near-normal level. A site-directed mutation at codon 129 of *cpcA* can suppress an interposon mutation in the *cpcE* gene. Systems for the analysis of site-directed mutations in the *cpcB*, *cpcA*, and *apcE* genes have been developed. The methylated Asn residue (Asn72) of the β^{PC} subunit has been changed to Asp and Gln. When the chromophore binding Cys-155 of β^{PC} was mutated to Ser, the resultant phycocyanin was still capable of assembling peripheral PBS rods. When the Cys-186 of the *apcE* gene product is replaced by Ser, the L^{99}_{CM} polypepude is no longer covalently chromophorylated thereby establishing that this cysteine is the site of phycocyanobilin attachment. PBS are still assembled in the mutant cells, and these PBS exhibit a major new fluorescence emission peak at 715 nm. Our working hypothesis is that the protein non-covalently binds the precursor of polypeptide-bound phycocyanobilin, which would be expected to contain one additional double bond and thereby account for the red-shift in the fluorescence emission. A double mutant strain (*apcD*$^{-}$ *apcE186Ser*) has been constructed and analyzed; the mutant is somewhat defective in PBS assembly and is severely impaired in energy transfer.

205 THE ACCLIMATION OF CYANOBACTERIA TO HARD TIMES. Grossman AR, DE Laudenbach, JL Collier, JM Ray and D Bhaya. Carnegie Institution of Washington, Department of Plant Biology, Stanford, CA 94305

Limiting cyanobacteria for macronutrients such as nitrogen, sulfur or phosphorus elicits both general and specific responses. Among the general responses are the breakdown of the light harvesting phycobilisome and the attenuation of photosynthetic membranes. This loss of pigmentation, or 'bleaching', gives the cells a yellow, chlorotic appearance. The responses that are uniquely associated with limitation for a single macronutrient aid the cell in the acquisition of that macronutrient. These responses include the production of specific periplasmic enzymes that catalyze the release of transport competent forms of the nutrient from molecules in the external medium (eg. alkaline phosphatase production during phosphate-limited growth), and the increased accumulation of specific transport complexes (eg. 10-20 fold increase in sulfate transport during sulfur-limited growth). I will describe the uses of molecular techniques to dissect both the general and specific responses. I will emphasize our work on the acclimation of *Synechococcus Sp.* PCC 7942 to sulfur-limited growth conditions and discuss both the function and regulation of genes that are activated during the acclimation process.

206 MOLECULAR MECHANISMS CONTROLLING ADAPTATION OF CYANOBACTERIA TO THEIR ENVIRONMENT. Nicole Tandeau de Marsac. Physiologie Microbienne, Institut Pasteur, 28 rue du Dr Roux, F-75015 Paris

Cyanobacteria, prokaryotes performing plant-type photosynthesis, exhibit unique physiological and morphological features in response to variations of environmental parameters. A great deal of information has been acquired on the expression of genes involved in photosynthesis, nitrogen metabolism and cell differentiation, but little is known about signal perception, the transduction pathways which lead to gene expression and the regulatory mechanisms which control the complex network of interactions between these fundamental biological processes. In this respect, the recent discovery of the genes encoding the PII (*glnB*) and NtrB (*ntrB*) proteins might be of importance. Highly identical to the PII protein which, in other bacteria, controls nitrogen assimilation *via* a two-component system (NtrB/NtrC) and depending on the availability of ammonia, its counterpart in the unicellular cyanobacterium *Synechococcus* PCC 6301 (Harrison *et al., FEBS Lett.*, 264, 25,1990) is covalently modified as a result of imbalance in energy transfer in photosynthesis where PSII is favored over PSI activity. Moreover, the presence of ammonia represses heterocyst differentiation and decreases phycoerythrin synthesis in *Calothrix* PCC 7601, a filamentous cyanobacterium able to undergo complementary chromatic adaptation. The expression of the genes encoding components of the light-harvesting antennae (phycobilisomes) have been studied in *Calothrix* cells grown under different light regimes (light intensity and red versus green light) or nutritional conditions. In most cases, the regulatory controls occur primarily at the transcriptional level. Two proteins, which behave as transcriptional activators of the genes encoding phycoerythrin, the major component of the phycobilisome rods in green-light grown cells, have been evidenced and partially purified. The DNA-binding ability of one of these proteins depends upon phosphorylation. Thus the PII protein, by controlling the phosphorylation state of transcriptional effectors and/or of modulators in the signal transduction pathways, may play a role in the fine tuning of gene expression and/or in the cross-talk between the regulatory mechanisms involved in nitrogen metabolism, light adaptations and cell differentiation in cyanobacteria.

207 CHARACTERIZATION OF RNA EDITING SITES IN MAIZE MITOCHONDRIAL *rps13* TRANSCRIPT AND IMMUNOLOGICAL DETECTION OF RPS13 PROTEIN

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A plant mitochondrial gene similar to *rps13* of *E. coli* is present in mitochondria of maize, *Oenothera* and *Daucus*, but not in bean or pea. Wheat also has the mitochondrial gene, but it is reportedly not expressed. Six RNA editing sites were determined in the maize mitochondrial *rps13* transcript by comparing genomic and cDNA clones. Six amino acids out of 116 residues are deduced to change by RNA editing, four are Ser-Leu conversions and two are Arg-Cys conversions. 62% of transcripts (13 out of 21 clones) are fully edited, 24% (5 of 21) are partially edited and 14% (3 of 21) are not edited. No critical order of RNA editing is found, but the C residue at nucleotide 26 is always converted in edited molecules. The tobacco *rps13* gene was expressed in *E. coli* using the pET5a vector and the resultant fusion proteins were used for immunization of rabbits. Anti-tobacco RPS13 antibodies were raised after 4 injections. A 13 kDa polypeptide of maize mitochondria crossreacted with the antibody; no chloroplast polypeptides crossreacted. These results suggest that the maize *rps13* gene is functional.

208 NUCLEAR-MITOCHONDRIAL GENETIC INTERACTIONS IN CYTOPLASMIC MALE STERILE COMMON BEAN. Sally Mackenzie, Meiqing Lu, Carol Johns, and Anna Lyznik. Purdue University, West Lafayette, IN 47907

Cytoplasmic male sterility in bean (*Phaseolus vulgaris* L) is associated with the presence of a 3 kilobase unique mitochondrial sequence designated *pvs* (P. vulgaris sterility sequence). At least two open reading frames are carried within the *pvs* sequence, 297 and 720 base pairs in length. Of particular interest to our laboratory is the effect of nuclear genotype on this *pvs* mitochondrial region. Introduction of nuclear gene *Fr* results in loss of a portion of the mitochondrial genome encompassing *pvs* and restoration of normal pollen development. *In situ* hybridization experiments suggest that this mitochondrial DNA loss occurs during pollen formation. Introduction of a different nuclear gene, *Fr2*, results in restoration of normal pollen development without loss of the *pvs* sequence. The structure of the mitochondrial genome in the male sterile line, expression of the mitochondrial *pvs* region, and interaction between the nuclear and mitochondrial genomes in this system will be described.

209 MITOCHONDRIAL MUTATIONS ASSOCIATED WITH ABNORMAL GROWTH PHENOTYPES. Kathleen Newton, Mary Schramke, Marjone Hunt and Jianying Gu. Division of Biological Sciences, University of Missouri Columbia, MO 65211.

Several maternally-inherited mutations characterized by reduced growth and yield have been associated with specific mitochondrial DNA rearrangements in maize. We have been studying one set of such mutants, known as nonchromosomal stripe (NCS). The mutations are usually lethal during kernel development and highly deleterious at other stages. Thus, plants carrying the mutant mitochondrial genomes are heteroplasmic and sectoring of defective and normal tissues results from the somatic sorting-out of mutant and normal mitochondria during development. Each mutation is correlated with a deletion in an essential mitochondrial gene. We have characterized two different mutations in the cytochrome oxidase subunit 2 gene (NCS5 and NCS6), one in the NADH-dehydrogenase subunit 4 gene (NCS2) and two in the co-transcribed, overlapping S3/L16 ribosomal protein genes (NCS3 and NCS4). The progenitor mitochondrial genotype of NCS2 and NCS3 is the cytoplasmic male sterile, cms-T, while the progenitors for NCS4, NCS5 and NCS6 are fertile derivatives of cms-S. NCS-like plants have also been observed in plants carrying non-male-sterile N cytoplasms. NCS mutants arise most often in plants carrying a WF9-derived nuclear genotype, so nuclear genes appear to be involved in the generation and/or recovery of mitochondrial mutants affecting plant growth. Each NCS deletion appears to be generated by a similar series of events, beginning with recombination between very short repeats. The sizes of the repeated regions at the recombination junctions vary from 6 bp to 31 bp. Only one of the two products expected from a reciprocal recombination event is recovered in each of the mutant mitochondrial genomes. However, each deletion appears to be relatively short, not extending into nearby genes.

SYMPOSIUM SPEAKERS

210 GENE EXPRESSION AND RESPIRATION IN CYTOPLASMIC MALE STERILE PETUNIA. Maureen R. Hanson, M. Connett, S. McEvoy, H.T. Nivison, K.D. Pruitt, C.A. Sutton, R. Wilson, *S. Izhar. Cornell University, Genetics and Development, Biotech. Bldg., Ithaca, NY 14853 and *Volcani Center, ARO, Bet Dagan, 50-250 Israel

A complex mitochondrial locus has previously been correlated with cytoplasmic male sterility. This locus contains three genes: *pcf*, *nad3* (NADH dehydrogenase subunit 3), and *rps12* (small subunit ribosomal protein 12). The expression of *pcf*, an abnormal fused gene comprised of portions of *atp9*, *coxII* and an unidentified reading frame, has been characterized at the RNA and protein level by RNA blot, S1 analyses, and cDNA sequencing and by immunological experiments. In the presence of a single dominant nuclear fertility restorer allele *Rf*, the abundance of a *pcf* transcript and of a 25 kD protein product is reduced. Sterile and fertile isonuclear and somatic hybrid lines differ in their utilization of the cytochrome and alternative oxidase pathways. The reduction of alternative pathway activity in sterile lines is not exhibited by lines carrying the *Rf* allele, providing a possible correlation of *pcf* expression with electron transport partitioning. Recent experiments concerning RNA editing and protein products of the *pcf* locus will be presented.

211 RNA EDITING AND GENE MIGRATION IN PLANT MITOCHONDRIA. Michael W. Gray and Patrick S. Covello. Dalhousie University, Department of Biochemistry, Halifax, Nova Scotia B3H 4H7 (Canada).

Since its discovery in 1989, RNA editing in plant mitochondria has been shown to affect many different mRNAs in a variety of mono- and dicotyledons. The basic features of this phenomenon (as we have studied it in the COX2 transcripts of wheat, maize and pea) are: (i) editing results exclusively in C-to-U changes in the mRNA; (ii) editing is predominantly at first and second codon positions, almost always producing a codon specifying a different amino acid; (iii) editing is distributed rather evenly throughout the coding region, rarely at adjacent C residues, with a few edited positions in the 5'-untranslated region; (iv) some partial editing occurs at third position (silent) sites; (v) editing makes the derived amino acid sequence identical (or at least more similar) to that of non-plant (fungal and animal) COX2 sequences at each changed position. We infer that at least some of the editing, and perhaps the majority of it, is required to produce a functional COX2 protein. Although there are some intriguing differences in editing at homologous sites in COX2 mRNAs of the three species studied, detailed comparisons of primary sequence and potential secondary structure have not revealed any obvious motifs in the mRNA that could serve to distinguish edited from non-edited C residues. In the course of these studies, we have determined that although soybean mtDNA contains what appears to be a "normal" COX2 gene (*cox2*), this gene is not expressed. Rather, the functional *cox2* resides in the nucleus in this plant. Nucleotide sequence analysis of a soybean COX2 cDNA clone (derived from poly(A)$^+$ RNA) clearly shows that evolutionary transfer of soybean *cox2* from the mitochondrial to the nuclear genome must have involved reverse transcription of an edited COX2 mRNA intermediate. Supported by a grant from the Medical Research Council of Canada.

212 RNA EDITING MODULATES GENE EXPRESSION IN WHEAT MITOCHONDRIA. Jean-Michel Grienenberger, José Gualberto, Lorenzo Lamattina and Géraldine Bonnard, Institut de Biologie Moléculaire des Plantes du CNRS, 12 rue du Général Zimmer 67084 Strasbourg cedex, FRANCE

Upon cDNA sequencing using reverse transcriptase on total wheat mitochondrial (mt) RNA, it was found that RNA sequences cannot be deduced from their template DNA sequences, due to RNA editing. This RNA editing phenomenon is usually the result of the conversion of a C (in the DNA) into a U (in them RNA) at specific positions along the message. The reverse editing conversion (a T residue into a C residue) can also occur infrequently. RNA editing was found in most of the wheat mt protein genes we have studied up to now (*coxII*, *coxIII*, *cob*, *nad3*, *nad4*, *rps12*) and also in unidentified, expressed ORFs. When the protein sequence deduced from the edited message is compared with the corresponding proteins, the edited message allows a higher similarity of the wheat mt protein sequence with non-plant sequences. RNA editing seems therefore to act as a correction mechanism. Up to 50 different cDNAs corresponding to the transcripts of the genes coding for *nad3* and *rps12* have been analysed. These genes have 27 editing positions. When the precursor transcripts of these genes were specifically studied, it was found that they are differentially edited, with no evident polarity of the process. The evolution of RNA editing was studied during the maturation of this transcript and it was shown that the mature RNAs present in the mt polysomal fraction are fully edited leading to the synthesis of a unique protein.

213 WHEAT MITOCHONDRIAL DISCONTINUOUS GENES AND THEIR EXPRESSION. Linda Bonen, Yvan Chapdelaine, Karen Williams and Sharon Bird. University of Ottawa, Department of Biology, Ottawa, Canada K1N 6N5

Protein-coding genes in wheat mitochondria can be categorized with respect to the complexity of their physical organization and transcript pattern. Some are simple, unique-copy genes with single, abundant mRNAs; others have complex, multiply-split structures that require cis- and/or trans-splicing for their expression. In the latter category are a number of genes encoding subunits of the NADH dehydrogenase complex, including *nad1*, *nad5* and *nad7*. All these genes contain group II-like introns, some of which have undergone breaks with the pieces being rearranged so that coding segments are widely dispersed in the mitochondrial genome. The expression of these genes involves complex transcriptional/RNA processing events and RNA editing is also required for maturation. The steady-state levels of excised introns and RNA precursors vary markedly among split genes in wheat mitochondria and developmentally-specific differences are also seen. Our observations illustrate the radical effects that mitochondrial DNA rearrangements can have on individual gene structure and expression. Supported by NSERC Canada.

214 GENE ORGANIZATION OF LIVERWORT *MARCHANTIA POLYMORPHA* MITOCHONDRIAL DNA. K. Ohyama, K. Oda, K. Yamato, E. Ohta, Y. Nakamura, M. Takemura, N. Nozato, K. Akashi, T. Kanegae. Department of Agricultural Chemistry, Kyoto University, Kyoto, 606-01 Japan

Analysis of liverwort *Marchantia polymorpha* mtDNA by electron microscopy and restriction endonuclease mapping indicated that the liverwort mitochondrial genome is a single circular molecule of about 180 kb in size. We have determined the complete sequence of the liverwort mtDNA. In the sequence of 186,608 base pairs (bp), we detected 93 possible genes. These included genes for three species of ribosomal RNAs, 29 genes for 27 species of transfer RNAs, 32 open reading frames for functionally known proteins, three ORFs with similarity to ORFs of unknown function in the mitochondrial genomes of other organisms. Furthermore, significant 25 ORFs, except ORF732 having homology to RNA maturase, were predicted as possible genes by using the index of G + C content in first, second, and third letters of codons (42.0%, 37.2%, and 26.3%, respectively) obtained from the codon usages of functionally known liverwort genes. Thirty-two introns belonging to either group I or group II were so far found in the coding regions of 17 genes including ribosomal RNA genes (*rrn18* and *rrn26*), a transfer RNA gene (*trnS*), and a pseudogene (ψ*nad7*). Nine introns contained open reading frames encoding proteins with sequences similar to RNA maturase. Liverwort mitochondrial genome is relatively small, its complete sequence did not reveal any incorporation of foreign genetic information, there is no apparent high frequency recombination to generate subgenomes, and there does not seem to be any need for an RNA editing system. This indicates that liverwort mitochondrial genome probably resembles the primitive form of mitochondrial genome.

POSTER ABSTRACTS

215 ADAPTATION OF THE CaMV VIRUS TO TEST FOR RIBOZYME ACTIVITY IN PLANTS.

Richard Tritz, Arnold Hampel, James Borneman and Mitchell Altschuler. Department of Biological Science and Plant Molecular Biology Center, Northern Illinois University, DeKalb, Illinois 60115-2861.

A number of novel RNA sequences have been shown to have catalytic properties. Mutational analysis of some of these RNAs have defined specific domains responsible for cleavage capacity and they can be engineered to carry out both *cis* and *trans* reactions *in vitro*. This has led to the speculation that, if this mechanism could be adapted and modified to function *in vivo*, a novel way to control gene expression would result. In order to test if cleavage events can occur in plants *in vivo*, we have modified the Cauliflower mosaic virus (CaMV) to include a sequence that encodes the self-cleaving hairpin catalytic domain. Following inoculation, this recombinant virus took over the plant metabolism to replicate both the altered viral DNA as well as transcribe the ribozyme RNA *in vivo*. Both Northern and S1 analysis have detected intact ribozyme sequence as well as *cis* cleaved RNA products. No products were detected in mock infected or CaMV viral controls. Reduced levels of viral infection were seen in plants infected with the ribozyme containing virus, consistent with a phenotypic reduction of viral infectivity caused by the ribozyme. This demonstrates that engineered ribozyme activity can occur in plants.

216 ALTERNATIVE TERTIARY STRUCTURE ATTENUATES SELF-CLEAVAGE OF BYDV SATELLITE RNA.

W. Allen Miller and Stan Silver, Department of Plant Pathology and MCDB Program, Iowa State University, Ames, Iowa 50011 USA

A satellite RNA of barley yellow dwarf virus (RPV serotype) is able to self-cleave in both the plus (encapsidated) and minus strands. Both strands can form hammerhead structures. However the plus strand, which cleaves very slowly, has unusual deviations from the consensus hammerhead. The most striking of these is that an additional helix is predicted which may result in formation of a different, more stable tertiary structure comprising an unusual pseudoknot containing three stacked helices. This conformation would be quite different from a hammerhead. Disruption of the additional helix by mutagenesis to favor the hammerhead conformation increased the self-cleavage rate dramatically. In one case, a single base change caused a 400-fold increase in the rate of self-cleavage. Restoration of the additional helix with a new primary sequence reduced self-cleavage greatly. When the additional helix and thus the novel wildtype tertiary structure could form, the self-cleavage rate was inversely proportional to the stability of the helix. Digestion of the RNA with structure-sensitive nucleases supported the formation of the alternative structure by the wildtype sequence, and formation of the hammerhead conformation by the rapidly-cleaving mutants. These results also showed that use of mutagenesis to disrupt and restore helices can lead to unexpected alterations in tertiary structure. We propose that the sequence comprising the self-cleavage structure serves multiple functions in the satellite RNA replication cycle. The hammerhead conformation would function in self-cleavage of multimeric satellite RNA, the alternative conformation would serve an as yet unknown function.

217 ANALYSIS OF THE FIRST INTERVENING TRANSCRIBED SPACER IN RICE rDNA BY PCR

Wenyuan Song, Gengyun Zhang, Lihuang Zhu, Institute of Genetics, Academia Sinica, Beijing 100101, China.

In the transcription units of ribosome RNA genes (rDNAs) of higher plants, the DNA sequences coding for 17s, 5.8s and 25s rRNA are conserved, but the sequences of internal transcribed spacers (ITS1 and ITS2) are divergent. According to the 3' end sequence of 17s rRNA gene and the 5'end sequence of 5.8s rRNA gene, a pair of primers was designed for amlification of the ITS1 of genomic DNA from several rice varieties by PCR. Under standard conditions only a 300bp fragment was amplified, while inclusion of 10% DMSO in PCR process resulted in an amplified fragment of 281 bp instead of 300bp. When amplification reaction was performed in the presence of 75% 7-Deaza+25% %dGTP, both the fragments were amplified. Sequencing analysis proved that the 281 bp fragment was identical with the known rice ITS1, described by Fumio Takava (1985). Although sequence homologies between these two fragments can be found in their 5' and 3' terminal regions, the 300 bp fragment has low G+C content value of 56.5%, which is typical of lower eukaryotes, in contrast to the 72.7% high G+C content in the 281 bp fragment. Furthermore, when the two fragments were used as probes in Southern blot hybridization with restriction enzyme digested rice genomic DNA, similar hybridization patterns were observed. We suppose that the 300 bp fragment amplified by PCR may represent a new type of ITS1 of rDNAs in rice genome.

218 ANALYSIS OF VIROID HYBRIDIZING RNA ISOLATED FROM PSTV TRANSFORMED PLANTS.

Carolyn J Meduski and Jeff P Velten NMSU, Molecular Biology Program, Las Cruces, New Mexico 88005

Viroids are a group of single stranded circular RNA molecules that are pathogenic to certain plant species. We transformed tomato plants with different cDNA constructions of Potato Spindle Tuber Viroid (PSTV). RNA isolated from these plants and used in Northern analysis against a PSTV probe revealed the presences of a hybridizing smear ranging in size from 0.36Kb up past 10Kb. Monomeric and dimeric PSTV bands are clearly seen, but greater molecular weight material appears as a continuous smear and not as discrete bands. Viroid replication is accepted to be via a rolling circle mechanism with the formation of concatamers occurring during the replication process. Material of 10 Kb would correspond to over 27 copies of PSTV as one unit. This hybridizing material selects on a cellulose dT column eluting with the poly-A mRNA. Plants infected with PSTV RNA gave similar results. The significance of this viroid hybridizing smear is under investigation.

219 AUTOCATALYTIC SPLICING OF A GROUP I INTRON FROM CHLOROPLASTS IN VITRO AND IN VIVO.

A. J. Thompson and D.L. Herrin, University of Texas at Austin, Department of Botany, Austin, TX78713.

The group I intron from the chloroplast rRNA large subunit of *Chlamydomonas reinhardtii* (Cr.LSU) undergoes protein free self-splicing *in vitro*. Cr.LSU displays a range of reactions typical of other group I introns of which the LSU intron from *Tetrahymena* has been most extensively studied. Under optimal conditions for self-splicing, Cr.LSU has a K_m for guanosine comparable to that of the *Tetrahymena* intron, but the requirement for high temperature and [Mg^{2+}] suggests involvement of additional protein factors *in vivo*. The positions of three cyclization sites of the free intron have been mapped. Two of these sites represent reactions mechanistically analogous to 5'-splice site cleavage, whereas the third is an example of G-exchange in which cyclization occurs by release of the 5'-terminal GTP.

Cr.LSU contains an ORF potentially encoding an 18.7 kDa polypeptide. The function of this ORF, likely to be either promotion of splicing or intron mobility, or both, in analogy with fungal mitochondrial group I intron ORFs, has been investigated by using chloroplast gene replacement via particle bombardment. We have shown that the ORF can be deleted from Cr.LSU *in vivo* or *in vitro*, without affecting splicing and it thus does not encode an essential splicing factor. We are currently using particle bombardment transformation to determine which regions of Cr.LSU are required for efficient splicing in the chloroplast and thus may potentially interact with protein factors.

220 CHARACTERIZATION OF THE MAJOR RNASES OF *ARABIDOPSIS*.

Christie Howard[1], Yang Yen[2] and Pamela J. Green[1]. [1]MSU-DOE Plant Research Laboratory and Department of Biochemistry, Michigan State University, E. Lansing, MI 48824-1312. [2]Present address: Department of Agronomy, University of Nebraska, Lincoln, Nebraska.

Ribonucleases (RNases) are considered to play a paramount role in mRNA metabolism. However, in eukaryotes, the RNases that participate in mRNA degradation have yet to be differentiated from those with other roles in RNA metabolism. As a first step towards understanding the function of distinct RNases in plants, we have begun to study the RNases of Arabidopsis. A substrate based gel assay has been optimized to resolve up to 16 RNase activities from *Arabidopsis* plants. Several of these RNases were found to accumulate to different levels in leaves and stems. The gel assay has also been used to monitor the divalent cation dependence, EDTA sensitivity, pH optima, and DNase activity of the Arabidopsis RNases. These analyses indicate that Arabidopsis contains representatives of at least three of the four major classes of RNA-degrading activities found in other higher plants (Farkas, 1982. In Encyclopedia of Plant Phys. Vol. 14B, pp.224-262; Wilson, 1982. In Izozymes: Current topics in Biological and Medical Research 6:33-54). Additional studies have focused on the association of individual RNases with specific subcellular components. We are particularly interested in identifying the RNases that are associated with polyribosomes because a current hypothesis is that polysome bound RNases participate in mRNA decay. Initial experiments have shown that one of the major RNases identified in the gel assay preferentially fractionates with Arabidopsis polysomes. Further analysis of this RNase, as well as others associated with other cellular components, will be discussed.

POSTER ABSTRACTS

221 DELETION ANALYSIS OF THE CAULIFLOWER MOSAIC VIRUS POLYADENYLATION SEQUENCE. Francois Guerineau, Andrew Lucy[1] and Philip Mullineaux[1]. Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK and [1] John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK.

Deletions were made in the cauliflower mosaic virus polyadenylation sequence which was cloned downstream of the gus gene. The effect of these deletions on the expression of the gene was measured by transient expression in tobacco mesophyll protoplasts. Deletion of the sequence located upstream from position -100 with respect to the main polyadenylation site did not reduce gene expression whereas deletion upstream from position -55 had a deleterious effect. Deletion of the sequence located immediately upstream of the AATAAA box had the same effect as deletion of the whole polyadenylation sequence: the specific GUS activity given by these constructs was 3% of the activity given by the construct harbouring the undeleted polyadenylation sequence. In comparison, deletion of the polyadenylation site had little effect on gene expression.

222 DEVELOPMENT OF AN *IN VITRO* DEGRADATION SYSTEM DERIVED FROM SOYBEAN SEEDLINGS. Matthew Tanzer and Richard Meagher. University of Georgia, Department of Genetics, Athens, Georgia 30602.

General mechanisms of messenger RNA turnover remain largely unknown in plant systems. In bacterial and mammalian systems degradation of several mRNAs is believed to occur in a net 3' to 5' direction. Previous work in our lab has shown that the mRNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase gene, SRS4, is found as a full length message and as several lower molecular weight (LMW) degradation products in total RNA from soybean seedlings. The LMW products generally lack the 3' end of the mRNA but have an intact 5' end consistant with data found in bacterial and mammalian systems. In order to elucidate both the direction and the determinates of SRS4 mRNA decay, we have developed an *in vitro* degradation system derived from soybean seedling polysomes. In this system, full length endogenous SRS4 mRNA levels decrease over a six hour period whereas the descrete LMW products do not decrease at the same rate. Similarly, *in vitro* synthesized runoff transcript of SRS4 mRNA added to this system degrades into a similar set of fragments. We are in the process of further characterizing this *in vitro* system and hope to show that it can be used to examine the degradation of a wide variety of mRNAs.

223 DISSECTION OF THE CAULIFLOWER MOSAIC VIRUS POLYADENYLATION SIGNAL. Hélène Sanfaçon[1,2], Helen Rothnie[1], Peter Brodmann[1] and Thomas Hohn[1]. Friedrich Miescher Institut, CH-4002, Basel, Switzerland [1] and Agriculture Canada Research Station, Vancouver, B. C., V6T 1X2, Canada [2].

Mutation studies of the cauliflower mosaic virus (CaMV) polyadenylation (polyA) signal revealed striking differences to vertebrate polyA signals. The AATAAA sequence is necessary for efficient cleavage at the polyA site, however point mutations in this sequence have less drastic effect than in vertebrates. In contrast to vertebrate polyA signals, sequences downstream of the cleavage site do not affect the efficiency of processing. Deletion of sequences **upstream** of the AATAAA reduced drastically processing at the CaMV site suggesting the presence of one or several positively acting elements. Moving these upstream sequences to downstream of the cleavage site abolishes processing at the CaMV site. An oligonucleotide consisting CaMV upstream sequences could induce the recognition of a normally silent exogenous polyA signal when inserted upstream of its AATAAA motif. Further mutagenesis of the CaMV upstream sequences is underway to identify more precisely the active element(s).

224 EXPRESSION AND ASSEMBLY OF U2 PRE-snRNA TRANSCRIPTS IN TRANSFECTED PLANT NUCLEI. Carl K. Sung, Brian A. Hanley and Mary A. Schuler. University of Illinois, Departments of Plant Biology and Biochemistry, Urbana, Illinois 61801

Relatively few studies have analyzed the expression of plant snRNAs and/or genes *in vivo*. In contrast to the single abundant form of each spliceosomal snRNA expressed in mammalian nuclei, multiple U1, U2, U4 and U5 snRNA variants and a single U6 snRNA exist in plant nuclei. Sequence analysis of the numerous U1-U5 snRNA variants expressed in pea nuclei has demonstrated that each of these spliceosomal snRNAs is represented by multiple sequence variants some of which are differentially regulated during plant development. A pea U2 snRNA gene has been cloned that contains promoter elements at -66/-78 and -28/-37 nearly identical to sequences present in the 5' flanking region of the *Arabidopsis* U2 and U5 snRNA genes. In addition to these promoter elements, a conserved motif CAN4-9AGTN(A/T)AA occurs immediately adjacent to the end of the plant snRNA genes. For future *in vivo* trans complementation of mutant introns with plant snRNA genes, we have expressed the pea U2 snRNA gene on an autonomously replicating vector designed to express pre-mRNA transcripts at high levels in plant nuclei. On this vector, the pea U2 snRNA gene is accurately initiated and terminated using promoter and terminator elements present in its flanking sequences. Pre-snRNA transcripts are properly translocated to the cytoplasm for hypermethylation of the guanosine cap structure and reimported into the nucleus for assembly into functional snRNPs. The role of the 3' conserved sequence in the production of pre-mRNA transcripts has been investigated by mutational analysis of the 3' conserved motif. These experiments help define the integrity of heterologous U2 snRNAs in transfected plant nuclei for trans complementation studies.

225 EXPRESSION OF ARABIDOPSIS U1a snRNA PROMOTER DELETION-GUS GENE FUSIONS IN TRANSGENIC TOBACCO. Ann Pizanis Sturtevant and Raymond E. Zielinski. Department of Plant Biology, University of Illinois, Urbana, IL 61801.

U1 snRNA is one of a group of spliceosomal snRNAs that function in the removal of introns from pre-mRNA. A U1 snRNA gene from Arabidopsis thaliana (U1a) was cloned and sequenced. U1a snRNA is unique in having an extra U residue at position +2 in the region of U1 snRNA that base pairs with the 5' splice site of introns. The promoter of U1a snRNA contains regulatory sequences previously described for other plant snRNAs: the CAAT box, -78 sequence, TATA box and 3' box. In addition, U1a contains sequence motifs resembling the CaMV 35S box II sequence and the AT box found in leaf-specific genes. U1a promoter deletion-gene fusion constructs using the reporter gene β-glucuronidase were used to determine the minimum promoter required for transcription. U1a promoter deletion-GUS gene fusion constructs were expressed at much higher levels in tobacco leaf tissues than in bud, flower or stem tissue. The U1a-GUS constructs were light-regulated in tobacco leaves. This is the first report of light-regulation of an snRNA gene.

226 ENZYME-CATALYZED PROCESSING OF POTATO SPINDLE TUBER VIROID (PSTVd) AND RELATED VIROIDS. H.L. Sänger, M. Tsagris, M. Tabler, W. Schiebel, A. Axmann, C. Anderl, W.R. Quaas and B. Haas, Max-Planck-Institut für Biochemie, Abteilung Viroidforschung, D-8033 Martinsried bei München, F.R.G.

In contrast to the hammerhead-mediated self-cleaving of the exceptional avocado sunblotch viroid, the precursor-like RNAs of the PSTVd-related viroids proper are evidently processed by host enzymes. In our attempts to characterize this patnway we have partially purified from cultured potato cells a pyrimidine-specific endoribonuclease that is capable in vitro of cleaving out from 1073 nt long multimeric PSTVd RNA transcripts 359 nt monomers which carry a 5'-OH and a 2',3'-cyclophosphate at their termini. The circularization of these monomers to the mature viroid molecules is ATP-dependend and catalyzed by a RNA ligase which has also been partially purified. In addition to this bi-enzymic pathway we also discovered a novel and mono-enzymic pathway of viroid precursor RNA processing. In this case the cleaving-out of the monomer and its subsequent circularization is catalyzed by single endoribonucleases such as RNase T1, A, Ac, Bc and N1 all of which are characterized by the reversibility of the 2',3'-cyclophosphate generating transesterification step of their cleavage reaction. In the reversed reaction this energy-rich endgroup is utilized to circularize the cleaved-out monomeric product.

POSTER ABSTRACTS

227 EVOLUTION OF MUTANTS OF A PLANT VIRUS SATELLITE RNA. Christophe Robaglia*, George Bruening§ Jim Haseloff¶, and Wayne Gerlach§. Laboratoire de Biologie Cellulaire INRA Versailles*, France, Department of Plant Pathology, UC Davis, USA§, Department of Molecular Biology, Massachusetts General Hospital, Boston, USA¶, and Division of Plant Industry, CSIRO, Canberra, Australia§.

The behaviour of linker insertion mutants Tobacco Ringspot Satellite RNAs expressed in transgenic plants has been studied after helper virus inoculation. One mutant inactivating the positive strand catalytic RNA was unable to replicate. One mutant has been shown to replicate unaltered and displays an altered ratio of monomeric and multimeric forms. All the others mutant were found to evolve into new molecules. In some cases several different molecular species were found to replicate in the same plant. Most sequences changes were rearrangement at the mutation point which may involve either complete deletion of the linker insertion and surrounding sequences or rearrangement of linker sequences. In some molecules the deleted sequence was found to be replaced by various type of sequence duplications. In one type of mutant a compensatory change occurs outside of the linker insertion restoring a potential secondary structure. These results demonstrate a great flexibility for the satellite molecule.

228 FATE OF UNSPLICED mRNA IN MAIZE. Juli Nash and Virginia Walbot. Stanford University, Department of Biological Sciences, Stanford, California 94025

Unspliced *Bronze 2* (*Bz2*) transcripts accumulate in purple husk tissues grown in both field and greenhouse conditions; this finding indicates that plant cells may be susceptible to the production of novel proteins as a result of splicing failure. We have used a protoplast transient expression system to examine the fate of unspliced *Bz2* mRNA and the effects of the production of aberrantly spliced mRNA in plant cells. Because unspliced mRNA that is available to the translational machinery is expected to produce abnormal proteins. we propose that the discrimination between spliced and unspliced mRNA species occurs prior to translational initiation and is likely to occur prior to the export of transcripts from the nucleus to the cytoplasm. The relative mRNA half-lives of spliced and unspliced *Bz2* mRNA is compared in a protoplast transient assay system. To determine if the unspliced mRNAs are translated, expression constructs are made in which the only available ATG for translational initiation is within an intron in the 5' untranslated region of the gene. Reporter gene expression from these constructs is indicative of the translatability of unspliced mRNA and is a measure of the degree of splicing failure in the cells.

229 THE FUNCTION OF FOUR STRUCTURAL DOMAINS IN THE RIBOZYME RNASE P. Sylvia C. Darr, Karen Zito*, Drew Smith*, and Norman R. Pace*. Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68583-0718 USA and *Department of Biology, Indiana University, Bloomington, IN 47405 USA.

Ribonuclease P is an endonuclease which catalyzes removal of the 5' leader from precursor tRNAs. In eubactena, RNase P is composed of an RNA and a protein subunit. The catalytic subunit is the RNA, approximately 375 nucleotides in size. A secondary structure model of the RNA has been proposed based on comparisons of the sequence of RNase P RNAs from several different organisms. We are defining the minimum structure required for catalysis by deleting variable domains in the RNA. Four domains were deleted from the *E. coli* RNase P RNA to create *min1* RNA. The *min1* RNA is active, however it is defective in several properties: 1) the *min1* K_M for precursor $tRNA^{Asp}$ is 100-fold greater than the K_M of the native RNA, 2) much more monovalent salt is required for maximum activity, and 3) the RNA is no longer activated by the protein subunit (Waugh et.al. Science 244:1569). We are analyzing the four *min1* deletions individually and in greater detail in order to identify the deletions that are responsible for these defects. We find that one of the deletions (helix 260-290) causes the increased K_M. We are attempting to repair the K_M defect by altering the sequence in the region of the deletion. Two of the other deletions confer the increased salt dependence of *min1*. This requirement for high salt is consistent with the possibility that these two deletions disrupt the structure of the RNA. We are currently measuring which of the four deletions causes loss of activation by the protein subunit.

230 GENES WITH HOMOLOGY TO S-GENE RIBONUCLEASES ARE EXPRESSED IN A SELF-COMPATIBLE SPECIES. Crispin B. Taylor[1], Pauline A. Bariola[2], Brett K. McLarney[2] and Pamela J Green[1]. [1]MSU-DOE Plant Research Laboratory and [2]Dept of Biochemistry, Michigan State University, East Lansing, MI 48824-1312. The S-gene ribonucleases of *Nicotiana alata* and many other solanaceous plants, which are involved in the expression of gametophytic self-incompatibility, share extensive amino acid sequence identity with a class of secreted ribonucleases from fungi (Haring *et al* Science 250, p.937). This similarity is especially pronounced in the vicinity of the active site of the fungal enzymes. However the role of these proteins in the self-incompatible response is unknown, and we were interested in finding out whether similar enzymes are expressed in self-compatible plants, which would suggest a more general role for this type of RNase. When DNA primers corresponding to amino acid sequences in the active site region were used to amplify an *A. thaliana* cDNA library using the polymerase chain reaction, three different putative RNases, termed RNS1, RNS2 and RNS3, were identified. Amino acid functional identities within the amplified region are between 34% and 57%, when comparing the sequences of individual RNS genes with those of fungal and S-gene RNases, but around 70% when comparing the RNS sequences to each other. Comparison of the deduced amino acid sequence at the N-terminus of RNS2 with the corresponding regions of fungal and S-gene RNases shows that, in contrast with the functional identities in the active site region, RNS2 is much more like the S-gene RNases than the fungal RNases (60%-66% for RNS2 vs *N. alata* S-gene RNases and 20%-24% for RNS2 vs fungal RNases). All three RNS genes are expressed in *A. thaliana* above-ground tissue, but at differing levels. Interestingly, analysis of RNA from *A. thaliana* leaves, stems and flowers has demonstrated that the expression of at least two of the RNS genes, RNS2 and RNS3, is restricted to flowers, as is the expression of the S-gene RNases in *N. alata*. The expression of similar proteins in the flowers of both self-compatible and self-incompatible species strongly suggests that this type of RNase has a more general role in RNA metabolism in plants, in addition to its specialized function in gametophytic self-incompatibility.

231 HEAT SHOCK: A SYSTEM TO STUDY RNA PROCESSING IN MAIZE PROTOPLASTS. Gabriella Di Cola[1,2], Irvin Mettler[1], Nelson Marmiroli[2] and Ralph Sinibaldi[1]. Sandoz Crop Protection, Plant Biotechnology Department, Palo Alto, CA 94304[1]. Institute of Genetics, University of Parma, Parma, Italy.[2]

We and others have previously reported that introns can enhance the expression levels of reporter genes lacking introns in maize and other monocots.These experiments have utilized a constitutive promoter (35S from CaMV), various introns, a β-glucuronidase reporter and the poly A region from the nopaline synthetase gene (Nos).Since the 35S promoter is constitutively expressed in maize protoplasts, we had been limited to investigating steady state levels of mRNA and pre-mRNA.The heat shock promoters from many organisms have been used to direct synthesis of several gene products in a heat dependent manner.However, heat treatment does inhibit splicing. We have cloned and characterized two different maize heat shock promoters (Hsp 17.9 and Hsp 82) that have optima of expression at 41°C. Utilizing these maize heat shock promoters, we have defined an experimental heat treatment (39° C), which induces new GUS mRNA and results in very little inhibition of intron splicing as measure by GUS expression levels. At 41°C there is a 50 to 90% decrease in GUS report gene activity.We feel this system will allow us to better investigate the mechanism by which introns enhance expression in maize protoplasts.

232 INTRON-EXON JUNCTIONS OF AN LHCPII PRE-MRNA ARE NOT CONSERVED IN EUGLENA

Umesh S. Muchhal and Steven D. Schwartzbach, University of Nebraska, School of Biological Sciences, Lincoln, NE 68588.

A 7.4 kB Euglena genomic clone encoding the 3' end of the polyprotein precursor to the light harvesting chlorophyll a/b binding proteins of photosystem II (LHCPIIs) was sequenced. Based on amino acid sequence homology to higher plant LHCPIIs, ten exons can be assembled into an open reading frame encoding 113 amino acids of C-terminus of one LHCPII unit followed by 4 complete LHCPIIs. At least one intron is present within the region encoding each of the LHCPII units. RNAse protection assays indicate that the cloned genomic sequence is expressed and confirm the intron locations. Polymerase chain reaction amplification of cDNAs has been used to determine the sequence at the 3' end of the mRNA and at the intron exon junctions. The conserved dinucleotide found at the 5' end (GT) and 3' end (AG) of most nuclear gene introns are not present at the ends of the Euglena introns. Sequences for 5 intron-exon junctions indicate that the Euglena introns contain a conserved dinucleotide (CC) 2 nucleotides from the 5' end of all introns. A conserved sequence motif is not present at the 3' end of the introns sequenced but a conserved dinucleotide (AC) is found 1 nucleotide from the 5' end of all of the exons. As found for other Euglena mRNAs, a consensus polyadenylation site is not present at the 3' end of the mRNA. Although based on a small sample set, it appears that the pre-mRNA processing signals in Euglena differ from those of virtually all other organisms.

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233 *IN VIVO* INACTIVATION OF A SELF-CLEAVING RIBOZYME WITHIN A POLYCISTRONIC mRNA GENERATED IN PLANT CELLS. Wesley B. Bruce and Randal Hauptmann. Plant Molecular Biology Center, NIU, DeKalb, IL 60115-2861

In an attempt to develop a functional polycistronic gene expression system in plants, we examined the usefulness of a self-cleaving ribozyme as a tool to separate a single polycistronic mRNA into multiple, functional mRNAs. We have interposed a self-cleaving version of a ribozyme ("hairpin ribozyme (HP), a gift from Dr. A. Hampel) derived from the negative strand of the tobacco ringspot virus satellite RNA between the CAT and GUS genes. This dicistronic gene is potentiated by the 35S promoter and terminated by the NOS polyA addition site. An *in vitro*-derived RNA that encompassed the CAT-HP-GUS genes can cleave itself under appropriate conditions to nearly 50% resulting in two products, a 0.9 kb (CAT) and a 2.0 kb (HP-GUS) size RNA's. We introduced this dicistronic gene into carrot and tobacco protoplasts, isolated total RNA 16 hours after electroporation and detected an abundance of the full length mRNA (3 kb) by Northern analysis. This suggests that little or no cleavage had occurred in the protoplasts. We further tested the protoplast-derived RNA in an *in vitro* cleavage reaction and demonstrated the inability of this 3 kb RNA product to cleave itself in contrast to the *in vitro*-derived, catalytically-active RNA. The reporter enzyme data also supports the Northern analysis results confirming that the self-cleavage reaction of the polycistronic CAT-HP-GUS mRNA is insufficient to generate detectable levels of functional GUS mRNA. These results suggest that a proportion of the ribozyme-containing dicistronic RNA synthesized *in vivo* is somehow modified, preventing the ribozyme from functioning in subsequent *in vitro* reactions. We are currently testing this polycistronic construct in stably-transformed tobacco plants to determine whether the state of the plant tissue influences the RNA cleavage reaction.

234 ISOLATION AND CHARACTERIZATION OF SUCROSE SYNTHASE cDNA CLONES FROM SUGARCANE (*SACCHARUM OFFICINARUM*). A. Suresh Kumar[1], S. Ramagopal[2], and P. S. Chourey[3]. [1]CMBRU, Hawaiian Sugar Planters Association, Aiea, HI; [2,3]USDA/ARS, Aberdeen ID; The University of Florida, Gainesville, FL.

Sucrose synthase catalyzes the reversible reaction of sucrose cleavage into UDP glucose and fructose, and is considered to be an important enzyme for sugar metabolism in sugarcane. Two sucrose synthase (SS) genes, Sus1 and Sus2 were identified and cloned from sugarcane (*Saccharum officinarum*) by using monoclonal antibodies (MAb) as well as cDNAs of two non-allelic genes of maize as probes. The two cDNAs, corresponding to maize Sh and Sus genes, hybridized to different restriction fragments on genomic Southerns of commercial sugarcanes. Northern blot analyses showed slightly different size transcripts with Sh and Sus cDNA probes. Five isozyme bands similar to those of maize controls were observed on native immunoblots stained with either SS1 or SS2 MAb. Different restriction enzyme sites on the two SS sugarcane cDNAs indicated that they are two independent clones. Sequencing these clones confirmed that the two cDNAs represent the products of two different SS genes. Nothern blot and denatured immunoblot analyses indicated differential expression of Sus1 and Sus2 genes between source and sink tissues of sugarcane. Transcriptional and post-transcriptional regulation of SS genes in sugarcane in response to anaerobic stress was similar to that reported for maize.

235 *IN VIVO* ANALYSIS OF PLANT RNA PROCESSING H. Lou and M.A Schuler, Department of Plant Biology, University of Illinois, Urbana, IL 61801

A number of published *in vivo* and *in vitro* studies have demonstrated that the intron splicing machineries are not interchangeable between plant and animal systems or between dicot and monocot systems. The overriding goal of our research is to define the critical differences between monocot and dicot introns that prevent efficient *in vivo* expression in transgenic plant nuclei. To this end, we have initiated a series of experiments in which monocot and dicot intron constructs were introduced into dicot nuclei on a geminivirus-based replicating expression vector. Maize Adh1 and pea Adh genes were chosen because of their similar organization. In full length constructs, the maize Adh1 intron 3 and pea Adh intron 2 were spliced at 90% and 80% efficiency, respectively, while maize Adh intron 2 and pea Adh intron 3 were spliced at 50% and 64% efficiency. Surprisingly, the first intron in both the maize and pea Adh genes was spliced at the very low efficiency(<25%). To determine the basis for these differences in splicing efficiency, we have made constructs in which sequences from the maize Adh1 intron 1 and 3 were interchanged. The initial experiments demonstrated that two cryptic 3' splice sites were activated in the maize Adh 3:1 fusion construct. Further analyses of these constructs which is in progress will allow us to localize the cis-acting sequences required for accurate and efficient splicing in this dicot system.

236 Mutational Analysis of Plant pre-mRNA Splicing. Andrew J. McCullough and Mary A. Schuler, Department of Plant Biology, University of Illinois, Urbana, Illinois.

Plant genes, in contrast to mammalian and yeast genes, have a high degree of sequence variability in their exon-intron boundaries. Plant 5' splice sites vary in the region which potentially interacts with U1 snRNA and even more prominent deviations occur on the proximal side of plant intron 3' splice sites which potentially interact with the U5 snRNP. Accordingly, plant snRNA populations are significantly more heterogeneous than the mammalian or *S. cerevisiae* snRNA populations Despite the widespread natural variation in plant splice sites, the sequence requirements for accurate and efficient splicing of plant pre-mRNAs have not been experimentally determined.

Using a geminivirus-based autonomously replicating plant expression vector, we have analyzed the splicing of several wild-type plant introns in transfected *Nicotiana benthamiana* leaf cells. Additionally, the first intron of the *Pisum sativum* rbcS3A gene was mutagenized to define critical nucleotides required for intron excision. Using PCR site-directed mutagenesis, the 5' splice site of this intron has been modified at positions predicted to base pair with U1 snRNA. Mutation of the G at position +1 in the intron to A abolished normal splicing and activated at least three cryptic 5' splice sites. In contrast, mutation of the position +5 G to A, C or T did not affect the accuracy or efficiency of splicing Analysis of additional single and multiple 5' splice site mutants is underway and will be presented

237 MOLECULAR COMPARISON OF MONOCOT AND DICOT U1 AND U2 snRNAs. Daniel B. Egeland, Michael A. Musci and Mary A. Schuler. University of Illinois, Departments of Biochemistry and Plant Biology, Urbana Illinois 61801 In order to further clarify differences between the pre-mRNA splicing machineries of monocots and dicots, we have cloned and characterized U1 and U2 snRNA variants expressed in wheat seedling nuclei. Comparison of these wheat sequences with the numerous U1 and U2 snRNA variants expressed in pea has defined the regions of primary and secondary structure variation. In U1 snRNA, the 5' end that is implicated in direct interactions with the pre-mRNA, via RNA-RNA interactions, is absolutely conserved. In U1 snRNAs significant differences between wheat and pea occur in stems I and II, which have been implicated in the binding of the U1-specific A, C and 70K proteins. Stem III, which differs more extensively than stems I or II, has been shown to be required for splicing complex formation in mammalian systems. In U2 snRNAs the first 71 nucleotides are absolutely conserved. This region, as in U1 snRNAs, contains sequences which interact directly with the pre-mRNA. The most significant variations are localized at the 3' end of U2 snRNA in stem IV, which has been implicated in the binding of the U2-specific proteins A' and B". Thus, the sequence variations between the monocot and dicot U1 and U2 snRNAs are localized in regions involved in snRNA:snRNP protein interactions and not in regions involved in direct snRNA:pre-mRNA interactions. These differences suggest that monocot and dicot U1 and U2 snRNPs represent functionally distinct entities that may have monocot or dicot-specific protein variants associated with each snRNA.

238 MULTIPLE UPSTREAM SEQUENCE ELEMENTS ARE REQUIRED FOR EFFICIENT mRNA 3' END FORMATION IN PLANTS, Arthur G. Hunt, Brad Mogen and Margaret H. MacDonald, Plant Physiology/Biochemistry/Molecular Biology Program, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091

We are studying the *cis*-acting signals that direct mRNA 3' end formation in three genes: the cauliflower mosaic virus (CaMV) 19S/35S transcription unit, a pea *rbcS* gene, and the octopine synthase gene. Deletion studies have led to the identification of a novel class of upstream element that is involved in functioning of these sites. This class of elements (termed here the "far-upstream element", or FUE) can be located as far as 100 nt from corresponding poly[A] sites, is needed for polyadenylation at more than one site in genes with multiple poly[A] sites, and is not related to the mammalian AAUAAA polyadenylation signal. We have found that the FUE from the *rbcS*-E9 gene can replace the corresponding element of the CaMV polyadenylation signal, and that the 3 end profile of a particular transcription unit is not determined by the FUE. A strict consensus motif is not apparent in the various FUEs thus far identified, but sequences related to AAUU and UGUGUU may be involved in the functioning of these elements. We have also identified another class of upstream *cis*-acting element involved in polyadenylation in plant genes. This class of elements (the "near-upstream element", or NUE) is located between 10 and 40 nt from poly[A] sites and is probably functionally analogous to the mammalian AAUAAA polyadenylation signal. We find that each poly[A] site in genes with multiple sites has a corresponding NUE. It is thus the number and relative location of NUEs that determines the 3 end profile of a given transcription unit. We suggest that interactions between factors that recognize FUEs and NUEs are needed for efficient mRNA 3 end formation in plants.

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239 NOVEL FEATURES OF psbA INTRON-2 SELF-SPLICING.
Yijia Bao and David L. Herrin. University of Texas at Austin, Department of Botany, Austin, Texas 78713

We have confirmed the self-splicing ability of psbA introns from Chlamydomonas reinhardtii (Herrin, D.L., Bao, Y., Thompson, A.J., and Chen, Y., submitted for publication). Further characterization of psbA intron-2 self-splicing has shown some novel features. The incubation of in vitro synthesized transcripts of pGEM.R14.2 (which contains the 3'-half of exon-2, intron-2 and exon-3) under standard self-splicing conditions showed that a high percentage of RNA precursors (>90%) underwent self-splicing or self-cleavage reaction. The self-cleavage (i.e. hydrolysis) of the psbA intron-2 precursor differs from that of Tetrahymena (which hydrolyze specifically at the 5' and especially the 3'-splice sites). It has been demonstrated that the self-cleavage reaction is GTP (or guanosine) dependent and decreases by increasing the pH (from pH 6.5 to pH 9.0). Primer extension revealed that the 3' self-cleavage sites were located in a multiple U region near 3'end of intron-2, two to five nucleotides preceding the last intron nucleotide (a conserved G). Intron sequence and putative secondary structure analysis suggested that an unusual P9.0 structure may facilitate this self-cleavage reaction. The alternative 3' cleavage may prevent the exon ligation and cause the accumulation of self-cleavage products. The exon-intron cleavage product was isolated (presumably without G at 3' end) and incubated under self-splicing conditions. Cyclized molecules were produced, a reaction similar to the normal Group 1 introns. RNA sequence analysis will be carried out to confirm that this cyclization reaction accurs without 3'-end guanosine.

240 PROCESSING OF CHIMERIC INTRONS IN PLANTS: EVIDENCE FOR A CLOSE COOPERATION BETWEEN 5' AND 3' SPLICE SITE. Andrea Barta and Elisabeth Waigmann. Institut fur Biochemie, Wahringerstr. 17, 1090 Vienna, Austria

Splice sites of vertebrate introns are in general not recognized in plant cells. Several lines of evidences led to the proposal that the mechanism of 3'splice site selection differs in plants and animals (K. Wiebauer, J.J Herrero, and W Filipowicz, Mol. Cell. Biol., 8:2042-2051, 1988). To gain better insight into the mechanistic differences in plant and animal splicing we constructed chimeric introns consisting partly of dicotyledonous plant and partly of animal intron sequences. Splicing of these chimeric introns was analyzed in transiently transfected tobacco protoplasts.

Surprisingly, in one construct an authentic vertebrate 3 splice site (h-Metallothioneine intron1) was recognized in context with a plant 5'splice site, although the intact h-Metallothioneine intron1 itself cannot be recognized in plants. The results show that there are no principal sequence or structural differences between the 3'splice regions of plants and animals

Furthermore, we present evidence that there is a clear preference in splice site pairing. This points to a close cooperation between 5 and 3' splice sites thus exerting influence on their mutual selection

241 RNA EDITING INTERMEDIATES OF cox2 TRANSCRIPTS IN MAIZE MITOCHONDRIA. R. Michael Mulligan and Austin J. Yang. Department of Developmental and Cell Biology, University of California, Irvine, CA 92717

RNA editing in higher plant mitochondria results in modification of the sequence of mitochondrial transcripts such that the sequence of the RNA differs from the DNA sequence from which it was transcribed. The sequence modifications in plant mitochondrial transcripts are limited to the substitution of uridine residues in positions that cytidine residues were encoded by the DNA, and occasionally the reverse substitution. Eighteen cytidines are changed to uridines in the coding sequence of transcripts for cytochrome c oxidase subunit 2 (cox2) in maize mitochondria. The mechanisms by which these modifications are made are unknown. In order to establish the temporal relationships of transcription, editing and splicing, the degree of RNA editing was assessed in unspliced and spliced cox2 cDNA clones from maize mitochondria. Sequence analysis of 20 unspliced cox2 cDNA clones indicated that the population of unspliced transcripts was heterogeneous with respect to the degree of editing. The unspliced cDNA clones ranged from clones with no edited nucleotides to completely edited forms. In contrast, sequence analysis of 15 spliced cDNAs indicated that 12 of the clones were completely edited, while 3 of the clones were incompletely edited at a single position.

These experiments indicate that editing can precede splicing of cox2 transcripts. In addition, the unspliced cDNAs reflect the editing status of the nascent population of cox2 transcripts and these incompletely edited cDNAs reflect intermediates of the editing process. Thus, RNA editing is not completed simultaneously with nucleotide insertion, but probably occurs post-transcriptionally. The pattern of editing of individual nucleotides among the RNA editing intermediates indicates that RNA editing proceeds without a strong directional bias, in contrast to the 3' to 5' progression of RNA editing in Trypanosome mitochondrial transcripts.

242 RNA RECOMBINATION BETWEEN SATELLITE RNAS ASSOCIATED WITH TURNIP CRINKLE VIRUS. P.J. Cascone, C. Zhang, C.D. Carpenter and A.E. Simon. Univ. of Mass., Biochem. Dept., Amherst, MA 01003

Turnip crinkle virus (TCV), one of the smallest single stranded RNA viruses known to infect plants, can support three different types of subviral RNAs: satellite RNAs (sat-RNAs D and F), defective interfering RNAs (DI RNA G and DII RNA), and chimeric RNAs containing segments of both satellite and TCV genomic RNAs (sat-RNAs C and CX and sat-RNA D/C recombinants). The DI and chimeric RNAs are thought to have been generated by a replicase driven, copy choice mechanism. The right side junctions in these molecules share one of three sequences (Motifs I, II and III). Two of these sequences (Motifs I and II) are present at the 5' termini of the TCV genomic and subviral RNAs as well as near the initiation site of the 1.7 Kb TCV subgenomic RNA. The third sequence (Motif III) is also located near the initiation site of the 1.45 Kb TCV subgenomic RNA. We have focused our studies on Motif I, which is targeted by the replicase in the non-homologous RNA recombination occurring between sat-RNA D and sat-RNA C in planta. With only three exceptions, over seventy recombinant RNAs analyzed contain different lengths of sat-RNA D joined to one of five consecutive bases within Motif I in sat-RNA C. In order to determine the effect of alterations within this motif on recombination, site-directed mutagenesis was used to introduce over a dozen point mutations into and around Motif I in sat-RNA C. All nucleotide changes, except those generated in the five consecutive bases, abolish recombination between sat-RNAs D and C. We are currently using a deletion analysis approach to determine the exact 5' and 3' borders of Motif I.

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SEQUENCE COMPOSITION DIFFERENCES IN MONOCOT AND DICOT INTRONS. O. White, P. Shanmugam, C.A. Soderlund and C.A. Fields, Computing Research Laboratory, New Mexico State University, Las Cruces, NM 88003-0001, USA.

Using computational methods, the DNA sequence composition of plant introns has been characterized. Significant DNA level differences between the evolutionarily distinct plant groups, monocots and dicots, have been observed. By comparing 256 dicot introns and 184 monocot introns, it is noted that:

- Splice site consensus sequences are different between dicots and monocots. Significant differences are observed in splice site sequence when introns are separated by size. Monocot introns >125 nt have a 71% occurrence of a G in the +5 position of 5' splice sites. Dicot introns of similar length, however, have a 44% occurrence of a G in the same position. Other differences in both 5' and 3' splice site composition of dicot and monocot introns are apparent.
- Measuring the non-random distribution of nucleotides at any single position yields a measure of "information" for an aligned set of DNA sequences. The overall level of single nucleotide position information of introns is reduced in monocots when compared to dicots. Additionally, all plant intron information contents are below that of introns from the invertebrate, *Caenorhabditis elegans*.
- Dinucleotides containing A or T occur with a 51% and 36% frequency in dicot and monocot introns, respectively.

These results suggest that intron recognition mechanisms may be different in monocots versus dicots.

244 STRUCTURE, FUNCTION AND EVOLUTION OF PLANT SMALL NUCLEAR RNAs AND THEIR GENES. T. Kiss, Ch. Marshallsay and W. Filipowicz. Friedrich Miescher-Institut, 4002 Basel, Switzerland.

Plant small nuclear RNA (U-snRNA) gene promoters contain two conserved elements, the plant snRNA gene specific Upstream Element (USE) and the TATA box. These elements are necessary and sufficient for transcription by RNA polymerases II and III. It is a one helical DNA turn difference in the spacing between these two elements that determines the choice of RNA polymerase (Waibel & Filipowicz, Nature 346, 199, 1990).

We have now characterized plant genes encoding the nucleolar RNAs U3 and MRP. In contrast to the U3 genes from other organisms, which are pol II transcribed, plant U3 genes are transcribed by pol III. This is the only reported gene which is transcribed by different polymerases in different organisms. Manipulation of the USE/TATA spacing converts the specificity of plant U3 gene to pol II. These results empahasize the similarities between the pol II and pol III transcription systems and their common origin (Kiss et al. Cell, in press).

In mammalian cells, a fraction of the nuclear encoded MRP (for Mitochondrial RNA Processing) RNA is located in mitochondria where it functions, as a component of the RNase MRP, during mitochondrial DNA replication (Clayton, TIBS 16, 107, 1991). The majority of mammalian and tobacco MRP RNA is located in the nucleolus. Using very sensitive assays we were unable to detect this RNA in purified mitochondria from tobacco cells.

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245 STRUCTURE AND EVOLUTION OF PLANT 5S RIBOSOMAL RNA. THE NEW MODEL OF SECONDARY AND TERTIARY STRUCTURE OF 5S rRNA. M. Nalaskowska, M. Szymański, M. Barciszewska and J. Barciszewski.
Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, 61-704 Poznań, Poland

Ribosomal 5S RNA (5S rRNA) is a component of the large ribosomal subunit and takes part in a number of interactions during protein biosynthesis. Its role is not completely understood. Recently, it has been suggested that ribosomal protein L5-5S rRNA complex is the recognition site for elongation factor 2. 5S rRNA interacts with transcriptional factor IIIA, a protein essential for polymerase III dependent 5S rRNA gene transcription and forms 7S and 42S particles in Xenopus laevis oocytes.
At present, over 600 nucleotide sequences of 5S rRNA are known, out of which 19 primary structures for higher plant 5S rRNAs have been determined. Nine of them have been solved in our lab. Their analysis suggests that 5S rRNA structure is strongly conserved during evolution. However, there are some nucleotides which one can use as evolutionary markers for various plant groups. On the basis of biochemical experiments, enzymatic digestions, chemical modification, free energy calculations, differential scaning calorimetry, nuclear magnetic resonance spectroscopy and computer modelling we proposed the new model of secondary and tertiary interactions of higher plant 5S rRNAs. The new model of plant 5S rRNA differs from those suggested previously for eukaryotic 5S rRNA in arrangement of the two domains close to each other by tertiary hydrogen bond interactions. The model for plant 5S rRNA is also valid for all eukaryotic 5S rRNAs.

246 CLONING OF A RIBONUCLEOPROTEIN GENE FROM *ARABIDOPSIS*. Dudy Bar-Zvi[1], Ulrike Schindler[2], Anthony R. Cashmore[2] & Tomer Shagan[1]. [1]Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel & [2]Plant Science Institute, Department of Biology, The University of Pennsylvania, Philadelphia, PA 19104, USA.

We have isolated a 1090 bp long cDNA clone encoding an RNA-binding protein in *Arabidopsis*. The clone was isolated by screening an *Arabidopsis* λgt11 expression library for the binding of ssDNA. The 310 amino acids protein encoded by the clone efficiently binds RNA, ssDNA, but much less dsDNA. The protein contains in its carboxy-terminus two adjacent RNP-80 motifs, conserved putative RNA binding domains identified in vertebrates, flies, yeast and plants. Proteins containing this motif are suggested to play an important role in RNA processing. Each RNP-80 motif includes RNP1, a highly conserved amino acid octamer and RNP2 a less conserved hexamer, which are separated by 33 amino acids. However, the amino-terminus of the protein does not resemble any of the proteins containing RNP-80 motif cloned thus far. We have found two types of repetitive sequences in the amino-terminus, each present seven times. The first is Histidine-Proline containing hexamer repeat. The second repeat is acidic and is composed of five amino acids. The structure and the potential role of the protein will be discussed.

247 INVESTIGATION OF CUCUMBER MOSAIC VIRUS RNA 4A AND ITS APPLICATION TO ENGINEERING RESISTANCE IN LUPINS. Beau J. Anderson*, T. J. Higgins# & Robert H. Symons~. *Charles Sturt Uni., Sch. of Sc. and Tech., NSW 2650, Australia. #CSIRO Div. of Plant Ind., ACT 2601, Aust. ~Dept. of Biochem., Uni. of Adelaide, SA 5001, Aust.

Cucumber mosaic virus (CMV) is an economically important pathogen of lupins. It is a positive sense RNA virus with a tripartite genome. RNAs 1, 2 and 3 (3389, 3035 & 2197 nt, respectively) are required for infectivity. The coat protein is expressed from subgenomic RNA 4 (1031 nt) which is derived from the 3' end of RNA 3. Gel electrophoretic analysis of purified Q-CMV RNA has consistently revealed the presence of an additional RNA (RNA 4A). Sequence analysis of 7 RNA 4A cDNA clones demonstrated that RNA 4A is a highly conserved molecule of 680 nt that has >98% sequence homology with the 3' terminal 682 nt of RNA 2. Northern analysis of several strains of CMV has indicated that RNA 4A is consistently present in subtype II strains of CMV, but not in subtype I strains. A homologous sequence of 138 nt exists at the 3' terminus of all CMV RNAs. This sequence can be folded into a tRNA-like structure which contains a replicase recognition site. We are investigating the ability of RNA 4A to create CMV resistance in transgenic plants based on the hypothesis RNA 4A is a Defective Interfering RNA that will compete with genomic RNAs for replication factors and result in reduced viral RNA levels and attenuation of symptoms.

248 THE ACTIVITY OF PROMOTER SEQUENCES DERIVED FROM THE RICE TUNGRO BACILLIFORM VIRUS. Maitrayee Bhattacharyya, J. Scott Elmer+, Gary Laco and Roger N. Beachy. Department of Biology, Washington University, St. Louis, MO 63130 and +The Monsanto Company, Chesterfield, MO 63198.

Rice tungro bacilliform virus (RTBV) is one of two viral components which together are the causal agents for rice tungro disease. Analysis of the DNA sequence of a genomic clone of RTBV (a double stranded DNA virus) revealed the presence of sequence elements similar to the major promoter regions of caulimoviruses. The 5' and 3' endpoints of a full length transcript of the viral DNA have been precisely mapped: the transcript termini overlap by 265/268 nucleotides. Segments of DNA from the RTBV promoter region were used to create several reporter gene constructs using β-glucuronidase (GUS). Two of these constructs contain 34 and 94 nucleotides downstream of the RTBV transcript start site linked to the GUS reporter gene. Expression of these GUS reporter genes are being analyzed by transient assays in protoplasts from monocot (rice and maize) and dicot (tobacco) suspension cells. These reporter gene constructs are also being analyzed in leaf tissue following particle bombardment. Our results indicate that these DNA sequences have a significant promoter activity resulting in GUS gene expression. The characteristics of this new viral promoter will be discussed.

249 *AGROBACTERIUM TUMEFACIENS* T-*CYT* GENE: *IN VIVO* AND *IN VITRO* ANALYSIS OF ITS PLANT PROMOTER. S.T.C. Neuteboom, R.A. Schilperoort & J.H.C. Hoge
Department of Plant Molecular Biology, Clusius Laboratory, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

The *Agrobacterium tumefaciens* T-*cyt* gene codes in plant cells for an enzyme, isopentenyltransferase, which catalyzes the first step in the biosynthesis of cytokinins. To determine which DNA sequences in addition to a TATA box in the promoter region of the T-*cyt* gene are required for T-*cyt* expression in plants, we made chimaeric gene constructs in which wild-type or mutant derivatives of the promoter region are linked in translational fusion to a ß-glucuronidase (GUS) reporter gene and introduced these constructs in tobacco plants. Histochemical analysis of transgenic plants obtained, showed that T-*cyt* promoter sequences located between positions -185 and -139 with respect to the T-*cyt* ATG are essential for GUS activity and hence T-*cyt* expression in leaves, stem and roots. Gel retardation and methylation interference experiments demonstrated that a tobacco nuclear protein factor binds to a 7-10bp. GC rich sequence stretch (-157/-148; ATGCCCCACA) located within this essential T-*cyt* promoter segment. Further studies will be focussed on cDNA cloning and characterization of this protein factor.

250 ANALYSIS OF OPAQUE-2 FUNCTION. Marlena Ketudat, Ronald Parsons, Milo Aukerman, Len Pysh, and Robert Schmidt. Dept. of Biology, and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093.

The Opaque-2 protein regulates the transcription of certain members of the zein seed storage protein gene family. We have analyzed in yeast the ability of O2 to activate expression of a *lacZ* reporter gene downstream of the O2 binding site. Orientation and number of binding sites were analyzed to determine what effect each had on the capacity for O2 to activate transcription. Whereas a single binding site provided only minimal enhancement above background, multimers of the O2 target sequence produced a 500-fold increase above background. In addition, mutations in the O2 target site and mutations in the bZIP domain of the O2 protein were analyzed for their effect on reporter gene expression. This same system was used to begin analyzing the effect of the 289 bp untranslated O2 leader sequence on O2 protein expression. This untranslated leader sequence may play a role in regulating the translation of the O2 message.

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251 ANALYSIS OF THREE TISSUE-SPECIFIC ELEMENTS FROM THE WHEAT *Cab-1* PROMOTER . Cecilia Gotor, Luis C. Romero and Eric Lam. Rutgers State University, AgBiotech Center,Waksman Institute, Piscataway, NJ 08855.

The genes enconding the major light-harvesting antenna chlorophyll protein (*Cab*) of higher plants are known to be regulated by light at the transcriptional level. A 268-bp fragment of the *Cab*-1 promoter from wheat functions as a light-responsive and organ-specific enhancer in transgenic tobacco. Using DNase I footprinting, we have characterized three different regions (*Cab*1-A, *Cab*1-B and *Cab*1-C) in this 268-bp fragment that bind to protein factors in tobacco nuclear extracts. In transgenic plants, synthetic tetramers of each of these regions are able to confer leaf expression when fused upstream of the -90 derivative of the 35S promoter from cauliflower mosaic virus. The expression conferred by these tetramers in this promotor context is light-independent. Thus, these regions may not function as light-responsive elements in tobacco. Histochemical localization of the reporter gene product suggests that the three tetramers direct expression in the apical meristem and cotyledons of seedlings, but in mature plants, the *Cab*1-B and -C tetramers confer expression in vascular elements as well as mesophyll cells. In contrast, *Cab*1-A appears to confer expression predominantly in the leaf lamina region only. Our data sugget that the *Cab-1* promotor contains at least three distinct elements that can confer tissue-specific expression in transgenic tobacco.

252 ANALYSIS OF THE EXPRESSSION OF A MAIZE PROMOTER FOR THE C4-TYPE PEP CARBOXYLASE IN TRANSGENIC TOBACCO. S. Flores[1], B. Brunner[1], S. Yanagisawa[2], K. Izui[2],and G. Neuhaus[1], Swiss Federal Institute of Technology, Zurich, Switzerland[1] and Kyoto University, Kyoto, Japan[2]. In C4 plants such as maize, CO_2 is initially fixed into a four-carbon compound, oxaloacetate, by the enzyme phosphoenolpyruvate carboxylase (Pepc). This enzyme shows a complex pattern of regulation. At the mRNA level, its expression is limited specifically to mesophyll cells and is stimulated by light. We were interested in analyzing the function of the promoter for the maize C4-type Pepc in stably transformed tobacco in order to determine which aspects of the regulation might be duplicated in a C3, dicot host. Either 1 kb or 4 kb of 5'-flanking sequence was fused to a Gus reporter gene and introduced into tobacco by direct gene transfer. Analysis of Gus expression in the primary regenerants indicates that the reporter gene is expressed only in photosynthetic cells, although the endogenous tobacco enzyme is not expected to play a photosynthetic role. Surprisingly, analysis of Gus expression at the mRNA level revealed that tobacco transcripts were initiated primarily within an AT-rich region of the promoter upstream of the predominant start site used in maize. We are in the process of analyzing effects of promoter mutations both on the magnitude of expression and choice of start site in tobacco.

253 ACETOHYDROXYACID SYNTHASE GENE: DEVELOPMENTAL AND TISSUE EXPRESSION PATTERNS IN TRANSGENIC PLANTS. David Murray, Geraldine Fleming, Susan Armour, Shivendra Sahi and George C. Jen. CIBA-GEIGY Agricultural Biotechnology Research Unit. P.O. Box 12257. Research Triangle Park. NC 27709-2257.

Acetohydroxyacid synthase (AHAS or alternatively known as acetolactate synthase [ALS]) catalyzes the first step in the synthesis of the branched-chain amino acids. AHAS is the target of agronomically important sulfonylurea and imidazolinone herbicides. There have been many studies of the AHAS enzymes and genes from wild-type and herbicide-resistant plants. Little, however, is known of the developmental and tissue expression patterns of AHAS genes in whole plants. This study addresses that lack of understanding. We have fused the *Arabidopsis* AHAS promoter to the β-glucuronidase (GUS) gene and introduced this chimeric gene into tobacco plants using *Agrobacterium*-mediated transformation. Our studies of 4 weeks-old and 10 weeks-old seedlings from several independent transformants found GUS expression solely in leaves and petioles. Interestingly, in all cases the GUS staining in the leaves was variegated in pattern. Thin-section studies of the stained leaves are in progress, as are expression studies of this chimeric gene in older plants. The results of these studies will be presented.

254 ANALYSIS OF GIBBERELLIN-REGULATED α-AMYLASE PROMOTERS IN BARLEY. Michael B. Lanahan, John C. Rogers, and T-H. David Ho, Washington University, St. Louis, MO 63130

Gibberellic acid (GA) transcriptionally activates α-amylase genes in barley aleurone cells. Abscisic acid (ABA) suppresses this expression. We have assayed nuclear extracts from aleurone cells for proteins which bind to sequences in high and low pI α-amylase promoters (Amy32b and Amy6-4). Our results indicate numerous specific protein/DNA interactions. DNase I footprinting analysis has demonstrated that the sequences TAACAGA (element I) and TATCCATGCAGTG (element II) interact with proteins in the extracts. These elements are highly conserved in GA-regulated α-amylase promoters. A GT BOX-like element (AGCGGTATT), as well as other DNA elements, also interact with proteins in the extracts. Various derivatives of the low and high pI α-amylase promoters, including a set of linker-scan deletions which eliminate or disrupt sites of protein/DNA interaction, have been generated and fused to the reporter gene β-glucuronidase. Similarly, oligonucleotides that have been shown to interact with nuclear proteins have been fused to a minimal promoter/β-glucuronidase construct. Constructs were expressed transiently in aleurone cells following particle-bombardment mediated delivery into aleurone cells. Simultaneous introduction of a ubiquitin promoter/luciferase construct allowed normalization of GUS activity from shot to shot. GUS expression from constructs containing as little as 350bp of promoter sequence were appropriately regulated by GA and ABA. A 30bp deletion encompassing element I and II abolished GA-dependent expression. A linker-scan mutation in element II (TCCATCTAGA) retained GA-responsive expression, but at a reduced level (approx. 20%). The roles various other protein/DNA interactions play in the transcriptional regulation of α-amylase promoters will be discussed.

255 *ARABIDOPSIS ADH* MEDIATES TRANSIENT EXPRESSION IN *ARABIDOPSIS* SEEDLINGS. William McKendree Jr. and Robert J. Ferl. University of Florida. Departments of Botany and Vegetable Crops, Gainesville, Florida. 32611. The *Adh* gene of *Arabidopsis* is expressed constitutively in immature seedlings and may be induced in roots of mature plants. The 5' flanking sequences of this gene were examined previously for putative regions of regulatory function. Several regions of protein-DNA interaction were identified, including the G-box element which has been identified in several light regulated (and other) plant genes. The 1.0 kb 5' flanking region of *adh* has been cloned into a GUS expression vector for analysis of transcriptional regulation. Entire seedlings (4 to 5 days old) were used for transformation via the mechanical methods of particle bombardment and silicon carbide fibers ("whiskers") Wild type GUS activity is detectable for the full length promoter and for 100 base deletions to -275. Further deletions result in significant decreases in activity. Additionally, *in-vitro* mutagenesis of the G-box reveals sequences essential for gene activity. Transient and stable transformation of *Arabidopsis* will address questions concerning the induction and developmental regulation of *Arabidopsis adh*.

256 ANALYSIS OF 5' FLANKING REGION OF MAIZE cab GENE SPECIFIC TO MESOPHYLL CELLS. Kailash C. Bansal, Jean Haley, Jean F. Viret and Lawrence Bogorad. Department of Cellular and Developmental Biology, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA.

Differential expression of maize cab genes encoding chlorophyll a/b binding protein of photosystem II in mesophyll and bundle sheath cells is well documented(1). An analysis of the promoter region (1.0 kb) of cab-m1, a gene specific to mesophyll cells, was carried out by making fusions of the promoter region with a GUS reporter gene. GUS expression in different cell types was studied by histochemical localization of GUS activity in tissues bombarded with chimeric gene constructs. The plasmid pBI221 (35S/GUS) served as control. The expression of the GUS gene under the control of cab m1 gene promoter was strong in mesophyll cells and poor in bundle sheath cells, whereas 35S/GUS expressed equally in the two cell types. Studies on the involvement of certain cis-acting regulatory elements responsible for mesophyll cell specific expression of maize cab-m1 gene will be discussed. KCB is supported by the Department of Biotechnology, New Delhi, India.

1) J-Y Sheen and Lawrence Bogorad (1986) Proc. Natl. Acad. Sci. USA 83:7811-7815.

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257 ANALYSIS OF PLANT HORMONE-REGULATED PROMOTER EXPRESSION IN TRANSGENIC TOBACCO. M. Bâga, W.H.R. Langridge & A.A. Szalay. Plant Molecular Genetics, Univ of Alberta, Edmonton, AB. Canada

We have previously shown that the dual *mas* promoters from the T_R region of the *Agrobacterium tumefaciens* Ti-plasmid are differentially regulated throughout development of transgenic tobacco plants and controlled by endogenous auxin/cytokinin levels in the plant tissue. Our results show that *mas* promoter expression is inhibited in the region of the apical meristem and in the immature inflorescence. A detailed functional analysis of the *mas* promoter region has been undertaken by fusing a series of *mas* promoter deletion constructs to the bacterial luciferase reporter fusion gene. Promoter activity was monitored by transient expression in tobacco protoplasts and in transgenic tobacco plants *in vivo*. From these studies we have identified several upstream activating domains for both promoters. Hormone regulation of the 1' promoter is localized to a 65 bp region and a non-overlapping 38 bp DNA sequence is essential for hormone-activation of the 2' promoter. Gel retardation assays using the regulatory DNA sequences as probe have enabled us to identify specific DNA-binding complexes formed *in vitro*. Nuclear extracts prepared from selected tobacco tissues were used to identify tissue-specific trans-acting factors.

258 ANALYSIS OF T-DNA-MEDIATED GENE FUSIONS TO A PROMOTERLESS GUS GENE IN TOBACCO PLANTS. P R Fobert, V.N. Iyer, and B.L. Miki*. Department of Biology, Carleton University, Ottawa, Ontario K1S 5B6 and *Plant Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6.

One thousand transgenic tobacco plants were regenerated from leaf discs infected with pPRF120, a T-DNA vector containing a promoterless GUS gene linked to the right border repeat. Selection for transformants was achieved using a separate kanamycin resistance marker. Leaves from these plants were analyzed for expression of T-DNA-mediated gene fusions, and activity was detected in 5% of the tissues. Histological and quantitative fluorogenic analysis of vegetative and floral organs, as well as developing seeds from 14 of the GUS+ individuals revealed a variety of expression patterns. However, no leaf-specific fusions were identified. Four seed-specific gene fusions were detected by assaying seeds from several hundred plants which did not express GUS in the leaf. GUS expression in one of these plants, T218, was detectable only after 9-17 days post-anthesis. Histological analysis revealed that activity was restricted to the seed coat. Using the T-DNA as a tag, the T218 gene fusion was cloned and reintroduced into SR1 tobacco. Seeds harvested from the resulting transformants all expressed GUS specifically in the seed coat. Southern hybridization of plants transformed with the cloned T218 gene fusion indicated that the T-DNA had integrated at different chromosomal locations, demonstrating that all sequences responsible for the regulated expression of GUS in plant T218 are contained within 2.5 Kb upstream of the GUS starting codon

259 ANALYSIS OF TRANSCRIPTION FACTOR BINDING SITES IN THE PEA LECTIN PROMOTER. B. Sylvia de Pater[1], Khanh Pham[1], Fumiaki Katagiri[2], Ben J.J. Lugtenberg[1], Nam-Hai Chua[2] and Jan W. Kijne[1]. [1] Center for Phytotechnology, Botanical Laboratory, Nonnensteeg 3, 2311VJ Leiden, The Netherlands. [2] Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021, USA.

The pea genome contains one functional lectin gene, which is highly expressed in seed and poorly in leaf, stem and root. The promoter region contains several sequences, which resemble binding sites for transcription factors. Several of these have homology to the ASF-1 binding site. By using Electroforetic Mobility Shift Assays (EMSA) , DNase I footprinting and Methylation Interference we showed that protein encoded by tobacco cDNA clone TGA-1a (which very likely is identical to ASF-1) binds to a region in the lectin promoter (-46/-37), which contains three overlapping motifs (TGAGT, TGACT and TGATG), and very weakly to a region (-301/-295) with two overlapping motifs (TGAGT and TGACT). EMSA with wild-type and mutant binding sites showed that motifs TGACT and TGATG are better binding sites than TGAGT. Different pea organs contain protein, which is immunologically related to ASF-1. Chimeric genes, containing the better ASF-1 binding site (-46/-37) of the lectin promoter, are being tested to determine whether this binding site is responsible for the (low) expression observed in vegetative tissues. Studies have been initiated to find sequences responsible for (high) seed expression.

260 OLIGODEOXYNUCLEOTIDE BINDING TO MAIZE *ADH1* PROMOTER REPRESSES *ADH1*-GUS GENE EXPRESSION. Guihua Lu and Robert J. Ferl. Vegetable Crops Department, University of Florida, Gainesville, Florida 32611

There is a 36 base pair tract of extreme homopurine/homopyrimidine asymmetry in the maize *Adh1* gene promoter (from -44 to -79) that is S1 hypersensitive under supercoil tension (Ferl et al.1987. Plant Mol. Biol. 8:299-307). Oligodeoxynucleotides from the tract were designed to examine the secondary structure and the possible role of the tract in gene regulation. On the basis of oligodeoxynucleotide band-shift and DNase 1 footprinting analyses, it was concluded that the homopyrimidine oligodeoxynucleotide can form a triple helix with the homopurine/homopyrimidine tract. Transient assays in protoplasts, suspension cells, and seedling roots showed that the homopyrimidine oligodeoxynucleotide is capable of repressing *Adh1*-GUS gene expression during co-transformation, presumably by the formation of a triple helix with the duplex homopurine/homopyrimidine tract. The complementary homopurine oligodeoxynucleotide would not form triple helix *in vitro*, nor would it repress *Adh1*-GUS *in vivo*. We propose that the triple helix formation is a potential regulatory phenomenon *in vivo*, and that an intraregion triple helix could occur within the *Adh1* promoter via the formation of H-DNA.

261 REPLICATION OF THE GEMINIVIRUS WHEAT DWARF VIRUS AND ITS RELATION TO *TRANS* ACTIVATION OF THE VIRION SENSE PROMOTER. Julie M.I. Hofer, Helen Reynolds and Philip M. Mullineaux John Innes Institute, Norwich.

We have replaced the coat protein coding sequence (V2) of WDV with the coding sequence of the *cat* reporter gene. This construct (pWDV2CAT) can still replicate as ds viral DNA in protoplasts of *Triticum monococcum* (TM) and CAT activity is a measure of virion sense promoter activity. In TM protoplasts inoculated with pWDV2CAT, maximum CAT activity was concomitant with the appearance of the replicative ds WDV DNA. High levels of CAT activity did not correlate with the presence of input DNA despite its persistence throughout the experiment, often at an apparent higher copy number than the replicating DNA forms. The introduction of a frame shift mutation in the C2 ORF of pWDV2CAT, abolishes replication and dramatically reduces CAT activity. These data indicate a strong linkage between virion sense promoter activity and ds WDV DNA replication However, deletion of the core TATA box retained replication but abolished virion sense promoter activity and a further deletion mutant, interrupting a conserved stem loop structure could not replicate but gave levels of CAT activity approaching that of pWDV2CAT, indicating that the *cis* sequences of the virion sense promoter and the origin of replication do not overlap. Removal of the complementary sense promoter abolishes replication and CAT activity was lower than the levels encountered with the C2 frame shift mutant. Taken together, these data indicate that the product of the C1:C2 fusion independently interacts, directly or indirectly, with the viral origin of replication and *cis* sequences in the virion sense promoter. In this respect, homology between the amino acid sequences of the highly conserved C2 ORF and other NTP-binding proteins is presented.

262 CHARACTERIZATION OF PROTEIN - DNA INTERACTIONS THAT MEDIATE TRANSCRIPTIONAL REGULATION OF A T-DNA PROMOTER. E. Adams and W.B. Gurley. Microbiology & Cell Science, Univ. of Florida, Gainesville, FL 32611. A protein present in nuclear extracts of pea and cauliflower that binds to the activator region (-440 to -229 bp) of the *780* gene promoter has been identified. DNase I footprinting and gel retardation assays using PCR generated 5' and 3' deletion fragments have been used to map in detail the specific sites of interaction. This activator binding protein is being purified through a variety of ion exchange columns and ultimately through affinity chromatography. The molecular weight was determined by Southwestern blotting and UV-crosslinking.

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263 IDENTIFICATION OF PROMOTER ELEMENTS REGULATING TRANSCRIPTION OF THE T-DNA GENE AGROPINE SYNTHASE. D. Baldwin and W.B. Gurley. Microbiology & Cell Science, Univ. of Florida, Gainesville, FL 32611. Site directed mutagenesis was performed to identify promoter sequences responsible for reduced transcription in 5' deletion mutants. A linker scan strategy was used to sequentially alter three base pairs at a time over a 25 bp region which shows homology to a known element in the promoter of nopaline synthase, another T-DNA gene. Relative transcription levels for each mutant were determined by measuring RNA from sunflower tumors. Tumors were incited using *Agrobacterium* to transfer a T-DNA based vector carrying both mutant and wild-type promoter constructs.

264 SITE-DIRECTED MUTAGENESIS OF THE ENHANCER REGION OF THE T-DNA GENE 780 PROMOTER. K. O'Grady and W.B. Gurley. Microbiology & Cell Science, Univ. of Florida, Gainesville FL. 32611. The promoter of the 780 gene of T-nght from *Agrobacterium tumefaciens* Ti-plasmid has been shown to have an upstream activator region with enhancer like properties [Bruce, W., Bandyopadhyay, R.& Gurley, W. B. (1988) Proc. Natl. Acad. Sci. USA 85, 4310-4314]. In order to more clearly delineate which sequences are involved in determining this enhancer functionality, site-directed mutagenesis of the enhancer region from -451 to -240 was performed. A total of 27 mutations were constructed and cloned into a T-DNA based vector. Transcriptional activity was assesed in sunflower tumors by S1 nuclease mapping utilizing a homologous reference gene as an internal standard.

265 PRODUCING FUNCTIONAL T7 BACTERIOPHAGE RNA POLYMERASE IN TRANSGENIC PLANTS. Jeff Velten[1] and Melvin J Oliver[2]. [1]NMSU, Molecular Biology Program, Las Cruces, New Mexico 88003; [2]ARS-Cropping Systems Reserach Laboratory, Lubbock, Texas 79401. The bactenophage T7 encodes its own RNA polymerase (T7 RNA pol) that recognizes a simple and specific promoter sequence to initiate mRNA synthesis. This promoter-polymerase combination has evolved to express genes within a prokaryotic system lacking eukaryotic-specific DNA modifications and DNA associated proteins (histones, etc.). Despite the different state of DNA within eukaryotes, The T7 RNA pol - promoter combination has been shown to function in yeast and animal cells and has been used to examine the mechanisms of DNA transcriptional regulatory sequences in yeast. We have initiated similar experiments in plants, constructing genes designed to express the T7 polymerase gene in transgenic plants. The plant-functional T7 RNA polymerase genes, and a reporter gene (ß-glucuronidase (GUS)) driven by a T7 promoter, have been co-introduced into tobacco plants. The chimeric T7 RNA polymerase gene is expressed (T7 RNA pol mRNA and protein detected) in the transformed plants and *in vivo* function of the T7 RNA polymerase gene has been demonstrated by detection of both GUS mRNA and enzyme activity. Experiments are currently underway to optimize T7 RNA pol expression and to localize the T7 RNA polymerase present within transgenic plant cells.

266 CLONING AND CHARACTERIZATION OF THE LACTATE DEYHDROGENASE GENE FROM MAIZE. A.G. Good and D.H. Paetkau, Dept. of Genetics, University of Alberta In cereal roots, lactate dehydrogenase (LDH) is induced under anaerobic conditions. The increase in LDH activity levels in roots are matched by increases in the amount of LDH protein, and by increases in the amount of LDH mRNA as judged by in-vitro translation and Northern blots. In barley and maize LDH is a tetrameric protein assembled from two different subunits. We have cloned and sequenced both Ldh genes from maize, by screening a maize genomic library with a barley cDNA. The 5' upstream regions of the Ldh1 gene has similarities to the maize alcohol dehydrogenase anaerobic regulatory element (ARE). We are currently determining whether the Ldh promoter will regulate a GUS reporter gene and are beginning to analyze this promoter using both Ldh/GUS fusion constructs and DNA binding studies.

267 NON-DESTRUCTIVE ANALYSIS OF 35S PROMOTER ACTIVITY IN TRANSGENIC ASPEN TREES. Olof Olsson, Ove Nilsson, Torsten Aldén, Folke Sitbon, C.H. Anthony Little, Vladimir Chalupa*, and Goran Sandberg. Department of Forest Genetics and Plant Physiology, The Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden and *Forestry and Game Management Research Institute, Praha 5, Zbraslav-Strnady, Czechoslovakia
Hybrid aspen, *Populus tremula x tremuloides* was transformed with *A. tumefaciens* carrying a binary disarmed vector containing a fused bacterial *luxAB* gene expressed from the 35S cauliflower mosaic virus promoter. Genetic transformation and stable integration of the heterologous DNA was confirmed by Southern hybridization. By enzymatic assay expression of the 35S promoter was anlysed in detail in roots, vascular tissue, bark, and in leaves of different age in several regenerated trees, as well as within leaves of similar age in the same plant. Variation of luciferase activity between plants but also between similar leaves in the same plant was demonstrated, emphasizing the importance of a non-destructive assay when fluctuations of gene expression in time is to be studied. Because the unique decanal substrate in the bacterial luciferase reaction can be applied as a vapor directly to leaves left attached to intact trees, luciferase expression can be monitored directly by image analysis without any further manipulations of the plants prior to assay. Repeated assays can also be performed with certain time intervals on a particular leaf, since leaves were not visibly affected during the 15 minute imaging period. This allows a continous study of gene expression in the same leaf during e.g. changing physiological conditions.

268 H1 HISTONE GENES OF *Arabidopsis thaliana*. Steve Gantt, Todd Lenvik and Sui-Wei Hsu. University of Minnesota, Department of Plant Biology, St. Paul, Minnesota 55108

Two full length cDNA clones, corresponding to two different H1 histones, H1-1 and H1-2, have been isolated from an *Arabidopsis thaliana* cDNA library. The H1-1 and H1-2 proteins are 274 and 273 amino acids in length, respectively. Unlike animal H1 histones, the two *A. thaliana* H1 proteins share little sequence similarity outside the protein's central globular domain. A pentapeptide that is extremely well conserved in animal H1 histones is not found in either of the plant proteins. Southern blot analysis suggests that *A. thaliana* has only two or three H1 histone genes. Genomic clones containing the entire H1-1 gene and the 3' portion of the H1-2 gene were isolated. The H1-1 protein is encoded by two exons that are separated by a 104 base pair intron. The site of transcriptional initiation of the H1-1 gene was mapped by primer extension analysis. An octamer motif, found in the 5'-flanking region of all plant histones that have been characterized to date, is located 101 nucleotides upstream of the initiation site. However, we have been unable to demonstrate any protein binding to this region. Both of the H1 genes are flanked at their 3' ends by a highly conserved, convergently transcribed, gene. The identity of these flanking genes is not known.

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269 CHARACTERIZATION OF AT-RICH SEQUENCE ELEMENTS UPSTREAM OF THE PEA *FED-1* GENE. Thomas J. Pedersen, Gerald Hall Jr., George C. Allen, Shannon Frances, Steven Spiker, and William F. Thompson. Departments of Botany and Genetics, North Carolina State University, Raleigh, NC 27695. USA.

AT-rich sequences in the 5' flanking regions of several plant genes have been shown to bind nuclear proteins, but the nature of these proteins has remained largely unknown. We have found that both plant high mobility group (HMG) chromosomal proteins can interact specifically with sequences contained within an AT-rich region located upstream of *Fed-1*. Preliminary experiments have also shown that, under certain conditions, the same region will also bind nuclear scaffold preparations. The HMG-DNA complexes were similar to one of the two types of *Fed-1* complexes we have observed with pea nuclear extracts. Binding experiments with a synthetic oligo(dA)•oligo(dT) probe and competition experiments with synthetic DNA polymers suggest that HMG binding may depend upon structural features of AT-rich DNA rather than being sequence specific. Previous investigations in both plant and animal systems have linked HMGs with transcriptionally competent chromatin. These experiments suggest a link between HMG binding, scaffold attachment, and chromatin structures establishing transcriptional competence of *Fed-1* and other genes. We are currently assessing the effect of this AT-rich region on the activity of chimeric genes introduced into transgenic tobacco

270 NUCLEAR SCAFFOLDS AND SCAFFOLD ATTACHMENT REGIONS (SARS) IN HIGHER PLANTS. Gerald Hall Jr., George C. Allen, Deborah S. Loer, William F. Thompson, Steven Spiker. Departments of Genetics and Botany, North Carolina State University, Raleigh, NC 27695 USA

DNA in the nuclei of eukaryotic organisms undergoes a hierarchy of folding in order to be packaged into interphase nuclei and metaphase chromosomes. This higher order packaging consists of loops of the 30-nm chromatin fibers attached to a proteinaceous nuclear scaffold by an interaction between the scaffold and specific DNA sequences called "scaffold attachment regions" or "SARs". Higher order chromatin folding is important not only structurally, but also functionally. It has long been realized that regions of DNase I sensitive chromatin, which contain transcriptionally poised genes, are not confined to the genes themselves, but rather extend over much larger areas. These DNase I sensitive domains have been shown to correlate with loop domains bounded by SARs. We have isolated scaffolds from tobacco nuclei using buffers containing lithium diiodosalicylate to remove histones followed by restriction enzymes to remove DNA not closely associated with the nuclear scaffold. The tobacco nuclear scaffold is visually (by fluorescent microscopy with DAPI), structurally (by SDS-PAGE of scaffold proteins), and functionally (binding of a well characterized yeast SAR) similar to scaffolds isolated from other sources. We have used the tobacco scaffold to identify endogenous tobacco SARs flanking three root-specific genes. SARs associated with two of these genes have been mapped to the 3' end of these genes. We are now searching for 5' SARs flanking these genes in order to study the chromatin domain defined by these SARs and to use them in transformation experiments to study their effects on gene expression *in vivo*

271 USING TRANSGENES IN FUNCTIONAL ANALYSIS OF PLANT NUCLEAR SCAFFOLD ATTACHMENT REGIONS. Lisa C. Childs, George C. Allen, Gerald Hall, Jr., Steven Spiker, and William F. Thompson. Departments of Genetics and Botany, North Carolina State University, Raleigh NC, 27695 USA

Variations in the level of gene expression that are independent of gene copy number are often ascribed to 'position effects'. Although not yet carefully studied in plants, these effects are believed to be caused by integration of the transgene into different chromatin domains which are correspondingly different in their availability for transcription. These domains are believed to be defined by scaffold attachment regions (SARs) flanking the gene of interest. Recent data in animal systems indicate that when transgenes which include flanking SARs are integrated, position effects are greatly reduced and the resulting levels of gene expression are dependent only on gene copy number. We are testing this hypothesis in several ways. First we are examining endogenous SARs. *In vitro* binding experiments have shown that the pea ferredoxin (*Fed-1*) promoter contains a potential SAR (see poster by Pedersen et al). Constructs containing the *Fed-1* promoter fused to the reporter gene *uidA* (GUS) have been transferred into tobacco suspension cells. Several lines vary in their GUS expression levels. We have measured the transgene copy number in these lines and isolated nuclear scaffold to determine whether the exogenously identified SARs correspond to endogenous SARs. To determine the size of the domain, we plan to map SARs downstream of the the reporter gene. A second approach is to make constructs with SARs in positions that create a putative loop domain when integrated into a chromosome. By including reporter genes in the domain we should be able to assess the effect of SARs on the variation of transgene expression levels.

272 CHARACTERIZATION OF ALDOLASE AND SUCROSE SYNTHASE GENE PROMOTERS FROM *ARABIDOPSIS*. Surinder Chopra, Jocelyne Dewulf, Rudy Dolferus and Michel Jacobs. Plantengenetica, University of Brussels, B-1640 Sint-Genesius-Rode.

We are interested in the regulation of gene expression under anaerobic stress conditions in *Arabidopsis thaliana*. To understand the molecular mechanisms underlying such anaerobic regulation of gene expression, we have studied the function and organisation of the alcohol dehydrogenase (Adh) gene promoter. To extend the study of anaerobically responsive promoter elements we have now characterized promoters of two more genes of *Arabidopsis* : fructose 1,6 biphosphate aldolase and sucrose synthase which have been shown to be anaerobically induced in maize. As a first approach to characterize the cis-acting promoter elements of both genes,we identified putative anaerobic response elements based on sequence similarity to the maize Adh1 and other anaerobically induced plant genes. Secondly a study of the aldolase promoter by 5' deletion mapping was initiated. Chimeric constructs containing deleted aldolase promoter fused to the Gus reporter gene, were assayed in transient expression experiments using protoplasts anaerobically induced or not. Efforts are also done to identify promoter regions which specifically interact with transcription factors by using nuclear extracts of various *Arabidopsis* tissues.

273 A MYB-RELATED POTATO CDNA CLONE ENCODING A PROTEIN THAT BINDS TO THE CAMV 35S PROMOTER. N.Baranowskii, I.Hemmerich, L.Willmitzer and S.Prat. Institut fuer Genbiologische Forschung, Ihnestr.63, 1000 Berlin 33, Germany

The CaMV 35S promoter is one of the best characterized and studied promoters expressed in plants. Within this enhancer two domains have been defined, domain A (-90 to +2) and B (-343 to -90), that direct expression primarily in root and leaf tissues, respectively. Potato clones encoding regulatory factors binding to domain A were isolated by South-Western screening of a cDNA expression library, using the multimerized domain A fragment. Sequence comparison from one of the clones (d13) shows partial homology to the highly conserved DNA binding domain of the c-myb protooncogene. Myb-related proteins, involved in cell growth and differentiation, have been previously identified in a wide variety of organisms, including plants. In animals the DNA binding region common to the Myb proteins comprises in general of three repeats of 51-52 amino acids each. In most of the plant myb homologues there are two of such repeats. Interestingly clone d13, although full length, codes for only one of these repeats. In Northern analysis high levels of d13 transcript was detected in roots. Structural and functional analysis of the regulatory role of this factor will be presented.

274 THE POTATO TGA1a-HOMOLOGUE d11 FUNCTIONS AS A TRANSCRIPTIONAL ACTIVATOR IN PLANT CELLS. S. Prat, N. Baranowskij, I. Hemmerich, J. Sánchez-Serrano and L. Willmitzer. Inst. für Genbiol. Forschung, Ihnest. 63, 1000 Berlin 33, Germany.

A 91 bp fragment deletion derivative (from -90 to +2 relative to the transcription start site) of the CaMV 35S promoter directs high levels of expression in the root tip. Nuclear factors, present in tobacco and potato extracts, bind to a TGACG-repeat in the -83 to -63 region of this truncated promoter and, apparently, are responsible for the tissue-specific expression associated to this regulatory element. A potato cDNA clone (d11) encoding a factor that specifically binds to this tandem motif was isolated by screening an expression library with the multimerized promoter fragment. Protein d11 includes a potential leucine zipper motif preceded by a small basic amino acid region homologous to the DNA-binding domains of the bZIP family of transcription factors such as Jun and GCN_4. Comparison of the amino acid sequence of this protein with other DNA-binding proteins from plants revealed a very high homology to the recently described TGA1a factor from tobacco. In transient expression experiments, where both a d11 expressing construct and a TGACG binding site-containing chimeric promoter were co-delivered to leaf-derived protoplasts or to entire leaf cells, the protein encoded by d11 was able to activate these chimeric constructs and thus, to function as a transcriptional activator in plant cells. Evidence for the involvement of this regulatory factor on the root-specific expression conferred by the CaMV minimal promoter, derived from the analysis of transgenic plants exhibiting either high levels of expression of the factor in leaves or antisense inhibition in roots will be presented.

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275 DIFFERENTIAL EXPRESSION OF ACTIN IN *ARABIDOPSIS THALIANA*. Shurong Huang and Richard B. Meagher, University of Georgia, Department of Genetics, Athens, Georgia, 30602.

Higher plants contain a highly divergent actin gene family composed of several ancient classes of genes. We have proposed that the ancient and divergent classes of plant actin genes have been preserved because they have unique patterns of gene regulation and/or encode actin proteins with unique functions. In order to elucidate the differennal expression of specific gene family members we are using polymerase chain reaction (PCR) to directly isolate plant actin cDNAs from tissues and cell types in *Arabidopsis thaliana*. The sequences of these amplified cDNAs are being determined by a PCR-mediated sequencing technique. Comparison of the cDNA sequences to available gene sequences unambiguously defines the mRNA phenotypes in the organs, tissues and cell types under study. At present degenerate actin-specific oligo nucleotides can be used to sequence actin RNA's from a few hundred cells from different organs. Ultimately we hope to use gene-specific oligos and determine mRNA phenotypes on a few cells from a single cell type or tissue. The relative expression of these actin genes in these tissue and cell types will also be quantified with internal PCR controls.

276 *MYB*-LIKE TRANSCRIPTION FACTORS FROM *ANTIRRHINUM MAJUS*. Francisco Culiañez-Macia, David Jackson, Steve Mackay, Andy Prescott and Cathie Martin. John Innes Institute, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

We have isolated six *myb*-like genes from the flowers of *Antirrhinum majus* (Snapdragon) containing sequences that encode the DNA-binding domain of the *Myb* protooncogenes. The proteins encoded by these genes have other features of transcriptional activators, including regions of amphipathic alpha-helix associated with transactivation domains (1). Analysis of the expression patterns of the *myb* genes with respect to organ-specificty, floral differentation and in response to light suggests that these genes are not involved in controlling anthocyanin biosynthesis, unlike the *myb*-related genes *C1* and *Pl* in maize (2, 3). Analysis of the activity of two of the *myb*-gene promoters by fusion to the Gus-reporter gene confirms this specific expression and gives some clues to the biological roles of these transcriptional regulators. To expand our understanding of *myb*-gene function we have transformed tobacco with gene constructs giving ectopic expression of each *myb*-gene, and ectopic expression of their antisense. The resulting phenotypes give further evidence of the function of these genes. It would appear that *myb*-homologous proteins represent a large class of transcriptional activators in plants controlling a range of metabolic processes.

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277 WHEAT DWARF VIRUS (WDV) ENCODES A TRANSCRIPTION ACTIVATOR. M. Kammann[1], S. Schaefer[1], V. Matzeit[1], B. Schmidt[1] and B. Gronenborn[1,2]

[1]Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, Germany. [2]Institut des Sciences Végetales, C.N.R.S., 91198 Gif sur Yvette, France.

The genome of wheat dwarf virus (WDV), a geminivirus of monocotyledonous plants, is transcribed bidirectionally from two promoters located in its large intergenic region. Using transient expression in cells of *Triticum monococcum* of a recombinant WDV genome carrying the neomycin phosphotransferase (NPT II) gene at the position of the viral capsid gene, we have shown an enhancement of NPT II expression by the ORF III/ORF IV protein(s) that are encoded on the complementary strand of WDV. In RNAse protection assays, the stimulation of the rightward (plus-sense) transcription was measured to be more than 50 fold. A less pronounced (about 15 fold) stimulation was also found when the 35S promoter of cauliflower mosaic virus (CaMV) was similarly assayed. Whether this transactivation is mediated by the 30kD protein of WDV ORF III alone or the 41 kD ORF III/IV fusion protein is currently being determined.

278 REGULATION OF mRNA LEVEL AND ACTIVITY FOR NITRATE REDUCTASE IN BEAN BY SUBSTRATE AND LIGHT.

Tine Hoff, Poul E. Jensen, Bjarne M. Stummann, Michael Kristensen and Knud W. Henningsen. Department of Molecular Biology and Ecology, Section of Genetics, The Royal Vet. & Agricult. Univ. DK-1870 Frederiksberg C, Denmark.

The regulation of mRNA expression and enzyme activity of a root specific and nitrate inducible nitrate reductase gene from bean (Phaseolus vulgaris) [Hoff et al, Physiol Plant 1991, in press] is studied. Northern blots show that plants grown without nitrate have no detectable NR mRNA. One hour after nitrate induction the NR mRNA reaches a maximum where it stays for 16 to 20 hours. Then the mRNA decreases to a low level. The gene can be induced in darkness as well as in light but the amount of induced mRNA in light is about two times as high as in darkness. No diurnal rythm has been detected. A low level of NR activity has been detected in non induced roots and leaves. 24 hours after induction the NR activity has increased 40-50 times. The induced NR is NADH-specific and has pH optimum 7.5.

279 FUNCTIONAL ANALYSIS OF THE PROMOTER FOR A ROOT SPECIFIC NITRATE REDUCTASE GENE FROM BEAN IN TRANSGENIC TOBACCO.

Poul E. Jensen, Tine Hoff, Bjarne M. Stummann, Michael Kristensen and Knud W. Henningsen, Department of Molecular Biology and Ecology, Section of Genetics, Royal Vet. & Agricult. Univ., DK-1870 Frederiksberg C, Denmark.

We have constructed a set of nested promoter fragments of lengths from 250 to 3500 bp. They all start immediately upstream of the translation start site of a previously characterized root specific and nitrate inducible nitrate reductase gene from bean (*Phaseolus vulgaris*) [Hoff et al, Physiol Plant 1991, in press]. These promoter fragments were cloned in front of the GUS gene in the binary transformation vector pBI101. These constructs were transferred to tobacco plants using *Agrobacterium*. Preliminary results have shown that the root specific expression and nitrate inducibility found in bean plants is qualitatively conserved in the tobacco plants transformed with a GUS gene preceded by a 250 bp promoter fragment. Further studies will be concerned with quantitative aspects of the regulation.

280 FUNCTIONAL ANALYSIS OF THE -300 REGION OF MAIZE ZEIN GENES. Tom Quayle* and Günter Feix. Biologie III, Universitat Freiburg, Freiburg, FRG. *Present address: Biological Sciences, Univ. of Wisconsin-Parkside, PO Box 2000, Kenosha, WI, 53141.

A 43 bp fragment containing the -300 region upstream control element common to the endosperm expressed zein genes of *Zea mays* has been analyzed by *in vivo* and *in vitro* techniques. Transient transformation studies with protoplasts from a maize endosperm culture indicate that the element positively affects a CaMV 35S promoter driven gene expression, and that this effect is both orientation and position dependent. Band shift experiments demonstrate that the element is specifically bound by different sets of DNA binding proteins from seedling and endosperm nuclei. A two base pair substitution within the most conserved region of the element both reduces the stimulative effect seen in transient transformation and alters the binding of nuclear proteins from both tissues. Southwestern experiments suggest a size for at least two DNA binding proteins. Work is currently in progress to clone the genes encoding these putative regulatory proteins.

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281 IDENTIFICATION OF AN ETHYLENE-RESPONSIVE ELEMENT IN THE CHITINASE 5B GENE. Karen Broglie, Dominique Roby[2] and Richard Broglie E. I. Du Pont de Nemours & Co., Agricultural Products Department, Wilmington, DE 19880 and [2] Laboratoire de Biologie Moleculaire, CNRS/INRA, Castanet Tolosan, France

An important method by which plants protect themselves against potentially pathogenic organisms is the production of lytic enzymes that are capable of cell wall hydrolysis. Among these is the enzyme chitinase which catalyzes the hydrolysis of chitin, a β-1,4-linked homopolymer of N-acetyl-D-glucosamine and a ubiquitous component of many phytopathogenic fungi. In healthy, uninfected bean plants, chitinase levels are low or undetectable. However, treatment with ethylene, oligosaccharide elicitors, or infection with fungal pathogens results in an increase in chitinase mRNA levels and an increase in enzyme activity. The expression of two genes, designated CH5A and CH5B, in transgenic tobacco plants was found to be dependent upon the application of exogenous ethylene (Broglie et al.(1989) Plant Cell 1: 599-607) or oligosaccharide elicitors. DNA sequences that are responsible for mediating this response have been shown to be situated upstream of the transcripuon initiauon site in the CH5B gene. Analysis of CH5B promoter deletions suggests that a 43 bp sequence that is precisely conserved between the CH5A and CH5B genes may play a role in transcriptional regulation of chitinase gene expression by the phytohormone, ethylene (Broglie et al. 1989). Consistent with this finding, we have identified a nuclear protein factor by gel mobility shift and DNAse I protection assays that binds to this DNA sequence element. Fusion of a 50 bp oligonucleotide containing this sequence to a truncated CaMV 35S promoter results in ethylene-dependent expression of this chimeric promoter.

282 A TRANSCRIPTIONAL REGULATOR ENCODED UPSTREAM OF RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE OPERON IN THE PHOTOSYNTHETIC BACTERIUM *CHROMATIUM VINOSUM*. Alejandro M. Viale[1], Steven Henikoff[2], and Hirokazu Kobayashi[3]. [1]Facultad de Ciencias Bioquimicas y Farmaceuticas, Universidad Nacional de Rosario, 2000 Rosario, Argentina; [2]Fred Hutchinson Cancer Research Center, Seattle, WA 98104, U.S.A.; and [3]School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka 422, Japan.

An open reading frame (ORF) encoding a polypeptide composed of 302 amino acid residues was identified 226 base pair (bp) upstream of *rbcA-rbcB*, the set of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) genes expressed in the photosynthetic purple sulfur bacterium *Chromatium vinosum* (A. M. Viale *et al., J. Bacteriol.*, 171, 2391-2400, 1989), which are distinct from cryptic RuBisCO genes (*rbcL-rbcS*) in the same bacterium (H. Kobayashi *et al., Gene*, 97, 55-62, 1991). Sequence comparisons and other features (size, divergent orientation with respect to *rbcA-rbcB*, and a predicted helix-turn-helix DNA-binding motif) reveal that this ORF encodes a member of the LysR family of transcriptional regulators. We also present evidence that an homologous protein is similarly encoded upstream of the RuBisCO operon in the chemoautotrophic bacterium *Xanthobacter flavus*. In both ORFs, as well as in most members of the LysR family, an anomalous lysine-to-arginine ratio was found. Expression in *Escherichia coli* of the *C. vinosum* ORF fused to the N-terminal region of β-galactosidase gene led to the reduced expression of *rbcA-rbcB* genes present in a compatible plasmid. Therefore, this newly described member of the LysR family in *C. vinosum*, designated *rbcR*, is likely to encode a *trans*-acting transcriptional regulator of *rbcA-rbcB*.

283 PROTEINS INTERACT WITH MAIZE *ADH2* PROMOTER SEQUENCES BOTH *IN VIVO* AND *IN VITRO* Anna-Lisa Paul and Robert J Ferl University of Florida, Department of Vegetable Crops, Gainesville Florida 32611

The *Adh2 (alcohol dehydrogenase-2)* gene of maize (*Zea mays*) is one of a suite of maize genes induced by hypoxic stress Several sequences within the 5' flanking region of *Adh2* interact with DNA-binding factors *in vivo* (Paul and Ferl, Plant Cell 3 159). In this report, the interactions of maize *Adh2* promoter sequences with cell extracts from maize suspension cultures were characterized by *in vitro* DMS (dimethyl sulfate) footprinting and gel retardation assays DMS footprinting was used to target *in vitro* protein binding sites in supercoiled and linear plasmids containing the *Adh2* 5' flanking region The DMS footprints generated *in vitro* with cell extracts were identical to the *in vivo* DMS footprints over the same sequences Gel retardation assays with oligonucleotides for the footprinted sequences indicate that the factors (partially purified over heparin agarose and FPLC HR 5/5 Mono Q columns) which preferentially bind to the footprinted *Adh2* promoter sequences centered around -160 and -212 are distinct from the factor complex which preferentially binds to the *Adh1* anaerobic response element (ARF-B$_2$) around -130

284 EXPRESSION OF ISOCITRATE LYASE GENES IN BRASSICA NAPUS James Z. Zhang, Charles G. Gasser and John J. Harada, Botany Dept., UC Davis, CA 95616

We are studying the regulation of genes encoding one of the glyoxylate cycle enzymes isocitrate lyase (IL) in order to understand the processes involved in glyoxysome biogenesis in seedlings of Brassica napus. Isocitrate lyase mRNA is present at low levels during late embryogeny, increases rapidly after imbibition, reaches maximum level at 4 DAI (days after imbibition) and is not detected in leaves of mature plants. There are approximately six isocitrate lyase genes in the B. napus genome, four of the them have been cloned, and they are divided into two subfamilies based upon the nucleotide sequence divergence in the 5' and 3' untranslated regions. We showed that both subfamilies are expressed in late stage embryos and germinating seedlings. The sequencing of one of the expressed isocitrate lyase genes and its flanking regions showed that there are four introns in the gene. Stable plant transformations and transient assays using a particle gun have been used in order to localize the important cis-acting sequences that are responsible for the expression of isocitrate lyase genes. A fragment of ca.250 bp in the 5' flanking region of the gene is shown to be necessary and sufficient to regulate isocitrate lyase gene expression in seedlings.

285 EXPRESSION OF THE DNA-BINDING DOMAIN OF GAL4 IN PLANTS. Sabine Rosahl, Dorothee Feltkamp and Jeff Schell. Max-Planck-Institut für Züchtungsforschung, Carl v. Linné-Weg 10, D-5000 Köln 30, Germany. We are interested in repression of gene expression in plants and want to analyze whether the DNA-binding domain of the transcriptional activator GAL4 from *Saccharomyces cerevisiae* can act as a repressor of transcription in plants. The N-terminal 147 amino acids of GAL4 which comprise the DNA-binding domain and the nuclear localization signal were cloned behind the 35S promotor. We used GUS, NPTII or DHFR as reporter genes and fused them to modified nos promotors which contained the recognition sequence of GAL4. Transgenic tobacco plants were obtained by *Agrobacterium tumefaciens*-mediated transformation and analyzed for expression of the marker genes in the presence or absence of the GAL4 gene. Our results so far show that there is no significant repression if the binding site of GAL4 is located 64 or 150 bp upstream of the transcription start point. Additionally, we have replaced sequences around the TATA box by the GAL4 binding site and are now attempting to increase the amount of GAL4 protein synthesized in the plant by creating fusions to known stable proteins.

286 EXPRESSION OF HOMEOLOGOUS AND GENOME-SPECIFIC GENES IN NATURAL AND SYNTHETIC AMPHIDIPLOIDS OF *BRASSICA*. Keming Song and Thomas C. Osborn, University of Wisconsin, Department of Agronomy, Madison, Wisconsin 53706. Previous studies indicated that the genome compositions of the three *Brassica* amphidiploid species *B napus*, *B. juncea* and *B. carinata* have changed compared to those of their parental diploid species, *B rapa*, *B. oleracea* and *B nigra*. In order to understand how these genome changes occurred, a series of synthetic amphidiploids have been developed These synthetic amphidiploids contained genome combinations analogous to those of natural amphidiploids and their reciprocal forms. Random genomic DNA and cDNA libraries for all of the the three parental diploid species used in synthesis of the amphidiploids have been constructed. The cDNA libraries are being screened for genes with either homeology or genome-specificity among the parental diploid species These genes will be used to hybridize with RNAs isolated from the diploid parents, synthetic amphidiploids and natural amphidiploids. The main goals of this study are to determine 1) if silencing of some homeologous genes from the diploid parents has occurred in natural amphidiploids and in synthetic amphidiploids during stabilization of the interspecific hybrids; 2) if the expression of genome-specific genes is different in amphidiploid species compared to their expression in diploid species, and 3) if the cytoplasmic genomes have any effects on expression of either homeologous or genome-specific genes. The results from this study will provide new information on molecular evolution of allopolyploid species, and information about the effects of genome interaction on gene expression in interspecific hybrids

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287 EXPRESSION OF A MAIZE UBIQUITIN PROMOTER-CHLORAMPHENICOL ACETYL TRANSFERASE CHIMERIC GENE IN ELECTROPORATED PLANT PROTOPLASTS. Alan H. Christensen and Donna M. Fox. George Mason University, Department of Biology, Fairfax, VA 22030.

A transcriptional fusion between the chloramphenicol acetyl transferase coding region and 0.9kbp of the 5' flanking sequence and the entire 5' untranslated region of the maize ubiquitin gene, Ubi-1, has been constructed. We have examined the expression of this construct and derivatives of it in electroporated protoplasts. Tobacco protoplasts electroporated with the Ubi-CAT chimeric gene contain only about one tenth of the CAT enzyme activity as similar protoplasts electroporated with a cauliflower mosaic virus 35S promoter-CAT chimeric gene. In contrast, introduction of the Ubi-CAT construct into maize protoplasts results in about ten-fold higher CAT activity compared to maize protoplasts electroporated with the 35S-CAT construct. Sequences required for this high level of expression are being identified. A set of deletions beginning at the 5' end of the ubiquitin promoter sequence and spanning the entire 0.9kbp promoter region have been constructed. Small internal and 3' deletions have also been constructed to further delineate sequence segments required for expression of genes fused downstream of this ubiquitin promoter.

288 REGULATION OF THE β-1,3-GLUCANASE B PROMOTER IN TRANSGENIC TOBACCO. Regina Vögeli-Lange, Craig Hart, Ferenc Nagy and Fred Meins. Friedrich Miescher Institute, Basel, Switzerland.

The basic, vacuolar β-1,3-glucanases are hormonally and developmentally regulated in tobacco. The enzymes are localized in the lower leaves and roots of the plant and are induced in leaves by treatment with the stress hormone ethylene or pathogens such as TMV. Although neither auxin nor cytokinin alone are sufficient, combinations of the two hormones block the ethylene-mediated induction in cultured cells. These β-1,3-glucanases are encoded by a small gene family with ≈ 4 members. We transformed tobacco cells with an expression vector containing 1.6 Kb of 5' flanking sequence of one member of this family, the β-1,3-glucanase B gene, transcriptionally fused to the coding sequence of E. coli β-glucuronidase (GUS). The GUS activity varied markedly in comparable leaves of plants regenerated from independent transformants (range 2.2-238 pkat/g FW, 21 transformants). Nevertheless, GUS activity and β-1,3-glucanase contents exhibited similar patterns of regulation. Therefore the 1.6 Kb promoter region must contain the elements necessary for correct developmental, hormonal and pathogen regulation.

289 ISOLATION OF THE ADENINE PHOSPHORIBOSYL TRANSFERASE GENE FROM *ARABIDOPSIS THALIANA*. E. McWhinnie and B. A. Moffatt. University of Waterloo, Waterloo, Ontario N2L 3G1.

Adenine phosphoribosyltransferase is an enzyme of the purine salvage pathway which converts adenine and PRPP to AMP. Due to its central role in nucleotide metabolism, the gene for APRT, *apt*, is expressed in all tissues. Although *apt* is a well characterized housekeeping gene in animals, very few constitutively-expressed genes have been isolated from plants. Moreover, the promoters of such genes have not been well defined. As part of our study of the role of APRT in plant development, we are investigating the regulation and expression of *apt*. Here, we report the isolation and preliminary characterization of the first gene for APRT from a plant, *Arabidopsis thaliana*. Initially, a partial *apt* cDNA from *Brassica juncea* was isolated using oligonucleotides based on the amino acid sequence of the purified protein. The *B. juncea* cDNA was used to isolate the *apt* cDNA from *A. thaliana*. Subsequently, the gene for APRT was identified by screening an *A. thaliana* genomic library. Using various fragments of this cDNA as probes, a physical map of the *apt* gene was constructed. Sequencing analysis was undertaken to characterize promoter elements associated with this gene.

290 REGULATION OF THE EARLY AUXIN INDUCIBLE GENE PSIAA4/5: ANALYSIS OF THE CIS-ACTING ELEMENTS IN PEA PROTOPLASTS (1) Nurit Ballas, Lu Min Wong and Athanasios Theologis. Plant Gene Expression Center, USDA-ARS, Albany, CA 94710, and Department of Plant Biology, University of California, Berkeley, CA 94720.

Two auxin responsive genes (PSIAA4/5 and PSIAA6) from pea have been cloned and characterized. Induction of these genes in elongating tissue of etiolated pea seedlings (3rd internode) occurs within 5-10 minutes after auxin application and does not require protein synthesis. Furthermore, protein synthesis inhibitors, *e.g.* cyclohexamide, can induce the PSIAA4/5 and PSIAA6 genes in the absence of auxin, suggesting that a labile repressor protein might be involved in the regulation of auxin responsive gene expression. To define the cis-acting elements that are responsible for the induction of the PSIAA4/5 gene, we introduced a 540 bp fragment of PSIAA4/5 promoter fused to the reporter CAT gene into protoplasts isolated from etiolated pea seedlings. Expression of this chimeric gene is highly dependent on auxin and it occurs specifically in protoplasts isolated from elongating pea epicotyl. Deletion analysis of the 540 PSIAA4/5 promoter region demonstrated that a 164 bp fragment, fused to the heterologous 35S CaMV core promoter, is sufficient to confer auxin inducibility to the CAT gene. The identification of trans-acting factors that interact with this region of the promoter is currently underway. (1) This work was supported by grants to A.T. from the NIH (GM-35447) and the NSF (DCB-8645952, -8819129, -8916286).

291 TRANSFER RNA GENE TRANSCRIPTION IN PLANTS. Michael G. Zwick, Scott E. Franklin, Julie M. Palmer and William R. Folk, Department of Biochemistry, 117 Schweitzer Hall, University of Missouri, Columbia, MO 65211

We are attempting to dissect transcription of plant tRNA genes by RNA polymerase III. A gel retardation assay has been used to detect a carrot nuclear protein which binds stably to a B box consensus sequence (a component of the internal control region of tRNA genes to which animal cell and yeast transcription factor IIIC binds). Two forms of this factor are seen in gel retardation assays and these can be partially separated by FPLC Mono Q chromatography. Protein blots of extracts containing TFIIIC activity were probed with B box multimers and we identified a DNA binding subunit of this protein with a M_r of 90 kD. The gel retarded complex has been footprinted by DNase I and appears to bind similar to the corresponding proteins from human and amoeba nuclear extracts. Currently, we are attempting to clone the various subunits of this protein to determine their function and interactions with each other and with the other RNA polymerase III transcription components. The eventual goal is to develop a well-defined *in vitro* transcription assay from plant nuclear extracts. Additionally, two $tRNA_i^{Met}$ genes, one from soybean and another from *Arabidopsis*, have been examined for their transcription activity in human 293 cell extracts. These genes are identical in their coding regions but have no homology in their flanking regions, yet the transcription activity of the *Arabidopsis* gene is much greater than that of the soybean. A tRNA processing activity from carrot cell extracts is able to process the *Arabidopsis* $tRNA_i^{Met}$ transcript, but not the soybean. Increasing the spacing between the A-B box promoter region still allows transcription of both these genes and we are transforming plants with these constructs to determine if these are also expressed *in vivo*. We believe this will be a means to define *cis* acting regulatory elements affecting tRNA expression.

292 CHARACTERIZATON OF *MYB* TRANSCRIPTION FACTOR HOMOLOGS FROM *A. thaliana* Peter W. Doerner, Yoshihiro Ozeki, Diane M. Rueznsky, Susan A. Hedrick and Christopher J. Lamb. Plant Biology Lab, The Salk Institute, 10010 North Torrey Pines, San Diego CA 92037

Homologs of the *myb* proto-oncogene have previously been identified in many species to be involved in the regulation of cellular differentiation, development and possibly in housekeeping functions. The gene products have been shown to bind DNA with a highly conserved DNA binding domain. The *Z mays* C1 gene is a *myb* homolog involved in the regulation of anthocyanin accumulation. The Arabidopsis *gl1* gene is a *myb* homolog regulating trichome development. Additional homologs have been isolated from maize, barley, antirhinum, petunia and Arabidopsis. Using the DNA binding domain of the *Z mays* C1 cDNA as a probe, we isolated 2 *myb* homologs from an Arabidopsis genomic library. Five additional homologs were isolated from this library using PCR generated probes. The deduced amino acid sequence of the DNA binding domain of these genes shares between 51-64% homology to the corresponding domain of the maize C1 gene. All *myb* homologs are single copy genes, however they are part of a larger gene family in the Arabidopsis genome. The genetic loci of two of these genes have been mapped by RFLP based methods. None of these map positions coincides with loci previously identified genetically. RNA slot blot analysis shows that two genes are highly expressed in floral tissues, one gene is induced by high intensity light, whereas all others are expressed at levels too low to detect. Results from analysis of expression at higher resolution using promoter GUS fusions will be shown. We are currently addressing the question of the function of these genes by introducing into Arabidopsis a series of constructs that are predicted to generate phenocopies of mutants. Data on these transgenic plants will be presented.

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293 PROMOTER CLONING AFTER RANDOM ß-GLUCURONIDASE GENE FUSIONS IN *ARABIDOPSIS THALIANA*. Sunee Kertbundit, Henri De Greve, Rosario Linacero, Marc Van Montagu and Jean-Pierre Hernalsteens. Vrije Universiteit Brussel, Laboratorium voor Genetische Virologie, B-1640 Sint-Genesius-Rode, Belgium

Vectors derived from the Ti-plasmid of *Agrobacterium tumefaciens* were applied for the isolation, *in vivo*, of random transcriptional and translational fusions of plant promoters with a promoter-less ß-glucuronidase (*gus*) sequence (Kertbundit *et al*., Proc. Natl. Acad. Sci. USA 1991, 88, in press). Different patterns of GUS expression were detected in the transgenic plants, due to the fusion of *gus* to the 5'-controlling elements of differentially expressed plant genes. The cloning of the promoter sequences mediating regulated *gus* expression is facilitated by the presence of a single full-length T-DNA copy in about 70% of the lines expressing GUS and by the favourable properties of the *Arabidopsis* genome.

The *Arabidopsis* line At3001 harbours a transcriptional *gus* fusion expressed in the phloem of all organs. This gene fusion was cloned in the bacteriophage lambda vector EMBL4, using the *supF* gene present in the T-DNA as a marker. The corresponding wild type sequence was isolated from a genomic library by conventional molecular cloning techniques, using the cloned gene fusion as a hybridization probe. The 3 kb *Arabidopsis* DNA sequence upstream of the *gus* gene in this clone was analyzed. After *Agrobacterium*-mediated introduction into tobacco and *Arabidopsis*, this sequence shows phloem-specific promoter activity as is observed in the original transgenic line At3001.

Oligonucleotide primers complementary to the *gus* sequence were applied for the cloning of some gene fusions by the inverse polymerase chain reaction. Three translational gene fusions were analyzed so far. In line At2324, the *gus* sequence is fused after the eighth amino acid of a long open reading frame. Similarly, in At6023, *gus* is inserted after the ninth amino acid. In the At2116, *gus* seems somehow to be fused to a truncated T-DNA copy.

These data prove that the random *gus* fusion technique is a useful tool for the rapid cloning of promoters mediating particular patterns of regulated gene expression.

294 ISOLATION AND CHARACTERIZATION OF cDNA AND GENOMIC DNA ENCODING WHEAT HMGR. Kazuko Aoyagi, Anne Beyou, Keith Moon, Larry Fang, and Tom Ulrich. Sogetal, Inc., 3876 Bay Center Place, Hayward, CA 94545.

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is a key enzyme in the isoprenoid biosynthetic pathway from which flow some of the precursors essential for plant metabolism, cell structure, and the regulation of growth and development. In order to study the expression and regulation of HMGR in wheat (*Triticum aestivum*), we have isolated partial cDNA fragments using the polymerase chain reaction (PCR) and degenerate oligonucleotide primers designed to regions of amino acid sequence homology that are highly conserved among several diverse organisms. Comparison of sequence data obtained from several of the cDNA shows that they represent a small number of discrete sets of HMGR genes which share a high degree of sequence homology among themselves as well as among other organisms including tomato, *Arabidopsis*, hamster, human, *Drosophila* and yeast. The developmental and environmental regulation of these cDNA and their tissue specific expression have been examined. The cloning strategy and characterization of these cDNA and the structural organization of an HMGR genomic clone will be presented.

295 THE BRASSICA ACETOHYDROXYACID SYNTHASE GENE FAMILY. STRUCTURE, EXPRESSION, EVOLUTION. Jiro Hattori, Robert Rutledge, Thérèse Ouellet, Bernard Baum, Douglas Brown and Brian Miki. Plant Research Centre and Biosystematics Research Centre, Agriculture Canada, Ottawa, Canada K1A 0C6. The acetohydroxyacid synthase (AHAS, ALS) gene family of the allotetraploid species, Brassica napus consists of 5 members of which 3 are expressed. AHAS1 derived from the C genome and AHAS3 derived from the A genome constitute housekeeping genes. RNAase protection studies indicate that they are constitutively expressed at low levels in all somatic and reproductive tissues. Both are very similar to each other and other plant AHAS genes in DNA sequence. AHAS2 however is distinct and is only expressed in certain reproductive tissues. Detailed analysis of the coding region sequences revealed that the divergence of AHAS2 occurred after the formation of the Crucifers. Transgenic plants with resistance to herbicides that inhibit AHAS were generated with AHAS3 genes.

296 A PLANT LEUCINE ZIPPER PROTEIN THAT RECOGNIZES AN ABSCISIC ACID RESPONSE ELEMENT. Mark J. Guiltinan*, William R Marcotte Jr. and Ralph S. Quatrano. *Department of Horticulture, The Pennsylvania State University, University Park, PA 16802. Department of Biology, University of North Carolina, Chapel Hill, NC 27599.

A putative plant transcription factor (EmBP-1) has been identified in wheat and rice cells which binds to an abscisic acid response element (ABRE) from the wheat Em gene (1). A two base pair mutation of the ABRE (mABRE) which is not recognized by EmBP-1 does not enhance gene expression in response to ABA in a protoplast transient assay system as does the wild type ABRE. These results strongly suggest that EmBP-1 is involved in the ABA signal transduction pathway. Via expression library screening, cDNAs encoding EmBP-1 were isolated and shown to belong to the basic-leucine zipper (bZIP) class of transcription factors. Partial cDNAs for three of these genes share >95% DNA sequence identity. The ABRE sequence recognized by EmBP-1 is similar to sequences from other plant genes which have in common a six base pair core sequence (CACGTG) known as the G-box. Several other bZIP cDNA clones have recently been isolated several of which encode G-box binding proteins. These proteins share little sequence similarity with EmBP-1, except in the basic region domain, the portion of the protein which recognizes and binds DNA. This is consistent with the similarity of their recognition sequences (the G-box), and raises the possibility that the different G-box binding factors may have different functional characteristics. These findings raise important questions about the evolutionary and functional relatedness of the various G-box binding proteins. Experiments which are in progress will be discussed including: Further characterization of the EmBP-1 gene family: Quantitation of EmBP-1 RNA levels during wheat development and in response to exogenous ABA application: Investigation of the binding specificity of EmBP-1 by mobility shift analysis with various heterologous G-box and mutant DNA sequences: Investigation of the functional properties of EmBP-1 *in vitro* and in transformed plant cells.

1) M.J. Guiltinan, W.R. Marcotte Jr. and R.S. Quatrano. Science 250:267-271

297 THE GENE FAMILY ENCODING THE SOYBEAN (Glycine max.) TRANSLATION ELONGATION FACTOR EF-1 . A. Spielmann, F. Aguilar, J. Hernandez Torres, S. Marc-Martin and E. Stutz. Laboratoire de biochimie, Université de Neuchâtel, CH-2000 Neuchâtel, Switzerland.

A cDNA and a genomic library were used to study a small gene family (3-6 members) coding for the -subunit of the translation elongation factor EF-1 . Several different clones were retrieved and two genes, called tefS1 and S2, analyzed in more detail (Aguilar et al., submitted). Both genes contain an open reading frame of 1341 nucleotides (nt), interrupted by a short single intron of 86 (S1) and 116 (S2) nt. The two genes diverge in 80 wobble positions (coding region), thus yielding an identical protein of 447 amino acids. Analyses of nearly full length cDNA clones show that the untranslated leader of both genes is interrupted by a large intron as was reported for A. thaliana (Curie et al., NAR 19:1305-1310, 1991) and tomato (Pokalsky et al., NAR 17: 4661-4673, 1989). In northern experiments a single transcript of about 1.9 kb interacts with S1 and S2 specific DNA probes.

We tested tef gene transcription in soybean seedlings exposed for various times to light. We noticed a sharp rise in the transcript level a few hours after onset of light (e.g. 3 h) and a decrease after prolonged light exposure. Both genes S1 and S2 behave in a similar fashion, but their transcription pattern is quite different from that of the small subunit of RUBISCO.

We are presently studying cis-elements upstream of the untranslated leader possibly involved in transcription regulation.

298 A 140 BP FRAGMENT OF A PLANT HISTONE GENE PROMOTER IS SUFFICIENT TO CONFER MERISTEM-SPECIFIC EXPRESSION IN TRANSGENIC *ARABIDOPSIS*. Rossitza Atanassova, Nicole Chaubet and Claude Gigot. Louis Pasteur University, Institute of Plant Molecular Biology, Strasbourg, France

Chimeric genes containing the ß-glucuronidase (GUS) gene under control of the histone H4A748 promoter from *Arabidopsis* was integrated into the genome of *Arabidopsis* via *Agrobacterium tumefaciens* using the root-infection technique established by Valvekens *et al*., (PNAS 85, 5536-5540, 1988). Plants were regenerated and the GUS activity was determined by fluorimetric measurements in different organs of plants of the F1 progeny. Significant levels of activity were found in all organs tested including non-proliferating tissues (adult leaves and stems). Young organs such as young leaves and flower buds show 4 times higher activity. These observations are in agreement with the amounts of H4 mRNAs detected in the same tissues by northern-blot hybridization. GUS activity visualized by histochemical tests is highest in meristematic tissues such as root-tips, budding rootlets, apical buds and the cambial tissues of the stems. As shown by fluorimetric estimations adult tissues often show significant GUS activity. These results confirm that the H4A748 promoter can drive gene expression in meristems as well as in non-proliferating-tissues (see poster by Lepetit *et al*.).

The promoter region of 900 bp was successively deleted from its 5' sequences into 430 (Δ Alu), 340 (Δ Xmn), 270 (Δ Nar) and 130 (Δ EcoRV) bp fragments upstream of the ATG initiation codon, chimeric genes were constructed integrated into the genome of *Arabidopsis*, and the GUS activities estimated as above. The GUS activities in different organs were progressively reduced from H4A748-GUS to Δ Nar-GUS and completely abolished with Δ EcoRV-GUS.

We conclude that the elements between the Nar site (+270) and the ECoRV site (+130) are sufficient to confer the replication-dependent activity to the promoter.

POSTER ABSTRACTS

299 COMMELINA YELLOW MOTTLE VIRUS HAS A STRONG PROMOTER WHICH IS ACTIVE IN VASCULAR TISSUE AND IN THE ANTHER. Scott Medberry, Ben Lockhart and Neil Olszewski. University of Minnesota, Departments of Plant Biology and Plant Pathology, St. Paul, MN 55108

Commelina yellow mottle virus (CoYMV) is a dsDNA virus that infects a monocot host *Commelina diffusa*. Other plant viruses with similar bacilliform virions and possessing a dsDNA genome (badnaviruses) include viruses infecting rice, banana, sugarcane, canna, cacao, yucca, *Kalanchoe* and *Shefflera*. CoYMV encodes a single genome length plus 120 bp transcript. A promoter fragment encompassing the region from -1029 to +11 relative to the 5'-end of the CoYMV transcript confers strong expression in both monocot and dicot suspension cells. Analysis of deletions of the CoYMV promoter in a transient assay system indicate that deletion of the region from - 1029 bp to -230 bp has no effect on activity. Deletion of a region (-230 to -200) with similarity to the as-1 enhancer of CaMV decreases promoter activity by 85%. In stably transformed tobacco plants, the -1029 to +11 CoYMV fragment promotes strong expression of a β-glucuronidase reporter gene in phloem or phloem associated cells and in axillary parenchyma. Additionally, intense activity was noted in and near the tapetum. The potential relevance of the promoter's activity to the biology of viral infection and the observation of seed transmissibility of the virus will be discussed.

300 **CHARACTERIZATION OF cDNAs ENCODING A HIGH MOBILITY GROUP (HMG) PROTEIN OF MAIZE.** Klaus D. Grasser and Günter Feix. Universität Freiburg, Institut für Biologie III, Schänzlestr. 1, D-7800 Freiburg, FRG.

HMG proteins represent a class of small chromosomal nonhistone proteins which have been isolated from a variety of eukaryotic organisms including several plants. They are frequently associated with transcriptionally active chromatin and are relatively more abundant in proliferating cells. As in animal systems, HMG proteins isolated from maize endosperm show four distinct bands in SDS-PAGE. They bind *in vitro* to A/T-rich stretches of duplex DNA and show a binding preference for the CCAAT- and TATA-boxes of a zein gene promoter fragment. Several cDNAs coding for one of the HMG proteins were isolated and sequenced. The deduced amino acid sequence revealed a "HMG-box" DNA binding motif and additional similarities to the animal HMG1 family.

301 ISOLATION AND CHARACTERIZATION OF CATALASE GENES. Sibel H Isin and Randy D. Allen. Texas Tech University, Department of Biological Sciences, Lubbock, Texas 79409

Catalase is a tetrameric, heme-containing enzyme which is located in peroxisomes. Catalase converts hydrogen peroxide into oxygen and water. Catalase is found in all aerobic organisms and is one of the key enzymes in the active oxygen scavenging system. We have isolated a full length catalase cDNA from a pea λgt10 library. The derived amino acid sequence of this cDNA shares significant homology with cotton and maize catalases. We have investigated the expression of pea catalase mRNA in germinated seeds and mature plants. Transgenic tobacco plants that express pea catalase constitutively have been recovered and analysis of these plants for increased resistance to oxidative stress is currently underway. Using this pea cDNA, we have isolated a soybean genomic clone that includes a catalase gene. This soybean catalase gene includes 2 kb of upstream sequence and the catalase coding region consists of 7 exons. Terminal deletions of 5' flanking sequences of this gene have been fused to the ß-glucuronidase gene. These reporter gene constructs have been introduced into tobacco plants in order to identify *cis*-acting transcriptional regulatory elements.

302 EXPRESSION OF HIGH- AND LOW-pI α-AMYLASE GENES IN INTACT GERMINATING BARLEY. Ronald W. Skadsen and Brian K. Tibbot. USDA, ARS, Cereal Crops Research Unit, 501 N. Walnut St. and Univ. of Wisconsin, Agronomy Department, Madison, WI 53705

Although much is known about the expression of barley α-amylase genes in isolated aleurones, little is known about their expression in intact seedlings. Using northern blot analyses, we have determined the relative levels of high-pI α-amylase (HAAM) and low-pI α-amylase (LAAM) transcripts in 1- to 5-day-old post-imbibition seedlings of a malting cultivar, Morex, a feed cv., Steptoe, and an extensively studied hulless cv., Himalaya. All displayed similar temporal changes in their levels of HAAM and LAAM transcripts, respectively, in total extractable RNA. HAAM RNA increased to a peak at day 3 and then declined, while LAAM RNA increased continuously to day 5. HAAM RNA was 20-fold more abundant than LAAM RNA. Polysomal mRNA was also analyzed; temporal patterns of HAAM and LAAM expression and the greater abundance of HAAM mRNA were very similar to the results for total extractable RNA. Transcript levels correlate with the levels of HAAM and LAAM enzyme activities (per g. fresh wt.) detected on IEF gels. LAAM enzymes increase gradually to day 5 and are much less abundant than the HAAM enzymes. However, HAAM enzyme levels continue to increase after day 3, even though its corresponding mRNA (as a percentage of mRNA mass analyzed) declines. This may result from the continued rise in overall HAAM levels, since total extractable RNA and polysome yields per g fresh wt. increase gradually through day 5. Steptoe had much lower levels of both mRNAs, relative to Morex and Himalaya, but displayed the same temporal expression profiles. Steptoe was found to have a 1000-fold lower α-amylase response to GA_3, relative to Morex, even though its HAAM and LAAM gene copy numbers and organization appear to be the same.

303 AN 80 BP CIS-ACTING ELEMENT CONFERS NODULE-SPECIFIC EXPRESSION ON THE *SESBANIA ROSTRATA* LEGHEMOBLOBIN glb3 GENE. Krzysztoph Szczyglowski[1,2], Laszlo Szabados[2,3], Pascal Ratet[2,4], Britta Grunenberg[2], Susan Fujimoto and Frans J. de Bruijn[1,2]. MSU-DOE Plant Research Laboratory, E. Lansing, MI, 48824, U.S.A.[1]; Max Planck Institute, 5000 Cologne 30, F.R.G.[2]; Present address: B.R.C., Szeged, Hungary[3] and ISV-CNRS, Gif-sur-Yvette, France[4].

A deletion/substitution analysis has been used to delimit a promoter-proximal 80-bp segment of the *Sesbania rostrata* leghemoglobin glb3 gene 5' region; which is capable of conferring infected-cell specific expression on chimeric glb3-uidA (glucuronidase) genes in nodules of transgenic *Lotus corniculatus* plants. This Nodule Specific Element (NSE) contains several highly conserved motifs found in (leg) hemoglobin genes of legumes and non-legumes. Base pair changes have been introduced in all these motifs to ascertain their role in nodule specific expression. The interaction of the NSE element and its mutants derivates, with specific trans-acting DNA binding proteins has been investigated and compared to other, previously characterized, protein - DNA interaction in the 5' regions of the glb gene and other lb genes. The results of these experiments will be discussed in the framework of our attempts to elucidate the molecular basis of the signal transduction pathways responsible for nodule-specific plant gene expression.

304 Characterization of the tobacco DNA-binding protein TGA-1B
Alexander R. van der Krol, Fumiaki Katagiri, Hillel Fromm and Nam-Hai Chua, Laboratory of Plant Molecular Biology, Rockefeller University, New York.

A partial cDNA encoding the DNA-binding protein TGA-1B was previously isolated from a tobacco cDNA library, using as a probe a conserved hexamer motif found in plant histone promoters (Katagiri et al., Nature 340: 727-730, 1989). Upon rescreening of a cDNA library we isolated several (near) full length (3 kb) cDNAs of TGA-1B and also cDNAs (TGA-1B') with an internal 1,2 kb deletion. Comparison of the sequence of cDNAs and TGA-1B genomic clones show the presence of a single intron in the gene. TGA 1B' mRNA seems to have derived from splicing at an alternative 5' splice junction site of the single intron. TGA-1B' mRNA encodes a protein with the same N-terminal acidic domain as full length TGA-1B but without the DNA-binding B-ZIP region. TGA-1B' mRNA comprises less than 10% of the TGA-1B mRNA population. The expression pattern as inferred from northern blot analysis is similar to that of TGA-1A and TAF-1 (two other tobacco B-ZIP proteins); TGA-1B is mostly expressed in roots and only at low levels in stem and leaf

Since TGA-1B binds to DNA it presumably functions and resides in the nucleus of plant cells. The monomeric form of TGA-1B is 82 kD and thus the protein should contain a plant nuclear targetting signal (NLS) to facilitate active nuclear import. We tested several basic domains of the TGA-1B protein for NLS function by fusing putative NLS encoding sequences in frame with the GUS reporter gene. Analysis of transgenic plants shows that of the different basic domains tested only the basic domain of the B-ZIP region contains a sequence that can direct chimeric GUS to the nucleus. This results indicates a very close association or overlap of the nuclear targetting and the DNA binding function and may account for the high homology in the basic domain of the B-ZIP proteins from different plant and mammalian systems.

POSTER ABSTRACTS

305 FUNCTIONAL ANALYSIS OF TWO rbcS PROMOTERS IN MAIZE RICE, AND TOBACCO. Jean-Frédéric Viret[1], Kailash C. Bansal[2], Michel Lebrun[2], Rodolphe Schantz[3] and Lawrence Bogorad[1]. [1]Harvard University, Department of Cellular and Developmental Biology, Cambridge, MA 02138, USA. [2]Rhône-Poulenc, 14-20 rue Pierre Baizet B P. 9163, 69263 Lyon, France [3]Institut de Biologie Moléculaire des Plantes, 12 rue du General Zimmer, 67084 Strasbourg, France.

We undertook the promoter analysis of a maize rbcS gene, which encodes the ribulose-1,5-bisphosphate carboxylase small subunit. The mRNA accumulation of the maize rbcS-m3 gene (1) is light regulated and bundle sheath specific (2). Various rbcS-m3 promoter-GUS constructs were assayed for transient expression in maize, rice, and tobacco leaves using microprojectile bombardment. As controls, we used the promoter of SRS1 (3), which is a strongly light regulated soybean rbcS gene, fused to GUS and the strong constitutive CaMV 35S gene promoter fused to GUS and the R locus Lc cDNA (4). We found that the rbcS-m3 promoter confers GUS expression in maize and rice leaves, but not in tobacco leaves. Conversely, the SRS1 promoter confers GUS expression only in tobacco leaves. We will present the histochemical localization in maize leaves of the GUS expression driven by the rbcS-m3 promoter.

This work is supported partially by Rhône-Poulenc. 1)Lebrun et al. (1987) Nucleic Acid Res. 15:4360 2) Sheen and Bogorad (1986) EMBO J. 5:3417-3422. 3) Berry-Lowe et al. (1982) J Mol. Appl. Genet. 1:483-498. 4) Ludwig et al (1989) Proc Natl Acad Sci USA 86:7092-7096.

306 FAST CLONING OF SUGARCANE SUCROSE SYNTHASE cDNA BY ANCHORED PCR. A. Suresh Kumar[1], Paul H. Moore[2], and A. Maretzki[1]. [1]CMBRU, Hawaiian Sugar Planters' Association, Aiea, HI, [2]USDA/ARS, Aiea, HI.

Sucrose synthase is an important enzyme of sucrose metabolism. Its role in sugar-storing plants is still not well understood. We have developed a fast cloning strategy based on the polymerase chain reaction (PCR) to amplify and construct full length sucrose synthase (SS) cDNA of sugarcane. Two internal consensus sequences were identified from published maize and wheat SS sequence data and used to synthesize internal PCR primers for sugarcane. Amplification of full length cDNA was achieved by an anchored PCR method utilizing internal and tailing primers which extend to 5' and 3' ends of specific cDNA. Two SS cDNAs were amplified: one with a 5' end (SSp1) and other with a 3' end (SSp2), using one internal SS primer and an anchored end primer. Overlapping fragments were identified by restriction mapping and the non-overlapping fragments were excised and re-ligated to reconstruct full length cDNA. Partial sequences of the reconstructed cDNAs (SS-5' & SS-3') were compared with the published SS sequences to confirm that the amplified DNA was, in fact, a copy of the SS transcript. The internal primers for SS were used simultaneously to amplify partial SS cDNA of 600 bp in order to esumate relative abundance of SS transcripts in various source and sink tissues of sugarcane. SS messenger RNA was the most abundant in cell cultures, followed by young stems and roots; it was the least abundant in mature stem internodes.

307 EVIDENCE OF CO-SUPPRESSION BETWEEN ENDOGENOUS SUCROSE SYNTHASE GENES IN MAIZE AT RNA TRANSCRIPT AND PROTEIN LEVELS. P S Chourey and E.W. Taliercio, USDA/ARS and University of Florida, Gainesville, FL 32611.

The two sucrose synthase isozymes, SS1 and SS2, encoded by molecularly homologous but non-allelic genes, Sh and Sus, respectively, are extremely similar biochemically; yet, each gene exhibits a high level of tissue and cell specificity. Whereas, both genes are expressed in young seedlings, storage cells in immature endosperm show only the Sh expression. The Sus expression in these cells is limited to sh mutants, marked by the loss of Sh expression. Unlike endosperm, immature embryos show only the Sus expression. The exception to this rule is the genotype with an inactive Sus (i.e. sus) gene, which shows no detectable levels of wild type SS2 protein in any tissue. Such genotypes (i.e. Sh sus) show altered tissue specificity as these are uniquely characterized by the Sh expression in embryos. Northern blot and in situ hybridization analyses show that suppression or activation of the Sh gene in embryos is trans-regulated at the RNA level by the Sus or sus gene, respectively. Increased steady state levels of Sh transcripts in sus relative to Sus genotypic embryos is indicative of, among other possibilities, a transcriptional mode of control in such expression An alternative possibility of decreased stability of Sh transcripts in embryos of Sus relative to sus genotype is, at present, not ruled out. Collective data from various genotypes will be presented to show that cross-regulation among molecularly similar genes such as Sh and Sus is regulated in a competitive fashion, modulated presumably by the tissue specific metabolic environment of the cell

308 CIS-ELEMENTS AND TRANSACTING FACTORS FROM AUXIN-RESPONSIVE GENES OF SOYBEAN. Jong Chan Hong, Virginia Gockjian, Ronald T. Nagao, and Joe L. Key, Department of Botany, University of Georgia, Athens, Georgia 30602.

Aux28 and Aux22 are members of two, related multigene families of auxin-responsive genes isolated from soybean. While these genes encode polypeptides of similar structure, the basal level of expression and the amount of induction by auxin treatment is different between the two genes. To understand the regulation of expression of these genes, DNase I footprinting and gel retardation analyses have been used to identify protein-binding DNA domains in the promotor sequences. The Aux28 gene was more extensively analyzed and showed multiple regions of protection, including eight binding sites in the -827 bp to +51 bp of the gene. The sequences of note include two AT-rich sequences, a TGACGACA sequence (as-1-like element), a CCACGTGT sequence known as a G-box motif, and two related sequences with a motif TAGTxCTGT. Two elements, D1 and D4, which are TAGTxxCTGT and TAGTxCTGT, respectively, differ only in the length of spacer between the TAGT and CTGT sequences. Gel retardation analysis using these two elements gave different patterns of gel shifting. D1 and D4 share the lower band while D4 has an additional upper shifted band. The binding activity represented in the upper band decreases as the region of hypocotyl section used to make extract goes from apical to mature tissue, the binding activities of other binding sites increased or same level. Aux22 also contains a D1/D4 related element which competes with the Aux28 element. We have isolated multiple cDNA clones which encode binding proteins for the as-1-like and G-box sequences from a lambda Zap library of soybean. The characterization and significance of these factors on the regulation of the auxin-responsive gene and the further isolation of other transcription factor clones are in progress.

309 SEQUENCE ANALYSIS OF THE SECOND LARGEST SUBUNIT OF RNA POLYMERASE II FROM *ARABIDOPSIS THALIANA*. Rob Larkin, Craig Chandler, and Tom Guilfoyle. University of Missouri-Columbia, Department of Biochemistry, Columbia, Missouri 65211.

RNA polymerase II is a multisubunit enzyme responsible for transcription of protein-encoding genes in eukaryotes. The enzyme is composed of two large subunits with molecular weights greater than 140kDa and six to ten smaller subunits with molecular weights ranging from 10 kDa to 40 kDa. A more detailed understanding of the functional contribution of each subunit to the transcription process is emerging from molecular biological studies of individual subunits. To more thoroughly understand transcription in plants, we are studying the molecular biology of plant RNA polymerase II subunits and general transcription factors. Using PCR and degenerate oligonucleotides designed from amino acid sequences derived from genes encoding the second largest (140 kDa) subunits of eukaryotic class II enzymes and the β subunits of prokaryotic and chloroplast enzymes, a segment of the gene encoding the second largest subunit of RNA polymerase II was amplified from *A. thaliana* cv Columbia genomic DNA. Using this PCR product, we have isolated overlapping fragments of *A thaliana* genomic DNA that represent a gene encoding the second largest subunit of RNA polymerase II. We are studying the structure and expression of this gene and other genes encoding RNA polymerase II subunits and general transcription factors

310 INTERACTIONS AFFECTING GENE EXPRESSION INVOLVED IN MAIZE STARCH BIOSYNTHESIS. Michael J. Giroux, Charles Bover(*), Gunter Feix(**), and L. Curtis Hannah. University of Florida, Vegetable Crops Department, Gainesville, FL 32611, (*)Penn State University, Department of Horticulture, University Park, PA 16802, and (**)University of Freiberg, Germany.

Transcript levels of *Sh1*, *Sh2*, *Bt2*, and *Wx* were analyzed on a total RNA basis from maize kernels isolated at 14, 22, and 30 days post-pollination (dpp). The W64A isogenic lines utilized were wild type, *sh1*, *sh2*, *bt1*, *bt2*, *o2*, *ae*, *du*, *wx*, and *aeduwx*. Through kernel development, transcript levels of the four monitored genes were elevated in the mutant backgrounds. The use of zein specific cDNA probes indicated that these transcripts were also higher in the mutant background both on a relative and an absolute level. In all mutant backgrounds the greatest relative increases were seen at 30 dpp. *Adh* and *actin*, two maize genes not involved in the production of endosperm storage products were relatively unaffected. The relative protein levels of *Sh1*, *Sh2*, and *Bt2* were also greater in the mutant backgrounds. Nuclear run-on assays performed with extracts isolated at 30 dpp suggested that the increases in transcript levels were the result of increased stability. Western blot analysis implied that the relative increases in protein levels also resulted from greater stability.

POSTER ABSTRACTS

311 INTERACTION OF SPECIFIC NUCLEAR FACTORS WITH THE 5'-UPSTREAM REGION OF THE RICE Wx GENE. Hua Jian, Wang Zong-yang, Zhang Jing-liu and Hong Meng-min. Shanghai Institute of Plant Physiology, Academia Sinica, 300 Fenglin Road, Shanghai 200032, CHINA

The rice Wx gene controls the production of amylose in the endosperm and pollen. Genomic clones of Wx gene of O.sativa subsp. Hangtong 0366 have been isolated and sequenced. The coding region of Wx gene is interrupted by 13 introns. The gene codes for a protein with a MW of 72kD. Putative TATA and Agga boxes are present in the 5'-upstream region. In order to identify trans-acting factors required for Wx gene expression, DNA gel electrophoresis mobility retardation assay was performed. Nuclear proteins were extracted from rice pre-mature seeds and reacted with several labeled DNA fragments spanning 950 bp of Wx gene 5'-upstream sequences. The results indicated that two DNA fragments formed protein-DNA complexes when reacted with nuclear extracts. When same unlabeled fragment DNA was added as comptitor, the binding activity of labeled DNA fragment decreased, but no decrease in binding activity was observed upon addition of calf thymus DNA and M13mp18 DNA.

312 DEVELOPMENT OF AN IN VITRO TRANSCRIPTION SYSTEM FROM CULTURED PLANT CELLS. P. Schweizer[1], E. Mösinger[1] and K. Hahlbrock[2]. [1]Sandoz Agro Ltd. CH-4108 Witterswil. [2]Max-Planck Institut für Züchtungsforschung, D-5000 Köln 30.

We are developing an in vitro transcription system which is based on a fractionated whole cell extract from cultured parsley cells (Yamazaki et al. PNAS USA 87,7035). This system was used to transcribe a G-free reporter cassette under the control of the parsley PR2 promoter which is strongly activated in vivo by a cell wall elicitor from Phytophthora megasperma (Van de Löcht et al. EMBO J 9,2945). Transcription in vitro was initiated at a single position 24 bp upstream of the in vivo cap site. Transcription from the fully elicitor responsive PR2 promoter was 5 to 10 fold stronger than from the core promoter which contained only the TATA element. Transcription showed a lag phase of 10 minutes indicating the formation of an initiation complex. The TATA element binding factor(s) seemed to be involved in complex formation, since transcription could be inhibited by addition of a doublestranded oligodeoxynucleotide spanning the TATA element of the cauliflower mosaic virus 35S promoter. Constructs containing the G-free in vivo cap sites of the parsley chalcone synthase-, the potato proteinase inhibitor 2- or mutated PR2 promoters did not direct major initiation from the respective in vivo cap site indicating that construction of the G-free cassette did not destroy a necessary "initiator" sequence of the PR2 promoter. We are presently exchanging the PR2 TATA element by the 35S TATA element.

313 POLLEN-SPECIFIC GENE EXPRESSION. Sean P. Davies, Hui-Ling Xu, Piyada Theerakulpusit, Andrew P. O'Brien, Mohan B. Singh and R.Bruce Knox. School of Botany, University of Melbourne,Parkville, Australia. 3052

In flowering plants the male gametes are housed within the haploid male gametophyte, the pollen grain. Haploid pollen-specific gene expression has been identified at various stages during pollen development. In this study, pollen-specific genes have been isolated and characterized from three plant species - *Brassica campestris*, *Lolium perenne*, and *Arabidopsis thaliana*. The genomic clones isolated from *B.campestris* and *L.perenne* are each members of gene families to which we have also isolated pollen-specific cDNA clones. Northern blotting and *in-situ* hybridization have been used to determine the temporal and spatial pattern of expression of the pollen-specific genes isolated from the latter two species. The genomic clones isolated from all three species are currently being analyzed by plant transformation studies in *Nicotianna tabaccum* and *Arabidopsis thaliana* in order to determine DNA sequences involved in the regulation of their expression.

314 ORGAN SPECIFIC EXPRESSION AND REGULATORY REGIONS OF THE TOBACCO *par* (protoplasts auxin-regulated) GENE. Yasuo Niwa, Yohsuke Takahashi* and Yasunori Machida. Department of Biology, Nagoya University, Chikusa-ku, Nagoya, Japan. *Department of Botany, Tokyo University, Bunkyo-ku, Tokyo, Japan

We isolated the 5'-upstream region of the *par* (protoplasts auxin-regulated) gene, which is shown to be expressed in the tobacco mesophyll protoplasts in response to exogenous 2,4-D. Organ specific expression of the *par* gene was examined by introducing *par*-GUS fusion genes into tobacco plants. The GUS reporter gene linked to the 1732 base pair 5' fragment of the *par* gene (-1679 to +53) was predominantly expressed in roots. In particular, strong expression was observed in root hairs and in root tip of seedlings. As the transgenic tobacco plant with that fusion gene grew, it was expressed in entire roots. The fusion gene was also expressed at a late stage of seed maturation, but the level of GUS activity was lower than that detected in roots. Deletion analysis of the 5'-upstream region showed that it was split into three regulatory domains. When the sequence between -1679 and -768 was deleted, GUS activity was entirely abolished. A low level of GUS activity was, however, detected when the deletion was extended to -328. In addition, the 5' sequence from +53 to -328 allowed the organ specific expression of the *par* gene as described above. Thus, this region seems to be enough for determining the specificity of the *par* gene expression. The sequence between -328 and -768 probably has a negative function for the expression and the further upstream region could be responsible for the high level of the expression of the *par* gene.

315 HBP-1a AND HBP-1b: LEUCINE ZIPPER-TYPE TRANSCRIPTION FACTORS OF WHEAT. Tetsuya Tabata*, Takuya Nakayama, Koji Mikami and Masaki Iwabuchi. Department of Botany Faculty of Science, Kyoto University, Kyoto 606, Japan

We have studied the wheat transcription factors HBP-1a and HBP-1b, which were identified by their binding to the cis-acting element with the hexameric core sequence ACGTCA of the wheat histone H3 gene promoter. HBP-1b also binds to the same hexamer motif of the CaMV 35S RNA gene promoter, whereas HBP-1a does not. The cDNA clones encoding HBP-1a and HBP-1b were isolated on the basis of their binding specificities to the hexamer motif. The deduced amino acid sequences indicate that both HBP-1a and HBP-1b belong to a leucine zipper class of transcription factors. Using these cDNAs, HBP-1a and HBP-1b were expressed in bacterial cells or in vitro to examine their properties. HBP-1a and HBP-1b form homodimers without binding to DNA, but not heterodimers. Mutational analyses revealed that truncated polypeptides containing the leucine zipper and basic regions were sufficient for DNA binding. Although the hexamer motif or its homologs exist in several plant genes, gel mobility shift assays using the various hexamer motifs as competitors indicated that HBP-1a and HBP-1b exhibited the highest binding affinity to the hexamer motif of the histone promoters, suggesting that both DNA binding proteins are involved in transcriptional regulation of wheat histone genes.

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316 CLONING AND CHARACTERISATION OF cDNAS ENCODING BOTH SUBUNITS OF GLUTATHIONE-S-TRANSFERASE ISOFORM II FROM MAIZE. Ian Jepson, David Holt, Venetia Lay, Simon Bright and Andrew Greenland. ICI Seeds, Plant Biotechnology Section, Jealott's Hill Research Station, Bracknell, Berks. RG12 6EY.

Glutathione-S-transferases (GSTs) in maize represent a family of enzymes which conjugate glutathione to several commonly used herbicides resulting in their detoxification. Total GST activity in maize can be enhanced by the application of herbicide safeners. In maize three isoforms have been identified. GST I and GST III are homodimers of 29kD and 26kD subunits respectively. Nucleotide sequences encoding these subunits have been reported by others (1, 2, 3). We are interested in a third isoform, GST II, which is induced by safener treatment. We have purified GST II to homogeneity by FPLC and affinity chromatography. GST II is a heterodimer composed of 29kD (GST II-29) and 27kD (GST II-27) subunits. We have shown by amino acid sequence analysis of tryptic fragments of GST II-29, that the 29kD subunits of GST II and GST I are identical.

We have separated the 27kD and 29kD subunits of GST II and raised polyclonal antisera in sheep. The specificity of these antisera for the 27kD and 29kD subunits respectively has been demonstrated by Western analysis. Immunoscreening of a maize seedling root cDNA library in lambda ZAP II has identified a full length cDNA encoding GST II-27. Oligonucleotide probes have been used to isolate a full length cDNA corresponding to GST II-29. Currently we are assessing the relative expression levels of each GST isoform.

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POSTER ABSTRACTS

317 THE PR-1a MINIMAL PROMOTER REGION CAN BE ACTIVATED BY HETEROLOGOUS ENHANCERS IN TRANSGENIC TOBACCO PLANTS Andrea Beilmann, Margit Mau, Konstanze Albrecht and Ursula M. Pfitzner. University of Munich, Department of Botany D-8000 Munchen, FRG

PR-1 genes are induced by various environmental stimuli such as pathogen attack or exposure of the plants to certain chemicals. To examine the regulation of these genes the 5 flanking regions of the PR-1a gene up to -335 were joined by a transcriptional fusion to the GUS reporter gene The constructs were stably integrated into the tobacco genome No indications could be obtained for the occurrence of a positively acting, (strong) autonomous regulatory element in the immediate PR-1a upstream regions mediating gene expression in response to environmental stimuli On the other hand, however, out of 38 transformants analysed two plants were found to exhibit considerable GUS activities without any inductive treatment of the plants Molecular analysis of these plants showed that the PR-1a/GUS hybrid promoter expression cassettes were most likely activated by heterologous enhancer elements dispersed in the tobacco genome To further investigate modulations of the transcriptional activity of the PR-1a minimal promoter region, we have joined various enhancer elements to the PR-1a sequences The data obtained with the endogenous enhancers and with chimaeric enhancer/PR-1a promoter expression cassettes in transgenic tobacco plants will be presented These results will be discussed with respect to functional domains o the PR-1a promoter and with respect to the suitability of the PR-1a sequences as a general minimal promoter element for the identification of putative enhancer elements

318 THE 5'-FLANKING REGION RESPONSIBLE FOR THE ENDOSPERM-SPECIFIC EXPRESSION OF A RICE PROLAMIN CHIMERIC GENE IN TRANSGENIC TOBACCO Yun-Liu Fan and Jianjin Zhou Biotechnology Research Centre, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Substantial progress about the cis-elements of the seed storage protein genes has been made in cereals such as Maize, Barley and Wheat etc. But the information about the cis-elements of rice seed storage protein gene is accumulated much more slowly. To date, only two papers about the cis-elements of rice glutelin gene are available. In my laboratory, the 5' upstream region (-690--+42) containing the complete signal peptide coding sequence of the rice seed storage prolamin gene was amplified in vitro with polymerase chain reaction from the genome of Chinese super rice cultivar Zhonghua 8. Physical map and DNA sequence analysis show strong homology with the 5'-flanking region of rice prolamin gene published by Okita in 1989. No change in the signal peptide coding sequence and a long leader sequence with several small ORFs were found. Chimeric genes containing 5'-flanking region of the prolamin gene have been transcriptionally or translationally fused with the β-glucuronidase reporter gene. The fusion junction was confirmed by both physical map and DNA sequence analysis. The transcriptionally fused chimeric gene was used to transform the tobacco explants by Ti binary system of Agrobacterium tumefaciens LBA4404. With dot and Southern blotting hybridization, three transgenic tobacco plants with the copies of chimeric GUS gene as high as 20 were obtained. Histochemical analysis revealed the GUS activity in the endosperm tissue of tobacco seed at the developmental stage about 20 DAP. No GUS activity was found in leaves, stems, roots and flowers of the transgenic tobacco plants. Therefore, we conclude that the 5' upstream cis-elements from -690 to -18 was enough to confer the endosperm-specific and temporal expression of rice prolamin gene. Functional analysis of the translational fused chimeric genes is in progress.

319 MOLECULAR EVOLUTION OF THE INTERGENIC SPACER OF THE rRNA GENES IN CUCURBITACEAE. U. Zentgraf, K. King, R. Torres, V. Hemleben. University of Tübingen, FRG

The intergenic spacer (IGS) of a 10 kbp repeat of the rRNA genes of Cucurbita pepo was completely sequenced and compared to the IGS of Cucurbita maxima and Cucumis sativus. Although the length of the IGS of C. pepo and C. maxima is different, the nucleotide sequence and organization is very similar. They are mainly composed of three different repeated elements interspersed into unique sequences: GC-clusters, a 422 bp element with the transcription initiation site (TIS), and 260 bp D repeats. The TIS for RNA polymerase I is duplicated in the 10 kbp repeat class of C. pepo as also described for C. maxima; the first one is predominantly used as was shown by mung bean nuclease mapping. The IGS of C. sativus is also composed of different repeated elements, however, obvious homology to the Cucurbita species only occurs around the TIS and the preceding AT-rich region. GC-rich regions with different primary sequences are present in the IGS of all three plants. Remarkably, the repeated elements in the IGS accumulate TG and TNG motifs, whereas CG and CNG motifs are reduced. This accumulation might be caused by the transition of ^{m}CG or ^{m}CNG into TG or TNG via deamination. Reversion of the TG or TNG back to CG or CNG increased the homology on the sequence level between the Cucurbita and Cucumis species. A common ancestor of the IGS for Cucurbitaceaes was constructed.

320 TRANSCRIPTIONAL REGULATION OF rRNA GENES IN TWO HIGHER PLANTS. K. Unfried, U. Zentgraf, K. King, V. Hemleben. University of Tübingen, FRG

The intergenic spacer (IGS) of the rRNA genes of higher plants shows a complex pattern of different directly repeated elements. Comparing the nucleotide sequence of the intergenic spacer of Cucumis sativus and Vigna radiata only the transcription initiation site appears to be conserved. The involvement of these repeated elements in transcriptional regulation was investigated by different methods. In C. sativus DNA-binding assays show a specific affinity of proteins of a crude nuclear extract to the transcription initiation site (TIS) as well as to the repeated elements in the external transcribed spacer downstream of this site. Competition assays indicated that corresponding proteins are involved in these binding reactions. At least two binding proteins (16 and 24 kD) could be identified by CNBr-activated sepharose affinity chromatography and by South-Western experiments. In Vigna radiata the functional relevance of repeated spacer sequences also including slightly differing copies of the TIS (P2 /P3) was assayed by in vitro transcription and gel retardation assays. Deletion of parts of these sequences resulted in changes in transcriptional activity; the deletion of the first TIS activates those copies positioned further downstream. A protein binding site is located upstream of the TIS.

321 REGULATION AND FUNCTION OF THE NITRATE ASSIMILATION GENES IN ARABIDOPSIS. Nigel Crawford, Jack Wilkinson, Sam Labrie, Yi-Fang Tsay. Dept. of Biology, UCSD, La Jolla CA 92093

Arabidopsis has two genes which encode nitrate reductase, the first enzyme in the nitrate assimilation pathway. By screening plants mutagenized with γ-rays for resistance to chlorate, we have identified three mutants in which the nitrate reductase gene NIA2 is deleted. These plants have only 10% the wild type level of leaf NR activity yet they grow as well as wild type plants even when nitrate is limiting. The mutant phenotype of these plants could be complemented by introducing the NR coding region fused to a CaMV 35S promoter. These NIA2 deletion mutations were found to be allelic to mutations at the chl3 locus; thus, the NIA2 and CHL3 genes are one and the same. Irrigating wild type plants with nitrate results in the accumulation of NR mRNA, protein and activity; thus, nitrate appears to act as an inducer of nitrate reductase. We have found that chlorate treatment also results in the accumulation of NR mRNA; however, NR protein and activity do not accumulate but in fact drop. It appears that chlorate treatment stimulates NR mRNA accumulation but leads to destabilization of the protein. This result is consistent with the observation that chlorate treatment of NR in vitro can result in the inactivation of the enzyme.

322 INDUCTION OF NPT II EXPRESSION IN TRANSGENIC TOBACCO. Wei Shao (1), Zhen Zhu (2) and Karen Hughes (1), University of Tennessee, Department of Botany, Knoxville, Tennessee 37996 (1) and Institute of Genetics, Academia Sinica, Beijing, China.

Tobacco protoplasts were transformed with the neomycin phosphotransferase II structural gene (Npt II) attached to a nopaline synthase promoter (PNos). In a portion of the transformed calli, the Npt II gene was silent. In a second group, the gene was expressed at a lower than normal level; However, in all calli, NPT II activity can be induced or increased with 5-azacytidine, a DNA methyltransferase inhibitor. The effective concentration of 5-azacytidine ranges from 3 - 30 uM with the optimal concentration for induction of gene expression at about 10 uM. Calli treated with 5-azacytidine for 5 days invariably show obviously higher NPT II activity than untreated calli. Calli treated with 5-azacytidine for 2 days do not show consistently higher NPT II levels. Auxin, especially 2,4-D, induces higher levels of NPT II in leaf slices within 24 hours of application, whereas 5-azacytidine is not able to induce NPT II expression in this non-dividing tissue indicating that auxin and 5-azacytidine induction of NPT II activity may involve different mechanisms. In contradiction to published reports indicating that treatment of calli with hydroxyurea (an inhibitor of DNA replication) inhibits 5-azacytidine-induced NPT II activity, we find that hydroxyurea by itself or in combination with 5-azacytidine induces NPT II activity while reducing total cellular protein. Mechanisms by which NPT II induction is obtained are being investigated.

POSTER ABSTRACTS

323 NOVEL TISSUE-SPECIFIC REGULATION OF MAIZE ANTHOCYANIN SYNTHESIS BY *Bh*, AN ALLELE OF *P1*. Suzy Cocciolone & Karen Cone. University of Missouri, Division of Biological Sciences, Columbia, MO 65211

Anthocyanins are purple pigments that are synthesized in specific tissues of the maize plant. The tissue-specificity of anthocyanin synthesis is dictated by the tissue-specific expression of a few regulatory genes that control the expression of a common set of structural genes. Two of the regulatory genes, *C1* and *P1*, perform identical functions but in different tissues. *C1* controls pigmentation of the kernel, and *P1* controls pigmentation in the vegetative parts of the plant, such as leaves, stems, and tassels. The functional identity of these genes is mirrored by 90% homology at the sequence level. Another potentially related gene, *Blotched (Bh)*, leads to blotches of pigmentation in the kernel, even in the absence of a functional *C1* gene. *Bh* has been mapped to a chromosomal location near *P1*, and our results suggest that *Bh* may actually be an allele of *P1* which is differentially expressed on a cell by cell basis in both the kernel and the plant. One line of evidence to support this idea has been the detection of *P1* mRNA on a Northern blot of RNA from blotched kernels. *P1* mRNA is not normally detected in kernels, but if it were expressed in this tissue, it could functionally replace *C1* to promote anthocyanin synthesis. Additionally, Southern blot analysis has shown that no other region in the maize genome shares significant homology with *P1* and *C1*, irrespective of whether the kernel phenotype was blotched or non-blotched. Further evidence supporting the idea that *Bh* is an allele of *P1* has resulted from phenotypic observations. Although *Bh* expression has only been reported for the kernel, we have also observed its expression in the following plant tissues: leaf blade--including mid-rib and leaf margin, sheath, husk, culm, anther, silk hair, kernel glume, and tassel glume face. This blotchy pigmentation in the plant co-segregates with *Bh* expression in the kernel and with the *P1* allele from *Bh* carrying maize lines--as determine by Southern hybridizations. Taken together, these results suggest *Bh* is a *P1* allele which is variably expressed not only in the kernel, but also in the vegetative plant tissues.

324 **ISOLATION AND CHARACTERIZATION OF A GENE ENCODING RICE CYCLOPHILIN.** Wallace G Buchholz, **Jonathan W Seay and Timothy C Hall.** Dept. of Biology, Texas A&M University, College Station, Texas 77843-3258. USA

Cyclophilin, an abundantly expressed protein found in all eucaryotes investigated, has peptidyl-prolyl *cis-trans* isomerase and protein folding activity *in vitro* and is thought to be involved in protein folding *in vivo*. As an initial step toward investigating the regulation and function of cyclophilin in plants, genomic and cDNA clones were isolated from rice (*Oriza sativa.*). Comparison of the encoded amino acid sequence with those of maize and human T-cell cyclophilins yielded over 70% identity. Whereas a single cyclophilin mRNA transcript is expressed in other organisms investigated, northern blot analysis of rice yielded two highly expressed transcripts of approximately 800 and 900 nt. The 800 nt band was seen only in root mRNA whereas the 900 nt band was present in both root and shoot mRNA. Southern blot analysis suggests the presence of a small (6 to 8 member) divergent cyclophilin gene family in rice. A chimeric gene was constructed by fusing a 2.5 kb genomic DNA fragment from upstream of the cyclophilin coding sequence to a *ß-glucuronidase* reporter gene. High levels of transient *ß-glucuronidase* activity were detected after electroporation of this construct into rice protoplasts. Experiments are ongoing to stably introduce this chimeric gene into rice and to determine if the promoter directs spatially and/or temporally specific gene expression.

325 ABA RESPONSIVE ELEMENTS FOR TRANSCRIPTIONAL REGULATION OF RICE RAB GENES

Takeshi Izawa[1], Akemi Ono[2], Ko Shimamoto[2], and Nam-Hai Chua[1]

The Rockefeller University, Laboratory of Plant molecular biology, NYC 10021[1], Plantech Research Institute, Yokohama, JAPAN 227[2]

We have previously shown that the expression of the rice rab genes can be induced by exogenous abscisic acid (ABA). By DNase I footprinting experiments, we have identified two conserved motifs, motif I & II in rab promoters, that can interact with rice nuclear factors. To see whether these motifs are involved in ABA regulation, we made chimeric promoters containing either motif I or II, or motifs I & II together. These constructs were assayed in rice protoplasts as well as in transformed rice calli. We found that motif I & II confer qualitative and quantitative functions, respectively, in transient expression assays. However, a combination of motifs I & II is necessary and sufficient for ABA-inducible expression in transformed calli. These results indicate that motifs I & II together function as ABA-responsive elements (ABREs) combinatorially and synergistically in rice cells.

327 IDENTIFICATION OF *cis*-REGULATORY ELEMENTS OF THE GST I PROMOTER OF MAIZE. J. D. Neill, M. C. Ross, B.A. Roth and J. A. Howard. Pioneer Hi-Bred Int'l, Inc., Johnston, IA 50131.

The maize glutathione S-transferase I (GST I) gene is constitutively expressed in germinating embryos and seedlings. This expression can be increased by the application of exogenous chemicals such as dichlormid, a herbicide safener. We have developed a transient expression system in an embryogenic suspensions cell line in which GUS expression was under the control of the GST I promoter. Cells were transformed by microprojectile bombardment and were assayed for GUS activity 24 hours post-bombardment. GUS activity was readily detected in untreated cells, but following treatment with dichlormid, enzymatic levels were increased up to five-fold. Expression plasmids containing deletions in the GST I promoter have been constructed. These constructs are being analyzed in this expressions system as well as by gel shift analysis and by DNA footprinting to identify *cis*-regulatory elements necessary for basal-level and chemically-inducible expression.

328 ARYLSULFATASE AS A REPORTER GENE IN *Chlamydomonas reinhardtii*. John P. Davies, Donald P. Weeks[1], and Arthur R. Grossman. Carnegie Institution of Washington, Department of Plant Biology, Stanford CA 94305 and [1]University of Nebraska, Center for Biotechnology, Lincoln NE 68583.

We are developing a reporter gene system in *C. reinhardtii* in which the coding region of the arylsulfatase (*ars*) gene is fused to a heterologous promoter such as that of the β_2-tubulin gene (β_2-*tub*). The rationale for using *ars* as a reporter gene rests on the finding that the endogenous *ars* gene is only transcribed when *C. reinhardtii* cells are starved for sulfur. Hence, in sulfur-replete medium the chimeric gene can be expressed when the endogenous gene is inactive. Furthermore, the Ars protein, located in the periplasmic space, cleaves sulfur from aromatic compounds, and can be easily assayed using the chromogenic substrate 5-bromo-4-chloro-3-indolyl sulfate (XSO_4).

To assess the usefullness of *ars* as a reporter gene, we are using a construct in which the promoter sequence from the *C. reinhardtii* β_2-*tub* was fused to the coding sequence of an *ars* genomic clone to form a β_2-*tub*/*ars* chimeric gene. The chimeric gene was introduced into CC425 (cw15 arg2) via cotransformation with the arginnosuccinate lyase gene (which complements the arg2 lesion) using the glass bead method. Transformants containing an intact copy of β_2-*tub*/*ars* exhibited constitutive expression of Ars. Expression of the chimeric gene was elevated immediately following deflagellation in a manner similar to the endogenous β_2-*tub* gene. This system is being used to assess the role of *cis*-acting sequences in the control of β_2-*tub* transcription.

329 CHARACTERIZATION OF RICE GLUTELIN TRANSCRIPTION FACTORS. Soo Young Kim, William D. Park and Ray Wu*, Dept. of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843 and *Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

Glutelin is the major seed storage protein of rice, constituting 80% of total endosperm protein of rice seed. Previously we cloned one of the glutelin genes, pGL5-1, and showed that several nuclear proteins of immature rice seed bind to the promoter region of pGL5-1. Two of the putative transcription factors, GT1 and GT3, bind to DNA sequences which are almost identical to each other and to the c-jun/GCN4 binding site. We investigated the two protein factors further. Competition gel-retardation assays where GT1 binding site was employed as a probe and GT3 binding site as a competitor DNA or vice versa, show that GT3 can bind to the GT1 site as well, whereas GT1 does not bind to the GT3 site. Gel-retardation assays using extracts prepared from leaf tissue indicate that GT3 is also present in leaf. As a preliminary study to clone the putative transcription factors we performed Southwestern blots of nuclear extracts prepared from immature rice seed. Our results indicate that factor GT1 is a 38 kd protein, which appears to be highly basic. Experiments to clone the gene encoding GT1 are now in progress and the result will be presented in the meeting.

POSTER ABSTRACTS

330 THE INCREDIBLE STABILITY OF HISTONE OCTAMERS IN PLANT NUCLEOSOMES: ROLE OF H2A AND H2B HISTONES. Charles P. Moehs and Steven Spiker. North Carolina State University, Department of Genetics, Raleigh, North Carolina 27695

The protein core of the nucleosome is composed of an octamer of histones (two each of H2A, H2B, H3 and H4) organized as an H3-H4 tetramer sandwiched between two H2A-H2B dimers. Our present knowledge of transcription dictates that "something" must happen to the octamer during transcription to allow polymerase to pass through a nucleosome. That is, the octamer must transiently be released from DNA and then reassociate, or the octamer itself must transiently dissociate and then reform. Evidence from vertebrate animal systems indicates that *in vitro* without DNA the octamer can exist as a discrete entity but comprises a *mixed associating system* in equilibrium between the octamers and the tetramers and dimers. We have used exclusion chromatography and equilibrium sedimentation to study the octamer isolated from wheat (*Triticum aestivum*). We have found that the plant octamer is incredibly stable, having an association constant approximately two orders of magnitude higher than vertebrate animal octamers. Because H3 and H4 histones are essentially identical molecules in plants and animals, we assume the enhanced stability of the wheat octamers is due to the properties of the evolutionarily variable H2A and H2B histones. We present evidence for this assumption based on reconstitution of octamers with wheat and chicken histones.

331 THE FEEDBACK CONTROL ELEMENT OF THE MAIZE SHRUNKEN GENE INTERACTS WITH A HOMEOBOX PROTEIN. Wolfgang Werr, Regina Bellmann, Christian Korfhage & Thomas Lugert. Institut fur Genetik, Universitat Koln, Weyertal 121, 5000 Koln 41, FRG.

A 26 bp region covering the transcription start site of the maize Shrunken promoter is sufficient for the initiation of transcription and determines the transcription rate according to the requirements of the cellular carbohydrate metabolism. Transcriptional activity is repressed at high sucrose concentrations and increases at high rates of UDP-glucose turnover. The underlaying regulatory principle explains the anaerobic induction of the Shrunken gene and is relevant for the partitioning of photoassimilate in the maize plant.

For the isolation of possible signal transducing DNA-binding proteins we have screened a lambda GT11 expression library for clones recognizing the 26 bp feedback element. Several clones encoding maize DNA-binding proteins have been identified interacting internally of the 26 bp transcription start region. In DNase I footprinting experiments one protein covers the transcription start site and a second one interacts immediately upstream. The transcription start factor does not show any homology to known DNA-binding protein sequence motives. The second maize nuclear protein shows homology to the DNA-binding homeobox motif and an overall structural relationship to the OCT2 transcription factor. As common to many transcriptional activators the N-terminal protein domain is highly acidic. Both proteins completely occupy the 26 bp feedback control element therefore two major components of the transcriptional feedback control may have been cloned.

332 POSITIVE REGULATORY ELEMENTS CONTROLING EF-1α GENE EXPRESSION IN *A thaliana* C. CURIE, M. AXELOS, T. LIBOZ, C BARDET, R. ATANASSOVA[1], N CHAUBET[1] and B. LESCURE. Laboratoire de biologie moleculaire des relations plantes-microorganismes CNRS-INRA, chemin de borde rouge, BP 27, 31326 Castanet-tolosan cedex FRANCE [1]Institut de biologie moleculaire des plantes, CNRS, 12 rue du general Zimmer, 67000 Strasbourg, FRANCE

In *A thaliana*, the translation elongation factor EF-1α is encoded by 4 genes. The activity of the A1 gene promoter deletions has been examined by transient expression in protoplasts, using the GUS reporter gene. One cis-acting domain, the TEF1 box, has been accurately mapped 100 bp upstream of the transcription initiation site. This domain can act in the two orientations and is the target for trans-acting factors identified in nuclear extracts. The four EF-1α genes contain two introns, one of which is located within the 5' untranslated region. In the A1 gene, deletion of this 5' IVS decreases by 10 to 20 fold the activity of the promoter. When the 5' IVS is replaced by the 3' short intron of the coding region, part of the promoter activity is restored. This implies that (i) the presence of an intron stabilizes the A1-GUS chimaeric mRNA, either by increasing the rate of transcript accumulation in the cytoplasm, or by protecting it from the exonucleases degradation; (ii) The difference of activation between the 5' IVS and the 3' IVS could be explained by the presence of a positive regulatory element. Moreover, addition of the A1 5' IVS in a 35S promoter context shows that this intron contains a regulatory element which only works in the A1 promoter context. In addition, neither of the two elements, the TEF1 box or the 5'IVS, is functional in the absence of the other, suggesting that they probably act in synergy to activate EF-1α A1 gene expression. Most of the deletions have also been tested in stably transformed plants and give activities in good agreement with results obtained in transient expression assays.

333 VARIABILITY IN THE EXPRESSION LEVELS OF GENES DIRECTED BY A ROOT SPECIFIC PROMOTOR. Antje Dietz, Cornelia van der Hoeven and Jorg Landsmann. Biolog. Bundesanstalt, Inst. f. Biochemie, W-3300 Braunschweig, FRG

Nicotiana tabacum var. W38 and Petunia hybrida var. RL01 were transformed by the Agrobacterium tumefaciens leaf disk method. Different vector constructions with the root specific PAR promoter (from the Parasponia andersonii hemoglobin gene) fused to CAT (chloramphenicol-acetyl-transferase) and GUS (β-glucuronidase) reporter genes were used. In 10 PAR-CAT transformed plants (out of 86) and in 28 PAR-GUS transformed plants (out of 140) the tissue specificity of the PAR promoter had been lost so that the reporter gene was expressed in leaves as well. The PAR-GUS tobacco plants are being tested for the effects of developmental state and gene copy number on the level of reporter gene expression. For some PAR-CAT tobacco plants investigated we found a correlation between the expression levels of the CAT gene and the linked selection marker gene NPT. To look for effects of plant sequences at the integration site on the expression of the introduced reporter gene, we constructed genomic clone banks of leaf expressing PAR-CAT transformants. Two different clones were isolated from the petunia clone bank, which contain the introduced genes plus plant DNA of the integration site. The T-DNA border sequences with the adjacent plant DNA are being sequenced.

334 THE INTERACTION OF THE OPAQUE-2 TRANSCRIPTION FACTOR OF MAIZE WITH TARGET PROMOTER SEQUENCES. Stefan Lohmer, Massimo Maddaloni, Hans Hartings, Mario Motto, Francesco Salamini, Richard Thompson. Max-Planck-Institut für Züchtungsforschung, W-5000 Köln 30, FRG

The Opaque-2 (O2) locus of maize has been shown to control the synthesis of a group of maize storage proteins, the 22 kD zein class, and a number of non-zein polypeptides including a 32 kD albumin, b-32. Molecular analysis of the O2 gene indicates that it encodes a DNA-binding protein. We demonstrate that the O2 gene product can bind in vitro, and activate in vivo target promoter sequences derived from a b-32 genomic clone, providing evidence that the O2-gene product is a transcription factor. The ability of O2 protein to activate transcription has been shown to depend on the presence of specific regions of the O2 gene sequence, corresponding to the likely site of the transcriptional activation domain.

The canonical binding site for the O2-protein is homologous to part of the -300 or endosperm box, a motif common to endosperm-specific promoters of cereals, and implicated by deletion analyses in transgenic plants with a role in conferring endosperm-specific expression.

335 ENHANCER ELEMENTS FROM HIGHLY EXPRESSED PHOTOSYNTHETIC GENES DO NOT FUNCTION WHEN CLONED INTO THE CAMV 35S AND NOPALINE SYNTHASE CONSTITUTIV PROMOTERS. Mark H. Harpster, Karin Breck, Vida Vameghi-Myers and Pamela Dunsmuir. DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608. Previous studies conducted in this laboratory have identified "enhancer-like" elements within the promoter regions of the most highly expressed ribulose bisphosphate carboxylase small subunit (SSU) genes and chlorophyll a/b binding protein (cab) genes of petunia. To determine whether these elements can be combined with constitutive promoters, we cloned these elements into the Cauliflower mosaic virus 35S promoter and nopaline synthase promoter, both of which were fused to a reporter gene; the chitinase B (chiB) gene of Serratia marcescens. For the analysis of chiB expression levels in transformants containing these enhancer element constructs, we extracted total RNA from a minimum of 10 independent transformants/construct and measured steady-state levels of chiB mRNA by the method of primer extension. Our results show no enhancement of gene expression compared to control plants which lack the SSU and cab enhancer elements within the constitutive promoters. This was true regardless of the number of elements, their orientation in the promoter and the age or growth conditions of the plant. These results indicate that putative "enhancer-like" elements cannot necessarily be transposed to foreign promoters and expected to retain their original properties.

POSTER ABSTRACTS

336 Agrobacterium Mediated Transformation in Some Legume Cultivars in Egypt.

MAHMOUD, S., SOLIMAN, M., ABOU-SALEH, A., and MADKOUR, M.A.

National Agricultural Genetic Engineering Laboratory (NAGEL), Giza, Egypt.

To develop a universal transformation system, *Agrobacterium tumefaciens* (C58)was used to infect the most commonly used cultivars of the major legumes in Egypt. The cultivars were *Vicia faba* cv. Giza2, *phaseolus vulgaris* cv. Giza6, *Vigna unguiculata* cv. cream7 and *Pisum sativum* cv. Little Marvel. The response of each cultivar to the infection by *Agrobacterium* Ti-plasmid was recorded as a number of days from infection to tumor formation, the size of the tumor and the distance between tumor location and soil surface. The results showed that there was no significant variation among legumes in regard to tumor location. However, tumor size and number of days to tumor formation varied considerably among the tested legumes. *Vicia faba* was found to be the most sensitive species whereas *Phaseolus vulgaris* was the least sensitive. *Agrobacterium* identification tests like 3-ketolactose, kado's and the ability not to grow at 37°C confirmed that *Agrobacterium* Ti-plasmid is the causative of the tumor. Southern blot hybridization proved that T-DNA was integrated into the nuclear genome of the infected plants.

337 ALTERED RIPENING OF TRANSGENIC TOMATOES EXPRESSING AN ANTISENSE GENE FOR ETHYLENE FORMING ENZYME (EFE). Steve Picton, Sarah Barton, Andy Hamilton and Don Grierson. University of Nottingham, Dept. Physiology & Environmental Science, LE12 5RD, U.K.

Ethylene is associated with many physiological processes in higher plants, including the onset of ripening in climacteric fruits. Ethylene forming enzyme catalyses the terminal step in ethylene biosynthesis but has not been well characterized. Using antisense expression we have identified a cDNA (pTOM13) from a ripening tomato fruit library that causes down regulation of EFE activity in transgenic plants. The antisense genes is stably inherited and causes an 87% reduction in ripening-related ethylene synthesis in fruit from hemizygous plants and a 97% reduction in homozygotes. Fruit homozygous for the antisense gene initiate colour change at a similar chronological stage to wild-type fruit but, on extended storage, show reduced reddening and an increased resistance to over-ripening and the associated problems of shrivelling and splitting of the fruit. Ripening can be accelerated by adding ethylene. Results will be presented and discussed showing the effects of antisense EFE and added ethylene on various aspects of fruit ripening, including pigment accumulation, expression levels of previously identified ripening-related mRNAs and changes in the protein profile during the ripening process.

338 ANALYSIS OF ELITE TRANSGENIC MAIZE PLANTS PRODUCED BY MICROPROJECTILE BOMBARDMENT. S. Jayne, M. Hill, G. Crawford, D. Murray, J. Dietz, J. Suttie, E. Dunder, K. Lewis, J. Reed, M. Koziel, G. Jen, G. Pace CIBA-GEIGY Agricultural Biotechnology, POB 12257, Research Triangle Park, NC 27709.

Transgenic maize plants of an elite B73 derived inbred line were produced by microprojectile bombardment. Plasmid DNA containing genes for hygromycin phosphotransferase (HPT) and beta-glucuronidase (GUS) was precipitated onto tungsten particles using standard methods, and delivered to embryogenic cultures. Transformed colonies were recovered after selection on hygromycin-containing medium for several weeks. Plants were grown to maturity and seeds were obtained. Southern analyses of one transformed line showed the presence of both genes in the T0, and T1 plants. Diagnostic Southerns showed the HPT gene was intact but the GUS gene was not. Consistent with this finding, HPT but not GUS enzyme activity was detected in the transformed plants. Southern analysis of the T2 progenies indicated that all the insert DNA was in three loci on one chromosome.

339 APH TYPE II GENE TRANSFERRED TO AMARANTHUS VIA RI PLASMID VECTORS. P.K.Chand and A.Pal. Botany Dept. Utkal University, Bhubaneswar-751004. India.

Engineered Agrobacterium rhizogenes: A4R 160-1 (pRiAub-pTK 291; co-integrate vector) and LBA 9402 (pRi1855-pBin 19; binary vector) harbouring Tn5-derived APH(3') typeII gene for aminoglycoside (Kanamycin) tolerance were employed in explant inoculation. Decapitated seedlings, internodes and leaf midribs of Amaranthus caudatus, A.dubius and A.hypochondriacus were used. The rooting characteristic viz. profusion, hairyness tropism and antibiotic tolerance was dependent on the plant species. Excised single roots were cloned separately on MSO agar medium minus phytonormones but with added cefotaxime (500ug/ml) and Kanamycin sulphate (100 ug/ml). All transformed root cultures tolerated the antibiotic upto 200 ug/ml. Acetocarmine preparation of transformed root tips of A.caudatus and A. hypocnondriacus revealed mitotic counts of 2n=32+1, whilst that of A. dubius was 2n=64+2. Plant regeneration was via an intermediate callusing stage. Regenerated plants survived on Kanamycin (50-200 ug/ml.). The presence of the foreign gene was detected by DNA-DNA hybridization and gene expression confirmed by opine (agropine and/or mannopine) positivity as well as aminoglycoside phosphotransferase (APH) activity.

340 Characterization of a manganese superoxide dismutase cDNA from Pea. Luis Wong-Vega and Randy D. Allen. Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409.

A full length cDNA which encodes the manganese containing superoxide dismutase (MnSOD) was isolated from a pea λgt10 library. This SOD isoform is localized in mitochondria where it functions in the scavenging of superoxide radicals that are produced in oxidative reactions. Sequence analysis of this cDNA indicates the presence of a mitochondrial transit sequence and signature sequences unique to MnSODs. Sequence comparison with other Mn and Fe SODs indicates strong similarity. Genomic Southern blot analysis, suggests that MnSOD is encoded by a single gene in the pea genome. Analysis of the expression pattern of the pea MnSOD gene in plants grown under normal and stressful conditions is underway. Attempts to develop transgenic tobacco and cotton plants that over-express pea MnSODs that are targeted to various cellular compartments are currently ongoing.

341 CHARACTERIZATION AND CELLULAR LOCALIZATION OF *BRASSICA* ANTHER SPECIFIC GENES. Jennie B. Shen and Francis C. Hsu, Agricultural Products, Du Pont Corp., Wilmington, DE 19880-0402

A cDNA library of *Brassica* was constructed using poly(A)$^+$-RNA isolated from developing anthers in 2-3 mm flower buds. Differential hybridization, using cDNA probes complementary to poly(A)$^+$-RNA purified from either the developing anthers or seedlings, was used for screening. Sixteen putative anther specific clones were isolated. Blots of RNA purified from developing anthers, pistils, seedlings, fully-developed leaves and stems were hybridized using labeled cDNA fragments as probes. Six clones (#42, 42W, 54, 73, 112 and 158) were characterized, and all showed anther specificity. The sizes of their messages ranged from 0.7-1.35 kb. The sequence of cDNA clone #42 shared significant homology with chalcone synthase from other plant species at both the nucleotide and deduced amino acid levels. Sequences of the remainder of the cDNA clones did not show homologies with any other known sequences. *In situ* hybridizations were performed to analyze mRNA accumulation at the cellular level. Pollen-specific, tapetum-specific and other cell types gene expression patterns will be presented.

POSTER ABSTRACTS

342 CHIMAERIC HEAT SHOCK-ADH GENES CAUSE HEAT INDUCIBLE ENZYME ACTIVITY IN TRANSGENIC *ARABIDOPSIS*.

Fritz Schöffl, Armin Wagner and Klaus Severin.
University of Tübingen, Biology, D-74 Tübingen, FRG

We have constructed gene fusions using either the soybean heat shock (hs) gene *Gmhsp17.6-L* or a synthetic HSE promoter and the *Arabidopsis* alcohol dehydrogenase (Adh) gene. A promoter fusion, transcriptional and translational fusion constructs were introduced via *BIN19* vectors into a Adh null line of *Arabidopsis*. Heat inducible Adh levels in leaves and germinating seeds vary for the different constructs. Trancriptional and translational controls may be involved in the regulation of gene expression. The capacity to exhibit heat inducible Adh activity correlates with the susceptibility of germinating seeds to allyl alcohol treatment. This heat inducible sensitivity of transgenic plants may have the potential to aid selection of *trans*-acting mutants affecting the heat shock response.

343 CLONING AND REGULATION OF A cDNA ENCODING THE BIFUNCTIONAL ENZYME ASPARTOKINASE-HOMOSERINE DEHYDROGENASE FROM CARROT. B.F. Matthews, B.J. Wilson, J.M. Weisemann. USDA, ARS, PSI, Plant Molecular Biology Laboratory, Beltsville, Maryland 20705

Aspartokinase (AK) and homoserine dehydrogenase (HSDH) are key enzymes in the biosynthesis of the aspartate family of amino acids. These two enzyme activities reside on different protein molecules in several prokaryotes. However, in E. coli, the two enzyme activities are present on the same protein forming a bifunctional enzyme. We have purified AK and HSDH activities from carrot cell suspension cultures. The two activities coelute from chromatography columns and are present at the same location on electrophoretic gels, as indicated by enzyme specific staining and immunodetection techniques. The purified protein was proteolytically digested and fragments were subjected to amino acid sequencing. One fragment was similar in sequence to a portion of the AK domain of E. coli AKI-HSDHI, while another fragment's sequence was similar to the HSDH domain. Through PCR amplification and cDNA library screening a carrot cDNA clone was identified that encoded a protein with 48% amino acid homology to the thrA gene product, AKI-HSDHI from E. coli. Carrot cell suspension cultures grown in the presence or absence of end products were analyzed for enzyme activities and amounts of AK-HSDH mRNA. Similar analyses were conducted on leaves and roots of carrots of different ages, grown under different conditions.

344 CLONING THE RICE ACC SYNTHASE GENES*

Thomas J. Zarembinski and Athanasios Theologis. Plant Gene Expression Center, ARS-USDA, 800 Buchanan St., Albany, CA 94710 and Dept. of Plant Biol., UC Berkeley, CA 94720.

Ethylene is a gaseous plant hormone which affects many aspects of plant growth and development. 1-aminocyclopropane-1-carboxylic acid (ACC) synthase catalyzes the key regulatory step in the ethylene biosynthetic pathway. It converts S-adenosylmethionine (AdoMet) to ACC, the precursor of ethylene. We have cloned three rice (*Oryza sativa* L.) ACC synthase genes using degenerate oligonucleotides and the Polymerase Chain Reaction (PCR). The protein sequence encoded by one of the rice genes (OS-ACC1) shares significant homology to the ACC synthase sequences from *Curcubita pepo*(1,2), *Lycopersicon esculentum*(3), and *Arabidopsis thaliana*(4). The sequence of the other genes has not yet been determined. Northern analysis shows OS-ACC1 expression is greatly enhanced by indole-3-acetic acid plus benzyladenine plus LiCl. The isolation and characterization of this gene is the first step towards understanding the regulation of ethylene production and ethylene-mediated shoot elongation in rice.

(1) Sato, T. & Theologis, A. (1989). *PNAS* 86, 6621-6625
(2) Sato, T., Oeller, P.W. & Theologis, A. (1991). *J. Biol. Chem.* 266, 3752-3759
(3) Rottmann, W.H., et al. (1991). *J. Mol. Biol.* submitted
(4) Liang, X., Keller, J. & Theologis, A. (1991). in preparation

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345 COLLAGENASE EXPRESSION IN TRANSGENIC PLANTS: AN ALTERNATIVE TO NEMATICIDES. P. Havstad, D. Sutton, S. Thomas, C. Sengupta-Gopalan, J. Kemp. New Mexico State Univ., Plant Genetic Eng. Lab., Las Cruces, NM 88003

The human gene for collagenase has been engineered into plants to generate nematicidal activity. This activity provides an alternative to chemical pesticides for the control of economically damaging plant parasitic nematodes. We have acquired the full length c-DNA clones for three different human collagenases each exhibiting different substrate specificity; a collagenase pCol 185.2, a stromelysin pStrom, and a gelatinase pGel 186.2 (Arthur Eisen, Washington University School of Medicine). These c-DNAs have been tailored by us for expression in plants by using the pMon 316 cloning vector (Monsanto Corp., St. Louis, MO) modified by adding the AMV translation enhancement sequence. Two constructs of each c-DNA were made; one retaining the sequences for the human signal peptide, procollagenase peptide, mature collagenase peptide, and human untranslated 3' poly-A (complete), and the second retaining just the mature collagenase sequence, with or without the human poly-A (mature). mRNA accumulates in leaves of tobacco transformed with both mature pCol 185.2 and mature pStrom, with the highest level of RNA accumulating in plants containing a mature pStrom and a plant poly-A. Western data are supportive of mature protein accumulation. We are currently analyzing plants transformed with the complete constructs of pStrom and pCol 185.2, and tailoring pGel 186.2 for expression in plants. Nematode-effect assays with *Meloidogyne* (rootknot) and *Pratylenchus* are currently underway, as are crosses between plants containing the different c-DNAs. Other agronomically significant crop plants, including cotton, have been transformed with the various collagenase constructs.

346 CONSTRUCTION OF A BINARY VECTOR TO REDUCE THE TRANSCRIPTIONAL INTERFERENCE FROM ENDOGENOUS PROMOTERS. Madan K. Bhattacharyya, Bruce A. Stermer and Richard A. Dixon. Plant Biology Division, The Noble Foundation, P. O. Box 2180, Ardmore, Oklahoma 73402, USA.

Transformation mediated by Agrobacterium tumefaciens is the preferred method for gene transfer in many plant species. However, functional analysis of promoters by this procedure can be very difficult due to the variability among the transgenic plants carrying the same construct. We propose that part of such variability could be explained by transcriptional interference. Koncz et al. 1989 (PNAS, 86: 8467-8471) concluded that on average 30% of the T-DNA insertions occur into transcriptionally active regions of both Arabidopsis and Nicotiana. Thus a significant proportion of T-DNA, upon insertion, is flanked by potentially active endogenous promoters. Transcription from such flanking promoters can cause transcriptional interference or promoter occlusion on the downstream promoters in the T-DNA. Expression of the gene inside the T-DNA also may be inhibited to a large extent by the transcription initiated from a flanking promoter if the 3' end of the T-DNA gene fuses with this promoter. Active transcription from an endogenous promoter that fuses with the 3' end of a T-DNA gene can (i) produce the antisense strand of the gene, and (ii) interfere the transcriptional machinery of the sense strand. A binary vector has been constructed to minimize these two possible kinds of transcriptional interference. Data from transgenic tobacco using this vector will be presented and discussed.

347 CRYOBIOLISTICS: THE BIOLISTIC TRANSFORMATION OF PLANT CELLS USING FROZEN DNA AS MICROPROJECTILES. Chris Brinegar[1,2], James Gilmore[1,2], Michael Johnson[2] and Nigel Walker[2].
San Jose State University[1], Department of Biological Sciences, San Jose, CA 95192 and NitroGenes, Inc.[2], 6385 Camino Verde, San Jose, CA 95119

The biolistic transformation method (Klein, et al., 1987 Nature 327:70-73) involves the high-velocity acceleration of DNA-coated microprojectiles into plant cells. These microprojectiles are typically micron-sized tungsten spheres coated with plasmid DNA. In this presentation we show that metal microprojectiles are not a necessary component of biolistic transformation. By freezing solutions of DNA into macroprojectiles and accelerating them with compressed air through a stopping plate, the frozen block of DNA can be shattered into pieces of ice small enough to penetrate individual cells and promote transient transformation as measured by the histochemical beta-glucuronidase (GUS) assay. We call this method "cryobiolistics." Preliminary results, using pBI121 (BUS/Kan^R) to transform cells from tobacco suspension cultures, show transformation rates up to 100 cells per shot with a plasmid concentration of 0.2 mg/ml and volume of 40 µl. Although this transformation efficiency is currently lower and more variable than the microprojectile method, cryobiolistics has several potential advantages, e.g., ease of preparation, capability of delivering more DNA per cell, better control of DNA concentration, and the ability to deliver other biologically active substances (enzymes, antibodies, viruses, etc.) to the cell.

POSTER ABSTRACTS

348 A COMPARISON OF TRANSFORMATION EFFICIENCY OF RICE PROTOPLASTS FROM CALLI AND CELL SUSPENSIONS. Maria-Jesus Cornejo, Diane Luth Olin D. Anderson, Ann Blechl and Frank C. Greene. USDA, ARS, Western Regional Research Center, 800 Buchanan, Albany, California 94710

We compared the transformation efficiency of Japonica rice (cv. Taipei 309, Calmochi 101) protoplasts prepared from both calli and cell suspensions and their subsequent competencies for callus formation and plant regeneration. Transient luciferase and GUS expression was used to evaluate the relative strength of three promoters: 35S CaMV, maize ADH and maize ubiquitin. Gene expression levels from the ubiquitin promoter and its intron were at least 7X the level obtained from ADH and its intron and 14X the level from 35S CaMV with the ADH intron. Thus, the ubiquitin promoter/intron greatly stimulates gene expression in Japonica cultivars, as shown for Indica rice by Wirtz and Baker (personal communication). Protoplasts derived from calli supported gene expression from the ADH promoter/intron GUS or luciferase at levels equal or greater than protoplasts from cell suspensions. Protoplasts from either source divided after PEG-mediated transformation and produced calli which differenciated into plants. Our results show that calli-derived protoplasts can be efficiently transformed using PEG, extending previous results using electroporation (Cornejo and Walbot, 1990). Protoplasts for rice transformation can be obtained directly from calli, thus eliminating the need for a cell suspension.

349 COMPARISON OF PERFORMANCE CHARACTERISTICS OF DIFFERENT BIOLISTIC DEVICES. Erik Dunder, Tim Harris, Art Weissinger, Martha Wright, Cindy Bowman, Karen Launis, Janet Reed, Jan Suttie, Susan Jayne, George Jen and Gary Pace. CIBA-GEIGY Agricultural Biotechnology, POB 12257, Research Triangle Park, NC, 27709.

Three different designs of the Biolistic device developed by Cornell University and DuPont have been evaluated over the last several years in our laboratory. The efficiency of target coverage and reproducibility of shot pattern were assessed for the older "dual chamber" device introduced by John Sanford, the PDS-1000 introduced by DuPont and the recently available PDS-1000/He, a device powered by compressed helium. Performance characteristics were determined using transient expression of a chimeric GUS gene delivered to different types of maize cell and tissue cultures. Shot patterns were also determined for the helium device using biotinylated DNA precipitated onto the gold particles. These patterns were then compared to the distribution of GUS foci developed in situ. Overall comparisons indicated that the helium device exhibited a 5 to 10 fold improvement in the delivery of "biologically effective" particles and a significantly greater target area compared to both of the devices based on gunpowder.

350 CONSTRUCTION OF A PROMOTER THAT RESPONDS TO LOW LEVELS OF TETRACYCLINE. Christiane Gatz, Claus Frohberg and Regina Wendenburg, Institut für Genbiologische Forschung, GmbH, Ihnestr..63.1 Berlin 33, FRG.

We have established the use of the Tn10-e ncoded Tet respressor-operator system to regulate the expression of a suitably engineered Cauliflower Mosaic Virus (CaMV) 35S promoter in transgenic tobacco plants. First, transgenic plants were generated which constitutively synthesize the Tet repressor protein. In a second transformation step, the β-glucuronidase (gus) gene under the control of a modified CaMV 35S promoter, containing two tet operators, was stably integrated into the plant genome of a tetR+ plant. Expression of the gus gene is repressed down to very low levels. Maximal induction is achieved already after 0.5h upon application of only 0.1 mg/l tetracycline (Tc) throughout the plant. This indicates that Tet repressor-operator complexes can form on plant chromosomes and block transcription. The fast and efficient induction makes the system extremely useful for specifically inducing expression of transferred genes at different stages of plant development without affecting other endogenous functions.

351 CORRECT EXPRESSION OF THE *GUS* GENE INTERRUPTED BY AN INTRON IN VARIOUS SOLANACEAE AND LEGUMINOSAE PLANTS. Edgardo Filippone, Teodoro Cardi*, Giulio Bile, Vittoria Iannamico, Monica Leone*, Maurilia M. Monti, and Raoul Penza. University of Naples, Department of Plant Genetics, Portici, Italy 80055; *Res. Ctr for Vegetable Breeding, Portici, Italy 80055.

To prevent the expression of the *gus* gene in bacteria, the *gus* coding region was interrupted by the IV2 intron from the potato gene ST-LS1 (Eckes *et al.*, Mol Gen Genet *199*: 216-224). The p35S CaMV GUS-INT binary vector (Vancanneyt *et al.*, Mol Gen Genet *220*: 245-250) was introduced into two *Agrobacterium tumefactens* strains (GV2260 and EHA105). Genetic transformation was carried out on leaf explants of some Solanaceae species (*Nicotiana tabacum, Petunia hybrida, Solanum commersonii,* and *Solanum melongena*) and on mature embryos of two Leguminosae species (*Cicer arietinum* and *Vigna unguiculata*). No significant difference in virulence of the two strains was observed. Genetic transformation, expressed as percentage of explants showing positive *gus* activity, ranked from 83% (*Nicotiana tabacum*) to 11% (*Cicer arietinum*). *Gus* activity was observed in proliferating explants, in regenerating tissues (either *via* embryogenesis or *via* organogenesis) and in regenerated plantlets. The results obtained indicate that the het RNA carrying this intron is corrected recognized and spliced in different taxa, in tissues at different level of differentiation, and in different organs.

352 DELETION OF A PROMOTER ELEMENT OF THE PATATIN PROMOTER INCREASED THE MAGNITUDE OF POSITION EFFECT IN TRANSGENIC POTATO PLANTS.

Yonatan Elkind[1], Andrzej Kilian[2] and Richard A. Jefferson[3]. [1]Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76-100, Israel. [2]University Slaski, Katowice, Poland. [3]Center for the Application of Molecular Biology to International Agriculture.

We analyzed approximately 400 potato plantsunder field conditions, representing replicates of 70 independent transgenic potato plants. Each of these transgenic plants contained one of four patatin promoter deletion derivatives fused to a GUS reporter gene. The distribution of GUS activity in tubers of independent transformants containing the shorter constructs was bimodal, as compared to a normal distribution in those with long constructs. While the mean GUS activity was lower in plants containing shorter promoter derivatives than that of transformants with the longer promoter, variation in the former, presumably due to position effect, was 10 times larger. This is consistent with a model in which cis acting element(s) in the patatin promoter interacts with elements of the insertion sites, which in turn reduces the magnitude of the effect on the expression of the inserted gene.

It is concluded that interaction between a promoter and regulatory elements characteristic to the insertion site (lack of additive effects) can be detected by heterogeneity of the position effect variation. This interaction can lead to misleading conclusions about the effect of promoter deletions based only on the mean gene expression of transgenic plants.

353 DELIVERY AND EXPRESSION OF PLASMID DNA IN Z. mays (MAIZE) MICROSPORES. Anne Fennell & Randal Hauptmann. Northern Illinois University, Plant Molecular Biology Center, Montgomery Hall, DeKalb, Illinois 60115.

To study gene expression in gametophytic tissue, we tested the ability to deliver and detect reporter gene activity in maize microspores, using promoters that are not pollen specific in activity. Expression vectors consisted of CaMV 35S, maize alcohol dehydrogenase 1 (Adh1), or maize ubiquitin (UBI) promoters fused to the coding region of the chloramphenicol acetyltransferase (CAT) gene and terminated by the polyadenylation signals of the nopaline synthase gene. These constructs were introduced into maize microspores isolated at the late uninucleate stage or maize protoplasts using electroporation or polyethylene glycol (PEG). Both electroporation and PEG were effective for delivering free DNA to microspores. The three promoters tested, CaMV 35S, Adh1 and UBI, have been shown to be active in maize protoplasts. The CaMV 35S promoter has been reported to have low activity in pollen and we found no activity in maize microspores. Adh1 has been shown to be actively expressed in pollen, however, it did not promote expression in maize microspores. The ubiquitin promoter produced high levels of CAT activity in maize microspores. These results demonstrate that foreign DNA can be introduced and expressed in microspores using promoters that are not pollen specific. In addition, this free DNA delivery to microspores may provide an efficient alternative strategy for plant transformation.

POSTER ABSTRACTS

354 DEVELOPMENT OF A CHEMICALLY-INDUCIBLE GENE EXPRESSION SYSTEM FOR TRANSGENIC PLANTS. Howard Hershey, Mark* Chapman*, Hugo Dooner*, James English*, Edward Ralston*, Sonja Schmitz, Timothy Stoner and James Wong. Du Pont Agricultural Products, Wilmington, DE 19880 and *DNA Plant Technology Corp., Oakland CA 94608

Several cDNA clones representing maize mRNA species which are induced by substituted benzenesulfonamides were isolated and used as probes to obtain the corresponding genomic clones. Studies examining the induction of these sequences showed good structural specificity for plant responses to inducers following chemical treatment and a rapid appearance and disappearance of induced mRNAs. Promoter sequences from two genes designated In2-1 and In2-2 were isolated and fused to a GUS reporter gene. The chemically-induced expression of the resulting genes was analyzed by transient assays using a monocot protoplast transformation system. In this manner, the locations of cis-acting elements within the inducible promoters that are necessary for chemical responsiveness were identified. These inducible promoter/GUS genes were also stably introduced into a number of plants species. The results of these studies indicate that the In2-1 and In2-2 promoters are chemically responsive in transgenic plants, indicating that they may be useful in developing a novel inducible gene expression system that may have both research and commercial applications.

355 DEVELOPMENT OF GENETIC TRANSFORMATION SYSTEMS FOR MICROALGAE. Kathryn G. Zeiler, Eric E. Jarvis, Terri G. Dunahay, Paul G Roessler and Lewis M. Brown. Solar Energy Research Institute, Golden, Colorado, 80401.

Several microalgal species have been identified that will accumulate lipids under conditions of nutrient stress. Our goal is to utilize genetic engineering techniques to optimize lipid production and growth characteristics in order to achieve economically viable liquid fuel production from algal lipids. As a preliminary step in the development of genetic transformation systems, a transient assay using the firefly luciferase gene was applied successfully to protoplasts of the green alga *Chlorella ellipsoidea* (Jarvis and Brown, 1991, Curr. Genet., in press). However, successful stable transformation in another green alga, *Chlamydomonas reinhardtii*, required the use of homologous genes as selectable markers, most likely due to a GC codon bias (Mayfield and Kindle, 1990, PNAS 87:2087). DNA from several green algae in our collection were also found to be GC-rich, up to 71%, indicating the need to develop homologous marker systems for these organisms. Putative nitrate reductase mutants were isolated from the green alga *Monoraphidium minutum* and the diatom *Cyclotella cryptica*. The mutants are stable and show the expected inability to utilize nitrate as a nitrogen source and are resistant to chlorate. We have also isolated putative orotidine-5'-phosphate decarboxylase mutants in *M. minutum*. These mutants require exogenously added pyrimidines for growth. Both sets of mutants are being characterized biochemically to determine if the lesions are in the structural gene. Heterologous probes and the DNA amplification are being used to isolate the corresponding wild-type genes from genomic and cDNA libraries for use in the development of microalgal transformation systems.

356 DEVELOPMENTAL REGULATION OF THE ACC SYNTHASE GENE FAMILY IN TOMATO *

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ACC synthase is the key regulatory enzyme in the ethylene biosynthetic pathway. Five genes which encode ACC synthase isoenzymes in tomato (*Lycopersicon esculentum* Mill) have been cloned, mapped, and structurally characterized[1]. Two strategies have been used to determine their developmental expression and the type of inducers that activate them. First, promoter sequences have been isolated from each gene, fused to the GUS reporter gene, and introduced into tomato by Ti-mediated transformation. GUS activity has been monitored during plant development. Second, the Polymerase Chain Reaction has been used to isolate cDNAs for each gene, following treatment of tomato tissues with known inducers of ACC synthase activity. Differential expression of these ACC synthase genes is believed responsible for the complex temporal and spatial regulation of ethylene biosynthesis in tomato.

[1] J Rottmann, et al. J. Mol. Biol. Submitted.

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357 DIRECT-GENE-TRANSFER TO RAPESEED PROTOPLASTS BY ELECTROPORATION. Per Bergman and Kristina Glimelius. Swedish University of Agricultural Sciences, Department of Plant Breeding, P.O Box 7003, S-750 07 Uppsala, Sweden.

Recent advances in tissue-culture and recombinant-DNA technology have made it possible to transform higher plants with genes of interest. One of the most widely used methods for gene transfer is based on *Agrobacterium tumefaciens* ability to transfer DNA to receptive tissues. However monocots (e.g. cereals) and many dicots cannot be efficiently transformed using *Agrobacterium* mediated gene-transfer. As a consequence several methods for direct-gene-transfer have been developed for a number of plant species. We describe here our efforts to develop a reproducible and efficient method for direct-gene-transfer to protoplasts isolated from *Brassica napus* hypocotyls. Protoplasts were transfected with plasmid DNA using the method of electroporation. To monitor plasmid transfer efficiency a plasmid was used which allows the expression of a screenable marker-gene for transient expression analysis (the ß-glucuronidase, "Gus"-gene), as well as a resistance marker gene for selection of stable transformants, the neomycin phosphotransferase (NPTII) gene. To determine the most suitable conditions for gene-transfer, parameters such as discharge potential, protoplast density, electroporation-buffer composition and plasmid-vector concentration were tested using transient Gus-assays. Recently we have used conditions defined above for gene transfer to determine suitable conditions for selection of stable transformants. Our results indicate that exponentially decaying pulses of about 700 V/cm result in frequencies of transient expression in protoplasts of about 0.1%, at a concentration of 1.5×10^6 protoplasts and 100 µg reporter plasmid per ml. Using these conditions, electroporated protoplasts were grown to calli under different selective regimes. After 8 weeks of selection for kanamycin resistance 1 - 20 growing calli were isolated per 10^6 electroporated protoplasts, depending on the conditions used. About 2% of the kanamycin resistant calli generated plants. To confirm their transgenic nature, NPTII-dot blott assays will be performed. The obtained results will be presented and discussed.

358 THE EFFECT OF CHROMOSOMAL SCAFFOLD ASSOCIATED REGIONS (SARS) ON TRANSGENIC POSITION EFFECTS Rosalind E. Slatter and J. C. Gray. Botany School, University of Cambridge, U.K.

The chromatin fibre of eukaryotic chromosomes is organised above the level of the 30nm fibre into domains or loops. Each loop of 5 to 200kbp is anchored at the base to a scaffold composed of non-histone proteins. SARs have been examined in many non-plant organisms and been found to have certain features in common. They generally cover several hundred base pairs, are composed of A/T-rich DNA, and contain sequences with homology to topoisomerase II binding sites. They have often been found to co-map with enhancer regions and in some cases with autonomously replicating sequences (ARSs). These features have lead to speculation that torsional stress regulating enzymes, transcriptional enhancer proteins and even replication complexes may bind to SARs to exert their effects on each loop and the genes therein, making each loop somewhat independent of its neighbours. In fact some non-plant experiments have shown that cloning SARs upstream and downstream of a reporter gene have lead to an alleviation of commonly observed position effects on expression of the transgene. Previous studies on pea (*Pisum sativum*) have allowed localisation of an SAR to a 77% A/T-rich region about 8kbp downstream of the single copy plastocyanin gene. This region has been shown to bind purified scaffolds *in vitro*. A 2.7kbp fragment containing this SAR has been cloned upstream and/or downstream of the GUS reporter gene in various combinations and orientations, and these constructs have been put into tobacco (*Nicotianum tabacum*), together with controls using λDNA fragments instead of the SAR. Analysis of these transgenic plants is currently underway.

359 EFFECT OF NUCLEASES RELEASED FROM GERMINATING POLLEN ON FOREIGN DNA DURING POLLEN ELECTROTRANSFORMATION. Sally L. Van Wert and James A. Saunders. USDA, Agricultural Research Service, Soybean and Alfalfa Research Laboratory, Beltsville, Maryland, 20705.

Pollen electrotransformation is a technique being developed in our lab to genetically modify plants by the electroporation of foreign DNA into germinating pollen. The reported release of DNA nucleases from germinating pollen grains implied that foreign DNA added to germinating pollen would be degraded within a few minutes. We added plasmid pBI221 DNA (20µ g) to germinating tobacco (*Nicotiana gossei*), corn (*Zea mays*) and alfalfa (*Medicago sativa* L.) pollen (4 mg) and observed the effect of released nucleases on the DNA by agarose gel electrophoresis and transient expression assays. Isolated DNA from the pollen/pBI221 suspensions showed increased degradation of pBI221 as incubation time with the pollen increased. Electroporation immediately after the addition of pBI221 had little effect on the amount of degradation. The addition of increasing amounts of EDTA or $MgSO_4$ to the pollen suspension after germination but before the addition of pBI221, followed by electroporation, resulted in decreasing DNase activity. Changing the germination medium prior to electroporation also decreased nuclease activity. Results of transient expression assays will be discussed. Electroporated tobacco pollen/pBI221 suspensions were placed on the stigmas of emasculated flowers and viable, transformed seed was produced. It was concluded that nucleases released from germinating tobacco pollen would not degrade all the foreign DNA in the time period necessary for the uptake of DNA during pollen electroporation. The potential for pollen electrotransformation in corn and alfalfa is currently under investigation.

POSTER ABSTRACTS

360 EFFECT OF 5-AZACYTIDINE ON THE EXPRESSION OF FOREIGN GENES IN STABLY TRANSFORMED BARLEY

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Using embryogenic callus from immature barley embryos and from mesocotyl explants, 6 cell lines from 5 cultivars were established. Protoplasts capable of sustained divisions could be isolated from cell lines of 4 cultivars (plating efficiencies 0.1 to 25 %). The morphogenetic response of well-growing callus derived from protoplasts is limited so far to abnormal leaf-like structures and proembryoids as could be shown by histological investigations.

Transformation of protoplasts was mediated by the PEG-method using the bacterial marker genes neomycinphosphotransferase (*npt*) and ß-glucuronidase (*gus*) under control of the 35S-promoter (selection by 25 mg/l G418). All selected colonies showed strong expression of NPT. However, GUS expression varied from no activity to high activities even in one and the same transgenic cell line. Results will be presented about the positive effects of 5-azacytidine as demethylation agent in this respect. PCR analysis demonstrated the presence of the expected *npt* and *gus* specific DNA-fragments. Genomic Southern blots of DNA from resistant calli confirmed these findings.

Data on transformation studies using phosphinothricine resistance as selectable foreign gene will be shown. Furthermore, results of microprojectile-mediated stable transformation of barley will be presented. Also in the latter case, 5-azacytidine was able to strongly increase the level of NPT activity in transgenic callus.

361 EFFICIENT GENETIC TRANSFORMATION OF THE TROPICAL FORAGE LEGUME STYLOSANTHES GUIANENSIS USING A BINARY Agrobacterium tumefasciens VECTOR.

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Genetically transformed plants of the forage legume Stylosanthes guianensis were regenerated from leaf disc explants inoculated with Agrobacterium tumefasciens strain EHA101 containing the plasmid pGV1040 (kindly provided by Plant Genetic Systems, Belgium, in a binary disarmed vector system. The T-DNA region of this vector contains the Neomycin Phosphotransferase II (NPT II) marker, the reporter gene Beta-Glucuronidase (Uid A) and the bialaphos resistance gene (bar gene) as additional selectable marker. Doses for Kanamycin and herbicide (BASTA) selection during plant regeneration were predetermined in vitro using non transformed tissues. Glasshouse tests of regenerated plants for herbicide resistance confirmed the data obtained under in vitro conditions and physical DNA transfer evidence was provided by Southern blot analysis of the glasshouse growing plants.

Work is underway to determine if the bar gene is stably transferred to the next generation.

362 ENHANCED OXIDATIVE-STRESS DEFENSE IN TRANSGENIC POTATO EXPRESSING TOMATO Cu.Zn SUPEROXIDE DISMUTASES.

Avihai Perl[1], Rafael Perl-Treves[1], Shmuel Galili[1], Dvora Aviv[1], Elisha Shalgi[2], Shmuel Malkin[2] and Esra Galun[1].
Departments of [1]Plant Genetics and [2] Biochemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

The two cDNAs coding for the cytosolic *(cyt)* and the chloroplast-located *(chl)* Cu.Zn superoxide dismutases (SODs) of tomato (Perl-Treves et al., 1988) were cloned into respective plasmids under the control of the CaMV 35S promoter. Each of the two resulting expression cassettes was then cloned into a binary vector with the *npt*II marker gene and mobilized into an *Agrobacterium* strain. Potato tuber discs were infected with either of the two agrobacterial strains. The integration of either of the *cyt* or the *chl* SOD transgenes was verified by Southern blot hybridization. The enzymatic activity of the additional tomato *chl* Cu.Zn SOD could be distinguished from the endogenous SOD activity because the latter isozyme migrated faster on SOD-activity gels. Several transgenic potato lines harboring either the *cyt* or the *chl* SOD genes of tomato showed elevated tolerance to the superoxide generating herbicide paraquat (methyl viologen). After exposure of shoots to paraquat, tolerance was recorded either by scoring symptoms visually or by measurements of photosynthesis using the photoacoustic method. Root cultures from transgenic lines that harbored the additional *cyt* Cu.Zn SOD gene of tomato were tolerant to methyl viologen up to 10^{-5} M. We assume that transformed potatoes with elevated levels of SOD may have an increased protection against photooxidative damage and that similar transformations could pave the way to confer paraquat resistance to other important crop plants.

363 ENZYMES AND GENES INVOLVED IN GENETIC RECOMBINATION IN CRUCIFERS.

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Genetic recombination is very poorly understood in plants, but there is a strong need for a better knowledge in that area if genetic engineering of plants is to be improved.

We have undertaken both a biochemical and a genetic approach. Two enzymes likely to be involved, namely DNA topoisomerase I and DNA recombinase (= strand-exchange protein) have been purified to near homogeneity from broccoli (*Brassica oleracea* var. *italica*), a species closely related to *Arabidopsis thaliana*. A gene for topoisomerase I has been cloned from *Arabidopsis thaliana*. Sequence information has been obtained from the recombinase and polyclonal antibodies are being raised against it in order to clone the corresponding gene(s) by oligonucleotide or antibody screening respectively.

We are also beginning to use a cDNA expression library both in *Saccharomyces cerevisiae* and *Escherichia coli* to complement mutations in genes involved in recombination. Strategies and progress will be described.

364 EFFICIENT PRODUCTION OF ACTIVE INDUSTRIAL ENZYMES IN PLANTS.

Jan Pen[1], Theo C. Verwoerd[1], Peter A. van Paridon[2], Peter J.M. van den Elzen[1], Albert J.J. van Ooyen[2], Krijn Rietveld[2], Peter C. Sijmons[1], Rob F. Beudeker[2] and André Hoekema[1].
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[2] Gist-Brocades N.V., R&D, P.O. Box 1, 2600 MA Delft, The Netherlands

There are several reports on the use of transgenic plants for the production of peptides and proteins, generally referred to as 'Plant molecular farming'. Plants provide us with an inexpensive source of biomass; its exploitation for economic production of proteins for *e.g.* food, feed, or processing industries would be very attractive.

As a first example, we have demonstrated the technical feasibility of the production of a high-value pharmaceutical protein in plants. In transgenic potato and tobacco plants, correctly processed, native human serum albumin was produced, opening up the way for plant molecular farming (Sijmons *et al.* 1990 Bio/Technology 8, 217-221). Despite this promising result, the required purity for the intended use may pose problems concerning purification steps and the costs involved. This is a disadvantage, as the economic production of this kind of proteins in plants may be offset by the costs for downstream processing.

The problem does not apply to proteins that don't require extensive purification, *i.e.* industrial enzymes. Competing with more traditional production systems for these enzymes, although these are very efficient and thus cheap, may be possible because economic production in plants may be combined with low downstream processing costs. We have assessed the feasibility of this approach. An example of the high-level production of an industrial enzyme in plants and its applications will be presented

365 EPIDERMIS - SPECIFIC GENES.

Anna M. Clark[1], Hans J. Bohnert[1] and Judith A.Verbeke[2]. University of Arizona, Department of Biochemistry[1], and Department of Plant Sciences[2], Tucson, Arizona 85711

The epidermis is a terminally specialized tissue originating from the L_1 layer of the apical meristem of plants. The distinct development and structure of this tissue is assumed to be a response to its location in immediate contact with the external environment. The molecular nature of the control elements that determine characteristics of an epidermal cell, and the signals that trigger its differentiation are unknown. Our initial approach to attack this problem was to isolate and characterize epidermis-specific mRNAs, identified by differential screening of a cDNA library established for the leaf epidermis from *Sedum* sp. To date, six different epidermis-specific mRNAs have been obtained. Four of the clones are abundant mRNAs. They are found exclusively in the epidermis. One transcript is present in the epidermis, and in much lower amounts in mesophyll cells. Finally, an epidermis-specific transcript of very low abundance has been obtained. *In situ* hybridizations confirmed the epidermal location of at least one of the messages studied. Additional characterization of the coding regions, for which we anticipate that they represent in part as yet unidentified genes, is underway. Furthermore, epidermis-specific genes are being employed as heterologous probes in screening of the *Arabidopsis* genomic library. Our ultimate goal is the dissection of promoter regions of the genomic counterparts of these cDNA clones. We expect that the information about the biochemical nature of these genes, and about functional domains of their control elements will allow us to determine what makes an epidermis.

POSTER ABSTRACTS

366 EVIDENCE THAT *Eu4* IS THE SOLE FUNCTIONAL UREASE-CODING GENE OF MATERNAL SOYBEAN TISSUES. RS Torisky, MA Holland, JD Griffin, JC Polacco. Department of Biochemistry, University of Missouri-Columbia; Columbia, MO 65211.

We tested the model that *Eu4* is the sole gene coding the ubiquitous urease in all soybean tissues by: (1) extending studies of its pleiotropy to developing embryos and seed coats, and (2) by determining its identity (or tight linkage) to cloned ubiquitous urease. The ubiquitous urease isozyme is found in all tissues but is absent in leaves, roots and seedlings of the *eu4/eu4* genotype. We report here that *eu4/eu4* lacks the ubiquitous urease in developing embryos and seed coats as well, although synthesis of the embryo-specific urease is normal. Double-mutant embryos, homozygous for *eu4* and the embryo-specific urease-null lesion (*eu1-sun*), lack virtually all urease. A complete urease genomic clone, LC4, was isolated and assigned to the ubiquitous urease by sequence and hybridization criteria. RFLP's revealed by an LC4 subclone are allelic or tightly linked to *Eu4*. The combined results favor allelism: that *Eu4* is the single functional ubiquitous urease coding gene. Less likely is the model that *Eu4* is a urease activation gene tightly linked to the LC4 structural gene: two other unlinked genes, *Eu2* and *Eu3*, are necessary for the activity of both urease isozymes making unlikely an *Eu4* activation function operative only on the ubiquitous urease. To confirm that *Eu4* contains the ubiquitous urease coding sequence found in LC4, we will attempt to correct the *eu4/eu4* lesion in callus with LC4, introduced by particle bombardment.

369 EXPRESSION OF GLUTELIN mRNA DURING RICE CARYOPSIS DEVELOPMENT. Hsin-Kan Wu, and Gunn-guang Liou. Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan, 11529 ROC

Based on the knowledge of rice caryopsis development we obtained in the past, an *in situ* technique was employed to monitor the spatial and temporal expression of the glutelin genes in O. perennis (genome AA), O. minuta (genome BBCC) and O. eichingeri (genome CC). The picked caryopses were cryo-fixed, cryo-substituted and finally embeded in K4M medium at -20 °C in order to preserve the samples almost alive. Hybridization of 0.5-1.0um sections of the caryopses with 35S-labelled antisense transcripts revealed that the expression of the genes is temporal different in the three wild rice species examined. Furthermore, their expressions are mostly limited to the peripheral cell layers of the endosperm. The expression of the glutelin genes is endosperm specific.

367 EVALUATION OF *bar* GENE AS A SELECTABLE MARKER IN THE PRODUCTION OF TRANSGENIC RICE PLANTS. Keerti S. Rathore, Vijay K. Chowdhury, Ruey-Chih Su and Thomas K. Hodges. Dept. of Botany and Plant Pathology and Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

The phosphinothricin acetyl transferase (PAT) gene *bar* , derived from *Streptomyces hygroscopicus*, has been reported to function as a selectable marker to obtain transgenic plants from maize, tobacco, potato and *Brassica* species and calli from rice. We are assessing its use in obtaining transgenic rice plants. We have made two constructs: pG35barA and pG35barB, each with a 35S promoter and *nos* polyA. In the pG35barA, the *bar* gene is in its original form, whereas in pG35barB the sequence immediately preceding the translation start codon of the *bar* gene was modified for optimal translation in eukaryotes (White *et al.*,Nucleic Acids Res.18:1062, 1990). Transformation of suspension culture-derived rice (cv. Nortai) protoplasts was carried out using PEG-mediated DNA uptake. Gene expression was monitored by analyzing PAT activity using the thin layer chromatography method. Transient gene expression assay on protoplasts, 48 hours after transformation, showed that PAT activity was much higher when pG35barB was used; henceforth, this construct was used to obtain stable transformation. Protoplasts were plated in Kao protoplast regeneration medium on filters over nurse cells for 3-4 weeks followed by transfer of filters to the N6 selection medium containing phosphinothricin. Resistant colonies were visible after 3-4 weeks on the selection medium. PAT assay on the resistant calli confirmed the expression of *bar* gene. Regeneration experiments and Southern blot analysis of the putative transformants are in progress.

370 EXPRESSION OF A BACTERIAL NITRILASE IN CANOLA PHOTOSYNTHETIC TISSUES CONFERS RESISTANCE TO BROMOXYNIL.

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Bromoxynil is a contact herbicide which acts by blocking photosynthesis in dicot plants. A bacterial gene coding for a bromoxynil specific nitrilase has been isolated (STALKER *et al.* 1987). The region coding for this enzyme has been put under the control of the promotor of a sunflower RuBisCO SSU gene and a nos terminator. This chimaeric gene has been introduced into Westar Canola using *Agrobacterium* and the internode technique described by BOULTER *et al.* 1990. The transgenic plants sprayed, under greenhouse conditions, with bromoxynil at doses up to 1,200 g a.i./ha show no sign of phytotoxicity. The progeny obtained after self-pollination has the same phenotypic behavior as the regenerating plants. Analyses of DNA, RNA and proteins are in progress to confirm the expression in various tissues of the plants. Field analyses will be performed to evaluate the agronomical value of these canola.

BOULTER M.E. *et al.*, Plant Science, 70 (1990) 91-99.
STALKER D.M. *et al.*, J. Bact. 169 (1987) 955-960.

368 EXTRACHROMOSOMAL DNA RECOMBINATION IN MAIZE PROTOPLASTS. Leszek A. Lyznik, David McGee, Po-Yen Tung, Jeffrey L. Bennetzen[a], and Thomas K. Hodges. Department of Botany and Plant Pathology, Department of Biology[a]Purdue University, West Lafayette, Indiana 47907

DNA recombination reactions in transformed protoplasts/cells are responsible for integration of a foreign DNA into the chromatin structure. While nonhomologous recombination process(es) is the main incorporation pathway of foreign DNA into mammalian and plant cell genomes, homologous recombination is also involved although at very low frequencies. Understanding the mechanisms of homologous recombination reaction in plant cells offers the possibility of gaining control over the integration and expression of foreign genes in transgenic material and for *in situ* modification of plant genes. We have designed a system to study the requirements for homologous recombination between plasmid DNA molecules in maize (*Zea mays* L.) protoplasts. Two plasmids were introduced into maize protoplasts: one plasmid (pBx26) contained a genomic clone of the *Adh*1 maize gene; the other plasmid (piGUS) was a promoterless construction containing part of intron A of the *Adh*1 gene fused to the *gusA* coding sequence. Thus, the two vectors shared an effective homologous region consisting of a 459 bp (*Hind*III-*Pvu*II) fragment of the *Adh*1 intron A sequence. An active *gusA* fusion gene would occur upon homologous recombination between the plasmids within the intron A sequence, and indeed GUS activity was observed in extracts following co-transformation of maize protoplasts with the two plasmids. For efficient homologous recombination, both plasmids had to be linearized. The recombination reaction was induced by restriction of the plasmid molecules either inside the effective homologous region or at the border sites of the intron sequence. However, the presence of even small terminal nonhomologous sequences at the 3' end of the pBx26 fragment inhibited the recombination reaction. Also, both ends of the linearized piGUS DNA molecules were involved in the recombination reaction. We did not find evidence that DNA polymerase was involved in recombination reactions. Experiments are underway to extend these observations to recombination between plasmid and chromosomal DNA.

371 EXPRESSION OF HUMAN α-INTERFERON GENE IN TRANSGENIC RICE PLANTS. Zhen zhu, Xiang-hui Li & Yong-ru Sun, Institute of Genetics,Academia Sinica, Beijing 100101, China

Use of lipofectin-mediated method we have delivered human α-interferon (Hu-α-IFN) gene into rice protoplasts, from which transgenic rice plants were regenerated. The expression of Hu-α-IFN gene in transgenic plants has been conformed. Donor DNA E.coli plasmid pIG3031 carried Npt-II gene and Hu-α-IFN structure gene under the control of T-DNA transcript 1 promotor. Lipofectin, a kind of cationic liposome could interact with DNA to form Lipofectin-DNA complex. Rice protoplasts isolated from suspension cell were mixed with lipofectin-pIG3031 DNA complex. PEG 6000 was added for enhancing delivery efficiency. Treated protoplasts were suspended in modified Km8p medium and cultured according to agarose beads method. Selection was performed 5-7 day after culture by adding 20mg/l G418 in fresh medium. After 6 weeks,Transformed colonies were counted basis on Npt-II assay, high transformation frequencies (>14%) were obtained. Transformed calli were transferred to differentiation medium, further more 5-6 weeks, whole plants were regenerated,some of them have already been transferred into greenhouse.Sourthern blot analysis indicated DNAs from transgenic plants present Hu-α-IFN gene sequence hybridizing to IFN cDNA sequence from plasmid. The result from RNA slot blot showed foreign IFN gene could transcribe in transgenic plant. The assay of biological activity in vitro conformed the presence of special IFN activity in extracts from transformed plants and calli, which could protect human amnion cell from attacking with vesicular stomatitis virus in vitro.The activities in transformed tissues arranged between 512-2048 international units pergram.The results imply under the control of plant promoter foreign Hu-α-INF could express efficiently in rice plant.

POSTER ABSTRACTS

372 EXPRESSION IN MAIZE PLANTS OF WHEAT GERM AGGLUTININ, A NOVEL SOURCE OF INSECT RESISTANCE. Maddock S.E., Huffman G., Isenhour D.J., Roth B.A., Raikhel N.V., Howard J.A. and Czapla T.H., Pioneer Hi-Bred International, Inc., 7300 N.W. 62nd Avenue, Johnston, Iowa 50131. The plant lectin wheat germ agglutinin (WGA) has been shown to have insect resistant properties in vitro. A cDNA clone of WGA isolectin D was modified by PCR procedures, and inserted into a plant expression cassette. This vector was co-transformed into embryogenic maize suspension cells with the herbicide-resistant marker genes BAR or ALS, using the particle gun for DNA delivery. Embryogenic colonies were selected in the presence of herbicide, and those containing and expressing the WGA gene were identified by PCR and ELISA techniques. Plants were successfully regenerated from these colonies. The integration and expression of the gene in transformed plants was confirmed by Southern and western blot analyses. Whole-plant and in vitro bioassays were conducted with neonate European corn borer larvae (a major insect pest of maize). Plants expressing WGA had reduced feeding damage to the leaves relative to controls. Insects reared on these plants and on tissue from them showed significant reduction in weight and higher mortality than those on control plants.

373 **EXPRESSION CHARACTERISTICS OF TWO 1-AMINOCYCLO-PROPANE-1-CARBOXYLATE (ACC) SYNTHASE GENES IN TOMATO.***

Jürg Oetiker, Allan D. Campell, Jim Lincoln and Athanasios Theologis. Plant Gene Expression Center, ARS-USDA, 800 Buchanan St., Albany CA 94710 and Dept.of Plant Biol., UC Berkeley, CA 94720.

ACC synthase is encoded by a multigene family in tomato (1). We have investigated the expression pattern of two genes expressed during ripening in intact and wounded fruit tissue. The rapidity of their expression by ethylene and the effect of cycloheximide on their induction has been studied to determine whether the hormonal response is direct. The physiological significance of the presence of different isoenzymes during fruit ripening will be discussed.

(1) W.H. Rottmann et al., 1991. *J Mol Biol.* submitted.

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374 EXPRESSION OF *bar* GENE DRIVEN BY *rbcS* AND CaMV *35S* PROMOTERS IN TRANSGENIC *ARABIDOPSIS* AND POTATO. Hiroaki Ichikawa[1], Yoshiyuki Tanaka[2], Kyoichi Matsuba[3], Makoto Matsuoka[2], Mitsumasa Satoh[1] and Teruo Ishige[2]. [1]National Inst. of Agro-Environmental Sciences, Tsukuba, [2]National Inst. of Agrobiological Resources, Tsukuba, and [3]Japan Turf Grass Inc., Narashino, Japan.

Expression pattern of each gene is controlled by its promoter specificity. Detailed characterization of each promoter enables introduced foreign genes to be expressed in tissue- and/or period-specific manner in transgenic plants. Bialaphos (Herbiace®, Meiji Seika Ltd.) is an inhibitor of glutamine synthetase in plants and bacteria, and used as a non-selective herbicide. A gene which confers bialaphos resistance (*bar*) has been introduced into several crops and was expressed under the control of CaMV *35S* promoter in those transgenic plants [M. De Block *et al.*(1987)]. Since bialaphos is usually treated on leaves and stems, and is degraded rapidly in soil, the expression of introduced *bar* gene may be required only in greening tissues of plants. We have isolated *rbcS* promoter regions from *Arabidopsis* genome and from cloned tomato *rbcS-3C* gene (kindly provided by Drs. M. Sugita and W. Gruissem) with PCR. The *rbcS* promoters were joined in front of the *bar* gene (kindly provided by Dr. H. Anzai). The plant transformation vectors containing *rbcS-bar* or *35S-bar* constructs were transferred to *Agrobacterium* by electroporation. The *bar* gene was introduced into *Arabidopsis* and potato by *Agrobacterium*-mediated transformation. Results of the expression patterns of the *bar* gene driven by different promoters will be presented.

375 EXPRESSION OF CHEMICALLY-INDUCED GENES IN MAIZE. Todd J. Jones, Sonja A. Schmitz and Howard P Hershey. DuPont Agricultural Products, Wilmington, DE 19880

We have identified several maize genes that are induced by treatment with substituted benzenesulfonamide herbicide safeners. Two of these genes, designated In2-1 and In2-2, have been examined in greater detail with respect to the specificity of induction and expression patterns within the maize plant. While induction of both In2-1 and In2-2 exhibits structural specificity for substituted benzenesulfonamides, differences in the relative responsiveness of the two genes were observed in roots treated with different compounds. Messenger RNAs for both In2-1 and In2-2 accumulate rapidly after chemical induction and disappear equally rapidly following removal of the inducer. Chemical-inducibility of these genes following foliar application has been demonstrated in vegetative and meristematic plant organs. We are currently examining the tissue-specificity of the induction response within these organs using *in situ* hybridization.

376 EXPRESSION OF FOREIGN GENES IN TRANSGENIC SWEET POTATOES. C. S. Prakash and Usha Varadarajan. Tuskegee University, School of Agriculture, AL 36088, USA.

Sweet Potato (*Ipomoea batatas* (L.) Lam.) cultivars Jewel and TIS-70357 were successfully transformed with foreign marker genes using the *Agrobacterium* vector approach. The disarmed *A. tumefaciens* strain LBA 4404 containing the binary vector pBI 121 was employed. The screenable marker gene *gus* A was controlled by the 35S promoter from cauliflower mosaic virus (CaMV) and the selectable marker *kan* was controlled by a promoter from the nopaline synthase gene. High frequencies of transformation were observed when leaf and petiole pieces were used as explants with two days of cocultivation. Histochemical assays of shoots regenerated on a kanamycin (50 mg/l) medium showed that most antibiotic-resistant shoots positively expressed the *gus* A gene. The evidence for the expression for the *kan* gene was obtained by the ELISA test for the enzyme neomycin phosphotransferase II. The presence of the foreign gene in the sweet potato plantlets was confirmed by the polymerase chain reaction amplification of the DNA fragment encoding the *kan* gene and by subsequent Southern analysis. Variation in developmental and tissue specific expression of the *gus* A gene due to CaMV and figwort mosaic virus promoters are now being comparatively studied in transgenic sweet potato plantlets.

377 FEW POTENTIAL TECHNIQUES FOR TRANSFER FOREIGN GENES INTO SOME CROP PLANTS. Li Bao-jian, Xu Yao, Shi Heping, Xu Xinping, Ke Xiayi, Yu Diqin. Zhongshan University, Biotechnology Research Center, Guangzhou P.R.CHINA

For overcome the host-range limitation of Agrobacterium tumefaciens, a phenolics-pretreated method for bacteria and then co-cultivated with suspension cereal cells(include rice, wheat & millet cells) had been established. Both NPT II gene and NOS gene were transferred and expressed in cultured cell of these crop plants. Integration of the foreign genes into genome of these plants was confirmed by Southern blot hybridization. The details of T-DNA transfer procedure into cells had been observed with the help of the EM autoradiograph technique. Using the electroinjection instrument, the NPT II gene has been sucessfully transferred into embryo cells of Oryza sativa indica and japonica. Transferred calli completed the regeneration via somatic embryogenesis and produced transgenic plants. It is demonstrated by Southern blot hybridization and NPT II dot assay that the foreign gene is stably integrated and expressed in the transformed rice plants. The use of partical gun in transfer foreign genes into cells of cereal crop plants has been also discussed.

POSTER ABSTRACTS

378 FIELD TEST RESULTS OF A CHEMICALLY-INDUCIBLE PROMOTER. Sandra S. Dincher, Eric Ward, John Rvais. CIBA-GEIGY Biotecnnology Researcn Unit, 3054 Cornwallis Rd., Research Triangle Park, North Carolina 27709. During tne summer of 1991, the first field test of a transgenic tobacco line carrying a cnemicallv-inducible promoter linked to a reporter gene was executed in North Carolina. The experiment itself included three inducing chemicals and two independent transformants of tne PR1 promoter linked to tne GUS reporter gene. The experimental design and preliminary results will be discussed.

379 A GEMINIVIRUS VECTOR FOR ANTISENSE RNA EXPRESSION. Christopher G. Miller and William E. Gardiner. Mississippi State University, Department of Biological Sciences, Mississippi State, Mississippi 39762.

A vector has been produced from the "A" genome component of Tomato Golden Mosaic Virus (TGMV) for the expression of antisense RNAs in plant protoplasts. It consists of one and one-quarter copies of a modified TGMV A component cloned into pUC118. The vector, once transfected into protoplasts, sponsors the release of a TGMV replicon from which a 528-bp fragment of the coat protein (CP) coding region has been removed. The remaining CP sequences include all of the 5' untranslated region (including the strong promoter), 74 bp of 5' coding sequence, 141 bp of 3' coding sequence (with a unique Csp45I restriction site) and all of the 3' untranslated region (including the polyadenylation signal). The remainder of the replicon contains all other TGMV A genes neccessary for viral replication and maximal expression of the CP promoter. A 542-bp fragment of the cDNA for a tomato chlorophyll a/b binding protein gene (CAB10b) has been cloned into the Csp45I site in both sense and antisense orientations. A replication-defective version of this antisense RNA vector has also been produced. Tests are underway to determine the activity of antisense RNAs expressed from these vectors in protoplasts.

380 GENE TARGETING IN *ARABIDOPSIS*. Stephan A. Ohl, Annette C. Vergunst, Paul J.J. Hooykaas and Peter J.M. van den Elzen. MOGEN International nv, Leiden and Leiden University, Clusius Laboratory, Leiden, The Netherlands

Gene targeting is becoming an increasingly important tool in determining the function of eukaryotic genes as it can be used not only for gene disruption but also to introduce subtle mutations at specific sites.

Recently we have been able to demonstrate gene targeting in tobacco using *Agrobacterium tumefaciens* (see poster by Offringa et al.)

The goal of our work is to establish systems for gene targeting in *Arabidopsis thaliana* and to investigate *Agrobacterium* as a vector system.

To this end we are developing a system for the efficient transformation of *Arabidopsis* protoplasts via *Agrobacterium*. Transformation frequencies obtained using PEG-mediated direct DNA transfer and *Agrobacterium*-mediated protoplast transformation will be compared.

As a first step to demonstrate gene targeting in *Arabidopsis* we will select for targeted restoration of a nonfunctional hygromycin resistance marker integrated in the *Arabidopsis* genome.

As a second step we will introduce by homologous recombination a specific change in the *Arabidopsis* RNA polymerase II gene (AtrpII), which confers resistance to the drug α-amanitin. To determine conditions for selection in a modelsystem *Arabidopsis* has been transformed with the α-amanitin resistant form of AtrpII. The poster will show and discuss our latest results in this area.

381 GENERATION AND CHARACTERISATION OF HERBICIDE TOLERANT CORN Ian J. Evans, Mairi J. Macdonald, Ming Tang Chang*, Sheila Baird, and Simon WJ Bright. Plant Biotechnology, I.C.I. Seeds, Jealott's Hill, Bracknell, UK and * Garst Seed Co., Slater, Iowa 50244.

Lines of an elite corn inbred that are tolerant to normally lethal concentrations of the ALS-inhibiting Imidazolinone herbicide 'Pursuit' (American Cyanamid), have been generated. Tolerant plants were identified from 100,000 seedlings in a glasshouse screen of an M1 population resulting from EMS pollen mutagenesis. The segregation ratios obtained upon screening the progeny of these plants indicated that tolerance was in most, if not all, lines, the result of a single dominant gene. Dose response and cross- resistance studies on both whole plants and isolated ALS enzyme indicate that the lines are probably the result of different mutations. Some lines are resistant to other classes of ALS-inhibitors. Two of the lines have been characterised at the DNA sequence level, ALS genes having been isolated from cDNA libraries constructed from homozygous plants. At least two classes of ALS genes are expressed in these corn lines. Generation of herbicide tolerance in an elite line permits the production of tolerant hybrids without the need for extensive backcrossing.

382 GENETIC INSTABILITY OF TRANSGENIC *Nicotiana plumbaginifolia*. * Wichai Cherdshewasart, ** Michel Jacobs and ** Ioan Negrutiu. * Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand. ** Plantengenetica, Instituut voor Moleculaire Biologie, B-1640, Belgium.

Analysis of large amount of transgenic *N.plumbaginifolia* via PEG-mediated gene transfer resulted in high percentage of inactivated progeny after F_2 generation. Increasing instability of the progeny is found to be related with increasing meiotic passages. Southern blot analysis proofs that most of the inactivated case is not due to loss of functional sequences. Rearrangement or amplification of the functional sequences is found in some case. Using isochizomeric enzymes, difference Southern blot patterns are confirmed among inactivated plant DNA samples. Treatments of inactivated seeds with 5-azacytidine shows significant recovering rates. This finding shows the influence of methylation upon transgene expression within the cellular environment of transgenic plants.

383 GENETIC TRANSFORMATION OF PEA (*PISUM SATIVUM* L.) Johanna Puonti-Kaerlas, Tage Eriksson and Peter Engstrom Dept. of Physiological Botany, Univ. of Uppsala, Box 540, S-751 21 Uppsala, Sweden

A transformation system based on *Agrobacterium* cocultivation was developed for pea. Explants from shoot cultures were cocultivated with non-oncogenic bacterial strains carrying hygromycin resistance as a plant selectable marker, and transgenic callus could be induced from the explants on selective medium. The calli could be induced to regenerate shoots after several passages on regeneration medium. Roots were induced on the regenerated shoots, and the plantlets were transferred into the greenhouse, where they proceeded to flower and produce seed. The cytological and morphological analysis of the transgenic R_1 and R_2 offspring will be presented, as well as molecular evidence of the stability of the inheritance of the hpt gene into the progeny of the primary transformants.

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384 GENOTYPE-INDEPENDENT TRANSFORMATION OF SUGARBEET USING *AGROBACTERIUM TUMEFACIENS* J.E.Fry, A.R.Barnason, M. Hinchee. Monsanto Co., Plant Protection and Improvement, St. Louis, Missouri 63198

A highly reproducible, genotype independent method has been developed for producing genetically modified sugarbeet plants from cotyledonary explants inoculated with *Agrobacterium tumefaciens*. Cotyledon explants were inoculated with *Agrobacterium tumefaciens* strains containing chimeric genes coding for kanamycin resistance and *β-glucuronidase* "GUS" activity or kanamycin resistance, GUS activity, and glyphosate resistance. After coculture of the cotyledons with the *Agrobacterium*, shoots are regenerated from cotyledons. The presence of the chimeric genes have been demonstrated by GUS histochemical assay, NPT dot blot and by 5-enopyruvylshikimate-3-phosphate synthase(EPSPs) assays. Progeny from genetically modified plants inherited the foreign genes in a Mendelian manner. Plants containing a mutant EPSPs gene have shown resistance when sprayed with glyphosate at levels that kill control plants. Several hundred plants of several different genotypes have been produced by this method.

385 GUS EXPRESSION IN RICE TISSUES USING *AGROBACTERIUM*-MEDIATED TRANSFORMATION. X-Q. Li, C. Liu, J. Peng, S.B. Gelvin, and T. K. Hodges. Purdue University, Botany and Plant Pathology Department (XQL, JP, TKH) and Biological Sciences (CL, SBG), West Lafayette, Indiana 47907.

Rice (*Oryza sativa*--indica and japonica varieties) seedlings were incubated with *Agrobacterium tumefaciens* containing a ß-glucuronidase (*gusA*) gene, and transient GUS expression was obtained at a high frequency. The *Agrobacterium* strain used (At565) was similar to that used by Rainers et al. (BioTech. 8:33-38. 1990) for transformation of rice callus. *A. tumefaciens* At565 contains the C58 chromosomal background and a disarmed version of the supervirulent plasmid pTiBo542. At565 also carries the binary vector (pKIWI105) obtained from B-J. Janssen (University of Auckland). This binary vector contains a *gusA* gene that does not express GUS activity in the bacterium. The explants used were obtained from young seedlings produced from either mature or immature embryos. Transient expression of GUS was observed in leaves, coleoptiles, mesocotyls, roots, and other cells associated with the embryo. GUS expression following *Agrobacterium* treatment was obtained in all 7 indica varieties tested, 6 out of 7 japonica varieties tested, and in 3 of 4 *Oryza glaberima* varieties. Indica varieties had the highest number of cells exhibiting GUS activity. The addition of 2,4-D to the medium used for germination was detrimental to GUS expression following treatment with *Agrobacterium*, but 2,4-D (2-6mg/l) was required in the medium during co-cultivation of the rice with *Agrobacterium* for GUS expression. Transient GUS expression was not influenced by growing the seedlings in the light or dark. Preinduction of *Agrobacterium* with acetosyringone, octopine, or glucose had no significant effect on the frequency of transformation. In summary, *Agrobacterium* has been shown to transform transiently several tissues of 16 varieties of rice .

386 A HIGHLY OPTIMIZED MONOCOT EXPRESSION CASSETTE: APPLICATIONS FOR BARLEY TRANSFORMATION AND BARLEY VIRUS RESEARCH. Christoph Maas, Andrew Davidson, Joachim Bendiek, Fred Jasper, Anke Sohn, Christoph Reichel, Jeff Schell and Hans-Henning Steinbiß. Max-Planck-Inst. für Züchtungsforschung, D-5000 Köln 30, F.R.G. ——— High level expression of introduced selectable marker genes as well as of agronomically important genes in plants is a crucial aspect of plant molecular biology. The most commonly used promoter for chimeric genes is derived from the Cauliflower Mosaic Virus (CaMV) 35S gene and is highly active in dicotyledonous plants. In contrast expression of chimeric 35S genes in monocots reaches only 0.1-1% of the level that can be observed in dicots. This low level expression can be overcome by insertion of exon1 and intron1 of the maize *Shrunken*1 (*Sh*1) gene, which enhances transient gene expression in barley as well as in maize and rice protoplasts up to 1000-fold (Maas et al., 1991 Plant Mol. Biol. 16:190). In an attempt to transform barley plants we are currently investigating the effect of enhanced gene expression during the selection process using the NPT II marker gene. In addition, enhanced gene expression by *Sh*1 intronic sequences should allow us to investigate more thorougly parameters affecting gene integration using promoterless selectable marker genes.

To demonstrate the applied aspects of this highly optimized monocot expression cassette we have started to investigate engineered resistance to Barley Yellow Mosaic Virus (BaYMV), an economically important barley pathogen. As the field isolate of BaYMV is able to replicate in barley protoplasts shortly after transfection we commenced studying replication of the virus by use of the protoplast system. CDNA's corresponding to RNA 1 and RNA 2 of BaYMV (Prols et al., 1990 J. Phytopath. 130:249; Davidson et al., 1991 J. Gen. Virol. 72:989) were cloned into the monocot expression cassette and transfected into protoplasts in order to obtain infectious RNA's. In an attempt to study inhibition of viral replication we have also inserted the coat protein coding region of BaYMV into our monocot expression cassette. We are currently studying the possibility of inhibition of viral replication by overexpression of BaYMV coat protein in transient gene expression assays and by the production of a transgenic barley suspension cell line overexpressing BaYMV coat protein.

387 INCREASING THE S-AMINO ACID CONTENT IN LEAVES OF ALFALFA AND TOBACCO. Suman Bagga, C. Sengupta-Gopalan & J.D. Kemp. New Mexico State University, Plant Genetic Eng. Labs., Las Cruces, NM 88003.

The quality of nutritional proteins can be improved by balancing its aminoacids for the needs of the intended user. Alfalfa is a major forage crop but its leaf proteins are low in the sulfur amino acids. In an attempt to raise the amount of cysteine and methionine in alfalfa leaves, we have introduced a gene that codes for a 15 KD zein protein which is very rich in S-amino acids. The coding sequence of the zein gene from corn was engineered behind the CAMV 35S promoter and introduced into alfalfa and tobacco. Our initial studies on tobacco shows high level accumulation of the 15 KD protein both in the leaves and seeds. This is in contrast to the *β*-phaseolin protein which accumulates to high levels in seeds but not in leaves of transformed alfalfa and tobacco. Differences in the accumulation pattern of the two proteins suggests that zein is probably targeted to a subcellular location in leaves where it is protected against proteolytic degradation. If zein protein were to accumulate to a level that represents 10% of the total leaf protein in alfalfa, then the overall protein quality would greatly improve.

388 INDUCIBLE EXPRESSION OF BACILLUS THURINGIENSIS TOXIN IN TOBACCO. Shericca C. Williams, Eric R Ward, John A. Ryals. CIBA-GEIGY Agricultural Biotechnology Research Unit, P. O. Box 12257, Research Triangle Park, North Carolina 27707 USA

Transgenic tobacco plants have been engineered to express an insecticidal toxin under external control. Tobacco plants that contain the truncated Bacillus thuringiensis (Bt) δ -endotoxin gene fused to the inducible PR-1a promoter express a low constitutive level of Bt toxin. Activation of the PR-1a promoter occurs when a CIBA-GEIGY inducing chemical is applied to the leaves. Untransformed Xanthi plants and two transgenic Bt lines were treated with H_2O or the inducing chemical, and infested several days later with tobacco hornworm larvae. Multiple assays were performed and monitored for at least two weeks each. Insects from the induced Bt lines typically died faster or weighed less by the end of the assays than hornworms feeding on uninduced transgenic plants or induced wild type control plants. Damage to the H_2O treated plants and the chemically treated Xanthi plants was more extensive than damage to the chemically induced plants. This inducible expression system may be a strategy for limiting the development of resistance to Bt toxin in natural insect populations. In addition, the PR-1a promoter should prove useful for conferring inducible expression on other genes of interest.

389 INHERITANCE, EXPRESSION, AND STABILITY OF GENES INTRODUCED INTO MAIZE. T. Michael Spencer, James V. O'Brien, William G. Start, Thomas R. Adams, William J. Gordon-Kamm, and Peggy G. Lemaux. Discovery Research, DEKALB Plant Genetics, Eastern Point Road, Groton, Connecticut 06340.

Transgenic A188 X B73 R_0 maize plants (regenerated from embryogenic suspension culture cells transformed by microprojectile bombardment) were backcrossed with nontransformed B73 plants. Inheritance of a selectable marker gene, *bar*, and a nonselectable marker gene encoding *β*-glucuronidase (GUS) was analyzed in progeny (R_1) representing four independent transformation events. Activity of the *bar* gene product, phosphinothricin acetyl transferase (PAT), was assessed in plants comprising the four R_1 populations. The number of R_1 plants containing PAT activity per total number of R_1 plants recovered for each population was 2/7, 19/34, 3/14 and 73/73. Molecular analysis confirmed the segregation of *bar* in three R_1 populations and the lack of segregation in the fourth R_1 population. GUS coding sequence appeared to be linked to *bar* in all four R_1 populations. Analysis of numerous R_2 plants derived from crossing transformed R_1 plants with nontransformed inbreds revealed a 1:1 segregation of PAT activity in three of four lines, including the line that failed to segregate in the R_1 generation. Integrated copies of *bar* in the fourth line appeared to be unstable.

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390 IN SITU GENE EXPRESSION IN MAIZE TISSUES BY PARTICLE BOMBARDMENT. Lisa Lee, Michael R. Movnihan and Nicholas P. Everett. Enimont Americas, Inc. Biotechnology Laboratory, 2000 Cornwall Road, Monmouth Junction, NJ 08852.

To examine whether particle bombardment can be used to study in situ expression, intact seedlings and various maize tissues were bombarded with a 35SCaMV-GUS gene using a biolistic PDS-1000 Particle delivery system. The use of a screen above the sample reduced the damage to the tissue in the target area. All the tissues bombarded with pEN14 DNAs expressed blue spots after X-Gluc staining although the number of spots varied from tissue to tissue. The stalk sections and tassels or tassel sections produced fewer than 10 spots per bombardment. These tissues showed considerable amount of browning during incubation. The inner leaves surrounding the tassel gave the highest number and density of spots (max. 400 spots/cm^2). More mature leaves produced intermediate amount of spots after clearing with ethanol (max. 40 spots/cm^2). Germinated seedlings also expressed GUS activities well with most of the spots in the shoots. The transient expressions of different promoter-GUS gene constructs were also compared.

391 INTEGRATION OF THE CYTOKININE SYNTHESIS GENE AND ITS EXPRESSION IN THE GENOMES OF COTTON. F.Muhamedhanova, M.Muminova, A.Tashpoulatov, A.Abdukarimov. A.S.Sadykov's Institute of Bioorganic Chemistry, Academy of Sciences Uzbek SSR, Tashkent, USSR.

The problem of plant regeneration in such valuable industrial crops as cotton is of great interest in science and in agriculture. The transformation of cotton leaf discs (No. 02906 of G.arboreum L. and the cv. Coker 201 of G.hirsutum L.) with A.tumefaciens LBA 4404, containing gene 4 from T-DNA region of pTiAch5 plasmid, the product of which initiates the synthesis of the cytokinin isopentyl adenosin, was carried out. A number of callus clones were obtained in the MS medium with 150 mg/l kanamycin, 500 mg/l carbenicillin and phytohormones for callus formation. It is shown that DNA dot-hybridization from transformed tissue with Ti-plasmid vir-region gave negative results, while the hybrid combination containing NPT II and isopentyl adenosin synthesis initiating genes and NPT II test gave positive results.

392 *IN VIVO* TARGETING OF TOBACCO MOSAIC VIRUS BY A HAIRPIN RIBOZYME. Scott Galasinski, Thomas Zinnen, and Randal Hauptmann. Plant Molecular Biology Center, Northern Illinois University, Montgomery Hall, DeKalb, IL 60115.

In vitro studies suggest that catalytic RNA may down regulate gene expression through cleavage of target RNA. We are evaluating this application by using a "hairpin" ribozyme derived from the negative strand of the satellite RNA of tobacco ringspot virus. This synthesized ribozyme targets the 542 position of the tobacco mosaic virus (TMV) replicase gene. As a control to verify catalysis as opposed to antisense binding, we are using a mutant ribozyme containing a substitution in the hairpin which destroys cleavage activity while retaining the target complement. These ribozymes have been cloned into a variety of expression cassettes to evaluate RNA stability. All constructions are expressed by the CaMV 35S promoter and have been mobilized into the binary vector pBI121. These vectors have been used in *Agrobacterium* mediated stable transformations of two *Nicotiana tabacum* cultivars: Xanthi and Xanthi-nc. These cultivars respond differently to TMV infection and will be used to evaluate ribozyme activity by monitoring changes in systemic spread and local lesion response, respectively. Ribozyme effects on TMV infection are being assessed through ELISA and RNA analysis. Data from this model may direct the application of the "hairpin" as a means to inhibit viral infection and affect gene expression.

393 ISOLATION AND TRANSIENT ASSAY OF TOBACCO ANTHER SPECIFIC PROMOTERS. Carl W. Garnaat and Gary Huffman. Biotechnology Dept., Pioneer Hi-Bred Intl. Johnston, Iowa 50131

In order to obtain regulated gene expression in anthers, an anther specific cDNA clone from tobacco was used to probe a tobacco genomic library to isolate homologous genes and the associated flanking regions. Two independent genomic clones were obtained and sequenced. These two genomic clones share 99.8% and 90% homology with the cDNA clone, respectively. The upstream regions of these genes have been identified and fused with a GUS reporter gene. Particle gun transient assays with these reporter constructs demonstrate that both of these promoters are able to confer GUS expression in tobacco anther tissue.

394 ISOLATION OF A cDNA CLONE CODING FOR *PHASEOLUS VULGARIS* POLYGALACTURONASE (PG) AND CHARACTERIZATION OF THE PG-EXPRESSION DURING POD DEVELOPMENT. Kees Recourt, Monique C.M. Ebbelaar and Cees van Dijk. ATO-Agrotechnology, P.O.Box 17, 6700 AA Wageningen, The Netherlands.

The quality of fruit and vegetables is determined in part by the texture of the plant tissue. Cell wall-modifying enzymes are considered to play an important role in determining the texture of tissue. For tomato, a correlation has been demonstrated between fruit firmness and levels of the activity of the cell wall-degrading polygalacturonase (PG). Further, measurements of PG mRNA using isolated PG cDNAs showed that PG mRNA is most abundant in soft, ripe tomato fruit (1).

In order to get insight in the role of cell wall-degrading enzymes during ripening of snap beans (*Phaseolus vulgaris* L.), PG gene-expression was investigated during pod development. For this purpose, specific oligonucleotides were synthesized based on homology between tomato and peach PG. By using these primers and the PCR technology, a pod-specific PG cDNA of 0.23 kB was isolated. This PG fragment was cloned and appeared to contain an additional domain homologous to PG cDNAs of tomato and peach. Results from the PG-expression studies will be presented and discussed.

1. Della Penna D, Alexander DC, Bennett AB: Molecular cloning of tomato fruit polygalacturonase: Analysis of polygalacturonase mRNA levels during ripening. Proc Natl Acad Sci USA 83: 6420-6424 (1986).

395 ISOLATION OF PROMOTER SEQUENCES FROM PLANTS. Lorenzo Frigerio and Corrado Fogher. Università Cattolica, Istituto di Genetica, 29100 Piacenza, Italy.

A method has been developed for the isolation of constitutive plant promoters, based on the use of GUS reporter gene and *in vitro* transcription-translation reactions. We have constructed the promoter-probe pLOX1 vector, wich carries a promoterless GUS cassette from pBI101[1] and Ampicillin resistance from pBR322. Total genomic DNA was isolated from tomato leaves, and after digestion with SauIIIA, the resulting fragments have been cloned into the BamHI site located upstream of the GUS cassette in pLOX1. Following transformation in E. coli, clones containing repeated sequences and organellar DNA have been eliminated[2] and plasmids isolated from the remaining checked for the expression. To perform *in vitro* transcription three systems have been assayed: a) nuclear extract from tomato leaves, unfractionated[3]; b) fractionated extract from tobacco cells (grown in suspension)[4]; c) HeLa whole cell exract[5]. These extracts were tested with pBI121[1](GUS cassette + CaMV 35S promoter) as a strong promoter source, and with pBI101 (GUS cassette); the transcription products were translated *in vitro* using a commercial extract from wheat germ, and the presence of GUS activity was detected by a spectrofluorometric assay, and quantified as percent relative fluorescence (100% r. f. = 10 nM 4-metyl-umbelliferone). No activity was detected with the use of extracts a) and b), while we found appreciable levels of GUS activity using the HeLa cell lysate. Each clone of the genomic library has been tested for GUS expression; clones showing relative fluorescence values above 30% of the negative control will be further characterized in terms of size and nucleotide sequence of the genomic inserts.

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396 MANIPULATION OF TRANSGENES: *LOXP*-CRE MEDIATED MARKER EXCISION. Sandra H. Russell, Joyce L. Hoopes, and Joan T. Odell. Du Pont, Central Research and Development and Agricultural Products, Wilmington, DE 19880-0402

Stably introduced *loxP* and *cre* elements derived from bacteriophage P1 were previously shown to be active in the plant genome resulting in site-directed recombination (Odell et al. MGG (1990) 223:369). *LoxP*, a 34 bp DNA sequence, is a substrate for the Cre recombinase protein. Cre was used to activate an inactive gene, that was integrated into the tobacco genome, by excising an intervening sequence bounded by *loxP* sites. We now demonstrate that two linked traits can be introduced into the plant genome, and then loss of one of the traits in re-transformants or in F1 and successive generations can be controlled by the introduction of *cre*. A sulfonylurea resistant acetolactate synthase (ALS) gene bounded by *loxP* sites was used as the selectable marker to produce transgenic tobacco and Arabidopsis plants. A β-glucuronidase (GUS) gene was linked to the ALS gene. Deliberate loss of the ALS selection marker through Cre-mediated excision was demonstrated by loss of resistance to sulfonylurea herbicides and molecular analysis, while the GUS gene remained intact. Factors affecting the efficiency and heritability of the controlled trait loss were analyzed. These and the previous results demonstrate that the *loxP*-Cre system allows manipulation of a transgene to either an active or inactive state; a transgene can exist in one expression state for any number of generations until *cre* is introduced, which then causes an alteration in the expression of the transgene.

397 A MODEL SYSTEM FOR HOMOLOGOUS GENE REPLACEMENT IN *NICOTIANA PLUMBAGINIFOLIA*. László Márton, Ivan F. Kanevsky, and Mihály Czakó. University of South Carolina, Department of Biology, Columbia, SC 29208.

The *101* recipient plant contained a single T_n-DNA copy from a ACH5 *Agrobacterium* strain in it's tetraploid genom, encoding mannopin/agropin biosynthesis in the plant cells. From the 5.4 kb *Eco*RI fragment of the T_n DNA a small central *Hind*III fragment was cut out, thereby truncating the mannopin/agropin genes, and was replaced by a selectable plant marker (a kanamycin cassette). The recombinant fragment was cloned into a binary vector and after *Agrobacterium* mediated transformation kanamycin resistant and mannopin/agropin deficient (AM^-) transformants were expected if homologous gene replacement took place. 738 transformants were analyzed: 42 were AM^-, and in 9 demethylation (treating the cultures with 5-azacytidine) did not restore AM synthesis, therefore they stayed in the putative homologous recombinant category. In one cell line the perfect replacement of the T_n-DNA by the recombinant fragment has been confirmed by Southern blots. Analysis of 5 other lines of that 9 is also presented. Since this result has been obtained in a relatively small transformant population the homologous gene replacement may be far more frequent in plant systems using the *Agrobacterium* mediated gene transfer technology than it was expected based on mammalian analogies.

398 MODULATION OF PLANT GENE EXPRESSION USING BACTERIAL REPRESSORS. Rob Wilde, *Diane Shufflebottom, Sue Cooke, William Brammar, *Mike Bevan and †Wolfgang Schuch. ICI Joint Laboratory, University of Leicester, University Road, Leicester, LE1 7RH, England. *John Innes Institute, Colney Lane, Norwich, NR4 7UH, England. †ICI Seeds, Plant Biotechnology Section, Jealott's Hill, Bracknell, RG12 6EY, England.

One of the major mechanisms acting to control gene expression in a wide diversity of organisms involves the interaction of regulatory proteins with specific DNA sequences. Although such proteins are at present poorly characterised in plants, the protein-DNA interactions between bacterial repressor proteins and their specific operators are well understood at the molecular level. This project investigates the efficacy of two important areas, a) can prokaryotic repressors be used to down regulate plant gene expression and b) does gene expression recover in the presence of inducer. Data will be presented on the effectiveness with which the E.coli lac repressor can down regulate operator containing B-glucuronidase constructs, with subsequent derepression in the presence of IPTG.

399 MOLECULAR BASES OF THE RESISTANCE TO SHIKIMIC HERBICIDES IN ADAPTED MAIZE CELLS LEBRUN M., FREYSSINET M., SAILLAND A., ROLLAND A. & FREYSSINET G. Rhône-Poulenc Agro, B.P. 9163, 69263 LYON Cédex 09, France.

Cells of the black mexican sweet (BMS) corn are highly sensitive to shikimic herbicides (fosametine and glyphosate). These herbicides act by blocking the enol-pyruvyl shikimate phosphate synthase (EPSP-S), an enzyme of the aromatic amino acid pathway. The cell growth is completely inhibited by a concentration of 0.8 mM. This inhibition is reversed by addition of aromatic amino acids. By successive transfers in medium containing increasing concentrations of one of the herbicides, cells have been adapted to resist a concentration of 15 mM fosametine and 50 mM glyphosate. These cells have retained this property when cultured for more than one year on herbicide free medium.

The bases of this increased resistance was determined at the enzymatic, gene and gene expression levels: Analysis of the EPSP-S reveals a 20-30 fold increase in activity in the adapted BMS cells compared to the sensitive ones. This increased enzymatic activity is solely due to a higher steady state level of the enzyme which otherwise keeps its kinetic properties unmodified. Part of a maize EPSP-S cDNA was used to determine the level of mRNA and the gene copy number. For both we found an amplification of 20-30 times, similar to the one observed for the enzymatic amplification.

400 MUTANT TRANSCRIPTION FACTORS AS DOMINANT NEGATIVE INHIBITORS OF PLANT GENE EXPRESSION. Ben Bowen, Pete Solan, Lynne Sims, Bruce Drummond, Sheila Maddock and Brad Roth. Pioneer Hi-Bred Int'l, Inc., P.O. Box 38, Johnston, IA 50131.

Anthocyanin biosynthetic genes in maize are coordinately regulated by R and C proteins, which share homology with the helix-loop-helix and myb families of transcriptional activators, respectively. Two types of vector which inhibit the trans-activation of anthocyanin biosynthetic genes in vivo have been developed. These express either antisense RNA (asR or asC) or mRNA which encodes an altered R or C protein (R-I or C-I). Using microprojectile bombardment, inhibition has been assayed either by counting the number of cells which turn red following transient expression of 35S::R and 35S::C in the presence of increasing amounts of inhibitor template or by measuring Bzl::luciferase activity. The ID_{50}'s (i.e. mole equivalents of template which reduce the control response by 50%) of our most potent R-I and C-I constructs are between 5- and 20-fold lower than the ID_{50}'s of asR or asC. Furthermore, R-I and C-I in combination act synergistically, whereas inhibition by asR and asC in combination is no better than when asR or asC is used alone. Whether R-I and/or C-I will specifically inhibit anthocyanin biosynthesis in stably transformed maize plants is currently under investigation.

401 NUCLEAR RECEPTORS AS MOLECULAR SWITCHES IN YEAST AND IN PLANTS. Cécile Bergmans, Patricia Kock, Peter Weisbeek and Sjef Smeekens. Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The expression of genes involved in plant developmental processes is controlled by transcriptional regulation. To study the *in vivo* function of these genes in transgenic plants an expression system which functions independent from the physiology of the plant is essential. For this purpose we are developing an inducible gene expression system for use in yeast and in plants. This system makes use of the hormone inducible human glucocorticoid and estrogen receptor. Chimaeric genes consisting of the DNA-binding domain of the yeast GAL4 transactivating protein and the transcription activating region of the respective nuclear receptors were cloned behind the yeast ADH1 promoter and the 35S CaMV promoter. As a reporter gene the GUS gene was cloned behind a minimal 35S CaMV promoter linked to four synthetic responsive elements of which the sequence was based on the UASg consensus sequence. Both the effector and reporter genes were transfected to yeast and tobacco plants. The hormone dependent induction of gene expression of the reporter gene is tested in a fluorometric assay for GUS activity and in a S1 nuclease assay for mRNA quantification.

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402 NUCLEAR TRANSCRIPTION ACTIVITY OF THE TOBACCO CHLOROPLAST rps12 GENE FRAGMENT. Anatoly P. Galkin, Paul G. Kovalenko, Nickolay N. Domansky and Fedor P. Galushka. Department of Bioengineering, Institute of Bioorganical Chemistry, Acad. Sci. of Ukrainian, Kiev.

A promoterless neomicyn phosphotransferase II gene has been used to identify tobacco DNA sequences with transcription-promoting activity both in bacteria and plants. One of the cloned fragments exhibiting cell-type specific expression in plants was examined. Sequence analysis revealed that this fragment represented exon 2 of the chloroplast gene for ribosomal protein S12 and adjacent sequences. Transcription start point in transgenic plant was localized within the rps12 DNA coding sequence. These results allow to suggest that silent regulatory elements exist in genomes which can be activated as a result of their translocation.

403 OPTIMIZED CONDITION FOR VIRULENCE GENE EXPRESSION AND AGROBACTERIUM-MEDIATED PLANT TRANSFORMATION. Guangbin Luo, Angus G. Hepburn, Jack M. Widholm. University of Illinois, Plant and Animal Biotechnology Laboratory, Urbana, Illinois 61801.

Agrobacterium-mediated plant transformation has been used extensively to produce transgenic plants. Unfortunately, this system has not been used successfully to transform monocotyledonous species. Even for some important dicotyledonous crops, such as soybean, this system is still very inefficient, mainly due to the low frequency of transformation. A successful transformation event mediated by *Agrobacterium* relies on the coordinated expression of several chromosomal genes of the Agrobacterium host (the *chv* genes) and a series of genes on the Ti-plasmid (the *vir* genes). Several factors, including the acidity of the culture conditions, phosphate starvation, and the presence of acetosyringone, hexoses, and opines, have been reported to cause the induction of the *vir* genes. In our experiment, *A. tumefaciens* strain A281 was pre-induced under different culture conditions and its ability to induce tumors on soybean cotyledons was determined. Our results indicate that pre-induction of *A. tumefaciens* strain A281 in the presence of arabinose, octopine and acetosyringone greatly enhances its ability to produce tumors on soybean cotyledons. This kind of pre-induction may be important in the *Agrobacterium*-mediated transformation of plant species which are relatively recalcitrant to *Agrobacterium* infection.

404 PARTICLE BOMBARDMENT MEDIATED GENETIC TRANSFORMATION OF WHEAT AND BARLEY. Ravindra N. Chibbar, Kutty K. Kartha, Nick Leung, Karen Caswell, Clifford S.Mallard and Lee Steinhauer. Plant Biotechnology Institute, National Research Council Canada, Saskatoon, CANADA.

The ability of high velocity microprojectiles to deliver DNA into intact cells has overcome the problems associated with delivery of DNA into cells capable of regeneration in cereals, such as wheat and barley. This technology has been used in our laboratory to deliver and express a variety of marker genes in cultured cells as well as immature zygotic embryos of wheat and barley. Embryogenic callus derived from the scutellar tissues can give rise to fertile mature plants in both wheat and barley. Constructs carrying hygromycin phosphotransferase (hph) gene driven by cauliflower mosaic virus 35S (CaMV 35S) promoter with an alcohol dehydrogenase 1 (Adh1) intron 1 cloned in between the promoter and coding region of the gene and a nopaline synthase (nos) terminator, when introduced into cultured barley cells and wheat immature zygotic embryos (scutellar tissue) gave rise to hygromycin resistant calli at frequencies up to 18 and 50 %, respectively. Of these hygromycin resistant calli 80 % in barley and 25 -30 % in wheat exhibited the hygromycin phosphotransferase activity, thus showing the expression of introduced gene. Southern analyses performed on a limited number of wheat calli showed the presence of an internal fragment of hph gene in some of them.

405 Production of transgenic plants with enhanced levels of resistance to phosphinothricin through insertion of a modified PAT gene. Raju S.S. Datla[1], Brigitte Bauer-Weston[2], Joe Hammerlindl[1], Colleen Bennett[2], Michael Oelck and Wilf Keller[1]. 1. Plant Biotechnology Institute, Saskatoon, Sask. S7N 0W9, Canada; 2. Hoechst Canada Inc., 106 Research Drive, Saskatoon, Sask. S7N 2X8.0

Phosphinothricin acyl transferase (PAT) confers resistance to L-PPT (Basta) in transgenic plants. We have modified the PAT gene by incorporating an optimal translation context and fusing it to a translational enhancer sequence containing a double CaMV 35S promoter. Agrobacterium binary vectors were constructed carrying the modified PAT expression cassette by itself and in combination with NPT-II and GUS marker genes. Transgenic plants were generated using tobacco and canola (Brassica napus) cultivars. Transgenic plants demonstrated high levels of PAT enzyme activity. When in vitro and soil grown plants were challenged with herbicide (L-PPT), a very high level resistance was observed compared to plants obtained using 35S derived constructs. We have also assessed the potential for generating transgenic plants employing the PAT expression construct without other selectable marker genes.

406 ROLE OF ENDOGENOUS BACTERIA IN MAIZE TRANSGENIC PLANT UNSTABILITY. Kosana Lj.Konstantinov, Miloje P.Denić Snežana D.Mladenović,Dragan R.Kovačević,Tijana Ž. Knežević, Bojana M.Tadić.Maize Research Institute 11081 Belgrade, Yugoslavia

Our recent results (in press) on maize transformation by bacterial genes showed that endosymbiotic P.maltophylia and A.calcoaceticus are responsible for non-mendelian inheritance of introduced genes.As it has been reported several years ago thatsome cultivars of ryegrass harbor seed-borne fungus which is present throughout the body of the plant,the general idea about the same localization and behaviour of endogenous bacteria came out. To test and experimentally support this idea, bacteria living in different maize plant tissue have been identified.Besides two above mentioned strains, E.cloace(API RAPID 20E,5265772)has been found in vascular tissue,leaf,pollen grains and anther tissue in both control and transgenic plants of B73 normal and opaque-2 version maize inbred line.Transgenic plants have been obtained by injection of NPT II gene in archesporial tissue,seed cocultivation in NPT II gene containing plasmide DNA solution and polen tagging of the same gene(M5,M2 and M2 plants respectivelly).Both gene expression (NPTII activity) and Southern analysis of transgenic plants confirmed both plant and bacterial genome transformation and non-mendelian gene segregation.Localization and cell compartmentalization of identified bacterial strains in analysed plant tissue by both light and electron microscopy is in progres.

407 "SAR"-SEQUENCES MEDIATE ENHANCED EXPRESSION OF A CHIMAERIC HEAT SHOCK GENE IN TRANSGENIC TOBACCO. Fritz Schöffl, Gerald Schröder, Mechthild Rieping and Manfred Kliem. University of Tübingen, Biology, Genetics Unit, D-74 Tübingen, FRG

DNA-sequences which facilitate the attachment of chromosomal loops to the nuclear scaffold are also frequently present in the flanking regions of soybean heat shock (hs) genes. A 395 bp fragment from the 3'flanking region of the gene *Gmhsp17.6-L* binds with a high affinity to isolated nuclear scaffolds *in vitro*. Constructions were made that contained the respective SAR fragment either at one side (5' or 3') or at both sides of a chimaeric hs promoter glucuronidase (GUS) reporter gene. A large increase of GUS activity was observed in plants containing constructs with SAR fragments at both sides, a moderate enhancement was caused when the SAR fragments were present at the 5' location. Expression levels were not stimulated by SAR fragments present 3' downstream from the test gene. A good correlation between gene dosage and expression levels suggests that position effects can be reduced for chimaeric genes flanked by SAR sequences.

POSTER ABSTRACTS

408 SITE DIRECTED MUTAGENESIS OF THE INDUCIBLE REGION OF A CHEMICALLY REGULATED CORN PROMOTER. Sonja A. Schmitz, Wayne Ho, Susan Baker, Laura Mercer, and Howard P. Hershey. Du Pont Agricultural Products, Wilmington, Delaware 19880-0402.

We have isolated a corn promoter designated In2-2 which is induced by substituted benzenesulfonamides. The promoter has been fused to a GUS reporter gene and shown to respond to chemical inducer in a rice protoplast transient assay system. This system allowed us to easily test manipulated promoters without having to go through stable transformation in plants. In order to define the sequences which were necessary for induction, a series of 5' and 3' deletions of the promoter were carried out. The deletion series showed the presence of a region from - 93b.p. to - 163 b.p. from the transcription start site which was necessary for chemical regulation. A random mutagenesis experiment was performed to locate regions within the inducible element which were sensitive to mutation. Based on the results obtained from the random mutagenesis, a 28 n.t. region of sequence was selected to perform site directed mutagenesis in order to specifically define the regions where changes could or could not be made. The results from these experiments are shown in this poster. A seven b.p. region from -108 to -114 was shown to be particularly sensitive to mutation. For the most part, transversions from a purine to a pyrimidine or vice versa, resulted in a loss of activity of seventy percent or more. However, transitional mutations within this same region affected both induced and uninduced levels of expression in such a way as to create new promoters which may have both research and commercial applications.

409 SITE DIRECTED MUTAGENESIS OF THE GENOME OF HIGHER PLANT CELLS VIA AGROBACTERIUM. Remko Offringa, Marcel J.A. de Groot Mirjam P. Does, Paul J.J.Hooykaas and Peter J.M. van den Elzen,

MOGEN International nv, Einsteinweg 97, 2333 CB Leiden and Leiden University, Clusius Laboratories, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

We determined whether *Agrobacterium* T-DNA is a suitable substrate for homologous recombination in the plant cell. Our results showed that homologous recombination occurs at high frequencies (1-4%) between T-DNAs that have been co-introduced from separate bacteria into tobacco protoplasts. More importantly, we demonstrated that a target locus in the plant genome is altered after homologous recombination with a T-DNA that was introduced into the plant cell via *Agrobacterium tumefaciens* (Offringa *et al.* "Extrachromosomal homologous recombination and gene targeting in plant cells after *Agrobacterium* mediated transformation, EMBO Journal; vol. 9, no. 10: 3077-3084). We will present a detailed molecular analysis as well as genetic data of one gene targeting event. Since this gene targeting occurred at a low frequency (10^{-5}), we are now optimizing our system to enrich for recombinants in a large pool of transformed cells. In our approach we have analyzed the potential of the aux-2 gene of the *Agrobacterium* T-DNA as a negative selectable or screenable marker. Moreover, in several experiments using the same target plant as mentioned above in combination with a repair construct containing the aux-2 gene we obtained recombinants of which we will present the molecular analyses. Our findings open the possibility to use the *Agrobacterium* transformation system for site directed mutagenesis (SDM) of the plant genome. The SDM-technique will have its implications for the use of reverse genetics in basic plant research as well as for the genetic engineering technology of crop plants.

410 SITE DIRECTED MUTAGENESIS OF THE GENOME OF HIGHER PLANT CELLS VIA ELECTROPORATION. Marcel J.A. de Groot[1], Remko Offringa Mirjam P. Does, Paul J.J. Hooykaas[2] and Peter J.M. van den Elzen .

1)MOGEN International nv, Einsteinweg 97, 2333 CB Leiden, The Netherlands. 2)Clusius Laboratories, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

In order to investigate mechanisms of homologous recombination in plants we performed both extrachromosomal recombination experiments as well as gene targeting experiments. For this purpose we constructed defective derivatives of the NPTII gene that contain a deletion or a frameshift mutation either in the 5' or the 3' part of the gene. Protoplasts of *Nicotiana tabacum* SR1 were transformed via electroporation. Depending on the combination of constructs that were cotransformed, restoration of Km' was found with a frequency of 1.1 up to 29.5% of the transformed calli. Transformation of each of the mutant NPTII genes separately did not give rise to any Km' calli. The presence of an intact NPTII gene formed via homologous recombination was confirmed through molecular and genetic analysis of plants regenerated from the recombinant calli. Currently we are investigating the use of ssDNA constructs in cotransformation experiments.

In addition we performed gene targeting experiments. A transgenic tobacco line containing a T-DNA insert carrying a defective NPTII gene with a 3' deletion was transformed with a defective NPTII repair construct. Experiments are in progress and results will be discussed. This combination of targetline and repair construct was also used for gene targeting via *Agrobacterium tumefaciens* (Offringa et al., EMBO Journal; vol 9, no. 10, p. 3077-3084) and therefore it will be possible to compare these two transformation methods with respect to gene targeting.

411 **STABLE CO-TRANSFORMATION OF GENES IN *TRITICUM AESTIVUM* CELLS AFTER PARTICLE BOMBARDMENT.** Stephen J. Meiza, Teresa Nguyen, Jonathan P. Driller, Leslie S. Walker, Tom Ulrich, and James R. Wong.

Sogetal, Inc., 3876 Bay Center Place, Hayward, California 94545

Evidence is presented for stable integration, expression, and co-expression of DHFR and GUS genes introduced into suspension culture cells of *Triticum aestivum* cv. Mustang via a high pressure, helium-propelled ballistic apparatus. Particles were coated with equal amounts of two plasmid constructs differing only in the marker genes (DHFR or GUS) driven by a 35S-promoter. Stably transformed calli started to appear after 45 days of culture on selective medium containing 1 mg/l methotrexate (applied 7 days post-bombardment). Cellular evidence for stable transformation with these two marker genes was the continued growth of calli on medium containing 5 mg/l methotrexate, and a visual GUS-positive response within 24 hrs. after staining cells with X-Gluc. Co-expression was observed from approximately 10% of the transformed calli. Molecular analyses of transformed calli to confirm transformation and compare co-integration to co-expression will be presented.

412 STABLE TRANSFORMATION OF SOYBEAN (GLYCINE MAX) PROTOPLASTS AND REGENERATION OF TRANSGENIC PLANTS. Sarwan K. Dhir, Seema Dhir and Jack M. Widholm. University of Illinois, Department of Agronomy, Urbana, IL 61801

Transgenic soybean (Glycine max (L.) Merr.) plants resistant to hygromycin-B have been regenerated from cotyledonary protoplasts following electroporation with plasmid DNA carrying linked chimeric genes for ß-glucuronidase (GUS) and hygromycin phosphotransferase (HPT) under the control of the CaMV 35S promoter. Over 93% of the hygromycin resistant (40 ug/ml) cells and colonies exhibited GUS activity indicating that the two genes were co-introduced and co-expressed. The transformation procedure reproducibly yields transformants at absolute transformation frequencies of 2.9-6.8 x 10^{-4} or relative transformation frequency of 23.0% counted after 6 weeks of selection. After repeated subculturing of callus on regeneration medium consisting of MS$_8$ salts+0.5 mg l^{-1} each of BA, KN, ZT+0.1 mg l^{-1} NAA+0.2 mg l^{-1} GA_3+50 mg l^{-1} asparagine and glutamine, 8.0% of the transformed calli formed shoots. Southern hybridization showed that both the GUS and HPT probes hybridized to DNA in all of the calli and leaves from the transgenic shoots which were tested. These shoots were rooted on 1/2 strength MS medium with 0.5 mgl^{-1} NAA. Co-transformation experiments performed with a selectable (HPT) and a non-selectable (GUS) gene on seperate plasmids, resulted in a co-transformation frequency of 64-70%. Hence, this approach can be used to introduce linked as well as unlinked genes at a reasonably high frequency into the soybean genome.

413 **STRUCTURE AND EXPRESSION OF 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE GENES FROM *ARABIDOPSIS THALIANA**** Xiaowu Liang, Julie A. Keller and Athanasios Theologis. Plant Gene Expression Center, ARS-USDA, 800 Buchanan St., Albany, CA 94710 and Dept. of Plant Biol., UC Berkeley, CA 94720

ACC synthase catalyzes the conversion of S-adenosylmethionine (Adomet) to 1-aminocyclopropane-1-carboxylate (ACC) - a key and rate-limiting step in the biosynthesis of plant hormone ethylene (1). Genomic sequences containing ACC synthase genes were isolated from *Arabidopsis thaliana* genomic libraries using heterologous cDNAs (2,3,4) as probes. The results revealed a multigene family consisting of at least 4 diverged members. The sequence of a developmentally regulated ACC synthase gene, AT-ACC2, has been determined. The corresponding cDNA has been isolated and characterized, and its authenticity confirmed by expression experiment in *E. coli*. Results will be presented to show the regulation of expression of this and other members of the gene family in response to environmental and developmental cues. Structures and organizations of ACC synthase genes from *Arabidopsis* and other plants in connection to another group of evolutionarily related genes (i.e. aminotransferases) will be discussed.

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POSTER ABSTRACTS

414 SUPPRESSION OF GENE EXPRESSION WITH ANTISENSE SEQUENCES TRANSCRIBED BY tRNA GENES. June E. Bourque and William R. Folk. University of Missouri, Coulumbia, Missouri 65211.

We have developed an expression system for non-coding (antisense) RNA utilizing polymerase III genes as constitutive promoter elements. As a model system, we studied suppression of chloramphenicol acetyltransferase (CAT) gene function. We have demonstrated that antisense sequences co-transcribed with the tRNA methionine initiator ($^{met}_i$) gene from soybean inhibits CAT expression in carrot protoplasts and appears to be more effective than previously reported antisense constructs from the Cauliflower mosaic (CaMV) 35S RNA promoter. Our studies indicate that the 3' portion of the CAT gene fused to the soybean tRNA$^{met}_i$ gene is highly effective. 100ug of this antisense DNA construct, electorporated simulataneously with 1ug of sense CAT DNA driven by the CaMV 35S RNA, always suppresses below basal level expression (not carrier enhanced) up to as much as 90%. This is substantially greater than that seen with antisense CAT clones containing sequences complementary to the 5' region of CAT or with the full length antisense CAT gene driven by the CaMV 35S RNA promoter. In processing experiments utilizing nuclear extracts prepared from carrot protoplasts, the tRNA$^{met}_i$, tRNA^{Tyr} and tRNA^{Leu} genes isolated from *Arabidopsis thaliana* and the tRNA$^{met}_i$ gene from soybean, all process at different levels of efficiency. All transcribe *in vitro* using nuclear extracts from human 293 cells. The portions of the CAT gene found to function best as antisense sequences have been cloned into vectors containing these genes. The effectiveness of each as an antisense promoter will be discussed.

415 SYNTHETIC B.T. GENE IMPROVED FOR HIGH EXPRESSION IN PLANTS. D Sutton, P Havstad, S. Bagga, J. Kemp. New Mexico State Univ., Plant Genetic Engineering Lab., Las Cruces, NM 88003

An 1800 bp. synthetic gene has been constructed from chemically sythesized oligonucleotides. The sequence of the DNA is based on the amino acid sequence of the*Bacillus thuringiensis* var tenebrionis [B.T.t] (V. Sekar, et al. 1987. PNAS 84, 7036) crystal protein toxin. This protein possesses insecticidal activity against the coleopteran order but not lepidopteran or dipteran species.

Previously, investigators have reported that transgenic plants express B.T. crystal protein genes at very low levels, both in the amount of protein produced as well as the amount of messenger RNA (F. Perlak, et al. 1990. Biotechnology 8, 939). This appears to be due to inefficient transcription and translation of the gene because of nucleotide sequences that mimic a number of putative eukaryotic signal sequences. These signals include those for poly-A addition, RNA degradation, and intron splicing. Specific alterations were made in the B.T.t. coding sequence to remove these signals while preserving the amino acid sequence. Further alterations included changing many amino acid codons to those commonly used by plants as well as the addition and elimation of restriction sites. The improved synthetic B.T.t. coding sequence has been placed behind the 35S promoter containing the AMV translation enhancer and transferred to various plant species, including potatoes, alfalfa and cotton. We also have engineered the synthetic gene behind the bacterial TAC promoter for expression in bacteria.

416 TESTING IN COTTON TISSUE CASSETE FOR THE REGULATED EXPRESSION OF FOREIGN GENES. F.Muhamedhanova, M.Muminova, A.Tashboulatov, A.Abdukarimov. A.S.Sadykov's Institute of Bioorganic Chemistry, Academy of Sciences Uzbek SSR, Tashkent, USSR.

The expression cassette consisting of D.melanogaster hsp-70 gene promoter and pTiC58 plasmid nopaline synthase gene transcription terminator was constructed. For the testing of this cassette as a marker the NPT II gene from the bacterial transposon Tn 5 was used. For the transfering of the construction to plant genome the binary system of transformation based on LBA 4404 was used. Transformants were selected on MS medium containing 100 mcg/ml of kanamycin, 500 mcg/ml of carbenicillin and phytohormones for cotton calli formation at 28°C at 16 hours photoperiod (4000 lux). The heat shock was performed at 40°C during 45 minutes.

417 TRANSFORMATION OF MAIZE CALLUS USING DIHYDROFOLATE REDUCTASE AS A SELECTABLE MARKER.

Jennifer M. Chase[1], Anne Fennell[1], Scott Galasinski[1], Mitchell Altschuler[1], Tom Ulrich[2], and Randal Hauptmann[1]. [1]Plant Molecular Biology Center, Northern Illinois University, Montgomery Hall, DeKalb, IL 60115. [2]Sogetal, Inc., 3876 Bay Center Place, Hayward, CA 94545.

One of the major difficulties in the isolation of transgenic cereals is the lack of an unambiguous selectable marker. However, one marker, methotrexate (MTX) has been shown to be a potent selection agent in cereals. Mouse, bacterial and synthetic dihydrofolate reductase (DHFR) genes were evaluated as alternative selectable markers. These three DHFR enzymes have reduced affinity for MTX and are tested in protoplast culture systems of *Zea mays*. The ability of the mouse DHFR gene (pMON806) has previously been shown to confer resistance to MTX in cereal protoplasts. We compared the mouse DHFR gene, a bacterial DHFR gene (R67 plasmid of *E. coli*) as well as a synthetic DHFR gene to determine the relative resistance conferred by each construct. The three genes were cloned into CaMV 35S expression cassettes using the nopaline synthase polyadenylation signal. The synthetic DHFR is based upon the R67 DHFR gene using the preferred codon usage of maize which favors a C or G nucleotide in the "wobble" position. The synthetic DHFR was generated by a modified PCR reaction using four oligonucleotides which spanned the complete 250 base pair sequence. These mutant DHFR genes were assessed for efficiency as selectable markers in the graminae.

418 TRANSFORMATION OF *TRITICUM MONOCOCCUM* L. CELLS AND ANALYSIS OF LONG TERM STABILITY OF FOREIGN GENES. Chen DF, Dale PJ and Mullineaux PM. John Innes Institute, Colney Lane, Norwich, NR4 7UH, UK

Transformed cell lines of *Triticum monococcum* L. were achieved by PEG (polyethylene glycol) stimulated DNA uptake into protoplasts or microprojectile bombardment of suspension cells using constructs containing both *gus* gene and a selectable marker genes (*aph* IV and *dhfr*, coding for resistance to hygromycin and methotrexate respectively). Initially, these transformed cells showed both antibiotic resistance and β-glucuronidase activity, and presence of *gus* and *dhfr* or *aph* IV gene was confirmed using Southern hybridization technique. After being kept on selection medium (Hygromycin B 100 mg/l or methotrexate 1.0 mg/l) for 18 to 24 months, some of these transformed cell lines were found to become GUS negative. Full length *gus* coding sequence was not detected in genomic DNA of these transformed cell lines using a standard polymerase chain reaction (PCR) procedure. However, amplification of *gus*cDNA prepared from RNA of these cell lines using the PCR 3' RACE (rapid amplification of cDNA 3' ends; Frohman *et al* 1988) indicated the presence of truncated *gus* transcripts i.e. a sequence smaller than expected for a complete *gus* mRNA. The data of genomic mapping and sequencing of *gus* cDNAs from these antibiotic resistant cell lines (including both *gus* positive and negative) will be presented. The stability of foreign DNA introduced by means of direct DNA delivery into plant cells will also be discussed along with experimental data.

Reference

Frohman MA, MK Dush and GR Martin. 1988. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligo-nucleotide primer. Proc. Natl. Acad. Sci. USA 85:8998-9002.

419 TRANSGENIC ARABIDOPSIS PLANTS OBTAINED BY A PNEUMATIC PARTICLE GUN. H. Morikawa[1], M. Seki[2], T. Yamashita[1], N. Shigemoto[1], Y. Yamada[2] & Y. Komeda[3]. [1]Fac. of Sci., Hiroshima Univ., Hiroshima 730, Japan. [2]Fac. of Agric., Kyoto Univ., Kyoto 606, Japan. [3]Mol. Genet. Res. Lab., Univ. of Tokyo, Tokyo 113, Japan.

Gene delivery to plant cells using particle gun devices that are driven by nitrogen gas pressure[1,2] and compressed air[3,4] has been studied. We previously reported[2] stable transformation of tobacco cells using these devices. We here report on stable transformation of Arabidopsis thaliana. Using partially optimized conditions, more than 5000 blue spots of GUS-expressing cells (per root tissues spread on a filter paper of 55mm diameter at 200 kg/cm) were obtained in Arabidopsis root cells. In the case of tobacco cells, more than 2000 blue spots (per 35mm diameter at 115 kg/cm) were observed. Cytological studies indicated that intranuclear gene delivery was essential for successful gene expression in tobacco cells. NPTII gene was delivered to root tissues of Arabidopsis and green calli were formed from the bombarded roots in the presence of 20 mg/l kanamycin or geneticin. Green shoots regenerated from the calli were transferred to root-inducing medium containing 20 mg/l geneticin. At present more than 150 plants were obtained and they set seeds. Ensyme assays and Southern blot hybridization confirmed gene expression of the foreign gene and its stable integration into the Arabidopsis genome. 1) H. Morikawa, A. Iida and Y. Yamada. Appl. Microbiol. Biotechnol. 31:320(1989). 2) A. Iida, H. Morikawa and Y. Yamada. Appl. Microbiol. Biotechnol. 33:560 (1990). 3) A. Iida, M. Seki, M. Kamada, Y. Yamada and H. Morikawa. Theor. Appl. Genet. 80:813(1990). 4) M. Seki, Y. Komeda, A. Iida, Y. Yamaka and H. Morikawa. Plant Mol. Biol. (in press).

POSTER ABSTRACTS

420 TRANSIENT AND LONG-TERM EXPRESSION KINETICS OF ß-GLUCURONIDASE IN SOMATIC VS ZYGOTIC EMBRYOS OF WHITE SPRUCE. D. Ellis[1], D. McCabe[2], S. McInnis[2], B. Martinell[2], D. Roberts[2] and B. McCown[1]. [1]University of Wisconsin-Madison, Madison, WI; [2]Agracetus, Inc., Middleton, WI; [3]Forest Biotechnology Centre, Vancouver, B.C.

The developmental stage of cells play an important role in the expression kinetics of introduced genes. We have used electrical discharge particle acceleration to introduce a CaMV-35s-GUS containing plasmid into somatic and zygotic embryos of white spruce. While zygotic embryos required a pretreatment on a bud induction medium for a high level of transient GUS expression, a pretreatment of somatic embryos on embryogenic medium had only a minor affect. Long-term GUS expression patterns also varied between the two types of embryos. In the zygotic embryos, maximal GUS expression was maintained for 7 days followed by a sharp decrease in GUS expression to a baseline level after 14 days. This level was maintained for over 56 days. In contrast, maximal GUS expression in somatic embryos was maintained for 28 days. Although the relative level of GUS expression was dependent on the developmental stage of the somatic embryo, the kinetics of expression remained similar for all embryo stages. The frequency of dividing cells expressing GUS was greater in the somatic embryos than the zygotic embryos. By maintaining this higher level of introduced gene expression in somatic embryos, we have been able to differentiate transformed organized proembryos. These results suggest that although the two explants are morphologically similar, the physiological and developmental conditions of the cells play a major role in the expression kinetics of introduced genes and the potential regeneration of transformed plants.

421 TRANSIENT GUS EXPRESSION IN MAIZE TISSUES USING *AGROBACTERIUM*-MEDIATED TRANSFORMATION. S. W. Ritchie, C. Liu, H. Kononowicz, T. K. Hodges, and S. B. Gelvin. Dept of Botany and Plant Pathology (SWR, HK, TKH) and Biology Dept (CL, SBG), Purdue University, West Lafayette, IN 47907. A recent report by Gould et al. (1991) [Plant Physiol. 95: 426-434] suggests that transformation of maize (*Zea mays* L.) apices with *Agrobacterium tumefaciens* is possible. In an effort to develop a defined and optimized system for *Agrobacterium*-mediated transformation of maize, we have histochemically investigated the transient expression of GUS activity in maize seedling tissue segments (1mm long) using the *Agrobacterium* strain At565. This strain contains a C58 chromosomal background, a disarmed version of the pTiBo542 supervirulent Ti-plasmid, and the binary vector pKIWI105 (Janssen and Gardner, Plant Mol Biol 14: 61-72) that does not express GUS in the bacterium. Three to five day-old sterile seedlings of maize genotype A188 were utilized as the source of tissue segments. At four days after inoculation, transient GUS expression was repeatedly found in mesocotyl segments taken from the intercalary meristem region. This expression was specific to the vascular cylinder and has not been found in the cortex or epidermal layers, nor was it found in mature mesocotyl tissue (segments 5mm below the coleoptilar node). We have also detected transient GUS expression in leaf and coleoptile tissue segments, although this is less consistent than was expression in the meristematic mesocotyl segments. We have never found transient GUS expression in the shoot apex tissues of genotype A188 or Funk's G-90 (Gould et al. 1991). Tests using a Ti-plasmid cured strain (C58 background) that also contains pKIWI105 have not shown GUS expression, indicating that *vir* gene activity was essential for GUS expression. The inclusion of 2,4-D (2mg/L) in the tissue culture medium during cocultivation was essential for GUS expression. Higher 2,4-D concentrations and the inclusion of light did not improve GUS expression. In conclusion, transient GUS expression can be detected in sterile mesocotyl, leaf, and coleoptile tissues of maize following transformation with *Agrobacterium tumefaciens*.

422 TRANSGENIC PLANTS EXPRESSING MICROBIAL GENES CODING FOR IMPROVED ENZYMES IN THE ASPARTATE FAMILY PATHWAY EXHIBIT OVERPRODUCTION OF EITHER LYSINE OR THREONINE AND SHOW A MODIFIED PHENOTYPE. G. Galili, O. Shaul and A. Perl, Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76 100, Israel.

Addressing the general problem of low proportions of the essential amino acids lysine and threonine in plants, we have transformed Solanaceae plants using chimeric genes containing the coding DNA sequences of the *dap*A and *lys*C genes from *Escherichia coli*. These bacterial genes code for dihydrodipicolinate synthase (DHPS) and aspartate kinase (AK), two key enzymes in the aspartate family pathway, whose activities are much less sensitive to inhibition by lysine and threonine than plant enzymes. A DNA fragment coding for a chloroplast transit peptide was also included in the constructs to direct the bacterial enzymes into the chloroplast, where lysine biosynthesis takes place in higher plants. Leaves of transgenic plants had high levels of DHPS or AK activities, which were much more resistant to lysine and threonine inhibition compared to non-transformed plants. These activities were largely compartmentalized within the chloroplast and were accompanied by significant increases in the levels of free lysine (DHPS) or threonine AK). No lysine or threonine overproduction was obtained in control plants in which the bacterial enzymes were expressed in the cytoplasm. Transgenicplants that accumulated the highest levels of lysine exhibited partial loss of apical dominance, delayed flowering, partial sterility and some abnormal leaf morphology. The possible relationship between the aspartate family pathway and these phenotypic alterations will be discussed. Our results indicate the possibility of improving the nutritional quality of plants by genetic engineering.

423 TRANSIENT EXPRESSION OF CHIMERIC GENE IN WHEAT BY COCULTIVATION WITH *Agrobacterium tumefaciens*. Ousama M. Faiz Zaghmout and Wen-Chung Wang. Texas Tech University, Department of Agronomy, Horticulture and Entomology, Lubbock, Texas 79409.

In wheat, no successful fertile plant regeneration and transformation protocols have been developed yet. Our interests, therefore, were directed toward developing such a system for this important cereal crop. *A. tumefaciens* has been used routinely in transferring genes into dicot plants where the frequency of stable transformation and fertile plant regeneration is much greater and more reliable than with any other gene transfer method. On the contrary, monocot species were not as responsive as dicots. In this study, we have used several wide-host range supervirulent stains of *A. tumefaciens*. Initially, to determine if these strains would actualy infect the wheat tissues, transient expression assays were carried out where the isolated protoplasts from wheat genotypes including 'Mit' (non-embryogenic cell type=NE), and 'Mustang' and 'Chinese Spring' (embryogenic cell lines=E) were co-cultivated separatly with *A. tumefaciens* strains each harboring the pK1w 105 plasmid which carries the encoding gene for B-glucuronidase and neomycine-phosphotransferase-II (20 ul of overnight grown bacterial culture/ ml of protoplast culture solution with a density of 10^6). The strains used in this study were A856, A281, C58, A4 and LBA4404. Results from these co-cultivation experiments shown that the level of GUS expression is greatly affected by the strain and the embryogenic nature of the tissue used. The level of GUS expression induced by A281(pK1W 105) found to be at least 75-100 folds over the control. In addition, more GUS activity was found in protoplasts infected with A281 than with any strain mentioned above. Furthermore, the level of GUS expression was twice higher in protoplasts originated from E cell types. Efforts are now underway to optimize the conditions for developing stable transformation in wheat with *A. tumefaciens* .

424 TRANSIENT GENE EXPRESSION UNDER CONTROL OF CaMV 35S AND NOS PROMOTERS IN A MARINE RED ALGA (RHODOPHYTA) Aaron L. Kurtzman, Uwe Klein*, and Donald P. Cheney. Dept. of Biology, Northeastern University, Boston, MA 02115. *The Biological Laboratories, Harvard University, 16 Divinity Ave., Cambridge, MA 02138

Very little is known about promoters and gene expression in multicellular algae. Homologous promoters have not yet been cloned for use in gene expression studies. To date, foreign gene expression has only been accomplished in a few unicellular (eg. *Chlamydomonas*) and coenocytic (eg. *Acetabularia*) algae. This study is the first to demonstrate transient gene expression of a foreign gene and to identify useful heterologous promoters in a multicellular red alga. Transient gene expression was observed in several different types of cells of *Kappaphycus alvarezii*. Three Plasmids, pVW426, pVW296 and pRAJ275, were introduced into plant sections using the Du Pont Biolistic/particle delivery system. The pVW426 incorporates a Cauliflower Mosaic Virus 35S promoter region upstream of a b-glucuronidase (GUS) marker gene, the pVW296 has a nopaline synthase (NOS) promoter upstream of the GUS gene and the pRAJ275 is a promoterless (control) GUS plasmid. Cells which received the 35S-GUS and the NOS-GUS constructs showed positive GUS staining in the presence of the substrate X-gluc. Whereas cells which received the promoterless control plasmid were not stained. Currently, the chlorophyll a/b binding protein (Cab) promoter from pea is being tested which has been shown to be light-regulated and tissue-specific in higher plants.

425 UNUSUAL REGULATION OF POTATO CYTOSOLIC PYRUVATE KINASE IN TRANSGENIC TOBACCO. SG Gottlob-McHugh, BL Miki, Plant Research Centre, Agriculture Canada,Ottawa,Ont,Canada, K1A OC6 RS Sangwan, SD Blakeley, GC Vanlerberghe, K Ko, DH Turpin, WC Plaxton, DT Dennis, Dept. of Biology, Queen's University, Kingston, Ont., Canada, K7L 3N6.

Pyruvate kinase is a key glycolytic enzyme that catalyses the formation of pyruvate and ATP from phosphoenolpyrvate (PEP) and ADP. To investigate the effect of overexpressing cytosolic pyruvate kinase (PKc) in transgenic plants, tobacco tissue was transformed with a potato PKc construct under the control of the cauliflower mosaic virus 35S promoter. Unexpectedly, several primary transformants lacked cytosolic pyruvate kinase in leaf tissue. Both potato PKc transgene transcripts and the endogenous tobacco PKc gene transcripts were suppressed within the leaves of these plants. Enzyme assays failed to detect PKc activity and no PKc protein was detected on western blots. Testing of progeny indicated that lack of leaf PKc is a stably inherited trait. Although these plants lacked PKc activity in the leaves, there appeared to be no difference in the viability and growth of the plants when compared to wild type. The leaves have normal rates of photosynthetic O_2 evolution and respiratory O_2 consumption. Metabolite analyses of leaf tissue indicated that these plants had levels of pyruvate similar to the wild type, but the levels of PEP and 3-PGA were substantially higher in the plants lacking PKc. Surprisingly, wild type levels of enzyme activity and protein were detected in the roots of these plants. This suggests that the co-suppression of PKc may not be occurring in the roots. We are currently analyzing additional plants to investigate this phenomenon.

POSTER ABSTRACTS

426 USE OF DIFFERENT SELECTION AGENTS TO PRODUCE MAIZE TRANSFORMANTS OF AN ELITE GENOTYPE USING MICROPROJECTILE BOMBARDMENT. Janet Suttie, Erik Dunder, Martha Hill, Vivian Higgins, Jon Dietz, Mike Koziel, Kelly Lewis, Ray Shillito and Gary Pace. CIBA-GEIGY Agricultural Biotechnology, POB 12257, Reasearch Traingle Park, NC 27709.

The selection agents paromomycin (neomycin E), G418 (geneticin), hygromycin and phosphinothricin were tested for their efficacy to produce maize transformants using microprojectile bombardment with an elite inbred culture. Sublethal and lethal levels of the different agents were determined in dose response experiments. The use of paromomycin, geneticin and phosphinothricin resulted in high frequencies of transformation and thereby low escape frequencies, producing a large number of transformants with each agent. Hygromycin was difficult to assess due to the high concentration required for lethal selection. GUS histochemical assays were done for each construct as an internal control of expression. Using phosphinothricin, "co-transformation" (bombardment with genes on different vectors) was compared to "co-integration" (bombardment with different genes on one vector only). Both methods produced equivalent numbers of transformants at high transformation frequencies.

427 VECTOR LINEARIZATION AFFECTS TRANSIENT GENE EXPRESSION AND TRANSFORMATION FREQUENCY IN BMS CELLS Joanne A. West, Irvin J. Mettler and Paul S. Dietrich. Sandoz Crop Protection Corporation, Plant Biotechnology Department, Palo Alto, CA 94304.

DNA topology is known to affect the expression of introduced genes in animals and dicot plants. To study this in a monocot system, both supercoiled and linearized plasmids were electroporated into Black Mexican sweet corn (BMS) protoplasts. Recipient cells were assayed for the transient expression of beta-glucuronidase and neomycin phosphotransferase reporter genes and for frequency of stable transformation. Linearization of DNA was found to differentially affect the level of transient expression, depending on the site of cleavage. However, all linearized plasmids supported a transformation frequency three to five-fold higher than their corresponding supercoiled structures. Results using the transformation method of particle gun bombardment will also be presented. Several factors may be important in determining the effect of vector linearization on transient gene expression and transformation frequency in corn.

428 WILDTYPE AND o2 MAIZE ENDOSPERM CELL CULTURES: THEIR USE IN STUDIES ON ENDOSPERM SPECIFIC GENE REGULATION. L.M. Manzocchi, S. Faranda, A. Genga and A. Viotti. Istituto Biosintesi Vegetali, C.N.R. - 20133 Milano, Italy.

Studies on the regulation of endosperm-expressed genes in maize have been hindered by the difficulties in obtaining fertile transformed plants and by the lack of homologous cell systems for transient expression studies. This has led to a renewed interest in tissue cultures from maize endosperms, which maintain in vitro most of the physiological features of the intact tissue (Lyznik and Tsai, Plant Sci. 63: 105-114, 1989; Quayle et al., Plant Cell Rep. 9:544-548, 1991; Manzocchi, Plant Cell Rep. 9:555-558, 1991).

We present here the characteristics of zein synthesis by endosperm cell cultures of A69Y wt and opaque-2 maize, stabilyzed in culture for more than 2 years. Cultured cells can be transformed by biolistic methods, and their protoplasts by electroporation or PEG-mediated DNA uptake. Both transient expression and stable integration of transfected genes are investigated.

429 THE RNA POLYMERASE II LARGEST SUBUNIT OF PLANTS IS PHOSPHORYLATED BY A CDC2-HOMOLOGOUS KINASE. László Bakó[1,2] & Christiane Nawrath[1,3], Jeff Schell[1], Dénes Dudits[2] and Csaba Koncz[1,2].

1) Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG, 2) Institute of Plant Physiology, Biological Research Center of the Hungarian Academy of Sciences, M-6701 Szeged, Hungary; 3) Plant Research Laboratory, MSU-DOE, East Lansing, MI, 48824, USA

The C-terminal domain (CTD) of the largest subunit of the RNA polymerase II of *Arabidopsis thaliana* consists of 16 conserved and 26 variant heptapeptides of the consensus sequence YSPTSPS. The complete CTD could be expressed as a protein A-CTD fusion protein with an unusual low electrophoretic mobility. A Ca^{2+}-independent protein kinase purified from alfalfa (*Medicago sativa*), which is able to phosphorylate histone H1, was found to phosphorylate *in vitro* the CTD of the fusion protein as well as the RNA polymerase II largest subunit isolated from tomato. The kinase shows a high affinity to $p13^{suc1}$-Sepharose beads and is recognized by the PSTAIR-antibody on Western blots. Based on these features and results from experiments using synchronized alfalfa cells this kinase is proposed to represent a cdc2-homologue.

430 GENE EXPRESSION FROM BRASSICA NAPUS TISSUE-SPECIFIC PROMOTERS IN TRANSGENIC PLANTS. Chris L. Baszczynski, Lynne Fallis, Sylvia Rozakis, Bin Huang, MaryAnne Arnoldo, Neil Maclean, Don MacRae, Rodolphe Boivin, [1]Guy Bellemare and Roger J. Kemble. Allelix Crop Technologies, 6850 Goreway Dr., Mississauga, Ontario, Canada L4V 1P1 and [1]Dept. de Biochimie, Universite Laval, Ste. Foy, Quebec, Canada G1K 7P4.

Promoters of Brassica napus genes which express in a tissue- or stage-dependent manner were recovered by first identifying cDNA clones with tissue-or stage-specific expression, then isolating, sequencing and characterizing the corresponding genomic clones. Promoters for the Brassica RUBISCO SSU and napin genes were introduced into binary vectors in front of the GUS reporter gene, as well as agronomically useful genes, and used to transform canola and tobacco. Expression data from the GUS vector constructs indicates that the SSU promoter shows the predicted tissue-specific and light-inducible expression, and that different levels of expression are attained with different length upstream regions. Expression from the napin promoter is very strong and specific; GUS activity was detected only in seed and embryo tissues and was observed to increase in the later maturing stages. For any one vector, absolute expression level varies among transgenics. The number of gene integration events also appears to influence the expression level. Microspore-derived doubled haploid progeny from the transgenics will permit analysis of the segregation of different combinations of the introduced genes in those plants with multiple integrations, and provide a measure of the effect of gene interactions. Recently, a root specific promoter was isolated and transferred into binary vectors, and plant transformations are underway. Tissue-specific promoters of other Brassica genes are at various stages of isolation or characterization.

431 THE CHLAMYDOMONAS REINHARDTII CHLOROPLAST *psb*B GENE: TRANSCRIPT MATURATION AND STABILITY DETERMINANTS. S.L. Berry-Lowe[1], C. Johnson[2], and G.W. Schmidt[2]. [1]Department of Biology, University of Colorado at Colo. Spgs., Colorado Springs, CO 80933. [2]Department of Botany, University of Georgia, Athens, GA 30602.

The chloroplast *psb*B gene encodes the 47-kilodalton chlorophyll-binding protein associated with the PSII reaction center. A photosynthetic mutant, GE2.10, of the green algae *Chlamydomonas reinhardtii* (Jensen et al, J.Cell Biol. 103:1315-1325, 1986) lacks the 47-kDa protein (and other PSII polypeptides) and its mRNA. Further analyses of GE2.10 have shown that the *psb*B transcript is unstable since its gene is transcribed but not accumulated (Sieburth et al, Plant Cell 3:175-189,1991). Several additional, smaller transcripts from the vicinity of the *psb*B gene were also shown to be unstable in GE2.10. These transcripts may be processing intermediates or derivatives (products of a large precursor transcript). Alternatively, they may be independent transcripts from other unidentified genes of the *C. reinhardtii* chloroplast genome. Because the chloroplast genome of *C. reinhardtii* is not conserved when compared to chloroplast genomes of other lower and higher plants, the identity of genes in this region of the genome needs to be determined. Nucleotide sequencing of the *psb*B gene and flanking regions has been carried out to clarify these issues. Immediately downstream of the *psb*B gene are several regions which may form stable hairpin structures in the transcript. These structures may be important in the stabilization or processing of the *psb*B transcript. Further sequencing of the 3' region is in progress as is the isolation of binding proteins which may interact with these regions in the 3' flanking region of *psb*B. Supported by NSF.

POSTER ABSTRACTS

432 AN ATTEMPT TO RECAPITULATE CAULIFLOWER MOSAIC VIRUS TRANSACTIVATION IN FISSION YEAST. J-M. Bonneville[c§], P. Schär[£], J. Kohli[£] and T. Hohn[c]. [c]Friedrich Miescher-Institut, Postfach 2543,CH-4002 Basel, [£]Universität Bern, Institut für allgemeine Mikrobiologie, Baltzerstr. 4, CH-3012 Bern, [§]present adress: CNRS/ISV, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France.

The product of the ORF VI of cauliflower mosaic virus (CaMV) allows a high expression of the downstream ORF of bicistronic mRNAs The protoplast transfection approach used so far to study this transactivation phenomenon is limited by: i) the lack of a genetic system where cellular mutants, e.g. for translation initiation, could be assayed and ii) the lack of an *in vitro* system. Yeasts may offer a possibility to overcome these problems.

Disruptions of the *ura4* locus of *Schizosaccharomyces pombe* have been performed with mono- or bicistronic transcription units containing the ß-glucuronidase (GUS) reporter gene and driven by the CaMV 35S promoter. The bicistronic GUS gene kept under detection threshold, while the monocistronic one was several hundred times above: the translational hindrance caused by the upstream ORF VII is clearly stronger in fission yeast than in plant cells.

Replicons from *Saccharomyces cerevisiae*, carrying the ORF VI under a dexamethasone inducible promoter, have been transformed into the GUS transgenic yeasts. A very slight induction of GUS activity was observed for the bicistronic GUS transcription unit. An attempt to express the ORF VI from a chromosomal location,using another inducible promoter, will be reported.

433 **TRANSLATIONAL REGULATION OF SEED STORAGE PROTEIN SYNTHESIS IN OATS** Scott K. Boyer, Mark A. Shotwell, and Brian A Larkins. Dept. of Plant Sciences, Univ. of Arizona, Tucson, AZ 85721

The high protein quality of oat seeds is largely a consequence of their unique storage protein composition. Between 70-80% of the seed protein is 12S globulin, which is structurally similar to the globulins of legumes, and only about 10-15% is avenin (prolamine). The relative synthesis of these two types of storage proteins is determined by the regulation of globulin and avenin gene expression during seed development. We have found that despite the predominance of globulin in mature seed, the steady-state concentration of avenin mRNA and globulin mRNA are approximately equal when expressed on a molar basis. An analysis of seed protein synthesis *in vivo* indicated that the regulation of storage protein synthesis in oats occurs primarily at the level of translation. *In vitro* translation of synthetic globulin and avenin transcripts showed no significant differences in the rates of synthesis of the respective storage proteins. This suggests that RNA secondary structure does not play a critical role in the regulation of storage protein synthesis in oats. An analysis of globulin and avenin gene sequences revealed an apparent difference in codons used to encode the two classes of storage proteins. Therefore, we are preparing a tRNA-dependent *in vitro* translation system with which to evaluate the function of the tRNA population of oat endosperm in the synthesis of globulins and avenins. An understanding of the regulation of storage protein synthesis in developing oats could provide the basis for future improvements in the nutritional qualities of oats and other agronomically important crops by genetic engineering.

434 REGULATION OF METHIONINE-RICH 10 kD ZEIN BY A TRANS-ACTING REGULATORY LOCUS, Zpr10/22 IN MAIZE. Sumita Chaudhuri and Joachim Messing. Rutgers, The State University of New Jersey, Waksman Institute, Piscataway, New Jersey 08855

The methionine-rich 10 kD zein in the line BSSS53 shows increased expression accounting for its greater than 30% higher seed methionine content in comparison to other lines tested in our laboratory. While the structural gene encoding this protein is located on chromosome 9, the regulatory locus (Zpr10/22) controlling its increased expression has been mapped to chromosome 4 suggesting a trans-acting mechanism for the control of expression. We have made polyclonal antibodies against the 10 kD protein isolated from BSSS53 in order to study the protein level in various genetic crosses. Surprisingly, our results indicate that the expression of the protein does not correlate to the dosage of Zpr10/22. In the F1 hybrids between the overexpression line BSSS53 and other low expression lines, the protein level is equivalent to the level of expression of the female parent. Estimation of the methionine content of these hybrids also shows the same maternal effect. That this phenomenon is not simply due to the requirement of a threshold for overexpression is substantiated by the observation that in one other line the maternal effect is absent and the expression level correlates with gene dosage. Currently, we are genetically characterizing this maternal effect. Another focus of our investigation is the long-range physical mapping of Zpr10/22 using a tightly linked RFLP marker which may finally enable us to clone this regulator gene.

435 CHAPERONINS AND RUBISCO GENE EXPRESSION. Lynn P. Cloney and Sean M. Hemmingsen. Plant Biotech.Inst., National Research Council, Saskatoon, Canada

Studies of rubisco assembly have contributed to the discovery of the role of **Chaperonin** (Cpn) function in post-translational gene expression (1988 Nature 333:330; 1990 Ann.Rev.Cell Biol. 6:125). We report here our further characterization of plant **Cpn-60** proteins and on the expression of cpn-60 and rubisco genes in E. coli. The post-synthetic folding-assembly of cyanobacterial rubisco subunits in E. coli requires endogenous **Cpn-60** (GroEL) and **Cpn-10** (GroES) activities (1989 Nature 342:884). Plant rubisco is an L_8S_8 enzyme very similar in structure to the cyanobacterial enzyme. Although the plant genes are transcribed and translated in E. coli, assembly into holoenzyme is blocked and rubisco L-subunits aggregate non-productively. We show that elevated levels of endogenous **Cpn** function rescue some plant L-subunits from aggregation but that further assembly is still blocked. Chloroplast **Cpn-60** is unique in that it is composed of two distinct subunit types, $p60^{cpn\text{-}60\alpha}$ and $p60^{cpn\text{-}60\beta}$, whereas other **Cpn-60** proteins (including GroEL) are composed of a single subunit type (1990 Gene 94:181). Plant rubisco gene expression may require homologous chaperonin functions. We have, therefore, co-expressed plant cpn-60α and cpn-60β and rubisco genes in E. coli however rubisco assembly remains blocked. Interpretation of these experiments is confounded since we observe that expression of plant cpn-60 genes perturbs the structure of endogenous **Cpn-60** protein.

436 LIGHT REGULATED RNA-BINDING PROTEINS: CANDIDATES FOR CHLOROPLAST TRANSLATIONAL ACTIVATORS. Avihai Danon and Stephen P. Mayfield, Dept. of Molecular Biology, Scripps Research Inst. La Jolla, California 92037

Translational regulation is a general mechanism for controlling gene expression in organisms as diverse as E.coli and mammals. Typically, regulatory proteins interact with the 5'-untranslated region (5'-UTR) of their target mRNA to control translation. Nuclear encoded factors required for the translation of specific chloroplastic messages have been identified by genetic dissection of *Chlamydomonas reinhardtii*. We have identified D1 mRNA specific RNA-binding activity that is composed of two proteins (47 and 60 kDa). These proteins were found to interact with a 36 base RNA, containing a stem-loop RNA structure, located upstream and adjacent to the ribosome binding site of the D1 message. The RNA-binding activity of these proteins is light regulated and correlates with the translational level of the D1 protein. The abundance of the 47 kDa protein is chloroplast development dependant. 2D-gel electrophoresis and immunoblotting analysis identify multiple forms of the 47 kDa protein, suggesting that post-translational modification are involved in the modulation of its RNA-binding activity. Uncovering the nature of these post-translational modification should help to elucidate the mechanism by which these regulatory proteins control translation.

437 RELEASE OF POLYSOMES FROM CYTOSKELETON FRACTIONS ISOLATED FROM PEA STEMS AND CORN ENDOSPERM. Eric Davies, Becky Fillingham, Shunnosuke Abe, Eric Comer, Jack Lionberger. School of Biological Sciences, University of Nebraska, Lincoln, NE 68588-0118.

In most animal cells, cytoskeleton-bound polysomes (CBP) comprise 70-90% of the total polysome population, but no comparable information exists for plant systems. Using pea stems and corn endosperm as model systems, we have been trying to determine whether such polysomes are present in plant cells. To this end we have developed a low ionic strength cytoskeleton stabilization buffer that prevents disintegration of the F-actin cytoskeleton that normally occurs during isolation. Low speed pellets (4,000 x g, 15 min) from pea stems subjected to sequential solubilization yield apparently different populations corresponding to free, membrane-bound, cytoskeleton-bound, and cytomatrix-bound polysomes. Although no mRNAs are localized exclusively in any one fraction, different mRNAs associate preferentially with different polysome populations. Ultra-low speed pellets (40 x g, 3 min) from corn endosperm furnish abundant protein bodies coated in actin and enmeshed in a network of fine actin filaments. Over 80% of the total polysomes, and almost 100% of the zein-synthesizing polysomes are present in this non-ionic detergent-resistant pellet. Treatment of these pellets with increasing concentrations of monovalent cation causes sequential release of monosomes, small polysomes, and large polysomes. Since polysomes can be differentially released by treatment with protease K, heparin, RNase, and deoxycholate, we postulate that most, if not all, of the zein-synthesizing polysomes are attached to both the ER (protein body) membrane and to the F-actin coating. We postulate further that many of these polysomes are attached to the cytoskeleton via their 40S sub-units and/or the nascent proteins, but not through their mRNAs. We are not yet in a position to postulate whether or not these cytomatrix-bound polysomes have an altered translational capacity.

POSTER ABSTRACTS

438 OVEREXPRESSION OF ß(1,3)-GLUCANASE IN TRANSGENIC PLANTS SUPPRESSION OF TRANSGENE EXPRESSION IN HOMOZYGOUS PLANTS. Fernanda de Carvalho, Godelieve Gheysen, Dirk Inzé, Marc Van Montagu, and Carmen Castresana*. Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium;* Centro de Investigaciones Biológicas, E-28006 Madrid, Spain

A chimeric construct containing a *Nicotiana plumbaginifolia* ß(1,3)-glucanase gene (*gn1*) under the control of the CaMV 35S promoter was used to transform *N. tabacum* SR1. All transformants were phenotypically normal showing high levels of expression of the chimeric *gn1* gene both at the RNA and protein levels. ß(1,3)-glucanase activity in transgenic plants was 14-fold higher than the endogenous activity in nontransformed SR1 plants. The protein encoded by the *gn1* gene has been identified as a basic ß(1,3)-glucanase isoform. Moreover, immunolocalization studies have shown that GN1 is localized in the vacuoles of the transgenic plants. Analysis of the progeny from a primary transformant with high levels of ß(1,3)-glucanase revealed an unexpected pattern of expression of the chimeric *gn1* gene. Plants heterozygous for the T-DNA locus contain high levels of *gn1* gene expression similar to those observed in the original transformant. In contrast, the homozygous plants had no detectable levels of neither *gn1* RNA transcript nor GN1 protein. In addition, the expression of the neomycin phosphotransferase II (*nptII*) marker gene present on the same T-DNA was not suppressed in any of the plants examined. We could demonstrate that suppression of the transgene expression is only associated with the homozygous genotype. The described phenomenon is stably maintained in the next generations. Run-on transcription assays revealed that the mechanism of suppression of gene expression in the homozygous plants is post-transcriptional

439 POST-TRANSCRIPTIONAL REGULATION OF RUBISCO SMALL SUBUNIT GENE EXPRESSION DURING ENVIRONMENTAL STRESS AND DEVELOPMENT. E. Jay De Rocher[1], Francıose Quigley[2], Regis Mache[2] and Hans J. Bohnert[3].[1]Dept. Mol. & Cell. Biol. & [3]Dept. Bioc., University of Arizona, Tucson, Arizona 85721 and [2]CNRS, Universite de Grenoble, France.

The Rubisco small subunit (SSU) gene family of *Mesembryanthemum crystallinum* (ice plant) has six members. We have determined the nucleotide sequences and mapped the genomic arrangement of these six genes on a series of overlapping lambda clones from a genomic library. Five genes are clustered within ~18 kb. The sixth gene is not closely linked to this array. All SSU cDNA clones isolated from leaf cDNA libraries correspond to only four of the SSU genes. These four genes have been used for studying ice plant SSU expression. We have found that ice plant SSU expression in response to salt stress and during development is mediated via changes in RNA turnover. Changes in SSU transcription rates and steady state mRNA levels in leaves were determined by nuclear run-on assay and northern analysis. When stressed with 0.5M NaCl, SSU mRNA levels drop over a period of five days to a new steady state level that is approximately five-fold less than in non-stressed control plants. SSU transcription, however, is not affected by the NaCl stress and remains constant during this period. During development, SSU mRNA levels in leaves decline with age. SSU mRNA amounts are approximately five-fold less in 9 week old plants than in 4 week old plants. Over this same period, SSU transcription is constant. The change in SSU RNA turnover in response to NaCl stress is itself developmentally regulated. Turnover of SSU mRNA is not increased by NaCl stress unless the plants are at least 6 weeks old. We are interested in understanding the mechanism of the regulated RNA turnover and the developmental induction of this mechanism.

440 TRANSACTIVATED TRANSLATION OF DICISTRONIC MESSENGER RNAs IN PLANTS/ TRANSSHUNT OF RIBOSOMES WITH CAULIFLOWER MOSAIC VIRUS LEADER ELEMENTS. Johannes Fütterer, Marc de Tapia, Carolien Zylstra, Jean-Marc Bonneville, Waltraut Schmidt-Puchta, and Thomas Hohn, FMI, Basel, Switzerland.

Dicistronic transcription units were constructed using the first 300 nts of the cauliflower mosaic virus (CaMV) 35S RNA as a leader and various sequences as intercistronic region. With simple intercistronic regions translation of the downstream reporter ORF required the presence of the transactivator TAV, the product of the CaMV 19S RNA. The leader fragment used is important for downstream ORF translation, its important elements seem to be its 4 small ORFs (sORFs). Deletion analysis show that only one of these is required, even this can be exchanged with a number of synthetic sORFs. The downstream reporter ORF can for a short distance overlap with the upstream one or can be separated from it by untranslated sequences or even those containing another sORF. However longer overlaps are not permitted.

A different situation is observed when the second 300 nucleotides of the CaMV leader are used as intercistronic region. In this case constitutive and host-specific translation of the downstream ORF occurs. Sequences in the first half of the leader had been shown to form on the computer screen a strong hairpin structure with the second half. This and mutation analysis promote a model according to which scanning ribosomes can bypass (shunt) most of the leader sequences including the upstream ORF. The shunt model is strongly corroborated by an observation that the two halfs of the leader can cooperate in trans.

We are characterizing the transactivator protein and searching for proteins involved in the shunt.

441 EXPRESSION OF CHIMERIC *GUS* FUSIONS IS NOT PROPORTIONAL TO THE STEADY STATE LEVELS OF mRNA. Lambert A.M. Hensgens, Maarten W.J. Fornerod, Saskia Rueb, Ron A.Winkler, Simone v.d. Veen and Rob A. Schilperoort. *Leiden University, MOLBAS, Department of Plant Molecular Biology, Wassenaarseweg 64, 2333AL Leiden, The Netherlands.*

Chimeric fusions between the GUS gene and the dual promoter fragment from gene 2' and 1' of the T_R DNA from *Agrobacterium* were made and transferred into tobacco. Expression of the chimeric genes was studied in hygromycin resistant progenies of reciprocal crosses between transgenic regenerants and non transformed *Nicotiana tabacum* cv. Petit Havanna SR1. Expression was studied by northern and western blotting analysis and measurement of GUS activities in tissues isolated from plants grown in the greenhouse and *in vitro*. No correlation was found between the steady state concentration of the GUS mRNA and GUS activities in preparations of the same tissues. Data are consistent with a model in which ultimate control of expression is at the level of translation, leading to higher expression levels in roots. An increased GUS activity is found in older tissues probably due to accumulation of the GUS enzyme in time. Using two different translational fusions it was observed that the initiation of translation is different between roots and leaves. The second initiation codon is exclusively used in roots in contrast with leaves.

442 **IMMUNOLOGICAL DETECTION OF NEPOVIRUS PROTEINS *IN VITRO* AND IN INFECTED PLANTS.** Laurence Hibrand, Gérard Demangeat(*), Olivier Le Gall, Thierry Candresse, Christiane Fritsch(*) and Jean Dunez. Station de Pathologie Végetale, INRA, BP 81, 33883 Villenave d'Ornon Cédex, FRANCE, and (*) IBMP-CNRS, 12 rue du Général Zimmer, 67000 Strasbourg, FRANCE.

Nepoviruses are economically important plant picoma-like viruses with bipartite, single stranded RNA genomes. Each genomic RNA is linked to a VPg at its 5' end, polyadenylated at its 3' end and translated *in vitro* into a large polyprotein cleaved by (a) virus-encoded protease(s) to yield the mature viral proteins. Grapevine chrome mosaic (GCMV) and tomato black ring (TBRV) are two closely related nepoviruses which are genetically compatible since viable pseudorecombinants can be obtained by genomic segment exchange. GCMV and TBRV cDNAs have been cloned in *Escherichia coli* and sequenced. Putative functions (helicase, protease, polymerase) have been attributed to regions of the viral polyproteins according to the presence of characteristic signature sequences but the exact mode of maturation of the nepovirus polyproteins remains largely unknown. The putative domains of the GCMV polyproteins have been expressed in *E. coli* fused to β-galactosidase, and peptides representing some of the TBRV domains have been synthesized. All these polypeptides have been used to immunize rabbits, which antisera thus allowed to characterize viral proteins among *in vitro* translation products as well as in extracts from infected *Chenopodium quinoa* and *Nicotiana clevelandii* plants or protoplasts. A putative scheme for the expression of the nepoviral genome can be derived from those results and will be presented.

443 THE NOVEL SMALL RNA FROM TRANSLATION PREINITIATION COMPLEXES. B.Iskakov,N.Nakisbekov,K.Madin,S.Zhanibekova,N.Polimbetova, Inst.Mol.Biol. Alma-Ata,12, USSR.

Relatively homogeneous 45S RNP particles containing newly synthesized rapidly labeled RNA, have been detected in cytoplasmic extract of germinating wheat embryos. It was shown that they represent native translation preinitiation complexes containing 40S ribosomal subunit, mRNP, initiation factors, GTP and initiator Met-tRNA. When RNA moiety of the particles was investigated several low molecular weight RNAs were revealed. One of these small RNAs- 5.3S -of about 130 nucleotides in length was the most abundant. It was shown that 5.3S RNA had several interesting properties. The amount of this RNA increased by 5-6 times under heat shock conditions (1 hour at 37° C). Under normal conditions (25° C) the 5.3S RNA localised in polyribosomes, 80S ribosomes and 45S RNP particles, whereas under heat shock conditions 5.3S RNA was found to be abundant in 45S RNP particles and also present in 80S ribosomes. This RNA was not found in ribosome-free cytosolic fraction. The 3'-end fragment of 5.3S RNA was sequenced and computer analysis showed that this RNA may represent a distinctive class of molecules. The results suggest that 5.3S RNA may be involved in regulation of protein synthesis in wheat embryos during heat shock at the level of translation initiation.

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444 A TRUNCATED PHENYLALANINE AMMONIA-LYASE GENE SEQUENCE IN TOMATO (Lycopersicon esculentum). S -W. Lee, E.J. Robb and R N. Nazar Univ. of Guelph. Dept. of Molecular Biology & Genetics, Guelph, Ontario, Canada. N1G 2W1.

Phenylalanine ammonia-lyase (PAL; E.C.4.3.1.5.) catalyzes the deamination of L-phenylalanine to yield cinnamic acid and NH_4^+ which is the first committed step in phenyl-propanoid synthesis. In tomato, genomic southern blot hybridization results indicate that there are at least five different genes for PAL. One of the tomato PAL genes (PAL5) was completely sequenced and found to contain one intron (712 bps) and two exons which encode a 722 aa polypeptide. Partial sequence analyses of two of the other genomic loci (PAL1 and PAL3) suggest that they also encode alternate PAL proteins. Of special interest is PAL1 which is highly homologous with other PAL genes but contains a stop codon within the second exon presumably encoding a 37 aa shorter polypeptide. The results suggest that previously reported heterogeneity in cellular PAL proteins may result from differences in protein size as well as the amino acid composition.

445 SAUR CODING REGION PLAYS A ROLE IN RAPID TURN-OVER OF SAUR. Will Gretchen Hagen and Tom Guilfoyle. Department of Biochemistry, University of Missouri, Columbia, MO 65211

SAURs or Small Auxin-Up RNAs are auxin and gravity responsive mRNAs, which have been previously shown to be degraded rapidly with a half life of several minutes. Induction of SAUR accumulation by protein biosynthesis inhibitors such as cycloheximide suggests that a rapidly turning-over nuclease(s) is responsible for rapid degradation of these mRNAs. We have constructed a variety of fusion genes with a SAUR leader, SAUR coding, or SAUR 3'-untranslated sequence, as well as some combinations, under the control of various promoters. The fusion genes were delivered into *Nicotiana tobacum* cv Xanthi by *Agrobacterium tumefaciens*-mediated transformation. F2 transgenic seedlings containing different fusion genes were used to test the cycloheximide inducibility of mRNA levels. We have found that the mRNA from the transgenic plants containing a SAUR leader, or a SAUR 3'-untranslated region alone, or in combination, are not induced by cycloheximide. The mRNA from the transgenic plants containing SAUR coding sequence fused with either a 35S-promoter or a Rubisco SSU-promoter and a 3'-untranslated NOS sequence can be induced by cycloheximide. These results suggest that the SAUR coding sequence plays a role in rapid turn-over of SAURs.

446 MUTANTS OF MAIZE INTRONS AFFECT BOTH RNA ACCUMULATION AND SPLICING EFFICIENCY. Kenneth R. Luehrsen and Virginia Walbot, Dept. of Biological Sciences, Stanford Univ. Stanford, CA 94305

Introns are present in most plant genes and their removal is required for proper gene function. There are many gene mutants of maize in which a transposable element has inserted into an intron to alter expression. To further explore the impact of foreign sequence on splicing, we have identified essential intron regions by simple deletion and by insertion of non-intron sequence, using a chimeric plasmid construct containing the *Alcohol dehydrogenase-1* intron 1 adjoined to the firefly luciferase reporter gene. We find that extensive deletion of internal sequences does not affect intron function. The insertion of non-intron sequences affects both transcript accumulation and splicing efficiency. These effects are position dependent and delineate two critical steps in splicing: intron recognition and splicing efficiency.

447 NUCLEAR-CHLOROPLAST INTERACTIONS IN THE REGULATION OF CHLOROPLAST mRNA TRANSLATION. Stephen P. Mayfield and Avihai Danon, Dept. of Molecular Biology, Scripps Research Institute, La Jolla, California 92037

Genetic analysis of nuclear and chloroplast mutants from the unicellular green algae *Chlamydomonas reinhardtii* has revealed that nuclear encoded factors are required for the translation of specific chloroplastic mRNAs. These nuclear encoded factors interact with stem loop structures contained within the 5' untranslated regions of chloroplast mRNAs, and act to regulate chloroplast mRNA translation. We have isolated two proteins which bind specifically to the 5' untranslated region of the D1 mRNA. These proteins bind to a 36 base RNA containing a stem loop structure. Site directed mutagenesis of this stem loop RNA reveals that bases within the stem are required for translation of D1 mRNA *in vivo*, while deletion of the loop showed no D1 translational reduction. The accumulation of at least one of the D1 RNA associated proteins (47 kDa, D1-RB47) is dependent upon chloroplast development. The D1-RB47 protein is encoded by a single nuclear gene but accumulates as a set of three or four proteins with an average M.W. of 47 kDA, suggesting post-translational modifications to the protein. Affinity of the D1-RB47 protein for the D1 mRNA is light modulated as is translation of the D1 mRNA. These data indicate that the D1-RB47 protein is a developmentally regulated protein which undergoes light activated modifications which alter its mRNA binding activity and thus regulate D1 mRNA translation. Molecular characterization of the light activated alterations to the D1-RB47 protein and further characterization of the RNA secondary structure should help dissect the mechanisms involved in light regulated translation of chloroplast mRNAs, and provide important insight into nuclear chloroplast interactions involved in coordinate gene expression.

448 UNUSUAL MECHANISMS CONTROL TRANSLATION OF BARLEY YELLOW DWARF VIRUS RNA.
W. Allen Miller, S.P. Dinesh Kumar, Veronique Brault, and Di Rong. Iowa State University, Plant Pathology Dept. and MCDB Program, Ames, Iowa 50011

We are studying how viral sequences control translation in plant cells. Barley yellow dwarf virus (BYDV) uses a variety of unusual mechanisms for translation of its genes. These include translational frameshifting (in the polymerase gene), internal initiation, and in-frame readthrough of a stop codon. The sequences and higher order RNA structures which induce the ribosomes to undergo these unusual events are being identified using a transient expression system in which the putative controlling sequence is linked to a reporter gene. Our results show that frameshifting occurs *in vitro*, in *E. coli*, and in plant cells. The frameshift-inducing sequence contains a "shifty heptanucleotide" followed by two possible pseudoknots similar to those found in frameshifting retro- and coronaviruses. To our knowledge, this is the first demonstration of translational frameshifting in plant cells. In addition we have identified a virus/vector chimaeric RNA structure at the frameshift region which induces unexpected, highly efficient internal initiation of translation in the GUS reporter gene. The BYDV coat protein and an overlapping reading frame are translated by initiation at two out-of-frame AUGs on the same subgenomic mRNA. We have precisely mapped the 5' end of the subgenomic mRNA and will present sequences and structures which regulate the ratio of initiation at the AUGs of both genes.

449 IDENTIFICATION OF AN INSTABILITY DETERMINANT DERIVED FROM AN AUXIN-INDUCIBLE TRANSCRIPT. Thomas C. Newman, Masaru Ohme-Takagi, Crispin B. Taylor, and Pamela J. Green. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, Mi. 48824-1312. The study of messenger RNA decay as a component in the regulation of plant gene expression is limited by a paucity of suitable *in vivo* systems for measuring mRNA half-lives. As a first step in identifying sequence elements that destabilize transcripts, we have developed a system to directly measure mRNA half-lives in stably transformed tobacco cells. *Nicotiana tabacum* cell suspension cultures are transformed with constructs that include, on the same T DNA, two reporter genes, both driven by the CaMV 35S promoter. One of the reporter genes, chloramphenicol acetyltransferase (CAT), remains unaltered in all of the constructs and serves as an internal reference. The other reporter gene, β-glucuronidase (GUS) contains restriction sites in the 5' and 3' untranslated regions into which potential decay-modulating sequences can be conveniently inserted. To measure message half-lives, RNA polymerase II transcription is > 95% inhibited using actinomycin D, and the decay of reporter gene transcripts is followed over a 150 minute time course. Using this system, we have determined that half-lives for unmodified GUS and CAT transcripts are 85 and 77 minutes respectively, whereas those for endogenous transcripts of the β-ATPase and HSP70 genes are > 5 hours. Moreover, insertion into the GUS 3' untranslated region of two tandem copies of DST, a sequence element conserved among all members of the Small Auxin Up RNA (SAUR) gene family from soybean (McClure et al. 1989. Plant Cell 1:229-239.), decreased the half-life of the GUS transcript approximately four-fold, relative to that of the CAT transcript.

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450 **BIOGENESIS OF COTTONSEED CATALASE.** Werting Ni and Richard N. Trelease. Arizona State University, Dept. of Botany, Tempe, AZ 8527-1601. Catalase is a constitutive enzyme of all peroxisomes. In cotyledons of cotton seedlings undergoing the transition from glyoxysomal to leaf-type peroxisomal metabolism, the temporal expression of five tetrameric isozymes is the consequence of temporal synthesis and interactions between two different charge subunits (SU 1, SU 2). Sequence comparisons of two distinct cDNAs encoding these two subunits, and genomic Southern analyses using these cDNAs as probes, indicated that two genes encode these subunits. The synthesis of SU 1 approx. 24 h before SU 2 results in a shift at day 1 from a predominance of isozyme A (a homotetramer of SU 1) to mostly isozyme E (a homotetramer of SU 2) at day 6; isozymes B, C and D (heterotetramers of SU 1 and SU 2) appear, then disappear between days 2-5. The steady state level of SU 1 protein (present at 12 h) increases through day 3 then declines linearly through day 6, while SU 2 protein (first detected at 24 h) increases continuously through day 6. Time courses for steady state levels of the corresponding mRNAs reveal two clearly separated peaks: the first at day 1 (SU 1) and the other at day 4 (SU 2). Accumulation of SU 1 mRNA is not light-dependent, whereas accumulation of SU 2 mRNA occurs only after exposure of seedings to light. The accumulation of the mRNAs by at least 24 h prior to accumulation of their corresponding proteins suggested temporal pretranslational regulation of synthesis of both subunits. Results from run-on transcriptional assays with isolated nuclei, however, revealed that transcripts encoding both subunits were synthesized together on days 1 through 5. Hence, it appears that accumulations of SU 1 and SU 2 mRNAs are controlled primarily at the posttranscriptional level, a conclusion which has not been reported for regulation of seedling catalase or any other eukaryotic peroxisomal enzymes. Supported by NSF Grant DCB-9018088.

451 EFFECT OF AU-RICH SEQUENCES ON mRNA STABILITY IN STABLY TRANSFORMED PLANT CELLS. Masaru Ohme-Takagi, Thomas C. Newman, Crispin B. Taylor, Christie Howard, Wan-Ling Chiu, and Pamela J. Green. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, Michigan 48824-1312. Over the past several years, the number of the genes considered to be regulated specifically at the level of mRNA stability has increased rapidly. However, little is known about the pathways for mRNA degradation and how these pathway are regulated. It has been reported that AU-rich sequences containing the pentamer motif "AUUUA" destabilize transcripts in animal cells. In plants, the AU-rich *Bacillus thurengensis* (BT) toxin transcripts have been hypothesized to be unstable because they fail to accumulate in transgenic plants even when strong promoters are used to drive their synthesis. To study the molecular mechanisms that control mRNA stability in plants, we have developed methods for measuring directly mRNA decay rates in stably transformed cells. Using these methods we have investigated the effect of AU-rich sequences on mRNA stability in tobacco. Sequences which include the AU-rich sequence from a human lymphokine transcript and a synthetic sequence containing a tandem array of AUUUA motifs have been inserted into the 3' noncoding region of reporter genes. Stably transformed tobacco cell lines harboring these constructs have been established and the measurement of the half-lives of the corresponding transcripts is in progress. Initial results indicate that AU-rich sequences can act to destabilize the mRNAs of reporter genes, but that some AU-rich sequences are more potent instability determinants than others. The significance of these results as well as the stability of transcripts containing BT-toxin sequences will be discussed.

452 STORAGE PROTEIN mRNAS ARE REPRESSED IN EARLY STAGES OF ALFALFA SOMATIC EMBRYO DEVELOPMENT.
Saroj K. Pramanik, Joan E. Krochko and J. Derek Bewley. Univ. of Guelph, Dept. of Botany, Guelph, ON N1G 2W1

During development on hormone-free media, somatic embryos pass through distinct morphological stages that superficially resemble those of zygotic embryo development (globular, heart, torpedo, cotyledonary stages). Despite these similarities they differ from zygotic embryos in the extent cotyledonary development and the patterns of synthesis and quantitative expression of seed-specific storage proteins (7S, 11S, 2S proteins). In vivo labelling of storage proteins and northern blot analysis of messenger RNA populations indicates that these abnormal patterns reflect differential transcriptional and post-transcriptional influences. Biochemical anomolies are evident from the earliest stages of somatic embryo development. In suspension culture and early globular stage embryos (days 0, 3) there is already significant accumulation of the mRNAs of the 7S, 2S and 11S storage proteins although at these early stages there is no evidence in vivo of storage protein synthesis or accumulation. The cytoplasmic distribution of these messages in somatic embryos at day 0 and day 3 occurs mainly as free mRNA-protein complexes (repressed message or inactive message) and is not associated with polyribosomes. Such behavior contrasts with that observed during zygotic embryo development.

453 THE EXPRESSION OF AN *ARABIDOPSIS* ADH GENE INTRODUCED INTO *NICOTIANA PLUMBAGINIFOLIA* BY *AGROBACTERIUM TUMEFACIENS* OR BY DIRECT GENE TRANSFER. Philippe Rousselin[1], Rudy Dolferus[2], Tahar Baya[2] and Michel Jacobs[2]. [1]Laboratoire de Biologie cellulaire, INRA, F-78026 Versailles and [2]Laboratorium Plantengenetica, University of Brussels, B-1640 Sint-Genesius-Rode.

The alcohol dehydrogenase gene is an interesting case of eukaryotic gene regulation by its tissue specific and developmentally controlled expression pattern and especially by its inducibility by plant hormones and environmental factors such as hypoxic conditions. With the goal to study and manipulate the expression of the Adh gene, an ethanol-resistant mutant of *Nicotiana plumbaginifolia*, deficient for the pollen and seed-expressed ADH activity, was transformed with a ADH gene of *Arabidopsis* under the control of its own upstream sequence or of the CAMV35S constitutive promoter. Restoration of ADH activity indicates interspecific complementation. ADH expression seems to be controlled at the translational and/or post-translational level. Inducibility properties by anaerobic stress and by the synthetic auxin 2,4-D will be compared in transgenic plants with the Arabidopsis gene driven by its own promoter or the CAMV35S promoter.

454 EXPRESSION OF A NONSTRUCTURAL PROTEIN OF MCMV IN BMS SUSPENSION CULTURE PROTOPLASTS. Kay Scheets[1] and Robert C. Nutter[2]. [1]Department of Microbiology, Oklahoma State University, Stillwater, OK 74078, [2]Department of Biotechnology Research, Pioneer Hi-Bred International, Inc., Johnston, IA 50131. Replication of maize chlorotic mottle virus (MCMV) in black Mexican sweet (BMS) suspension culture protoplasts can be followed by probing for MCMV genomic and subgenomic RNA on northern blots and by detecting the 25 kDa coat protein by western blots or PAS-ELISA. Continuous in vivo labelling of infected protoplasts that have been irradiated with UV light or incubated in the presence of low levels (30-45 ug/ml) of actinomycin D detects only ^{35}S-methionine labelled coat protein and smaller coat protein products. In the presence of high levels (90-180 ug/ml) of actinomycin D, pulse labelled protoplasts produce a 30 kDa protein which is visible 10 hr post-inoculation (hpi) and peaks at 24 hpi. Coat protein is detectable at 15 hpi and continues at a high level of production 20-48 hpi. The 30 kDa protein comigrates with a minor band from in vitro translated MCMV RNA. UV light irradiation or low levels of actinomycin D slightly increase the levels of viral RNAs in infected protoplasts, but high actinomycin D levels increase the amount of viral RNA by as much as five fold.

455 REGULATION OF HIGH METHIONINE ZEIN PRODUCTION IN MAIZE INBRED LINE Mo17. Hedva Schickler and Joachim Messing. Rutgers, The State University of New Jersey, Waksman Institute, Piscataway, New Jersey 08855

The methionine content in maize kernels is largely determined by the level of a methionine rich 10 kD zein protein, coded by the gene Zps/10(22). Overproduction of the 10 kD zein in the inbred line BSSS-53 correlates with at least two trans-acting factors. One appears to control mRNA levels, and another, designated Zpr10/(22), controls protein level. The methionine content in Mo17 kernels is very low relative to most inbred lines. It contains the same 10 kD protein, as shown by two dimentional gel analysis, but its level is extremely low. 10 kD mRNA levels in Mo17 exhibited a notably different developmental pattern relative to BSSS-53. At all stages, its level in Mo17 was lower than that in BSSS-53. Nuclear run-on experiments suggested post-transcriptional regulation for the observed differences in mRNA levels among inbred lines. In in vitro translation assays, equivalent amounts of 10 kD mRNA from BSSS-53 and Mo17 yielded similar amounts of 10 kD protein. These results suggest possible trans-acting factors controlling mRNA levels in Mo17, that may be amenable to biochemical analysis.

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456 INHERENT INSTABILITY OF OAT PHYTOCHROME mRNA. Kevin A. Seeley, Linda J. Barnes, and James T. Colbert. Iowa State University, Department of Botany, Ames, Iowa 50010

The mRNA encoding the type I phytochrome (*phy*) protein decreases in abundance, with a half-life of about 1 h, after production of Pfr by exposure of etiolated oat seedlings to red light (2). Pfr production also results in dramatically decreased transcription of the oat *phy* genes (3). The rapid decline in *phy* mRNA levels following Pfr production could be explained by either: a) inherent instability of *phy* mRNA; or b) a Pfr-induced destabilization of *phy* mRNA. Detection of putative *in vivo* *phy* mRNA degradation products in etiolated oat seedlings prior to light treatment supports the hypothesis that *phy* mRNA is inherently unstable (1). Subsequently, we have observed that similar *phy* mRNA degradation products are present in transgenic tobacco plants containing a transcriptionally active oat *phy* gene. To further investigate the hypothesis that oat *phy* mRNA is inherently unstable, we have estimated the half-life of *phy* mRNA in etiolated oat seedlings prior to Pfr production. To obtain this estimate we excised etiolated oat seedlings, using an infra-red viewing system, and exposed the excised seedlings to the transcription inhibitor cordycepin for various lengths of time in total darkness. These experiments lead to an estimate of about 1 h for the half-life of *phy* mRNA in etiolated oat seedlings, consistent with the hypothesis that *phy* mRNA is inherently unstable.

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457 MOLECULAR CLONING OF PLANT PROTEINS CONTAINING THE PROPOSED CATALYTIC SITES OF VERTEBRATE PROTEIN DISULFIDE ISOMERASE

Basil S. Shorrosh and Richard A. Dixon, Plant Biology Division, The Noble Foundation, P. O. Box 2180, Ardmore, OK 73402.

In order to elucidate the molecular mechanism of prolyl-hydroxylase regulation in plants, we attempted to clone its alpha and beta subunits. In vertebrates, the beta subunit of prolyl-hydroxylase is identical to protein disulfide isomerase (PDI) which is also a component of the microsomal triglyceride transfer protein complex. PDI is a microsomal enzyme that is implicated in the de novo folding of nascent polypeptide chains. PDI shares sequence similarities at the amino acid level with thioredoxins and phosphoinositide specific phospholipase C (PI-PLC).

Screening an alfalfa cDNA library with a human PDI sequence led to the isolation of two clones, B2 and G1. G1 encodes a smaller protein than B2 but both contain two active sites identical to the proposed PDI catalytic sites. The catalytic sites of G1 are closer together than in B2. In this respect, G1 resembles ERp72, a vertebrate endoplasmic reticulum protein with three PDI like catalytic sites which may function as a "glucose regulated protein". B2 encodes a protein that is highly similar to mammalian PDI in its size, amino acid sequence, and the location of the catalytic sites. We compare the alignment of the B2 and G1 deduced amino acid sequences with PDI, ERp72, PI-PLC, and other related proteins and describe the genomic organization, developmental regulation and inducible expression of B2 and G1.

458 ABSENCE OF TRANSLATIONAL CONTROL BY PHOSPHORYLATION OF eIF-2 AND EF-2 IN PLANT CELL. S.Smailov, S.Shaikhin, A.Lee, B.Iskakov. 480012, Inst.Mol.Biol., Alma-Ata, USSR.

Phosphorylation of two key translation factors of animal cells-eIF-2 or EF-2 leads to the block of protein synthesis. However, little is known about modulation of eIF-2 and EF-2 activities in plant cells by phosphorylation. It was shown that wheat germ eIF-2 (WGeIF-2) can be phosphorylated in hemin-deficient rabbit reticulocyte (RR) lysate, but protein synthesis was not affected. Similar results were obtained using partially purified RR DAI-kinase. In animal cells eIF-2 phosphorylation inhibits mechanism of GDP/GTP exchange and eIF-2 recycling. Our data suggest that the nucleotide exchange on WGeIF-2 can take place without any additional factor and apparently is independent of whether eIF-2 is phosphorylated or not. We have also studied the existence of EF-2-phosphorylation system in plant cells. It was shown that WGEF-2 can be strongly phosphorylated with EF-2-kinase isolated from RR. Moreover, phosphorylated WGEF-2 becomes inactive in *in vitro* translation as well as its analogue from animal sources. Thus, the activity of plant EF-2 may be potentially regulated by phosphorylation. However, we couldn't observe any endogenous phosphorylation of EF-2 in many plants and plant tissues tested. These results suggest another regulation mechanisms for eIF-2 and EF-2 activities in plant cells, than phosphorylation.

459 FUNCTION AND REGULATION OF PHOSPHOPROTEIN PHOSPHATASES IN PLANTS. Robert D. Smith and John C. Walker. Department of Biological Sciences, University of Missouri, Columbia, MO 65211, USA

A cDNA clone (*ZmPP1*) that encodes the catalytic subunit of a type 1 phosphoprotein phosphatase (PP1) has been isolated from a *Zea mays* . The deduced amino acid sequence of *ZmPP1* and the activity of ZmPP1 protein expressed in *E. coli* demonstrate that the structural and biochemical characteristics of the plant phosphoprotein phosphatases are remarkably similar to the phosphoprotein phosphatases found in mammalian tissues. Northern blot analysis show that *ZmPP1* mRNA is present at similar levels in all maize tissues examined indicating that it may play a fundamental role in cellular homeostasis. To further characterize PP1 in higher plants we have initiated a series of studies to elucidate the physiological function(s) and *in vivo* regulation of PP1 activity.

Our initial goal has been to assess whether plants contain regulatory subunits that modulate and/or target the activity of the PP1 catalytic domain to specific substrates, analogous to PP1 regulatory mechanisms in mammalian tissues. We have raised antibodies against synthetic peptides corresponding to the amino and carboxyl termini of ZmPP1 and are using these antibodies to characterize the subunit structure of the maize type 1 phosphoprotein phosphatases in various tissues. To further define potential physiological roles of PP1 in higher plants we are expressing the *ZmPP1* clone in transgenic plants to determine potential effects of increased phosphatase activity on developmental patterns and metabolic regulation. Conversely, a truncated *ZmPP1* cDNA clone that encodes a protein lacking phosphatase activity but which may retains the capacity to bind regulatory subunits has also been introduced into transgenic plants.

460 EXPRESSION STRATEGIES OF THE POTATO LEAFROLL LUTEOVIRUS

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A subgenomic 2.3kb RNA (sgRNA1) serves to express the gene cluster (CP, 17K, 56K proteins) located on the 3'half of the 5.8kb single-stranded RNA genome of potato leafroll virus (PLRV). Its start site of transcription maps in an intergenic region 40 nucleotides upstream of the CP AUG codon (Tacke et al., J. Gen. Virol. 71: 2265-2272, 1990). Translation mechanisms for the expression of the three ORFs were studied by transient expression in potato protoplasts with constructs bearing regulatory PLRV sequences in translational fusion with the GUS reporter gene. Translational initiation at the AUG of the 17K gene, although nested within the CP ORF, exceeds translation of the CP gene by a factor of seven. Furthermore, suppression of a UAG amber stop codon separating the CP and 56K ORFs occurs at a frequency of 1%. A tRNA fraction from potato has been identified that mediates suppression also in vitro.

The putative viral replicase gene (ORF2b) located in the 5'half of the genome presumably is expressed by -1 frame shift during ORF2a translation. The region of ribosomal frame-shifting was identified by deletion analysis and has the potential to form a stem-loop structure.

461 USE OF THE CAB-1 PROMOTER FOR MEASUREMENT OF mRNA HALF-LIVES IN TRANSGENIC PLANTS. Crispin B. Taylor and Pamela J. Green. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312.

Regulation of the expression of a number of eucaryotic genes has been shown to be controlled, at least in part, by post-transcriptional processes, such as those affecting mRNA turnover. In order to define *cis*-acting sequences that affect the stability of mRNAs, a system for measuring directly mRNA half-lives in transgenic tobacco seedlings has been designed. The system makes use of a natural transcriptional switch, the wheat chlorophyll a/b binding protein (Cab-1) promoter, fused to the reporter genes chloramphenicol acetyltransferase (CAT), β-glucuronidase (GUS) and β-globin, and allows the measurement of message decay in completely unperturbed plants. Transcription from the Cab-1 promoter is regulated by an endogenous circadian clock in transgenic tobacco, such that mRNA synthesis starts in the morning and shuts off about midday. The half-lives of reporter gene messages are measured in cultured transgenic tobacco seedlings after transcription from the Cab-1 promoter has ceased. Each construct that is transferred to plants via *Agrobacterium*-mediated transformation contains, on a single plasmid, both an unmodified transgene (the reference gene) and one containing a potential mRNA decay-modulating sequence in the 5' or 3' untranslated regions (the test gene). This provides an internal standard that facilitates the comparison of the decay rates of different test genes. In our initial experiments we confirmed that CAT, GUS and $\beta_1$globin transcript levels fluctuate as expected, rising in the morning and falling during the afternoon. In addition, CAT and GUS messages disappear at approximately the same rate, consistent with our finding that CAT and GUS transcripts decay with similar half-lives in cultured cells. Preliminary experiments, using a steady-state analysis of relative levels of reporter and test gene transcripts, suggest that sequences that decrease the half-life of the GUS message in the tobacco cell culture system may have a similar effect on this transcript in whole plants.

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462 THE LEADER FROM A SUBGENOMIC CAPSID mRNA OF MCMV ENHANCES CHIMERIC GENE EXPRESSION. Tom Ulrich, Mike Wach and David Korman, Sogetal, Inc., 3876 Bay Center Place, Hayward, CA 94545

In vitro translation of the p25 subgenomic RNA present in Maize Chlorotic Mottle Virus (MCMV) virions (Lommel *et al.* 1991,Virology 181: 382.) suggests that it possesses a strong translational enhancing element which may be similar to the TMV Ω element. We fused a portion of the p25 leader to the GUS reporter gene in order to determine the contribution of the viral leader to the overall enhancement of chimeric gene expression. Transient expression was assayed in corn (*Zea mays*) and wheat (*Triticum aestivum*) cells transformed with GUS chimeric genes containing this p25 MCMV leader as well as other leader sequences. The level of expression of the GUS gene was higher in cells transformed with the construct containing a portion of the p25 leader than in cells transformed with either a construct containing a mainly synthetic GUS gene leader or the TMV Ω leader. These results support the hypothesis that the p25 MCMV RNA leader possesses a strong translational enhancing element. In contrast, in corn and wheat we found that the TMV Ω leader did not boost gene expression above that of the synthetic GUS gene leader.

463 TRANSLATIONAL CONTROL IN POTATO TUBERS SUBJECTED TO ENVIRONMENTAL STRESSES Michael E. Vayda and John S. Crosby, Biochemistry, Microbiology and Molecular Biology, University of Maine, Orono, ME 04469 USA

Potato tubers exhibit two distinct responses to the environmental stresses of wounding and hypoxia, both include the induction and selective translation of stress-response RNAs. Messages that are newly synthesized in response to wounding, such as PAL, HRGP and GRP, are immediately bound to polysomes. By contrast, mRNAs present prior to wounding, such as patatin and the proteinase inhibitors, are displaced from polysomes within 1h of wounding, and are degraded in the free RNA pool within 4h. The response to hypoxia includes induction of a set of RNAs distinct from the wound response RNAs. Translation of the hypoxically-induced RNA set occurs at the same time that translation of the wound-response RNAs is inhibited. However, wound-response RNAs remain bound to polysomes during hypoxic conditions although they are not translated *in vivo*. Thus, translational control during wounding stress and hypoxic stress must operate by two different mechanisms.

Several proteins have been detected by SDS-PAGE that may be involved in this regulation. A 32kD protein is associated with polysomes isolated from wounded tissue, but not nonwounded tissue. This protein is phosphorylated immediately upon wounding. The kinase activity responsible for phosphorylation of this protein diminishes by 32h after wounding, although the protein remains polysome bound for at least 48h. The 32kD protein is not associated with polysomes isolated from hypoxic, nonwounded tubers, or hypoxic tubers subsequently wounded. However, polysomes isolated from hypoxic tissues display novel 48kD, 45kD and 90kD proteins that are not phosphorylated. Polysomes isolated from wounded, hypoxic tubers display both sets of polysome-associated proteins, although the kinase activity which phosphorylates the 32kD protein is not active in hypoxic tissues. These three proteins may mediate binding of specific messages to polysomes or regulate the efficiency of the translational machinery during stress conditions.

464 HOW MANY HISTONE H3 VARIANTS IN PLANTS ?
Jakob H. Waterborg, University Missouri-Kansas City, Div. Cell Biol. Biophys., Kansas City, Missouri 64110

Histone H3 proteins were extracted by BioRex-70 chromatography from guanidine.HCl extracts of whole plant cells, purified and fractionated by reversed phase hplc and acid urea Triton (AUT) PAGE. Five distinct histone H3 variant forms were identified among 5 dicot and 4 monocot species by Triton affinity in AU PAGE, reversed phase hydrophobicity, SDS and AU PAGE. They were named variant I through variant V according to increasing mobility in AUT gels. Variants I and II differ at residue 90. Variant III differs from these at residues 31, 41, 87 and 90 and is identical to alfalfa histone H3.2 which appears to be a replacement type histone with transcription independent of DNA replication. Variant III is the only form present in each plant species and in each species this H3 form is acetylated to a higher level than any other H3 isoprotein. Levels of histone H3 acetylation among plant species appear inversely related to genome size, suggesting that a relatively larger fraction of smaller plant genomes is transcriptionally competent.

Plant species	H3 Variants
Arabidopsis, Tobacco, Carrot, Rice :	I - III - -
Alfalfa, Soybean :	- II III - -
Maize :	I II III - V
Barley :	I II III IV -
Wheat :	- II III IV V

465 The Arginine Decarboxylases of *Arabidopsis* and *Avena*. Mark B. Watson and Russell L. Malmberg. University of Georgia, Department of Botany, Athens, Georgia 30602. The biosynthesis of polyamines in plants can be carried out by two different pathways. Arginine decarboxylase (ADC) catalyzes the first step in the production of putrescine from arginine. Degenerative oligonucleotide PCR primers were constructed from conserved regions of derived amino acid sequences from *E. coli* and *Avena sativa*. These primers were used to amplify a region of ADC from *Arabidopsis* genomic DNA. The PCR product was subsequently labelled and used to isolate a cDNA clone. Sequence analysis and regulation will be discussed. Antibodies were generated against different portions of the *A. sativa* ADC. Using these antibodies we have evidence that the *A. sativa* ADC is cleaved from a precursor protein into 2 subunits that remain together in the active soluble enzyme.

466 PURIFICATION AND CHARACTERIZATION OF CYTOPLASMIC POLY(A) BINDING PROTEIN FROM PEA (*PISUM SATIVUM*), Jianjun Yang and Arthur G. Hunt, Plant Physiology/ Biochemistry/Molecular Biology Program, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091

Poly(A) binding proteins (PABPs) are important regulatory factors for gene expression. PABP was purified from cell-free extracts of pea seedlings (*Pisum sativum*) by ammonium sulphate precipitation (25% - 60% saturation), Affi-gel Blue affinity chromatography and poly(A) Sepharose-4B affinity chromatography. The final elution from poly(A) Sepharose-4B column yielded a single polypeptide (~70kD) on SDS-PAGE gels and contained high poly(A) binding activity. Activity of the purified protein was independent of ionic strength, and showed temperature and pH optima of 37°C and pH 6.4, respectively. Specificity studies indicated that the pea PABP was most active with poly(A), showed some activity with poly(G), and did not bind to poly(C). However, the binding of PABP with poly(A) of varying lengths showed different profiles, that is, the PABP bound A_{10} very poorly, bound A_{25} in a linear manner as a function of [PABP], and bound A_{300} sigmoidly with increasing [PABP]. These properties are all similar to PABPs isolated from other sources. We conclude that we have identified, purified, and characterized a plant polyA binding protein similar in properties and function to those described in yeast ansd mammalian systems.

467 CELLULAR PROTEINS BIND TO THE LEADER REGION OF THE CAULIFLOWER MOSAIC VIRUS 35S RNA. Waltraud Schmidt-Puchta, Johannes Futterer and Thomas Hohn Friedrich Miescher-Institut, Basel, Switzerland

The 35S RNA of Cauliflower Mosaic Virus (CaMV) covers the entire viral genome and is thought to serve as a template for reverse transcription and as a polycistronic mRNA for translation of most of the viral proteins. At the 5'-end the RNA contains a 600 nt long leader sequence with several sORFs which normally reduce the efficiency of translation of the first ORF downstream of the leader. Transient expression assays of reporter genes in host plant cells showed, however, that there exist three sequence elements S1, S2, and S3, which permit a relatively efficient translation of the first ORF despite the presence of the entire leader. The two regions S2 and S3 seem to interact with each other and the stimulatory effect is more efficient in host than in non-host cells. A ribosome shunt model was proposed in which the regions S2 and S3 together with cellular proteins would form a bypass complex to transfer ribosomes from the 5'- end of the leader to the 3'-end.

To investigate interactions between the leader region of the 35S RNA and cellular proteins UV crosslinking experiments were performed using cytoplasmic protein extracts from turnip (host) and *Nicotiana plumbaginifolia* (non-host) and several RNA probes comprising the entire leader as well as different parts of it. In both extracts, two RNA binding proteins were detected. A 35 kD protein binds unspecifically to any RNA and a 49 kD protein binds specifically to two regions of the leader sequence. We will determine whether one and the same protein binds to two regions or wether two different 49 kD proteins bind to one of the regions each. Involvement of the 49 kD protein in translation has still to be proven, however, the binding sites reside in regions that are required for efficient translation downstream of the leader.

POSTER ABSTRACTS

468 EFFECTS ON FLORAL DEVELOPMENT OF NOVEL EXPRESSION PATTERNS OF A HOMEOTIC GENE. Robert C. Elliott, Sandra Doyle, Jose M. Romero, Sabine Hantke, Rosemary Carpenter, and Enrico Coen. John Innes Centre, Colney Lane, Norwich NR4 7UH, ENGLAND.

In the *floricaula* (*flo*) mutant of *Antirrhinum* the transition from inflorescence meristem to floral meristems is blocked, resulting in the substitution of inflorescences for flowers[1]. The *flo* gene was cloned by transposon-tagging with a Tam3 transposon[2]. The FLO protein contains features often found in DNA-binding proteins, such as acidic/basic regions and a proline-rich region. However, FLO has no overall homology to any of the sequenced DNA-binding proteins in current databases. The *flo* gene is transiently expressed only in very young floral primordia. The expression pattern of *flo* and the phenotype of the mutant suggests that *flo* may have a direct or indirect role in several aspects of floral development, including floral evocation, phyllotaxis, internode length, determinacy, inflorescence shape. *flo* may also interact with the genes controlling organ identity[1]. By introducing the *flo* gene under the control of its own or various other promoters (CaMV 35S; heat shock) we hope to explore the role of *flo* in floral development.

1) Carpenter and Coen (1990). Genes and Dev. 4:1483-1497.
2) Coen *et al.* (1990). Cell 63:1311-1322.

469 NOVEL STYLAR BIOENERGETICS REVEALS A UNIQUE INTERACTION BETWEEN STYLES AND POLLEN TUBES. Marcel Jansen, Guido Sessa, [1]Shmuel Malkin and Robert Fluhr. Weizmann Institute of Science, Department of Plant Genetics, [1]Department of Biochemistry, Rehovot, Israel 76100

Styles of angiosperms function to nurture and guide pollen tubes to the ovules. Recent molecular studies have focused on stylar function in incompatability reactions and tissue specific expression of its genes, yet fundamental aspects of stylar metabolism remain obscure. We therefore chose to investigate some of the basic bioenergetic considerations of style-pollen tube interaction. The styles of *N. tabacum*, a C3 plant, enclose a strand of metabolically active transmitting tract cells that are the essential functional component. We show that these cells are replete with well developed chloroplasts which have normal ultrastructure and can maintain electron transport. Surprisingly, unlike normal C3-type chloroplasts, they lack RUBISCO activity and were found by immunological methods to contain less than 10^{-3} of the normal amounts of RUBISCO polypeptides. Despite this lack, intact styles were photosynthetically active and fixed carbon via carboxylation of phosphoenolpyruvate to oxaloacetate and reduction to malate. The key step we found to be mediated by PEPCase, raising the question of the special function of this pathway in styles. We hypothesize that the biological significance of this system lies in the fact that excess CO_2 can be detrimental to certain plant cellular processes. In this context, PEPCase acts as a scavenger and detoxifies the cells from excess CO_2 produced by the respiring transmitting cells and pollen tubes, which cannot otherwise be dissipated through the impermeable stylar periphery. In line with this possibility, both germination and elongation of pollen tubes *in vitro* were found to be sensitive to relatively low physiological levels of CO_2. Interestingly, correlated with the accumulation of malate in the transmitting tract cells we detect a dramatic morphological transformation in elasticity that occurs in the style on the day of anthesis. Taken together these results highlight hitherto unknown aspects of stylar metabolism that play a unique role in angiosperm fertilization and stylar development.

470 **TRANSIENT GUS EXPRESSION IN ISOLATED WHEAT MICROSPORES VIA PARTICLE BOMBARDMENT.** Stephen J. Mejza, Denise E. DiBona, and James R. Wong. Sogetal, Inc., 3876 Bay Center Place, Hayward, California 94545

Transient expression of a 35S-promoter-driven GUS gene was achieved in isolated wheat microspores. This GUS gene was contained within a plasmid vector which was coated onto particles and subsequently introduced into wheat microspores by a high pressure, helium-propelled ballistic apparatus. Upon staining with X-Gluc, certain microspores gave a positive GUS response. The frequency of transient expression varies from 1/25,000 to 1/10,000 of the cells shot, and with manipulations can be as great as 1/1000. The possible limitations of transient expression in wheat microspores are discussed as well as the potential of using this system for production of stably transformed plants.

471 RFLP ANALYSIS IN *SOLANUM CHACOENSE BITT.* Sylvain R. Rivard[1,2], Marc Saba El Leil[1], Charles Després[1], Mario Cappadocia[1] and Benoît S. Landry[1,2]. [1]Institut de Recherche en Biologie Végétale de l'Université de Montréal, 4101 Est, rue Sherbrooke , Montréal, Québec, Canada H1X 2B2. [2]Agriculture Canada Research Center, 430 Blvd Gouin, St-Jean-sur-Richelieu, Québec, Canada J3B 3E6. In this study, we are using RFLP analyses to draw the genetic architecture of individual plants produced by selfing and by *in vitro* anther culture of two highly heterozygous plants of *Solanum chacoense* (2n = 2x = 24). Our objectives are: 1) to tag chromosomal areas or blocks of genes involved in anther culture response; 2) To monitor the spontaneous production of new S-alleles seldom occuring after several generations of forced selfing and; 3) to construct a genetic map for this species based on both gametic and zygotic segregation of RFLP markers (*see also Saba El Leil et al. and Després et al. , this meeting*).

472 ANALYZING THE REGULATORY DNA SEQUENCES OF BRASSICA POLLEN-SPECIFIC GENES IN TOBACCO. Ravinder Sardana, Diego Albani*, Steven Fabijanski*, Paul Arnison* and Illimar Altosaar. Dept. of Biochemistry, University of Ottawa, Ottawa, ONT, K1N 9B4, Paladin Hybrids Inc., 2475 Don Reid Dr., Ottawa, ONT, K1H 8P5, Canada.

The aim of this study was to define the regulatory DNA sequences of Brassica napus pollen-specific genes Bp10 and Bp4. The genes have been characterized and are expressed during pollen development. The Bp10 gene shows some degree of homology to ascorbate oxidase from cucumber. Presumed regulatory DNA elements were cloned and fused separately to a bacterial marker gene, beta-glucuronidase (GUS). The DNA constructs were transferred into tobacco using a standard Agrobacterium transformation procedure. A total of 5-10 regenerated transgenic plants containing 1-3 copies of each of the inserts (Bp10 and Bp4) were analyzed for GUS activity both histochemically as well as spectrophotometrically. Such plants were analyzed for GUS activity in leaves, petals, sepals, stamen, pistill and stem. The GUS activity was detected only in the developing pollen grains. The results show that a 500 bp fragment of Bp10 and 450 bp fragment of Bp4 gene promoter contain sufficient information to programme pollen-specific gene expression in tobacco plants. Deletion analysis of these fragments conferring pollen-specific expression is in progress.

473 HOMEOBOX CONTAINING GENES IN ARABIDOPSIS. Kyle A. Serikawa, Erik Vollbrecht, Sarah Hake and Patricia Zambryski. Plant Biology, University of California, Berkeley, CA 94720

Plant development is thought to depend upon unique temporal and spatial patterns of gene expression at specific points during growth. Genes displaying such specific patterns have been isolated and characterized in recent years. To date, however, few plant genes involved in large scale transcriptional regulation have been described. We are attempting to find genes that may play this role. Our strategy is to look for genes containing homeoboxes. Homeobox genes are involved in many different developmental processes in a wide variety of organisms We have screened cDNA libraries at low stringency with the maize *Kn* homeobox (obtained from Dr. Sarah Hake's lab) in an attempt to clone similar genes in Arabidopsis. We have begun the initial characterization of clones from our first set of screens and are examining these clones on the levels of sequence homology and expression patterns

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474 ISOLATION AND CHARACTERIZATION OF A PROLINE-RICH GENE. HIGHLY EXPRESSED IN YOUNG TOMATO FRUIT. Yehiam Salts[1], David Kenigsbuch[1], Ruth Wachs, Rivka Barg[1], Mohamad Abu-Abied[2], Daniel Zamir[2] and Wilhelm Gruissem[3]. [1] Dept. of Plant Genetics, The Volcani Center, ARO, POB 6, Bet-Dagan 50250, Israel. [2] Faculty of Agriculture, The Hebrew Univ., Rehovot 76100, Israel. [3] Dept. Plant Biology, Univ. of California, Berkeley, CA 94720, USA

A cDNA library was constructed in λgt10, from poly (A)$^+$ RNA isolated from young tomato fruits (6-8 mm in diameter, about one week after anthesis). Differential screening with tomato seedlings cDNA was used to select clones that are differentially expressed in young fruit. One of these, a 1.3 kbp cDNA clone 1-36-1, is expressed in young fruit at a level that is 2000, 1000, 60 and 30 times higher than in mature-green fruit, leaf, root and stem, respecuvely. It's level of expression is already high in ovaries at anthesis. Another 1.4 kbp cDNA clone, 1E1, and a 12 kbp genomic clone corresponding to 1-36-1 were isolated. Sequence analysis indicated: a. the existence of an intron in the 3' untranslated region of the gene; b. an identity between the shared regions of the 1E1 cDNA and the genomic clone; c. cDNA clones 1-36-1 and 1E1 are both truncated and lack the 5' terminal region, they differ at the 3' ends, both harbor a poly-A tail but 1-36-1 terminates at a position which is 75 bp upstream of 1E1; d. the open reading frame would encode a new proline-rich protein composed of repeating amino acids blocks; e. the ORF exhibits a codon bias in which there is a discrimination against the use of G or C at the third position. Using RFLP analysis cDNA clone 1-36-1 was mapped to a single locus on the tomato chromosome VII, between markers TG143 and TG166. Promoter analysis is performed by the study of transient expression and stable transformants

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475 PLANTLET REGENERATION AND IN VITRO FLOWERING IN SAFFLOWER, Carthamus tinctorius. P.Seeta and S.Y. Anwar, Department of Genetics, Osmania University, Hyderabad-500 007, India.

Plantlet regeneration was achieved in a commercial cultivar Mangira. Plantlet regeneration in safflower is a two step process, the shoot regeneration and rhizogenesis. The callus induction was observed on MS medium supplemented with 2 mg/l 2,4-D and 15% v/v coconut milk. Shoot regeneration was observed on MS medium supplemented with 1.0 mg/l BAP and 1 mg/l adenine sulphate with subsequent rooting on MS basal medium. High frequency of in vitro flowers were induced in cv Mangira (50%), on MS basal medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA.

476 ANALYSIS OF TOBACCO MUTANTS THAT ALTER FLORAL DEVELOPMENT. Melanie C. Trull and Russell L. Malmberg. University of Georgia, Department of Botany, Athens, Georgia 30602.

Mgr21 and *Mgr27* are developmental mutants of tobacco that were obtained by uv-mutagenizing cell cultures and selecting for resistance to an inhibitor of an enzyme in the polyamine biosynthetic pathway. Both *Mgr21* and *Mgr27* produce flowers with altered morphology and displaced tissue. *Mgr21* has a complex flower that may be indeterminate while *Mgr27* has stigmatoid anthers. Preliminary data also indicate differences in the vegetative meristem and the pattern of vegetative growth in *Mgr21* as compared to wild type. The *Mgr21* floral meristem typically produces a cylinder of tissue inside the stamens that contains additional organs. In some *Mgr21* flowers examined by SEM, the floral meristem appears to split after producing sepals and petals. The floral development of *Mgr21* will be examined by SEM and *in vitro* culture and the identity of the cylinder will be examined by light microscopy, SEM, and the use of a carpel-specific monoclonal antibody. Polyamine levels of the mutant lines will be compared with wild type. At the SEM level, the vegetative meristem of *Mgr27* appears similar to wild type. Stigmatoids appear on the anthers of *Mgr27* flowers at an early stage of development.

477 CHARACTERIZATION OF A FLORAL SPECIFIC *C-MYB* HOMOLOG FROM *ARABIDOPSIS THALIANA*. Diane M. Ruezinsky, Susan A. Hedrick, Richard A. Dixon*, Christopher J. Lamb, and Peter W. Doerner. Plant Biology Laboratory, The Salk Institute for Biological Studies, P.O. Box 85800 San Diego, CA 92138-9216; *Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

Members of the *c-myb* proto-oncogene family have been shown to be required for cell differentiation and proliferation in several species. The protein product of the first *c-myb* homolog identified in plants, the maize C1 gene, activates transcription of genes involved in anthocyanin biosynthesis. An additional six *c-myb* homologs were isolated from an *Antirrhinum* flower cDNA library. We have cloned *c-myb* homologs from an *Arabidopsis thaliana* genomic library. The amino terminal end of the protein encoded by one of these genes, Atmyb4, shares 64% identity with the maize C1 protein and 56-73% identity with the *Antirrhinum* genes. In addition, the intron/exon boundaries of C1 and Atmyb4 are positioned at the same amino acid residues. RNA slot blot analysis has shown that Atmyb4 is expressed predominantly in floral tissues (buds, flowers and siliques). To determine more precisely the expression pattern, the Atmyb4 promoter was linked to the β-glucuronidase (GUS) gene and introduced into *Arabidopsis*. In order to determine what role the product of this gene plays in floral development, a series of antisense, dominant negative, and overexpression constructs are being introduced into *Arabidopsis* Data on these transgenic plants will be presented.

478 GENES EXPRESSED DURING MICROSPORE DEVELOPMENT IN *BRASSICA NAPUS* AND *ARABIDOPSIS THALIANA*. Gary D. Foster, Michael R. Roberts, Robert P. Blundell, Susan W. Robinson, John Draper and Rod Scott. Botany Department, University of Leicester, Leicester LE1 7RH, U.K.

The expression of genes during the development of the male gametophyte, the microspore, is of interest both academically and commercially. The cytological aspects of pollen development have been studied at length but little is known about the genes expressed specifically during microspore development. We have cloned and sequenced six microspore-specific cDNAs (F2sL, F2S, E2, I3, CEX1 and CEX2) from *Brassica napus*. The predicted polypeptides of the I3 and CEX1&2 cDNAs are proline-rich and contain putative N-terminal membrane-spanning domains. Transcripts are detected in buds and anthers of male fertile plants, but not in green tissues, roots or in cytoplasmic male sterile buds. The I3 and CEX genes are expressed following microspore release and expression continues until the onset of pollen grain formation in *B. napus*. The corresponding *Arabidopsis thaliana* gene (APG) for one of these cDNAs (CEX1) was isolated (using the related I3 cDNA as a probe) and the coding and upstream regions identified. The putative microspore-specific promoter within this upstream region was linked to GUS and transformed into tobacco and *Arabidopsis*. Expression of GUS in tobacco was not detected in green tissues, stems or roots, but was present in anthers. GUS activity appeared in the microspores shortly after their release from the meiotic tetrads, and was maintained until the interphase after the first pollen mitosis. Strong GUS activity was also detected in the tapetum for a short time prior to this mitosis. Northern analysis of the E2 transcript also showed high expression in developing microspores as well as in the tapetum, as detected by *in situ* hybridisation. Current work includes functional analysis of these cDNAs by sense and antisense constructs with the *A thaliana* promoter in transgenic plants.

479 MOLECULAR ANALYSIS OF GENES EXPRESSED DURING ANTHER DEVELOPMENT IN *BRASSICA* Wyatt Paul, Rachel Hodge, Dawn Worrall, Kenan Turgut, Diane Hird, Sarah Smart, John Draper and Rod Scott, Botany Dept, Leicester University, LE1 7RH, UK.

In order to understand the nature and regulation of events that occur during anther differentiation we have made anther cDNA libraries that cover specific developmental phases (sporogenesis and microspore development) in *Brassica napus*[1]. The gene expression programme that supports androgenesis is large such that 10,000 (40%) of the transcripts present in the tobacco anther are organ-specific[2]. In agreement with this estimate, we have determined that within the sporogenesis library 34 of 100 cDNAs derived from low abundance messages represent anther-specific transcripts. Conventional differential screening of the anther libraries has led to the isolation of a set of anther-specific cDNAs; eight have been extensively characterised and represent abundant transcripts with unique temporal expression patterns collectively spanning premeiosis to the second microspore mitosis. Seven different groups of cDNAs have been isolated from the sporogenesis (premeiosis to tetrad dissolution) library. So far, three (A3, A8, A9) have been tested for spatial specificity by *in situ* hybridisation, and are confined to the tapetum. Representatives of each group have been sequenced. Two, A8 and A9, share structural features with certain plant protease inhibitors and 2S seed storage proteins, one probably plays a direct role in tetrad dissolution; one is homologous to a member of the flavanoid biosynthesis pathway; and three gave no data base matches. Four encode an hydrophobic N-terminus. Promoters for the A9 and A3 genes have been isolated from *Arabidopsis thaliana* and linked to the GUS reporter gene. Experiments in transgenic tobacco plants show complete conservation of developmental specificity. Promoters of two further genes are under analysis. Although these genes have similar expression patterns, no obvious homology exits between the promoters of these and other anther-specific genes. [1]Scott *et al* (1991) Plant Mol. Biol. in press. [2]Kamalay and Goldberg (1984) PNAS USA 81, 2801-2805

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480 ISOLATION AND EXPRESSION OF TOBACCO ANTHER-SPECIFIC GENES. Ann Tuttle, Linda Mercer, Sue Kadwell, Susan Jayne and Lyle Crossland. CIBA-GEIGY Corporation, P.O. Box 12257, Research Triangle Park, NC 27709-2257. A number of anther-specific cDNA clones have been identified by differential screening of a subtracted, anther cDNA library. Ten of these clones have been further characterized by DNA sequence analysis and determination of timing of expression. The mRNA from which the clone Ant32 was derived has been shown by *in-situ* hybridization to be expressed only in the tapetal cells of the anther. The gene encoding this mRNA has been isolated and sequenced, and gene fusions of the Ant32 promoter region with the coding region of the ß-glucuronidase gene have been transformed into tobacco plants. ß-glucuronidase activity has been assayed fluorometrically and histochemically and been shown to be present only in the tapetum and, to a lesser extent, in the pollen of transgenic plants.

481 EXPRESSION OF ADENINE PHOSPHORIBOSYLTRANSFERASE DURING POLLEN DEVELOPMENT IN *ARABIDOPSIS*
Sharon M. Regan and Barbara A. Moffatt. Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

In order to understand the role of the purine salvage pathway in plants, we are examining a mutant deficient in adenine phosphoribosyltransferase (APRT), an enzyme responsible for the conversion of adenine to AMP. In *Arabidopsis*, the mutant is male sterile due to aborted pollen development. Cytochemical analysis has shown that the course of pollen development in the mutant begins to diverge from that in the wild type soon after the dissolution of the callose wall and the release of the microspores. The pollen wall does not develop normally and the appearance of the vacuole is irregular. Many of the mutant microspores fail to undergo mitotic divisions and the activities of two enzymes, alcohol dehydrogenase and esterase, are lost. Although the majority of the morphological changes in the mutant appear in the developing pollen, the region directly affected by the APRT deficiency could be any of the tissues of the anther. We are currently using *in situ* hybridizations to localize APRT transcripts during pollen development in an attempt to gain insight into the mechanism by which the APRT deficiency causes male sterility.

482 Expression and functional analysis of a flower-specific putative thionin gene in *Nicotiana tabacum*.
Qing Gu and Alice Y. Cheung, Dept. of Biology, Yale Univ. New Haven, CT 06511

A flower-specific cDNA (49A) encoding a cysteine-rich protein has been isolated from *N. tabacum*. Northern blot analysis revealed that 49A mRNA was present in petal, stamen and pistil of the flower, but absent in sepal. The messenger was also temporally regulated. Spatial distribution of 49A mRNA is being determined currently by *in situ* hybridization and/or tissue printing techniques. An open reading frame has been identified in the cDNA sequence, which encodes a 105 a.a. cysteine-rich polypeptide consisting of 9 cys residues. Sequence homology search revealed 49A polypeptide has significant homology to several cysteine-rich proteins, including wheat gamma-purothionins (ref.1) and a putative potato proteinase inhibitor p322 (ref. 2). Detailed sequence comparisons will be presented. Transgenic plants with 49A over-expression and with 49A anti-sense constructs are being generated. Other biological assays will also be carried out to determine if 49A polypeptide display any proteinase inhibitor or thionin activities. Results on these functional analysis will be discussed.

1 Mendez, E. et al (1990). Eur. J. Biochem. 194: 533-539.
Colilla, F. J. et al (1990). FEBS Lett. 270.191-194
2. Stiekema, W. J. et al (1988). Plant. Mol. Biol. 11:255-269

483 POLLEN-SPECIFIC EXPRESSION OF α-TUBULIN GENE.
Younghee Kim, Seong-Ryong Kim, Wenbiao Chen, David Finkel, and Gynheung An. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Microtubules play an essential role in eukaryotic cells in many processes such as cell division, motility and morphogenesis. The major component of microtubules is tubulin, a heterodimer formed by two subunits, alpha and beta. The genome of *Arabidopsis thaliana* contains at least four copies of α-tubulin genes. We have studied one of the genes, α1-tubulin gene, by generating transgenic plants carrying gene fusions between the 5' control region (2.2 kbp) of the tubulin gene and a reporter (GUS or chloramphenicol acetyltransferase) gene. Expression of the reporter genes was observed only in mature pollen of transgenic tobacco and *Arabidopsis* plants. Regulatory elements involved in pollen-specific expression of the α1-tubulin gene are currently investigated by mutation analysis of the promoter. In an attempt to isolate a similar pollen-specific gene from monocot species, we have cloned three α-tubulin genes from rice. We are characterizing the expression pattern of these genes in various organs.

484 ISOLATION AND CHARACTERIZATION OF P-PROTEIN GENES.
Gary A. Thompson and Brian A. Larkins. Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Two major pumpkin phloem proteins, or P-proteins, have been characterized. PP1 is an 80-kD cysteine-rich protein that forms polymeric filaments *in vivo*, and PP2 is a 48-kD dimeric lectin. To obtain cDNAs encoding these and additional P-proteins, we raised polyclonal antibodies against total phloem exudate from pumpkin seedlings. Western blot analysis with this complex antiserum showed little cross-reactivity with proteins from pumpkin callus. A pumpkin cDNA expression library was screened with the complex antiserum and three groups of closely related or identical cDNA clones were isolated. cDNAs with the largest sized inserts were designated cPC2 (670 bp), cPC7 (1.38 kbp), and cPC13/20 (13 is 1 kbp and 20 is 850 bp). To identify the corresponding P-proteins the β-galactosidase-clone fusion proteins were used to purify specific antibodies from the complex antiserum. Western blot analysis of phloem exudate proteins showed that cPC7 encodes a protein the size of PP1, and cPC13/20 encodes a protein the size of PP2. The cDNA, cPC13, was used to probe a pumpkin genomic library, and four genomic clones were isolated that correspond to cPC13.

485 ISOLATION OF MOLECULAR MARKERS FOR SEX-DETERMINATION IN *Asparagus officinalis* L. David Bouchez, Christophe Robaglia (Laboratoire de Biologie Cellulaire) and Catherine Rameau (Station d'Amélioration des Plantes). INRA, 78026 Versailles Cedex, France

Cultivated asparagus (*A. officinalis* L.) is a monocot, dioecious species. Genetic studies indicate that sex determination in this species is apparently monogenic, the male allele (M) being dominant on the female one (m). This locus has been assigned to chromosome L5. Male plants are heterogametic Mm, while female plants are homogametic recessive mm. The presence of the M allele always leads to staminate flowers, but the development of pistillate flowers is more variable and also depends on environmental conditions. Evolution genetics models predict the existence of a super-gene where male and female sex-determining functions would be clustered. A number of secondary sexual traits are of agronomical importance (precocity, yield, ...).
In the course of our breeding programs, we have constructed a series of male/female quasi-isogenic lines to the fifth to sixth backcross. We are currently using these isogenic couples to perform subtractive cloning of sex-specific cDNAs and genomic DNA, in order to obtain molecular markers that would allow early sex-determination in this species.

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486 A GENE EXPRESSED SPECIFICALLY IN MAIZE POLLEN. Sue Kadwell, Ellis Merlin, Isabelle Dupuis and Lyle Crossland. CIBA-GEIGY Corporation, P.O. Box 12257, Research Triangle Park, NC 27709-2257 A maize pollen-specific cDNA has been isolated and sequenced. It is 1349 base pairs in length, with a 406 amino acid open-reading frame and a 24 base pair poly-A tail. The transcription start site has been determined by primer extension to be 41 bases upstream from the putative translational initiation codon. The deduced protein encoded by this gene has two domains, each related to known eukaryotic signal transduction proteins. Genomic DNA blot hybridizations using the entire cDNA as probe show this gene to be a member of a large multigene family, yet when the 3' end of the cDNA is used as a probe, only 2-3 bands per restriction digest are seen. Accumulation of the mRNA encoding this protein begins at the early to mid trinucleate stage of microspore development., and the mRNA is abundant in mature pollen.

487 DEVELOPMENTALLY REGULATED EXPRESSION OF THE TUBULIN GENES OF *ARABIDOPSIS THALIANA*. D. Peter Snustad, Jeffrey L. Carpenter, Steven D. Kopczak, Nancy A. Haas, Russell H. Goddard, Soon-Ok Cho, Susan M. Wick and Carolyn D. Silflow. Univ. of Minnesota, Depts. of (1) Genetics and Cell Biology and (2) Plant Biology, St. Paul, MN 55108. The rigid walls of plant cells largely preclude cell migrations. Thus, morphogenesis in plants is controlled primarily by the factors that establish (1) the planes of cell division and (2) the axes of cell expansion. The cytoskeleton of the plant cell plays a central role in both of these processes, and two arrays of microtubules unique to plants — phragmoplasts and preprophase bands — are directly involved in these key morphogenetic events. We are studying the expression of the individual tubulin genes of *Arabidopsis* in an attempt to begin to understand the genetic control of the cytoskeleton in a higher plant. To our surprise, the small *Arabidopsis* genome was found to contain a very large tubulin gene family. Five α- and nine β-tubulin genes have been cloned and sequenced to date. Work on a probable sixth α-tubulin gene is in progress. Noncoding 3' or 5' gene-specific hybridization probes have been constructed for all 14 sequenced genes and used to study their patterns of expression. In addition, the 5' regions of selected tubulin genes have been fused to the *E. coli* β-glucuronidase (GUS) reporter gene and introduced into *Arabidopsis* plants by *A. tumefaciens*-mediated transformation. The developmental profiles of gene expression have been studied in transgenic plants by histochemical GUS assays of various organs and tissues and compared with the expression patterns observed by northern blot hybridization studies. The *TUA1* gene is expressed primarily in male flower tissues and at especially high levels in pollen. The *cis* elements that control the pollen-preferential expression of *TUA1* are located within a 550-nucleotide-pair region 5 to the *TUA1* gene. A deletion analysis of this 5' controlling region is in progress. Antibodies specific to the α1-tubulin isoform have been prepared and are being used to investigate the utilization of this specific tubulin *in vivo*.

488 FM-98, A GENE REQUIRED DURING EARLY FLORAL DEVELOPMENT IN *ARABIDOPSIS*. Judith L. Roe, Allen Sessions, *Kenneth A. Feldmann, Patricia Zambryski. University of California, Department of Plant Biology, Berkeley, CA 94720 and *University of Arizona, Department of Plant Sciences, Tucscon, AZ 85721

The fm-98 flower mutant was originally isolated from a population of T-DNA transformed *Arabidopsis thaliana* seeds. Fm-98 mutant plants display flowers with abnormal numbers of floral organs and a partially unfused carpel. One of the T-DNAs present in the fm-98 line co-segregates with the mutant phenotype. The genomic DNA surrounding the T-DNA insert has been cloned and transcripts from the region are being analyzed by the isolation of cDNA clones and Northern blot analysis. Mapping studies indicate that one of the cDNAs derives from sequences flanking both sides of the T-DNA insert and has presumably been interrupted in the fm-98 mutant. To define the functional region of the gene disrupted in the mutant, sequencing of this cDNA and the corresponding genomic region is in progress, as are complementation studies. The fm-98 gene product appears to be required early in normal flower development. Scanning electron microscope analyses of mutant fm-98 buds has revealed that normal development is altered early in flower formation. Double mutants with fm-98 and another *Arabidopsis* flower mutant, ap-1, have been constructed, and the loss of both gene products results in plants which can no longer form floral structures. This suggests that the products of the ap-1 and fm-98 genes function early in floral development (and possibly interact), and together are necessary for normal floral development.

489 MEIOTIN-1: A CONSERVED, MEIOSIS-SPECIFIC PROTEIN WHICH ASSOCIATES WITH NUCLEOSOME STRINGS. C.Daniel Riggs and Clara A. Hasenkampf. Department of Botany, University of Toronto, Scarborough, Ontario Canada

By exploiting the natural synchrony of Lilium meiocytes, we have identified several meiosis-specific nuclear proteins. One of these, which we call meiotin-1, has been purified and an antiserum has been raised against the protein. The antiserum demonstrates that this abundant protein is a meiosis-specific protein which first appears during premeiotic interphase. Interestingly, this is a critical period during which the irreversible commitment to meiosis occurs. Complete turnover of the protein is observed by the end of the meiotic divisions. The antiserum also recognizes epitopes in meiocyte-enriched extracts prepared from maize, tobacco and tomato. Somatic cells from these organisms apparently do not contain the cross-reacting epitope(s). Chromatin fractionation studies indicate that the protein is a chromatin-associated protein that has an unusual stoichiometry relative to the other protein components of the nucleosome. On average, meiotin-1 is found once per 5 - 7 nucleosomes. Such a distribution can be interpreted in the context of the solenoid model of chromatin structure to suggest that meiotin-1 in some way serves to assist the organization of chromatin in the meiotic nucleus. Such a role has implications for synaptonemal complex structure and for recombination. Recent progress will be presented.

490 CHARACTERIZATION OF MALE-STERILE ARABIDOPSIS LINES GENERATED BY SEED TRANSFORMATION Christopher A. Makaroff[1], Nancy Smith-Huerta[2], Connie S. Bricker[2], and Kenneth A. Feldmann[3] Departments of Chemistry[1] and Botany[2], Miami University, Oxford, OH 45056 and Department of Plant Sciences[3], University of Arizona, Tucson, AZ 85721

Male-sterile mutants of *Arabidopsis thaliana*, which have been generated by T-DNA insertional mutagenesis, are being characterized to identify factors that control tapetum differentiation and to better understand the role of the tapetum in microsporogenesis. Over 8,000 transformed lines were screened for the reduced fertility phenotype. Approximately 140 lines were found to exhibit reduced male fertility. Twenty male-sterile lines, which exhibit relatively normal stamen development and alterations in microsporogenesis, have been chosen for further study. Anatomical and cytochemical studies are being conducted to determine at what stage the block in pollen development is first evident and to possibly identify the nature of the alteration. Both molecular and genetic cosegregation analyses are being used to identify those mutants that have resulted from insertional inactivation by the T-DNA. To date, strict cosegregation between the T-DNA insert and the male-sterility phenotype has been demonstrated for three lines. Segregation ratios for the lines indicate that the sterility phenotype is inherited by a single recessive gene. These results indicate that insertional inactivation by the T-DNA is most-likely the cause of the observed phenotypes in the three lines. Preliminary cytochemical analyses indicate that the three lines each differ in the timing of microsporogenesis breakdown. The mutants exhibit the following phenotypes: a) persistent tapetum, b) premature degradation of the tapetum, and c) normal tapetum. Experiments are currently under way to use the T-DNA as a tag to isolate the gene responsible for the observed phenotype in the three lines. These studies should allow us to identify and isolate genes involved in the control of tapetal cell development.

491 A FLOWER-INDUCING POLYPEPTIDE OF HIGH MOLECULAR WEIGHT FROM HIGHER PLANTS. Akiko Kozaki, Takasi Kido[1], Osamu Tanaka[2], Zenzaburo Kasai[1], Go Takeba*. Kyoto Univ., [1]Kinki Univ., [2]Konan Univ., *Kyoto Prefectural Univ., Kyoto, Japan. A flower-inducing substance of high molecular weight was found to be distributed widely in higher plants. It induced flowering of *Lemna paucicostata* 151 at a very low concentration. It was denatured by boiling of the crude extract for 5 min but the florigenic activity was stable to heating. The fragments produced by proteinase K also showed florigenic activity. The molecular mass of the factor was estimated by gel filtration to be about 120 kDa (Plant Physiol. 94:1677, 1990). It was extracted from *Lemna paucicostata* 441 and purified to homogeneity by gel filtration, ion-exchange chromatography, and reverse-phase chromatography. The purified active fraction generated only a single band (at about 22 kDa) after electrophoresis on a SDS-PAGE and stained by Coomassie brilliant blue (CBB). The CBB-stained material had florigenic activity. The amino terminal of this compound was determined to be Leu-Val-Gly-Asn-Thr on a protein sequencer. It is, therefore, clear that this compound is a polypeptide. The lowest concentration of the compound to show florigenic activity in our assay system was estimated to be about 10^{-10} M, if we assume that the molecular mass of the active agent is 22 kDa. We are now investigating the possibility that the 120-kDa polypeptide is broken down to a physiologically active fragment under various flower-bud-inducing conditions.

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492 A CONSTITUTIVE FLOWERING MUTANT OF ARABIDOPSIS
Alemu T. Belachew, Ralph Bertrand-Garcia, and Z. Renee Sung
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Berkeley. CA 94720

Arabidopsis is a quantitative long day plant. Environmental factors such as vernalization and day length affect the timing of flowering. However endogenous or genetic factors determine the process of flowering, because some races, e.g., *A. thaliana*, columbia, will flower without vernalization and under short day conditions. Late flowering mutants have been isolated that are being characterized to elucidate the sequential steps of flowering. We have recently isolated an early flowering mutant, which flowered upon germination and appeared to skip the vegetative growth. Genetic analysis indicates that the trait segregates as a single, recessive gene. We postulate that the mutant is either altered in a gene that controls the rate of meristem development (a heterochronic mutant), or in a gene that normally inhibits the transition from vegetative to reproductive growth. The developmental anatomy, physiology and genetic characterization of the mutant will be discussed.

493 EARLY EVENTS IN FLOWERING OF *ARABIDOPSIS THALIANA*.
Eva Huala and Ian M.Sussex. University of California at Berkeley,
Dept. of Plant Biology, Berkeley, California 94720.

Although recent work on mutations which alter floral organ identity has shed light on the molecular basis for the differentiation of floral organs, the process by which a vegetative meristem becomes florally determined remains poorly understood. The *leafy* mutation in *Arabidopsis* appears to affect an early step in the process of flower development. A severe *leafy* allele causes the conversion of flowers to vegetative branches and structures intermediate between flowers and branches. These intermediate structures show some degree of internode elongation and fail to suppress axillary bud development but terminate in one or more carpels. A mild *leafy* allele produces flowers with sepaloid petals, a reduced number of stamens, and extra carpels, suggesting that expression of *apetala3* or *pistillata* may require a fully active *Leafy* gene product. Mutations causing phenotypes similar to *leafy* have been studied in pea and tomato as well as snapdragon, from which the corresponding gene, *floricaula*, has been cloned and sequenced (Coen, E.C. et al. (1990). Cell 63:1311-22). We are currently investigating the function of the *leafy* gene through SEM studies of the apical meristem of *leafy* mutants. In addition, we are constructing double mutants to examine the role of *leafy* in the control of floral organ identity genes such as *ap1*, *ap2*, *pi* and *ag*.

494 CHARACTERIZATION OF LATE FLOWERING MUTATIONS AT THE fve LOCUS OF ARABIDOPSIS.
Cruz-Alvarez, M., J.A. Jarillo, A. Leyva,and J.M. Martínez Zapater. CIT-INIA. Ctra. La Coruña Km 7. 28040 Madrid, Spain.

We are interested in the molecular characterization of floral induction in Arabidopsis. Previous analysis of late flowering mutations, isolated at the Genetics Department of the Agricultural University of Wageningen, suggested that some loci (fca, fe and fv) are involved in the positive promotion of flowering through a pathway independent from cold induction. Mutants at the fve locus behave in a similar way. Two mutations have been isolated that produce different delays in flowering time . Both mutations are recessive and the late flowering phenotypes can be completely rescued upon vernalization. By constructing double mutants, homozygous for mutations at the fve locus and deficient or insensitive to plant hormones, we are analyzing the role of ABA and GA in the vernalization process. In order to obtain more information about the function of this locus in floral induction , we are currently pursuing the cloning of the correspondent DNA sequences using chromosome walking techniques. We have selected a collection of recombinants between the fve locus and linked morphological markers on chromosome 2. These recombinants are being used to integrate the morphological markers and the RFLP maps in that region of the chromosome, and to select the closest RFLP to initiate the walk.

495 VEGETATIVE STORAGE PROTEINS AND REGULATION OF FLORAL SET IN SOYBEANS
[1]C.D. Dybing, [2]R.N Reese, and [2]J.E. Peterson. [1]Plant Science-USDA-ARS, [2]Biology-Microbiology Dept., South Dakota State University, Brookings, SD 57007

Most of the flowers located distally on the racemes of soybeans are destined to abort, but they can be induced to set by removal of proximal flowers or treatment with benzyladenine. We hypothesized that low growth rate in aborting ovaries is causal to abscission of the flowers, inducing production of enzymes for wall-weakening processes in the abscission zone at the base of the pedicel. One dimensional SDS-PAGE of total protein extracts of setting and aborting raceme tissues reveals 5 major bands and more than thirty minor bands that are differentially expressed in the tissues during the course of development. Time course studies showing both translation and expression of proteins in raceme tissues of setting and aborting flowers show there is at least one protein specifically associated with abortion of flowers and several bands associated with flower set. Western Blot Analysis using antibodies for soybean vegetative storage proteins (VSP) and fluorography of in vivo labeled racemes clearly show that flower set is predicted by translation of a 30 kDa VSP within 48 h after anthesis. Lack of expression of this protein in raceme tissues predicts flower abortion. Regulation of expression of this protein by cytokinin and the role of this protein in flower set are under investigation and will be discussed.

496 TOWARDS ISOLATION OF THE *ARABIDOPSIS* FLOWERING-TIME GENE *FG*
Joanna Putterill, Francis Robson, Karen Ingle and George Coupland. JI Institute for Plant Science Research, Norwich, Norfolk, NR27UH, UK.

Mutations at the fg locus of Arabidopsis flower approximately 20 days later than wild-type plants when growing under long day conditions. However, when growing under short day conditions mutant and wild type flower at the same time. Therefore the product of fg appears to be involved in the control of floral induction in response to daylength. The gene is located on chromosome 5 at 16.4cM. We have initiated a chromosome walk from the ft-4 marker which is 2cM distal to fg and encodes the enzyme chalcone synthase. The EG YAC library was screened with 5 RFLP markers which are closely linked (within ca. 2cM) to CHS and therefore are in the vicinity of fg. Later, cosmid contigs in this area were provided by Brian Hauge (Boston) and these were also used to screen the EG YAC library. Total DNA was extracted from positively hybridising colonies and analysed by pulsed field gel electrophoresis followed by Southern transfer and hybridisation to appropriate cosmid probes. This procedure confirmed that 14 YACs do indeed hybridise to these probes and that they range in size from 50-240kb. Restriction mapping of these YACs and isolation of IPCR-generated end probes has resulted in the identification of three YAC contigs. One of these includes CHS and 6833 and is ca. 600kb in length while the other two are around 160kb long and contain either pCIT1243 or 5962. Currently we are trying to join these contigs by extending them in both directions. To locate fg on the YAC contig we have screened for recombinant plants derived from different marked Arabidopsis lines. These recombinants carry cross-overs very closely linked to fg. We are in the process of analysing DNA from these recombinants.

497 THE ISOLATION AND CHARACTERISATION OF POLLEN-SPECIFIC POLYGALACTURONASE GENES FROM MAIZE.
R.L.Allen, [1]H.J.Rogers and D.M.Lonsdale. Cambridge Laboratory, IPSR, John Innes Centre, Colney Lane, Norwich, U.K. [1]Biochemistry Department, Royal Holloway and Bedford New College, Egham, Surrey, U.K.

We are interested in those genes expressed postmeiotically in the male flowers of maize, which may have a role in the germination of the pollen grain and/or the development of the pollen tube. The isolation of such genes has been achieved by the differential screening of pollen and coleoptyle cDNA libraries of maize. Several cDNA clones which exhibited expression in developing tassels, reaching a maximum in mature pollen, and having no expression in sporophytic tissue were obtained. Nucleotide sequence data from these clones revealed that one of them had homology in its predicted amino acid sequence to that of the tomato polygalacturonase gene (1) involved in fruit ripening. *In situ* hybridisation studies have been carried out on various maize tissues to determine the spatial and temporal localisation of the transcript of this clone. Hybridisation has been observed only in the developing microspores within the anther and in mature pollen. No hybridisation has been detected in premeiotic microspores or any sporophytic tissue. The cDNA clone was used as a probe to screen maize genomic libraries and four clones were isolated. Nucleotide sequence data revealed that these are highly homologous within the coding region, differing more in the 5' upstream regions. Studies are underway to characterise the promoters of these genes further by examining the activity of these promoters fused to the *E.coli* reporter gene β-glucuronidase in transgenic plants.

(1) Rogers *et al* (1991) Biochemica Biophysica Acta (In Press)

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498 MOLECULAR ANALYSIS OF THE APETALA3 GENE OF *ARABIDOPSIS THALIANA*.
Thomas Jack, Laura Brockman, and Elliot Meyerowitz.
Division of Biology, California Institute of Technology, Pasadena, California

Mutations in the APETALA3 (AP3) gene of *Arabidopsis thaliana* result in homeotic transfomations of stamens into carpels and petals into sepals. We have cloned the AP3 gene from *Arabidopsis* by using the cDNA of the DEFICIENS (DEF) gene of *Antirrhinum majus* as a molecular probe. The sequence of two *ap3* mutant alleles and RFLP mapping data show that this gene is AP3. The AP3 gene product contains a MADS box and likely acts as a transcription factor. AP3 also shares extensive similarity with DEF in protein coding regions outside the MADS box. In situ hybridization to wild-type flowers shows that AP3 transcripts are expressed only in stamens and petals, the organ types that fail to form correctly in mutants. In the homeotic flower mutants *agamous*, *apetala2* and *pistillata*, the AP3 expression pattern remains confined to second and third whorl primordia.

499 GENE EXPRESSION DURING PETAL SENESCENCE. Edwina C. Cornish, Michael W. Graham, Stanley C. Baudinette, Michael Z. Michael and Keith Savin. Calgene Pacific Pty Ltd, 16 Gipps Street, Collingwood, Australia 3066.

The process of petal senescence is associated with well defined biochemical and physiological changes. For both carnation and petunia flowers the onset of petal senescence is controlled by ethylene. Treatments which prevent ethylene biosynthesis by the corolla delay senescence, while exogenous application of ethylene accelerates the process.

cDNA clones of genes that are differentially regulated during petal senescence have been isolated from both carnation and petunia. These include cDNAs that show extensive homology to ACC synthase (1) and the tomato ethylene-related gene TOM13 (2). In addition, a petal-specific, senescence-associated gene has been isolated from carnation. The promoter region of this gene includes three 17bp sequences that have marked similarity to tomato sequences that have been shown to be involved in ethylene-mediated gene expression (3).

1 Van Der Straeten *et al* (1990). Proc. Natl. Acad. Sci. USA 87: 4859-4863.
2. Holdsworth *et al* (1987). Nucleic Acids Res. 15: 10600.
3. Cordes *et al* (1989). The Plant Cell. 1: 1025-1034

500 IDENTIFICATION OF TISSUE-SPECIFIC GENES BY T-DNA TAGGING.
Keith Lindsey, Jennifer F Topping, Michael C Clarke, Wenbin Wei, Hayley F McArdle and Leonie M Rooke. Leicester Biocentre, University of Leicester, Leicester, LE1 7RH, UK.

T-DNA and transposon-mediated insertional mutagenesis provide a means of identifying genes which play regulatory roles in plant development, by creating mutant phenotypes. The physical linkage of the inserted DNA to the native gene can facilitate its isolation. We are investigating modified T-DNA gene tagging systems for identifying not only regulatory genes but also genomic sequences which direct tissue- or organ-specific gene expression. The strategy involves the introduction of either of two types of gene construction. The first is a 'promoter trap', which comprises the gusA reporter lacking a promoter; expression in transgenic plants is therefore the consequence of fusion between the gusA coding region and native promoter, followed by transcriptional readthrough. Observed tissue-specific GUS expression is a reflection of regulatory properties of the 'functionally tagged' promoter, which is also genetically tagged. A second transforming vector (an 'enhancer trap') comprises the gusA coding region linked to a weak promoter, which can be activated by nearby enhancer sequences in the absence of direct gene disruption; again, these sequences are functionally and genetically tagged. High frequency activation of both transforming vectors ('interposons') has been found to occur, suggesting preferential insertion of T-DNA in active or competent chromatin. The use of this system to identify cell-type-specific markers will be described.

501 STRUCTURE OF A cDNA ENCODING A POLLEN-SPECIFIC ZINC FINGER-LIKE PROTEIN IN SUNFLOWER.
Rachel Baltz, Claire Domon and André Steinmetz.
Institut de Biologie Moléculaire des Plantes, Strasbourg, France

We have isolated and sequenced a full-length pollen-specific cDNA (SF3) from sunflower which encodes a 219 amino acid-long polypeptide displaying the basic features of a transcription factor: a putative DNA-binding domain consisting of two zinc fingers, two motifs rich in basic amino acids (K, H and R) which could represent nuclear location domains and/or another DNA binding domain, and a putative activator domain consisting of a sixfold repeat of the pentapeptide sequence (S,T or A)(E or D)TQN. The mRNA coding for this polypeptide is easily detected in mature free pollen grains but not in immature flowers; its size was estimated by Northern hybridization at 1100 nucleotides. Southern hybridizations suggest that this putative transcription factor is encoded by a small gene family. A genomic copy of the gene was isolated from a nuclear DNA library and was found, by comparing its nucleotide sequence with that of the cDNA, to be interrupted by four short (80 to 132 bp) AT-rich introns.

502 TWO ANTHER-SPECIFIC GENES FROM SUNFLOWER SHARE A 300 BP REGION INCLUDING THE FIRST EXON CODING FOR A SIGNAL PEPTIDE. Claire Domon, Jean-Luc Evrard and André Steinmetz.
Institut de Biologie Moléculaire des Plantes, Strasbourg, France

Two anther-specific cDNAs from sunflower, SF2 and SF18, displaying the same expression pattern (peripheral anther cells in the late developmental stages), contain a highly conserved (>95%) 5' terminal region, whereas the homology in the remaining part of the coding sequence is only about 42%. Translated into amino acid sequence this conserved 5' terminal region corresponds to a hydrophobic domain typically found in amino terminal signal peptides. The unconserved region of the two cDNAs codes for different but apparently functionally related polypeptides: they share a domain containing the same pattern of 7 cysteine residues as well as a carboxy terminal domain rich in proline residues. To see whether the same organization of conserved and unconserved regions in the mRNAs is also present at the gene level, or whether it is the result of alternative splicing or trans-splicing, we have isolated and analyzed a genomic clone for each of the two cDNAs. We found that the signal peptide coding sequence is present in the two genes, and that each of the two genes is split by an intron, 2615 bp long in the case of the SF2 and 212 bp in the case of SF18. In the two genes the left splicing junction is located at the same position, which is one nucleotide after the last codon of the signal peptide coding sequence. A further examination of the nucleotide sequence of the two genes showed that the region of high sequence homology is not restricted to the signal peptide coding region but that it extends over 300 bp, also including 80 bp upstream of the transcription start site, as well as 150 bp of the intron. We propose that this conserved region originated by a recent duplication and that the two copies have been transferred by exon shuffling into the upstream proximal region of the two ORFs encoding the mature part of the SF2 and SF18 proteins. Experiments are in progress to determine whether this region also contains cis-acting regulatory elements determining the spatial and/or temporal expression pattern of the two genes.

503 BARLEY POLLEN cDNAs HOMOLOGOUS TO KENTUCKY BLUEGRASS GROUP IX POLLEN ALLERGENS. James D. Astwood, Shu Qunfang, Shyam S. Mohapatra, and Robert D. Hill. Department of Plant Science, University of Manitoba, Winnipeg, MB., Canada, R3T 2N2.

Barley (*Hordeum vulgare*) pollen is not known to contribute to the population of aero-allergenic proteins dispersed by pollen. Nevertheless, several barley pollen proteins were shown to react with IgE prepared from allergic humans. The DNA sequences of several *Poa p* IX isoallergens of kentucky bluegrass (*Poa pratensis*) have recently been deduced (*J.B.C.* 266: 1204-1210). Northern analysis of RNA obtained from barley pollen using *Poa p* IX cDNA clones as probes revealed RNA species of similar size to those found in kentucky bluegrass. Subsequent southern blots of barley genomic DNA using the kentucky bluegrass cDNAs as probes suggested that there may be several genes homologous to the *Poa p* IX isoallergens present in the barley genome. To study the biological and evolutionary significance of these allergen related proteins found in barley, a cDNA library was prepared from mature barley pollen mRNA. A number of barley pollen cDNA clones have been obtained and the expression of one, hvp101, has been examined. Northern analysis of gametophytic tissues (pistils, anthers, and pollen) and sporophytic tissues (leaves and roots), showed that hvp101 was expressed only in pollen.

POSTER ABSTRACTS

504 THE *STUD* GENE IS REQUIRED TO TERMINATE STAMEN SYNTHESIS IN FLOWERS OF *ARABIDOPSIS THALIANA*. Elizabeth Schultz and George Haughn. Biology Department, University of Saskatchewan, Saskatoon, SK, S7N 0W0, Canada

A. thaliana plants homozygous for a recessive mutation (*stud*) in the *STUD* gene, fail to form a functional gynoecium at the appropriate time and place during flower development. Instead, additional stamens are produced followed by one or more organs which can best be described as intermediate between a stamen and a carpel. Thus the *STUD* gene appears to be required to stop stamen production and/or initiate carpel production at the appropriate time. The interactions between *STUD* and other floral homeotic genes were investigated by analyzing double mutants homozygous for *stud* and a recessive allele of one of each of the floral homeotic genes *AG*, *AP2*, *AP3* and *PI*. Both *pi* and *ap3*, which are required for petal and stamen development, were found to be epistatic to *stud*. The flowers of a *stud/ag* double mutant have one whorl of sepals followed by multiple whorls of only petals, thus lacking the internal sepal whorls of a *ag/ag* homozygote. One hypothesis consistent with these data is that the *STUD* gene negatively regulates *AP3/PI* in order to terminate stamen production during development of wild type flowers. Finally, the *ap2-6/stud* double mutant phenotype showed the combined effects of the *ap2-6* and *stud* single mutants (perianth organs to reproductive organs, carpels to stamens) except that there was a partial suppression of the carpel to stamen transformation. This suppression could be due to a decrease in *PI* expression in *ap2-6/ap2-6* homozygotes.

505 MEGASPOROGENESIS: A POLLINATION REGULATED DEVELOPMENT PROCESS. Sharman D. O'Neill and Zhang Xiansheng. University of California, Department of Botany, Davis, California 95616

Many flowers undergo an extensive post-pollination syndrome of developmental changes. These include perianth senescence, changes in pigmentation and development of the ovary in preparation for fertilization and embryogenesis. Some flowers are long lived, with either the entire ovary or the ovules maintained in an immature state prior to pollination. Following pollination, megasporogenesis, the process of ovule development, is triggered in preparation for fertilization, which may occur days, weeks, or months later. We have characterized the post-pollination syndrome of developmental events in orchid flowers and will present: 1) characterization of ovary development over a period of three months after pollination during which time the megagametophyte is formed, matures and is fertilized, 2) the effects of pollination, auxin, ethylene and various hormone antagonists on megasporogenesis in orchid flowers, and 3) patterns of expression of ethylene biosynthetic genes that serve to trigger many, if not all post-pollination developmental events. We will present data suggesting that ovule development is an auxin- and ethylene-regulated process requiring the action of genes regulated by these hormones.

506 GENES EXPRESSED DURING FLOWER MORPHOGENESIS IN *BRASSICA NAPUS*. Jacqueline A. Pallas and Gareth I. Jenkins. Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

Studies of floral homeotic mutants in *Arabidopsis* and *Antirrhinum* show that diverse species have similar mechanisms of flower development. Several of these homeotic genes have been cloned by gene tagging. An alternative approach to isolate floral-specific genes is differential screening of cDNA libraries derived from floral tissue. This approach is difficult in *Arabidopsis* due to the small size of the flowers. We decided to use *Brassica napus* to clone genes involved in early flower morphogenesis and to use these clones to isolate the corresponding genes from *Arabidopsis*. Differential screening of a cDNA library made from stem apical tissue exhibiting floral buds with sepal primordia yielded 10 positive clones which are specific to floral tissue. Two cDNAs, pLF3A and pLF10B, are expressed throughout flower development. The expression of clone pLF2 is limited to floral apex and immature flower buds and the cDNA pLF7 is expressed in floral apices alone. All the cDNAs are expressed in *Arabidopsis* floral tissue and are present in the *Arabidopsis* genome at a low copy number. The cDNA pLF2 has been subcloned in both sense and antisense orientations into an expression cassette containing a CaMV 35S promoter and transgenic *Arabidopsis* plants are being generated. Current work includes sequencing of the cDNAs and *in situ* hybridisation to study their temporal and organ-specific expression.

507 ISOLATION OF PETUNIA FLORAL SPECIFIC GENES HOMOLOGOUS TO FLOWER MORPHOGENESIS GENES. Gerco C Angenent[1], Marco Busscher[1], John Franken[1], Joseph N.M. Mol[2] and Arjen J. van Tunen[1]. [1]Centre for Plant Breeding Research (CPO), PO Box 16, Wageningen, The Netherlands. Fax: 31-8370-18094. [2]Free University Amsterdam.

Homeotic genes regulating flower morphogenesis have been isolated recently from Arabidopsis and Antirrhinum. They most likely encode transcription factors and share some common features such as a conserved DNA binding part which is also found in animal transcription factors. With the use of a degenerated oligo nucleotide primer designed to the region corresponding to this DNA binding part, a cDNA clone was isolated from RNA isolated from young *Petunia hybrida* flowerbuds. With the use of this cDNA as a probe a flowerspecific cDNA library was sreened and a full-size cDNA clone, designated as *fbp* (floral binding protein) was isolated. The *fbp* cDNA showed a high degree of homology to the *agamous* and *deficiens* genes wich was largely restricted to the DNA binding part. Southern blot analysis revealed that FBP is most likely encoded by a single copy gene. Using the *fbp* cDNA as a probe it was determined that the corresponding gene is only expressed in whorls 2 and 3 (petals and anthers). The expression of the *fbp* gene was also determined in floral organs of two *Petunia hybrida* homeotic flower mutants. In the "Blind" mutant, where the limbs are transformed into antheroid structures, expression of the *fbp* gene was found in the tube, the normal anthers but also in the antheroid structures. In the "Green Petals" mutant in which the petals are transformed into sepaloid structures the *fbp* gene is expressed in the anthers but not in the sepaloid structures. Taken together these results suggest that *fbp* is a gene involved in the regulation of petal and anther formation. Analysis of the mode of action of the *fbp* gene is in progress.

508 CLONING *fca*, A LATE-FLOWERING LOCUS OF *ARABIDOPSIS THALIANA* (L.) HEYNH. Lore Westphal, Robert M. Ewing, Ian Bancroft and Caroline Dean. Cambridge Laboratory, John Innes Centre for Plant Science Research, Colney Lane, Norwich, Norfolk, NR4 7UJ, UK.

The late-flowering mutant *fca* has been isolated in the early *Arabidopsis thaliana* ecotype Landsberg *erecta* following EMS mutagenesis. Compared to the wild-type the mutant reveals a delay in flowering, represented by an increase in flowering time as well as leaf number. The wild-type phenotype can be completely restored by a vernalization treatment at 4°C for 8 weeks. Genetic and linkage analyses have shown that the mutation is recessive and inherited as a single locus (*fca*) on chromosome 4 at 23.5cM. In order to estimate the position of *fca* relative to genetic markers in this region of chromosome 4, crosses were made between the mutant line and the early flowering ecotypes Columbia and Niederzenz, respectively. In the F_2 progeny, late-flowering plants (homozygous for *fca*) were selected and selfed. DNA of these approximately 200 F_3 families was extracted and used in Southern hybridizations with RFLP markers (Meyerowitz and Goodman) and end fragments from YAC clones. Those F_3 families which showed recombinant hybridization patterns allowed us to estimate the map position of *fca* relative to the genetic markers used. The YAC clones integrated in our mapping approach are part of a large YAC contig established by Dr. Renate Schmidt and Gerda Cnops (this lab). This contig represents 600-700kb on chromosome 4 and spans 6cM. The information provided by the fine-mapping of *fca* onto the YAC contig will identify which YAC clone/subclone we will use in complementation experiments aimed at identifying the *fca* gene product.

509 GENES CONTROLLING FLOWERING TIME IN ARABIDOPSIS THALIANA. M. Koornneef, C.J. Hanhart, E.P. van Loenen Martinet, A.J.M. Peeters and J.H. van der Veen. Wageningen Agricultural University, Department of Genetics, 2 Dreijenlaan, 6703 HA Wageningen, The Netherlands.

Monogenic mutants of the early ecotype Landsberg 'erecta' were selected on the basis of late flowering under long day (LD) conditions after treatment with ethylmethane sulphonate (EMS) or irradiation. In addition to later flowering, the number of rosette and cauline leaves is proportionally higher in all mutants, although the regression of the two parameters is not the same for all genotypes. Forty-two independently induced mutants were found to represent mutations at eleven loci. The loci were located at distinct positions on four of the five Arabidopsis chromosomes. The recombinants of mutants at different loci flower later than or as late as the latest parental mutant. This distinction led to the assignment of the loci to several epistatic groups. In wild type, vernalization promotes flowering to a small extent. For mutants at the loci fca, fve, fy and fpa, vernalization has a large effect both under long day (LD) and short day (SD) conditions, whereas co, g1, fd and fwa mutants are almost insensitive to this treatment. SD induces later flowering except for mutants at the co and g1 loci, which flower with the same number of leaves under LD and SD conditions. This differential response of the mutants to environmental factors and their subdivision into epistatic groups is discussed in relation with a causal model for floral initiation in Arabidopsis thaliana. Progress on the cloning of one of these genes (fwa located on chromosome 4) will be reported.

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510 *IN VITRO* FLOWER BUD FORMATION: ISOLATION AND CHARACTERIZATION OF cDNA's SPECIFIC TO *IN VITRO* FLOWER BUD INITIATION IN TOBACCO.

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Flower bud formation on thin cell layer explants of tobacco (TCL) (consisting of epidermis and a few subepidermal cell layers) is regulated by auxins and cytokinins. There is some kind of interaction between auxins and cytokinins which is not on the level of uptake and metabolism. Cytokinins play a pivotal role in the initiation of this process probably by inducing cell divisions. We have isolated a group of homologous cDNA's of which expression is very high during the initiation and early development of flower buds on the TCL explants. Expression of these gene(s) during vegetative bud formation is at much lower level suggesting an involvement of these genes during *in vitro* flower bud formation. *In vivo*, expression only occurs in roots and, at a much lower level, in the apex meristem, in both organs just before the plants flower. *In situ* hybridization showed that the expression of one of these genes is localized in the epidermal cells of the tissue. Sequence analysis revealed a great homology to a tobacco extensin gene. Genomic analysis showed that the gene is present in tobacco, tomato and Arabidopsis.

511 **A BASIC CHITINASE IS EXPRESSED AT HIGH LEVELS IN THE TRANSMITTING TISSUE OF TOMATOES.** K. Harikrishna, Rachanee Jampates-Beale and Charles S. Gasser.Department of Biochemistry and Biophysics, University of California Davis, Davis, CA 95616. A pistil predominant gene (pMON 9617) isolated by differential screening of a tomato pistil cDNA library has been found to encode a protein with significant similarity to a basic chitinase. *In situ* hybridizations using an anti-sense copy of the cDNA showed localized expression in the transmitting tissue of the style. Northern analysis has demonstrated that the gene is expressed in mature pistils and at lower levels in mature anthers. As this gene is a member of a small gene family, we are analyzing gene specific expression in detail. An expression cassette was constructed using 1.4 kb of 5' flanking sequence fused to a GUS coding region and a NOS polyadenylation site. Plants transformed with this construct show the correct expression and localization of GUS staining. Bacterial expression vectors were built to produce the protein encoded by the cDNA and activity studies have been undertaken.

512 Gene Expression During Microsporogenesis in *Brassica napus*. Steven F. Fabijanski, Diego Albani, Aimin Li, Paul G. Arnison. Paladin Hybrids inc., 2475 Don Reid Dr., Ottawa, Ontario, K1H 8P5.

We have isolated a number of pollen specific genes from *Brassica napus*. One of these genes is a member of a gene family which codes for low molecular weight proteins that are apparently pollen wall associated. Promoter fusions with GUS constructs have revealed pollen-specific expression in both stable and transient heterologous systems. Similar promoter fusions and results have been obtained with two other different pollen specific genes. One of these genes codes for a protein which shares homology with pectinesterase. The other gene is a member of a pollen-specific gene family which shows homology to ascorbate oxidase. Expression of the ascorbate oxidase related coding region in bacteria has provided supporting data for an oxidative function. Experiments using antisense RNA directed against this pollen-specific oxidase have been used to define the role in microsporogenesis. The importance of this gene in pollen development is underscored by the presence of strongly homologous counterparts in the pollen of other non-related crop species.

513 Temporal and Spatial Regulation of Flower Specific Glycine-Rich Proteins Encoding Genes from Arabidopsis thaliana. Dulce E. de Oliveira[1], Luciana O. Franco[1], Cristina Torres[1], Johan Botterman[2] and Marc Van Montagu[3]. [1]Federal University of Rio de Janeiro, Dept Genetics and Biochemistry, C.P. 68011-21941 Rio de Janeiro-RJ, Brazil. [2]Plant Genetic Systems, J. Plateaustraat 21, B9000 Ghent, Belgium. [3]Rijksuniversiteit, Lab of Genetics, Ledeganckstraat 35, B9000 Ghent, Belgium.

We have isolated and characterized three glycine-rich proteins (GRP) encoding genes from Arabidopsis with specific expression in flowers. They have very similar structure and are disposed in tandem in the plant genome. There are similarities among the non glycine-rich domains of their encoded proteins while the glycine-rich regions are very distinct. RNA blotting experiments revealed a coordinated temporal regulation of the three genes with preferential expression in the male organ. The tissue specificity of the promoters were evaluated by in situ hybridization in the homologous system and by histochemical assay of the product of the reporter gene in heterologous systems. Antibodies were made against their encoding GRP in order to proceed the cellular localization of the genes products.

514 SEX DETERMINATION IN THE DIOECIOUS MELANDRIUM . CLONING OF MALE-SPECIFIC SEQUENCES AND PRELIMINARY RESULTS ON SORTING THE SEX CHROMOSOMES

I. Negrutiu[(1,2)], C. Lacroix[(1)], A. Truong[(1)], J. Veuskens[(2)], S Brown[(3)], A. Mouras[(4)], P Installé[(1)], M. Heinderickx[(1)]
[(1)] Phytotec SA, Belgium; [(2)] Free University of Brussels, Belgium; [(3)] CNRS LP40, Gif-sur-Yvette, France; [(4)] Université de Bordeaux II, France

Systematic physical mapping and cloning of genes involved in sex determination and/or differentiation will be more efficient if done on the basis of chromosome-specific DNA. As a preparatory work for sorting we have isolated male specific probes by subtractive cloning performed in total genomic libraries. 30 male specific clones were identified so far (insert size from 6 to 0.1 kb). Melandrium appears to be remarkably appropriate for flow cytometry. The modelisation indicated that the sex chromosomes are almost ideally placed and that the sorting of X and Y may only require one fluorochrome. Y-chromosomes with up to 25 % deletions or translocations on the X chromosome could still be sorted.
Y-deletion mutants with specific modifications in sex expression have been produced. Wild type and mutant lines are being used for sorting and library construction.

515 CHLAMYDOMONAS TELOMERE STRUCTURE: DNA FOLDING AND PROTEIN INTERACTIONS. Marie E. Petracek and Judith Berman. University of Minnesota, Department of Plant Biology, St. Paul, MN 55108.

Telomeres, the physical ends of linear chromosomes, contain short blocks of repeated DNA sequences with a bias for guanines (Gs) on the 3' end strand. The 3' strand terminates in a single-stranded overhang containing two telomeric repeats. Recent models, based on the properties of G-rich telomere repeats of Tetrahymena $(TTGGGG)_4$ and Oxytricha $(TTTTGGGG)_4$, propose that the G-strand telomeric overhang can form a G-quartet structure, as a result of intramolecular hydrogen-bonding interactions between 4 G residues. Because G residues are crucial for the formation of this structure, we have asked whether the G-poor sequence of Chlamydomonas $(TTTTAGGG)_4$ and Arabidopsis $(TTTAGGG)_4$ telomeres form similar folded structures. On nondenaturing polyacrylamide gels at 4°C, $(TTTTAGGG)_4$ and $(TTTAGGG)_4$ have altered mobilities although the changes in mobility are less dramatic than changes exhibited by either $(TTGGGG)_4$ or $(TTTTGGGG)_4$ oligonucleotides. Data from methylation interference and UV crosslinking of $(TTTTAGGG)_4$ suggest that although intramolecular G-quartets are formed by G-poor telomeres, they are less stable than the G-quartets formed by more G-rich oligonucleotides. We also demonstrate that double-stranded *Chlamydomonas* telomeric oligonucleotides with a two repeat single-stranded overhang (CDS) can dimerize to form intermolecular G-quartets. Unlike the analogous Tetrahymena molecules (TDS), CDS dimer formation is concentration dependent. Both TDS and CDS require high concentrations of sodium to form dimers. We have also identified a telomeric single-stranded binding protein in *Chlamydomonas*. This protein binds to the G-strand overhang and does not require the double-stranded telomere sequence for binding *in vitro*. We are presently characterizing and cloning this protein.

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516 A SINGLE MAS PROMOTER ELEMENT CONTROLS A COMPLEX PATTERN OF GENE EXPRESSION. Derek W.R. White, Paul M. Ealing, Roy Meeking and Jacqui Hannagan. Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand.

Complex spatial and temporal patterns of plant gene expression are often controlled by the combined action of a number of promoter *cis* regulatory DNA elements. The mannopine synthase (*MAS*) promoter, of the divergent 1′ and 2′ genes of *Agrobacterium* T-DNA, controls an intricate tissue and organ-specific pattern of expression during plant growth and development. In order to define regulatory element(s) within the *MAS* promoter we have constructed a series of deletions and rearrangements by cloning defined PCR fragments upstream of a promoterless β-glucuronidase (GUS) reporter gene. These constructs were tested for GUS expression in both an *Agrobacterium*-mediated transient assay and in transgenic tobacco plants. We have defined a central 36 bp responsive element, active in either orientation and in varying spatial arrangements, which is essential for transcriptional activation. This responsive element, together with either a 1′ or 2′ gene TATA box segment, gives a low level of activation as a monomer and a higher level of activation as a dimer. Flanking this central element there are single enhancer sequences. Also, a complex spatial and temporal pattern of expression from both full length and truncated promoter-GUS fusions was observed during floral and seedling development. These results suggest that the complex pattern of 1′ and 2′ gene expression in transgenic tobacco plants is controlled by a single, discrete, *MAS* promoter responsive element.

519 EXPRESSION OF A PROTEINASE INHIBITOR IN THE FEMALE REPRODUCTIVE TISSUES OF *NICOTIANA ALATA*. A. Atkinson, A.E. Clarke and M.A. Anderson Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia

The mature pistil of *Nicotiana alata* consists of a wet stigma and a solid style with a transmitting tract through which pollen tubes grow during pollination. Both the stigma and the transmitting tract are composed of secretory cells which release a mucilage into the extracellular spaces formed as the pistil matures. The secretions of the stigma and style are rich in carbohydrates, lipids and proteins, however, few molecular components of these fluids have been fully characterized. We are interested in the components of the mucilage which may have a role in the fertilization process. Our approach is to isolate cDNA clones corresponding to genes which have an elevated level of expression in the pistil. On this basis, we have isolated a clone from a style cDNA library encoding a protein with extensive homology to the proteinase inhibitor II of potato and tomato. Two RNA species homologous to this clone are expressed at high levels in the mature style of *N. alata*, and are also present at low levels in ovaries, petals and sepals. Non-wounded leaves do not express these messages, however, 24 hours after mechanical wounding both messages are detected in this tissue. *In situ* hybridization experiments show that the cells of the stigma express high levels of RNA homologous to the cDNA clone. No expression was detected in the transmitting tract or the cortical tissue of the style.

The type II proteinase inhibitors from potato and tomato leaves are potent inhibitors of the serine proteases, trypsin and chymotrypsin. In *N. alata* we have shown that extracts of stigmas also contain potent inhibitors of these proteases, and have a higher activity than other tissue extracts tested. We have isolated and characterized the protein encoded by the isolated *N. alata* cDNA clone.

The inhibitors of serine proteases isolated from potato and tomato are believed to act as protective agents to discourage insect pests, herbivores and microorganisms. The putative *N.alata* proteinase inhibitor may also have a role in defense, protecting the reproductive tissues to ensure their integrity for successful fertilization.

517 IDENTIFICATION OF A CHALCONE SYNTHASE ACTIVATOR REGION (CAR). Faktor, O., Halkier, B. A., Lamb, C.J. & Dixon, R. A.* Plant Biology Laboratory, Salk Institute, 10010 North Torrey Pines Road 10010, La Jolla, CA 92137.; *Plant Biology Division, The Samuel Roberts Noble Foundation, P.O Box 2180, Ardmore, OK 73402.

Promoter analysis of the chalcone synthase CHS15 gene of French bean (*Phaseolus vulgaris*) fused to the β-glucuronidase (GUS) reporter gene has identified a short region of 39 bp (-80 to -40) necessary for maintaining the pattern of expression of the full promoter in transgenic tobacco plants (*Nicotiana tabaccum*.) A construct with a tetramer of this activator region (CAR) has been shown to confer high and specific levels of expression when linked to the minimal CHS promoter. The correct CHS pattern of expression can also be conferred by the CAR when linked to the minimal promoter (-46) of CAMV 35S. This pattern includes high level of expression or activity in the pigmented part of the tobacco flower and in the root , medium levels of expression in the non-pigmented part of the flower, and low levels of expression in sepales, leaves and stem. The CAR multimer enhances the activity in the non-pigmented part of the flower, sepales, stem and leaves to a relatively higher level. The importance of this region for the response of CHS to environmental stimuli is currently being evaluated. CAR contains DNA-binding protein sequence. Thus, a CACGTG sequence is present which is a potential site for a G box factor. Several copies of the sequence CCTACC which is a highly conserved region found in many different CHS's, is present. Base shift mutations in each of these potential sites have demonstrated these elements to be essential for CHS promoter activity. In vitro binding studies using electrophoretic mobility shift assays show a complex interaction between the activator region and cognate DNA binding proteins. We are currently isolating cDNA clones encoding the corresponding DNA-binding proteins that bind to CAR.

520 A HOMOLOG OF THE SUBSTRATE ADHESION MOLECULE VITRONECTIN OCCURS IN FLOWERING PLANTS. L. Sanders, C.S. Wang, L. Walling and E. Lord. University of California, Dept. of Botany and Plant Sciences, Riverside California 92521.

The extracellular matrix (ECM) has been implicated in the primary developmental processes of many organisms. A family of secretory adhesive glycoproteins, called substrate adhesion molecules (SAMs), are believed to confer these dynamic capabilities to the ECM in animals. Here we report the existence of SAM-like genes and gene products in flowering plants. Hybridizations with a human vitronectin cDNA probe and genomic DNA from broad bean, soybean and tomato revealed vitronectin-like sequences. Human vitronectin antibodies cross-react with a 55 kD protein in leaf and root protein extracts from lily, broadbean, soybean and tomato. In addition, immunocytochemical staining of frozen sections of lily leaves and broadbean gynoecium demonstrated that vitronectin-like proteins were localized to the ECM on the cell surface, with the most intense labeling residing in the transmitting tract of V.faba gynoecium. A model is provided for how this plant SAM could function to facilitate pollen tube extension in the stylar matrix.

518 SNAPDRAGON CHALCONE SYNTHASE PROMOTER ACQUIRES ALTERED ORGAN SPECIFICITY IN TOBACCO. David B. Wing, [1]Klaus Fritze and Akira Kato. National Institute of Agrobiological Resources, Tsukuba, Ibaraki, 305 Japan. [1]Max-Planck-Institut, D-5000 Köln 30, Germany.

The organ-specific expression of the chimeric gene fusing 1.2 kbp of the snapdragon chalcone synthase (chs) promoter to the β-glucuronidase reporter gene was analyzed in transgenic tobacco. The results were compared to endogenous chs expression of both snapdragon and tobacco. During the development of the flower petal, chs is similarly expressed in both plants and the chimeric gene parallels that pattern. Tobacco seeds 1 - 2 days after pollination (dap) expressed levels of chs comparable to that observed in the petals, whereas snapdragon seeds did not up to 10 dap. The snapdragon promoter however was active in tobacco seeds 3 - 5 dap, and appeared to be co-regulated with the endogenous chs genes. Roots of 4 week old tobacco and snapdragon seedlings express little or no chs, but the chimeric gene was active there. This illustration of altered organ-specificity of the promoter in the heterologous host seems due to a combined absence of homologues of possible snapdragon repressors and the presence of tobacco activators recognizing foreign promoter elements.

521 ANALYSIS OF S-LOCUS STRUCTURE AND FUNCTION IN *PETUNIA HYBRIDA*. John J. Okuley, K. Reed Clark, and Thomas L. Sims. Ohio State University, Department of Molecular Genetics, Columbus, OH 43210.

Gametophytic self-incompatibility is characterized by the interaction of S-alleles expressed in both mature styles and in germinated pollen tubes. The style-expressed gene has been cloned from several species, and encodes a ribonuclease (S-RNase). To date, however, the pollen component remains unidentified. In an attempt to determine if other genes are closely linked to the S-RNase, we have undertaken chromosome walking experiments to identify transcripts in regions flanking the S1 allele. We have identified four sequences that are closely linked to the S-RNase gene, and that are expressed in other organs of *Petunia*. One of these sequence regions encodes a 2.2 kb transcript that appears to accumulate to low levels in germinated pollen. We are using RNAse protection, DNA sequencing, and RNA blot hybridization to further characterize this linked sequence. We are also seeking to identify 5' flanking regions of the S1-allele that are required for the correct developmental expression of this gene. A series of 5' promoter deletion constructs have been fused to the β-glucuronidase (GUS) reporter gene and used in particle bombardment studies of transient expression. Full length promoter constructs are expressed in style, ovary, and petal tissue, but not in leaves or pollen. Expression of deletion constructs having only 69 base-pairs 5' to the mRNA start site can be visualized by histochemical staining. We are presently using fluorescence assays with GUS and luciferase constructs in an attempt to identify quantitative regulatory elements governing the expression of the S-RNase.

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522 ACTIVITY OF AN S-LOCUS GENE PROMOTER IN PISTILS AND ANTHERS OF TRANSGENIC BRASSICA. T.Sato, M.K.Thorsness, M. K. Kandasamy, T. Nishio, M. Hirai, J.B.Nasrallah and M.E. Nasrallah. Cornell University, Section of Plant Biology, Ithaca, New York 14853

The pollen-stigma interaction of self-incompatibility in crucifers is mediated by glycoproteins localized in the cell wall of stigma papillae and encoded by the S-locus glycoprotein gene (*SLG*). When fused to the ß-glucuronidase reporter gene, the 5' upstream regulatory region of *SLG* directed high level expression in the papillae of transgenic Brassica plants. In addition to the stigma papillae which were the primary site of gene expression, ß-glucuronidase activity was also evident in the transmitting tissue of stigma, style and ovary. Moreover, in anthers of the self-incompatible kale line of B. oleracea, the promoter was active in the nurse cells of the tapetum and in microspores commencing at the binucleate stage. This pattern of expression is indicative of sporophytic as well as gametophytic expression for *SLG* . Since sporophytic control is thought to have evolved from gametophytic *S* -gene systems, we infer that sporophytic control was acquired without loss of gametophytic function.

523 COMPARATIVE STUDY OF S-ALLELES AND S-GLYCOPROTEINS BETWEEN TWO WILD BRASSICA CAMPESTRIS POPULATIONS. OGUNI-JAPAN AND BALCESME-TURKEY

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S-alleles and S-glycoproteins were compared between Oguni-Japan and Balcesme-Turkey populations, which are considered to be independent each other. From diallel pollination test and S-glycoprotein analysis by immunoblotting with anti-S8-glycoprotein-antiserum on the selfed progenies of wild plants, 35 S-homozygotes were isolated from each population, respectively. From diallel pollination between these S-homozygotes, 16 different S-alleles were identified in Oguni population and 18 S-alleles in Balcesme one. It was estimated that each population is composed of about 30 independent S-alleles. Four S-alleles were commonly found in these two populations. Therefore, the variation of S-alleles in total B. campestris population was considered to be abundant. Different S-alleles had different S-glycoprotein profiles, but the identical S-alleles had similar profiles according to IEF-PAGE-immunoblotting, even though two S-alleles were taken from different populations. Southernblot analysis were also conducted for the comparison of DNA sequences.

524 EXPRESSION ANALYSIS OF SELF-INCOMPATIBILITY GENES OF SOLANUM TUBEROSUM IN TRANSGENIC PLANTS.

Hans-Hubert Kirch, Helgard Kaufmann and Richard Thompson. Max-Planck-Institut fur Zuchtungsforschung, 5000 Köln 30, FRG.

Solanum tuberosum displays monofactorial, gametophytic self-incompatibility (SI). Analysis of major pistil proteins of potato showed that these included S-allele associated glycoproteins (SLGs) related to those characterized in other solanaceous species. Two genomic clones representing the SLG-S1 and S2 alleles have been sequenced revealing a high level of interallelic heterogeneity within the coding part and in particular in the 5' flanking promoter region of both genes. A crucial experiment to uncover the SI-mechanism is to show, that the characterized SLGs are really responsible for the S-phenotype. To address this question, genomic S1-DNA fragments with up to 3 kb 5' and 3' flanking sequences were re-introduced into tobacco and appropriate potato plants via A. tumefaciens-mediated transformation, and analysed for expression of the transferred S1-allele and an altered SI-phenotype. Additionally, deletions of the 5' flanking region of the S1 and S2 allele fused to reporter genes have been constructed for characterization in transient assays using the particle gun bombardment, to identify Cis-acting elements which are important for temporal and tissue specific expression of the S-genes.

525 MOLECULAR STUDIES ON MALE GAMETOPHYTE DEVELOPMENT IN Zea mays.

Patricia Bedinger, Anne Broadwater, Christopher Chay, and Ann Stapleton. Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

Several aspects of maize pollen development are being studied. We have devised a method for the preparative isolation of staged microspores. Protein analysis indicates that one set of proteins is expressed throughout early stages of development, up until the time of microspore mitosis. A developmental switch then occurs, such that about one half of the silver-stain detectable proteins differ between early and late stages. We have been successful in culturing maize spikelets using a liquid medium, wherein normal development proceeds from premeiosis to mature pollen. We are using this system to study the developmental switch by inhibiting microspore mitosis. We have constructed cDNA libraries using RNA from developmentally staged microspores and young pollen, and have isolated sequences expressed at specific times in microsporogenesis. One type of gene in which we are especially interested is that encoding protein components of the pollen wall, an extremely specialized extracellular matrix. We have isolated very pure pollen walls and find only seven proteins tightly associated with these structures. Antibodies to these proteins will be used to screen our cDNA libraries. Finally, we are using transposon tagging to isolate male-sterility genes. We have three mutations derived from Robertson's Mutator maize lines that segregate for a male-sterility trait. We are currently analyzing these mutants at the cytological and molecular level.

526 S- AND NS-GLYCOPROTEINS OF BRASSICA CAMPESTRIS.

A.Isogai*, S.Yamakawa*, M.Watanabe*, K.Hinata* and A. Suzuki*. *University of Tokyo, Department of Agricultural Chemistry, Tokyo 113, Japan. *Tohoku University, Faculty of Agriculture, Sendai 981,Japan.

The self-incompatibility of Brassica species is under the control of multi allelic genes at the S-locus. In B. campestris three S-glycoproteins, S_8-, S_9-, and S_{12}-glycoproteins, were isolated and their amino acid sequences were partly established. Those are strictly segregated with S-genes. On the other hand, NS-glycoproteins, which were closely related with S-glycoproteins in terms of the structure and distribution pattern in plants, were detected commonly in S_8- and S_9-homozygotes of B. campestris. By screening of the cDNA library obtained from the stigmas of S_8-homozygote with synthetic DNA probes, two clones encoding S_8-glycoprotein and NS-glycoprotein, respectively, were isolated. The cDNA sequence of the clone of the NS-glycoprotein revealed that the NS-glycoprotein is highly homologous with S-locus-related-glycoproteins of B. oleracea. Other NS-glycoproteins with different pI values were detected in several homozygotes of B. campestris by the antiserum against the NS-glycoprotein. Northern blot analysis of S_9-homozygote using the cDNA of the NS-glycoprotein also revealed the presence of a similar mRNA. These NS-glycoproteins in B. campestris were not segregated with S-genes in crossing experiments.

527 EXISTENCE OF S-GLYCOPROTEIN-LIKE-PROTEINS IN ANTHERS OF SELF-INCOMPATIBLE BRASSICA SPECIES

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An experimental evidence is presented that there is an anther protein which is reactive with a polyclonal antiserum against the stigma S-glycoprotein of S^8-homozygote in Brassica campestris. The antiserum did not react with extracts of any other organs. Furthermore, since this antiserum was not reactive with the similar polysaccharide residues to S-glycoprotein, it was considered capable to identify the S-glycoprotein-like proteins in anther (SA-protein: S-glycoprotein like anther protein). The SA-protein exhibited a single distinct band at about pI 5.0 on IEF-gel blot and three spots at 29kDa and 83kDa on two dimensional-gel blot which were different from those of stigma S-glycoprotein. Allelic difference of pI point was not found in the SA-protein. The SA-protein appeared in anther at uninucleate microspore stage which is earlier than the appearance of the stigma S-glycoprotein. It exists in anther wall rather than in pollen of mature anther. The SA-protein is considered to play an important role in sporophytic control of self-incompatibility.

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528 VARIATION OF S-GLYCOPROTEINS IN TWO WILD POPULATIONS OF SELF-INCOMPATIBLE BRASSICA CAMPESTRIS L.
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Variation of S-glycoprteins and stigma proteins were studied on 31 S-alleles isolated from two wild self-incompatible B. campestris populations in Japan and Turkey. Of 31 S-homozygotes, 15 were isolated from Oguni-Japan population and 16 were from Balcecme-Turkey one. S-glycoproteins were detected by immuno-blotting with polyclonal antibody against S^8-glycoprotein that was a strain of Oguni population. It was pointed out that a stigma involves a number of proteins which are cross-reactive with the antiserum, but different of pI points, and many of these proteins were heritable in correlation with each other. The content of these protein was variable and we classified major and minor S-glycoproteins according to their stainability. The major bands were generally detectable by Coomassie Blue Staining,too, but the minors were difficult to be identified by CBS. Presence of heterozygote specific proteins was pointed out, suggesting occurrence of post-translational modification of these proteins. The pI values of major S-glycoproteins range from 5.0 to 9.0 and those at 7.0 - 9.0 were frequent.

529 ISOLATION AND CHARACTERIZATION OF SELF-INCOMPATIBILITY ALLELES INTROGRESSED INTO BRASSICA NAPUS. D.R.Goring[1], C.L.Baszczynski[2], L.Fallif[2], and S.J.Rothstein[1]. [1]University of Guelph, Department of Molecular Biology and Genetics, Guelph, Canada N1G 2W1. [2]Allelix Crop Technologies, 6850 Goreway Dr., Mississauga, Canada L4V 1P1.

Several self-incompatible (SI) B. napus lines have been established by Dr. Beversdorf at the University of Guelph through the introgression of S-alleles from diploid Brassica species. We have choosen two of these lines (W1 and T2) to isolate the active S-alleles and study the mechanism of SI in B. napus. cDNA libraries were constructed from stigma RNA isolated one day prior to anthesis. These libraries were screened with a heterologous S-allele probe, 29-2, isolated from B. oleracea by Trick and Flavell (Mol. Gen. Genet. 218:112 1989). From the T2 library, four different cDNAs (A10, A14, A26, A34) were isolated and sequenced. Based on sequence homology, these alleles were found to fall into two distinct classes. The first class (A10, A14) had a high degree of similarity to 29-2 and the S-alleles reported by Nasrallah *et al.* (Nature 326:617 1987). The second class (A26, A34) was very similar to the pollen-recessive S-alleles isolated by Chen and Nasrallah (Mol. Gen. Genet. 222:241 1990). While stigma-specific RNA transcripts could be detected for three of these alleles (A10, A14, A26), preliminary evidence suggested that the A14 allele is responsible for the SI phenotype in the T2 plants. From the W1 library, two cDNAs were characterized and found to be the same as the A10 and A26 cDNAs. Neither of these alleles segregated with the SI phenotype in the W1 plants. However, under reduced stringency conditions with the A14 probe, three bands which segregated with the W1 SI phenotype could be detected on genomic blots. We are currently using the polymerase chain reaction with general S-allele primers to isolate candidate S-genes for the W1 line.

530 CHARACTERISATION OF TAPETAL-SPECIFIC cDNAs. Susan J Crossley, Andrew J. Greenland* and Hugh G. Dickinson. Dept. of Botany, Univ. of Reading, U.K. *Plant Biotech. Group, ICI Seeds, Jealotts Hill, Berks., U.K.

Microsporogenesis is a complex developmental process which, despite current intensive studies at the molecular level [1], remains poorly understood. Conventional molecular methodology is not readily applicable since the anther comprises a number of cell populations following very different developmental pathways. A number of stage-specific *Lilium* meiocyte cDNA libraries have thus been generated using subtractive hybridisation and we report here the temporal and spatial expression of three cDNAs from one of these libraries. Interestingly, *in situ* hybridisation shows the cDNAs to be specific to the tapetum; a specialised layer of cells investing the meiocytes, which is essential for pollen development. Northern analysis reveals that the mRNAs corresponding to the cDNAs are expressed from prophase I, throughout meiosis, and become degraded when the microspores are released from the callosic tetrad wall. Sequence data indicate that the three cDNAs are related, and have some homology to a family of seed proteins. Southern blot hybridisation demonstrates the cDNAs to possess homologies with genomic sequences from a wide variety of plant species. By raising antibodies to the *in vitro* expressed protein of one of these cDNAs we will be able to analyse further the biochemical characteristics of this tapetal product.

1. Mascarenhas, J.P. (1990) Annu. Rev Plant Physiol. Plant Mol. Biol. 41:317-38

531 MUTANTS OF ARABIDOPSIS THAT ARE DEFICIENT IN FERTILIZATION. Dapnne Preuss and Ronald W. Davis. Stanford University, Department of Biochemistry, Stanford, CA 94305.

Because *Arabidopsis thaliana* reproduces by self-pollination, plants that are sterile, and thus fail to produce seeds, can be isolated readily. Several mutants with alterations that affect fertility were identified, and partially sterile mutants that are deficient in either male or female functions, as well as fully sterile mutants that produce neither gamete were characterized. Some of these mutations affect flower structures, while others result in plants with apparently normal morphologies. Analysis of pollen tube growth, both on agar and within wild-type and mutant pistils, will be presented. In addition, genetic tests that are useful in characterizing the mutant defects, such as dominance, complementation, and epistasis, are currently in progress.

532 EVIDENCE FOR RECOMBINATION SUPPRESSION IN THE S-LOCUS. Craig E. Coleman, Andrew G. Clark and Teh-hui Kao. Penn State University, Department of Molecular and Cell Biology, University Park, PA 16802

Theoretically, a self-incompatibility system can be established and maintained with a minimum of three alleles at the S-locus. However, estimates of the number of S-alleles in self-incompatible species range from 50 to as many as several hundred. It is not known at the molecular level how new alleles are generated, because standard mutagenesis fails to produce functionally distinct alleles. As a first step toward addressing this question, we have analyzed nucleotide and amino acid sequences of a number of S-alleles from 4 solanaceous species. The phylogenetic relationship of these alleles and the extraordinarily high level of intraspecific sequence diversity suggest that S-allele polymorphism predates speciation in Solanaceae. Sensitive statistical tests of clustered polymorphic sites indicate a remarkable lack of intragenic recombination in the S-locus. We further characterized the flanking regions of two alleles of the S-gene in *Petunia inflata*. These regions contain several classes of repetitive sequences; the more removed from the coding region, the more highly repetitive the sequences are. Remarkably, no significant sequence similarity was found in the flanking regions of these two Petunia alleles other than the putative TATA box sequence. This extreme sequence divergence further suggests a recombination suppression mechanism operating at the S-locus which prevents homogenization of S-alleles. An interesting analogy can be drawn between our finding at the S-locus and the lack of recombination between X and Y chromosomes. The role of the S-locus in self-incompatibility and its apparent lack of recombination are consistent with the highly polymorphic nature of the S-locus and the extreme sequence diversity between alleles.

533 MULTIGENIC CONTROL OF SELF-INCOMPATIBILITY IN *PETUNIA*. Yunjun Ai and Teh-hui Kao. The Pennsylvania State University, Department of Molecular and Cell Biology, University Park, Pennsylvania 16802

Self-incompatibility in Solanaceae has generally been thought to be controlled by a single locus, the S-locus. However, classic genetic studies did reveal the possible existence of modifier loci. We chose to address this question by examining the cause of breakdown of self-incompatibility in a commercial cultivar of *Petunia hybrida* to determine whether mutation(s) occurred in the S-locus or elsewhere. We took advantage of reciprocal compatibility between this cultivar and self-incompatible *Petunia inflata* (S2S2 genotype) to raise sixty F1 hybrids. Analysis of their pistil protein pattern revealed that two *P hybrida* proteins segregated in the F1's in an approximately 1:1 ratio. The amino-terminal sequences of these two proteins share very high sequence similarity with all the S-proteins of self-incompatible solanaceous species. They also have ribonuclease activity comparable to that of *P. inflata* S-proteins. Thus, the S-alleles are not only retained, but also expressed, at least in the pistil of self-compatible *P. hybrida*. These two alleles are designated So (encoding a 31 kDa protein) and Sx (encoding a 24 kDa protein). Genetic analysis revealed that all 23 F1's with S2So genotype are self-compatible, while half of the F1's with S2Sx genotype (19 plants) are self-incompatible, and the other half (18 plants) are self-compatible. Our results suggest that functional S-alleles are necessary, but not sufficient, to elicit a self-incompatibility response. We propose that the defective So allele and a defective modifier allele contribute to the breakdown of self-incompatibility in *P. hybrida*. Our model implies that the full manifestation of self-incompatibility in *Petunia*, and perhaps in other solanaceous species, requires the presence of both functional S-alleles and modifier alleles.

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534 EXPRESSION OF *PETUNIA INFLATA* S-PROTEINS IN THE BACULOVIRUS EXPRESSION SYSTEM J. Mu, A. Singh and T. -h. Kao, Dept. of Molecular and Cell Biology, Penn State University, University Park, PA 16802

S-allele-associated proteins (S-proteins), which have been identified in a number of solanaceous species displaying gametophytic self-incompatibility, are thought to be responsible for recognition and rejection of self-pollen tubes during sexual reproduction. All the solanaceous S-proteins characterized so far share sequence similarity with two fungal ribonucleases, and some of them have been shown to indeed have ribonuclease activity. In order to determine whether the ribonuclease activity of the S-protein is essential to its biological function, we have set out to express both wild-type and mutant S-proteins in a baculovirus expression system, and then to test their ability to reject pollen tube growth by an *in vitro* bioassay. cDNAs encoding S2- and S3-proteins of *Petunia inflata* were inserted into the pAC373 vector, and then introduced into baculovirus genome by cotransfecting the insect host cells with the recombinant plasmid and baculovirus genomic DNA. Recombinant viruses carrying S2- or S3-cDNA were purified by several runs of serological screening using the S2- and S3-cDNA probes. Two forms each of the S2- and S3-proteins were detected, a 24 kDa form identical in size to the S2- and S3-protein previously determined, and a 23 kDa form. Both forms of the S2- and S3-proteins expressed in insect cells exhibited ribonuclease activity, and cross-reacted with polyclonal antibodies raised against S2- and S3-proteins. The S-proteins were expressed at a low level, and were not secreted into the medium. We are in the process of purifying S2- and S3-proteins from large scale suspension cultures. Experiments are underway to mutagenize the two conserved histidine residues which were thought to be essential for ribonucleolytic activity. The mutant constructs will be similarly expressed in the baculovirus expression system, and the ribonuclease activity of the expressed protein will be tested. The functional significance of S-protein ribonuclease activity will be examined by comparing the ability of the wild-type and mutant S-proteins to inhibit growth of *in vitro* germinated pollen carrying the same or a different allele.

535 ß TUBULIN EXPRESSION IN THE MAIZE MALE GAMETOPHYTE H.J. Rogers[1], A. Greenland[2] and P.J. Hussey[1]. [1]Royal Holloway and Bedford New College, University of London, Egham, Surrey; [2]ICI Seeds, Jealott's Hill, Bracknell, Berks.

Microtubules are involved in several key processes in plant cell morphogenesis. Their major component is a heterodimer of α and ß tubulin. Maize ß tubulin is encoded by a large gene family with at least nine members. We are interested in the expression of this gene family in pollen and germinating pollen. A cDNA library constructed to mRNA from maize germinating pollen has been screened with the full length maize ß2 tubulin cDNA clone. Ten cDNA clones have been isolated and shown, by sequence analysis, to be homologous to known ß tubulins. These 10 clones fall into three groups based on their 3' coding and noncoding region sequences. Members of each group are identical except for the site of polyadenylation. Group 1 has six cDNA clones, a representative of which we have called ß3. Group 2 has three cDNA clones, a ß tubulin cDNA clone from this group is referred to as ß4. Group 3 has only one component, the ß5 cDNA clone. We have prepared gene specific probes using the 3' noncoding regions of each of the three ß tubulins. We will present data comparing the expression patterns of each gene.

536 FUNCTIONAL ANALYSIS OF POLLEN SPECIFIC-PROTEINS Guy Vancanneyt, Judy Yamaguchi, Steven Feist and Sheila McCormick, Plant Gene Expression Center, 800 Buchanan Street, Albany CA 94710 USA

Several tomato genes (LAT52, LAT56, LAT59) have been isolated which are predominantly expressed in the male gametophyte. Both LAT56 and LAT59 show significant sequence similarity to the major Short Ragweed pollen allergen (antigen E) and to a cDNA (9612) derived protein expressed around the transmitting tract in the pistil of tomato flowers. In addition this protein family reveals some sequence similarity to pectin metabolizing enzymes in both eucaryotic (*Aspergillus*) and procaryotic (*Erwinia*) organisms. The LAT52 cDNA is very similar with respect to amino acid sequence and expression pattern to the maize clone Zmc13. Both clones encode a cysteine-rich protein of about 20Kd. Several inhibitors of trypsin and α-amylases have partial similarity to these proteins. In order to characterize these LAT proteins, antisera were raised. Immunoblot analysis revealed that both LAT 52 and LAT56 derived proteins are being actively translated prior to pollen germination. These proteins have been further characterized with respect to their stability, state of glycosylation and expression in sporophytic tissues. Both the LAT52 and LAT56 derived proteins are relatively insensitive to heating and are probably exposed outside the pollen grain. Further immunolocalisation of these proteins at the cellular and intracellular level might give us a clue to a possible role of these proteins during gametogenesis and pollen germination.

537 **EVOLUTIONARY ANALYSIS OF POLLEN-SPECIFIC GENES.** Keith Hamby, Judy Yamaguchi and Sheila McCormick. Plant Gene Expression Center, USDA-ARS, Albany, CA 94710.

Our lab studies the regulation of pollen-specific gene expression in tomato. Previous analyses of three different pollen-specific genes (LAT52, LAT56, and LAT59) revealed minimal promoter requirements and various *cis* elements within these promoters which affect levels of expression, but were unable to identify a *cis* element common to all three promoters. These promoters can be faithfully expressed in heterologous tobacco and Arabidopsis systems. The protein encoded by LAT52 shows amino acid sequence similarity to certain trypsin inhibitors. LAT56 and LAT59 proteins share similarity with some pectin degrading enzymes, with pollen allergens from ragweed and also with a recently identified pistil-specific protein from tomato. Comparisons with similarly-regulated genes from other plants may help both to identify the *cis* sequences critical for pollen-specific expression and to assign a function to the gene products. Toward this end, we are sequencing the genes homologous to LAT52, LAT56 and LAT59 from various plant species. We also are sequencing the homologs of a fourth pollen-specific gene, LAT51. The aligned nucleotide sequences and the relative conservation of intron placement also can be used to postulate the evolutionary relationships among the plant species. Analyses of amino acid conservation in the proteins encoded by the homologous genes in other plant species will help in determining functionally important domains.

538 CONSTRUCTION OF A TOMATO YEAST ARTIFICIAL CHROMOSOME (YAC) LIBRARY TO CLONE MALE STERILE GENES. Salma Talhouk, Sally Thorson, and Sheila McCormick, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710

Male flower development includes anther initiation, meiosis, microsporogenesis, and pollen grain maturation. Male sterile genes are known to play critical roles in pollen development. Since male sterility is mostly expressed in homozygous recessive mutants, it is assumed that male sterile genes act in sporophytic tissues of the anther, but affect the development of microspores. In tomato, over 40 male sterile loci have been described genetically and physiologically, but little is known about the products of these genes. Tomato, (*Lycopersicon esculentum*), offers an excellent system for isolating male sterile genes, because it has well defined classical genetic linkage and RFLP maps. Fifteen male sterile loci have been mapped to 7 of the 12 chromosomes in tomato, with chromosomes 2 and 11 having 4 male sterile loci each. We are constructing a YAC library with the aim of walking towards *ms* genes on chromosome 11. We have been using a novel vector that allows for the selective amplification of YACs in primary yeast transformants. With this new system, identification of YAC clones containing specific sequences by colony hybridization is greatly improved because of enhanced signal strength. RFLP probes from chromosome 11 will be used to screen the YAC library. In addition, we are specifically amplifying tomato DNA fragments adjacent to the YAC vector arm using the "vectorette method". This new technique allows us to generate terminus specific probes from YAC clones without requiring any cloning. The end probes will be used for chromosome walking, and locations on chromosome 11 will be confirmed by hybridization to southerns prepared from alien addition lines. Ultimately, the confirmation that a YAC clone carries the *Ms* locus will be obtained by transformation of *ms* tomato plants.

539 PATTERN OF SYNTHESIS AND ACCUMULATION OF A PROTEIN ENCODED BY A MAIZE POLLEN-SPECIFIC GENE. Joseph P. Mascarenhas and Andrea C. Scheewe. Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222.

Pollen grains of maize and other plants, contain stored mRNAs that appear on the basis of indirect evidence, to be utilized in protein synthesis in the germinating pollen grain and tube. The genes for these mRNAs are activated late in pollen development after the completion of microspore mitosis. We asked the following questions: Are these presynthesized mRNAs stored for use only during and following germination or are they translated before anthesis? If translated, when does translation occur? Are the mRNAs in the mature pollen grain just left over from a previous developmental program or are they translated during pollen germination and tube growth? Are the proteins synthesized from some of the pollen-specific mRNAs, conserved in other species? A pollen-specific maize gene Zm13, was selected as being representative of the late expressed class of genes. Polyclonal antibodies made to a synthetic peptide of Zm13, have been used in Western blots of extracts of pollen of different stages of development and from different plants. To determine whether translation of the Zm13 mRNA occurred during germination, proteins were labeled with ^{35}S-methionine during germination followed by immunoprecipitation with the antibody, gel electrophoresis and autoradiography. Zm13 mRNA is translated very late in pollen maturation just before anthesis. The mRNA is in addition used in new protein synthesis in germinating pollen. Proteins that cross-react with the Zm13 antibody are present in pollen of other species. The results will be discussed with respect to pollen function. Supported by NSF Grant DCB880165.

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540 EXPRESSION OF A POLLENSPECIFIC GENE AND ITS INVOLVEMENT IN POLLENWALL SYNTHESIS. JAM Schrauwen, MMA van Herpen, MT Oldenhof, KAP Weterings, PFM de Groot, WH Reijnen, GJ Wullems.Department of Molecular Plantphysiology, University of Nijmegen, Toernooiveld, NL-6525 ED Nijmegen, The Netherlands

A pollen specific cDNA clone (pNTP303) was isolated to study relations between gene expression and pollen development. NTPc303 was obtained through differential screening of a cDNA library prepared against mRNA from mature tobacco pollen. Characterization of this clone, by Nothern blot analyses and *in situ* hybridization, showed that NTPc303 is exclusively expressed in pollen from mono- and di-cots. The expression of this clone arises in tobacco pollen after the first haploid mitosis, accumulates thereafter and is expressed also during the first hours of germination. *De novo* transcription of NTP303 in germinating pollen was confirmed by *in vivo* labeling of the newly formed RNA in combination with liquid hybridization procedure to an antisense pNTPc303 transcript.

The nucleotide sequence analyses of NTPc303 showed a full length frame of 1960bp and the carboxy terminal of the protein has the characteristics of a leader peptide. The open reading frame encodes for a protein that shows homology to ascorbate oxidase and laccase. Both enzymes are thought to be involved in cell wall formation. So it is likely that NTP303 is highly conserved during evolution and has a main function in pollentube wall synthesis, a process with a long evolutionary history.

541 CYTOLOGY OF MALE STERILE MUTANTS OF ARABIDOPSIS THALIANA J Fuller, Z A Wilson, L G Briarty and B J Mulligan. University of Nottingham, Department of Botany, Nottingham NG7 2RD, U K

Allelism tests are currently being carried out on a collection of recessive nuclear male sterile (ms) mutants of *Arabidopsis thaliana* var Landsberg *erecta* generated by seed mutagenesis with EMS and X- and γ-irradiation. Such mutants fail to release viable pollen but show normal female fertility (seed set), while in other respects the ms plants are very similar to fertile Landsberg *erecta*. So far, six different *ms* loci have been identified. These are currently being mapped. Cytological analysis and pollen germination tests are being used to identify the stage at which pollen development fails. For example, in one line, tetrad formation occurs but microspores show abnormalities even before release. Another mutation is allelic to *ms1*; in this line pollen decays at a late stage after release of apparently normal microspores Two other other apparently leaky alleles of *ms1* have been obtained. One results in complete male sterility in plants up to about 6 weeks old, but these become fertile thereafter. Another permits production at a very low frequency of viable pollen, and thus occasional seed set occurs. As another aspect of this project, our progress with chromosome walking to isolate the *ms1* locus will also be presented.

542 MOLECULAR CHARACTERIZATION OF S-GENE SPECIFIC cDNAs IN *SOLANUM CHACOENSE* Bitt. Charles Després, Marc Saba El Leil, Sylvain R. Rivard, David Morse and Mario Cappadocia. IRBV, Université de Montréal, 4101 est rue Sherbrooke, Montréal, Canada, H1X 2B2.

Styles mRNA from two self-incompatible, intercompatible genotypes of *Solanum chacoense* (S1S2 and S3S4) were used to construct cDNA libraries. S-gene specific probes synthesized by PCR using oligonucleotides derived from conserved amino-acid sequences of *N alata* and *S. tuberosum* S-genes, were used to screen the cDNA libraries. Positive clones produced the same size fragments after PCR using the same oligonucleotides. Sequencing is underway and should permit the characterization of the alleles present in the parental genotypes and in selected plant material generated via anther culture, selfing, crosses, etc. For haploids and homozygous diploids, in particular, our strategy includes the use of PCR-amplified S-locus genomic DNA to rapidly detect the possible generation of new S alleles.

543 SELF-INCOMPATIBILITY STUDIES IN *SOLANUM CHACOENSE* BITT Marc Saba El Leil, Charles Després, Sylvain Rivard, David Morse, and Mario Cappadocia. Institut de Recherche en Biologie Végétale, Université de Montréal, 4101 est rue Sherbrooke Montréal, (Qc) Canada, H1X 2B2.

Solanum chacoense Bitt. (2n = 2x = 24) is rapidly becoming a choice material to study self-incompatibility since i) haploids can be produced (Cappadocia 1990), ii) RFLP markers are available (Rivard *et al.* 1989) and construction of the linkage map is underway, and iii) the sequence of two S-alleles has recently been reported (Xu *et al.* 1990). In order to create suitable material for genetic and molecular analyses, we have produced: i) pollen-derived haploids, homozygous diploids and 2n pollen-derived diploids in several self-incompatible clones, ii) pollen-derived progenies of selected F1 hybrids between these clones, iii) tester stocks of known S-alleles, and iv) inbreds via bud pollinations. In addition, we have also selected the v) inbred progeny of one of our clones that underwent a cycle of spontaneous selfing. Using S-specific cDNA derived probes (see Després *et al.*, this congress), the segregation analysis of the S-alleles in the plant material mentioned above can be performed even in genotypes where classical analyses cannot be made (e.g. haploids, nonflowering homozygous diploids, sterile inbreds, etc.). This approach should help in shedding more light on phenomena so far not well understood, such as the spontaneous generation of new S-alleles in inbred genotypes.

544 GENETIC EVIDENCE FOR THE OVERALL INVOLVEMENT OF RIBONUCLEASES IN GAMETOPHYTIC SELF-INCOMPATIBILITY. SEQUENCING OF S-ALLELES FROM APPLE. Wim Broothaerts, An De Bondt, Bruno P.A. Cammue, Jozef Vanderleyden and Willem F. Broekaert. F.A. Janssens Laboratory of Genetics, Katholieke Universiteit Leuven, Leuven, Belgium.

Gametophytic self-incompatibility is under the control of the S-gene encoding allele-specific glycoproteins (S-proteins) that are expressed in the style. The sequences of over 15 S-alleles from five species, all belonging to the Solanaceae, have been reported and appear to contain several highly conserved regions. Two of the conserved regions are also present in the sequences of the fungal ribonucleases T2 and Rh; these regions include histidine residues that are part of the active site in the fungal RNases. Moreover, the S-proteins have been shown to be RNases in Nicotiana alata and Petunia hybrida. We have used PCR to amplify genomic sequences encoding S-alleles from apple (*Malus x domestica*). This was accomplished by priming with synthetic oligonucleotides complementary to the two regions of homology around both active-site His-residues. The PCR products obtained were further used to identify and isolate genomic DNA sequences. The amino acid sequences of S-alleles from apple will be originally presented. Experiments are in progress to support the hypothesis that the proteins encoded by the isolated clones are involved in self-incompatibility.

545 GENE PRODUCTS ACTIVATED AT FERTILIZATION IN WHITE PINE MEGAGAMETOPHYTES. Joe C. Kamalay, Stokes Baker, Clayton Rugh and Bill Whitmore, Agronomy Dept. and Forestry Division,Ohio State Univ., Columbus, OH 43210

To study molecular events which may be significant in early embryo development, we constructed and screened a cDNA library from post-fertilization ovule mRNA. Messenger RNA levels of these sequences indicate that the initiation of their expression in ovule tissues of Pinus strobus L. (eastern white pine) appears to coincide with fertilization. (1) Two distantly related multigene families code for 18-20 kd peptides; sequence homology searches have revealed no relationship to any known sequences in the GENBANK or EMBL databases (2) The largest group of sequences encodes a family of legumin-like proteins, showing 30-40% identity to the known angiosperm legumins. We have constructed a phylogentic tree based on the conservation of the globulins in gymnosperms and angiosperms. Cloned cDNA sequences hybridized to 8-10 distinct genomic DNA restriction fragments under moderate stringency conditions and strongly hybridized with 3 bands. (3) A partial cDNA clone encodes a peptide homologous to the COOH terminus of rat and rabbit NADPH:cytochrome P-450 reductase Hybridization to genomic DNA revealed homology to one or two restriction fragments in the Pinus strobus L. genome, indicating that the gene is probably present as a single locus. The expression of this mRNA sequence appears to be modulated in ovule tissues following fertilization. To date, clones from (2) and (3) were used to examine tissues within the pine ovule by in situ hybridization and mRNA blots of dissected tissues Each of these sequences was detected in the haploid prothallium

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546 A STYLE SPECIFIC HYDROXYPROLINE-RICH GLYCOPROTEIN FROM *NICOTIANA ALATA*. Jan Lind, Adrienne Clarke and Marilyn Anderson. University of Melbourne, School of Botany, Melbourne, Australia, 3052.

During pollination in *Nicotiana alata*, pollen grains land on the stigma surface, hydrate and form pollen tubes which grow extracellularly through the transmitting tissue of the style to the ovary where fertilization occurs. We are interested in the structure and function of components of the transmitting tissue extracellular matrix, as they are in close contact with the growing pollen tubes and may play a role in pollination. We have isolated and characterized an abundant hydroxyproline-rich glycoprotein that is secreted by the transmitting tissue cells into the extracellular matrix of *N.alata* styles. The glycoprotein (Mr 120 KD) is approximately 65% protein and is positively charged (pI>10). The protein core of the molecule is rich in hydroxyproline, proline, lysine and serine and the carbohydrate side chains contain arabinose and galactose. In *N.alata* the glycoprotein is style specific and developmentally regulated. The glycoprotein is most abundant in mature styles. We have detected related glycoproteins in the styles of other plants.

547 HORMONAL REGULATION OF SEX EXPRESSION IN *CERATOPHERIS RICHARDII*. Jo Ann Banks[1], Josephine Ho[1], Leslie Hickok[2] and Todd Cooke[3]. [1]Department of Biology, McGill University, Montreal, Que. H3A 1B1, [2]Department of Botany, University of Tennessee, Knoxville, TN 37996, [3]Department of Botany, University of Maryland, College Park, MD 20742. We are using the homosporous fern *Ceratopteris richardii* as a model system for the study of plant gametophyte development and sexual differentiation in particular. *C. richardii* has several useful features for this purpose: 1) a short life cycle; 2) autotrophic and free-living gametophytes; 3) it can be easily selfed and outcrossed; 4) sexual differentiation of gametophytes is controlled by the pheromone antheridiogen, a gibberellin-like substance; and 5) selection of mutations affecting haploid gametophyte development is rapid and efficient. In an effort to understand the molecular mechanism that determines the sex of the gametophyte (i.e. male or bisexual), we have first characterized the response to, and secretion of, antheridiogen in *C. richardii* and have found that each developing gametophyte passes through 3 discrete phases. During the first phase (1-4 days after inoculation of spores) the germinating spore is responsive to antheridiogen but does not secrete it; the gametophyte will become male (produce only antheridia) only if exposed to antheridiogen during this phase. During the second phase (4-7 days after inoculation), the germinating spore is neither sensitive to nor secretes antheridiogen, hence, the mitotically active gametophyte becomes bisexual even if antheridiogen is added during this phase. During the third phase (7-14 days after inoculation) the gametophyte secretes antheridiogen yet is insensitive to it as it continues to grow in size. We are attempting to isolate genes that are involved in transducing the signal (antheridiogen) to promote a specific response (maleness) by isolating and characterizing a number of mutations that do not respond to antheridiogen, as well as suppressors of these mutations. By constructing subtracted cDNA libraries (wild-type minus mutant cDNA libraries, for example) we hope to identify genes that are immediately expressed in response to antheridiogen or are required for the antheridiogen response.

548 cDNA CLONING OF A CELL DIVISION CYCLE RELATED CALCIUM-BINDING PROTEIN FROM *ATRIPLEX NUMMULARIA*. J.K. Zhu, R.A. Bressan and P.M Hasegawa. Purdue University, Department of Horticulture, West Lafayette, Indiana 47907

An atriplex cDNA of 970 bp that likely encodes a calcium-binding protein was isolated. The cDNA comprises an open reading frame that encodes a 167 amino acid, 20.3 KD polypeptide. The predicted protein is hydrophilic, has a theoretic pI of 4.66 and a helix-turn-helix secondary structure typical of calcium-binding proteins. The deduced amino acid sequence has 62% identity to the *Chlamydomonas* caltractin (a basal body-associated calcium-binding protein), 46% identity to the *Chlamydomonas* and potato calmodulins, 48% identity to animal calmodulins, and 42% identity to the yeast *Saccharomyces cereyisiae* CDC31 gene product required for spindle pole body duplication. Genomic DNA blot analysis revealed that the calcium-binding protein is probably encoded by a multi-gene family. In RNA blot analysis, the cDNA hybridized to two mRNAs, a larger 2.3 Kb message, and a smaller 1.1 Kb message. The 2.3 Kb mRNA has a maximum level of expression at a very early stage of the cell growth cycle, at the beginning of cell division. The level of the 1.1 Kb mRNA remains constant throughout the growth cycle. The correlation between the expression of the 2.3 Kb mRNA and the mitotic activity of the cells suggests that a calcium-binding protein or a closely related protein encoded by the cross hybridizing 2.3 Kb mRNA has a role in cell division.

Supported by Purdue University Agricultural Experiment Station Funds and McKnight Foundation grant

549 ISOLATION AND CHARACTERIZATION OF CELL CYCLE CONTROL GENES FROM *ARABIDOPSIS*. Adriana S. Hemerly, Paulo C.G. Ferreira, Marc Van Montagu, and Dirk Inzé. Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

In recent years the mechanisms that regulate cell division have been intensively investigated in yeast and animal systems. In contrast very little is known about cell division in plants. Some unique characteristics of higher plants, such as the presence of meristems and the phenomenon of totipotency make it a very attractive system to study cell division. We are interested in studying how cell division is regulated during plant development. As a first step, we have cloned and characterized a cDNA encoding a *cdc2* homolog from *Arabidopsis*. When expressed in a temperature-sensitive *CDC28 Saccharomyces cerevisiae* mutant strain, this cDNA can partially complement the deficiency at the restrictive temperature, thus confirming that the gene identified is a *cdc2/CDC28* functional homolog [Ferreira *et al.* (1991) *Plant Cell* 3:531-540]. Subsequently, we isolated and sequenced a genomic fragment containing the coding region of the *Arabidopsis cdc2* gene. In order to address the question of tissue-specific expression of the *cdc2* gene, we fused its promoter region to the reporter gene β-glucuronidase and this construction was introduced into *Arabidopsis* and tobacco. Recently, using polymerase chain reaction, we have amplified two different fragments encoding cyclin homologs from *Arabidopsis* and these have been used to screen a cDNA library in order to isolate full-length clones. The characterization of the cyclin clones will be reported.

550 Isolation and characterization of a cdc2 genomic sequence from *Arabidopsis thaliana*. Steven J. Szarka and Maurice M. Moloney. University of Calgary, Department of Biological Sciences, Calgary, Alberta, Canada T2N 1N4

Genetic determinants of cell division cycle (cdc) progression are being identified and analyzed in fungi and animal systems. The cdc2 gene product, p34, plays a major role in regulating passage through the cell cycle; from G1 into S phase and from G2 into M phase. $p34^{cdc2}$ has protein kinase activity which is regulated by both protein - protein interactions and its state of phosphorylation. Proteins which interact directly with $p34^{cdc2}$ include cyclin (cdc13) and p13 (suc1). The p34 / cyclin complex, also known as MPF (maturation promoting factor) becomes active at the G2 / M phase transition and is responsible for inducing cellular changes associated with M phase through phosphorylation of specific substrates.

Conservation of these cell cycle factors is extensive as homolgs of cdc2, cyclin, and p13, have been identified in yeast, insects, amphibians, birds, and humans. More recently, homologs of cdc2 have also been isolated from plants. Although post-translational activation of p34 is being extensively studied, very little is known about transcriptional regulation of cdc2. Northern blot analysis has already shown that the level of total cdc2 mRNA in plants varies depending on the tissue type. To further characterize cdc2 transcriptional regulation in plants, a genomic clone from *Arabidopsis* was isolated. Promoter analysis is being performed by expression of the reporter gene β-glucuronidase and identification of the cis-element containing regions.

551 IDENTIFICATION OF *CIS*-ELEMENTS FOR PHENYLALANINE AMMONIA-LYASE EXPRESSION IN VASCULAR TISSUES. Antonio Levva[1], Armand Séguin[1], Richard A. Dixon[2] and Christopher J. Lamb[1]. [1]Plant Biology Laboratory, Salk Institute for Biological Studies P. O. Box. 85800, San Diego, CA 92037, [2]Plant Biology Division, Samuel Noble Foundation, P. O. Box. 2180, Ardmore, OK 73402

Phenylalanine ammonia-lyase (PAL) catalyzes the first reaction in the biosynthesis of a wide variety of phenylpropanoid natural products including lignin, flavonoid pigments, and phytoalexins. PAL activity and expression is highly regulated during development and by many environmental stimuli. We have shown previously that a 1.2 kb PAL2 promoter fragment from bean fused to GUS gene is regulated appropriatly in transgenic tobacco plants. High levels of GUS expression are observed in protoxylem cells. To analyze the PAL2 promoter we generated 5' and 3' deletions and studied their effect on the observed pattern of GUS expression in transgenic tobacco plants. Deletion of upstream sequences to -613 and -440 progressively reduced the level of extractable GUS activity in stems but histochemical analysis of GUS activity in these plants resulted in the same pattern as observed with the full promoter. An internal deletion from -74 to -119 maintained this spatial pattern of expression in the stem vascular system. However, deletion from -74 to -135 led to a new pattern of expression where strong GUS staining was observed in the internal and external phloem while weak GUS staining in the xylem could still be observed. Further deletion from -74 to -289 abolished expression in xylem, but strong expression in phloem tissues was retained. Finally, deletion from -74 to -480 caused the loss of all expression in vascular tissues. The same altered expression patterns were also observed in the vascular tissues of roots and petioles. We will present a more detailed analysis of the *cis*-elements involved in specifying this pattern. Isolation of *trans*-acting factors that interact with the PAL2 promoter is in progress.

POSTER ABSTRACTS

552 CELL-CYCLE-DEPENDENT AND -INDEPENDENT EXPRESSION OF SEVERAL HISTONE GENE PROMOTERS IN TRANSGENIC PLANTS

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After 24 h of culture tobacco mesophyll protoplasts show an increase of histone mRNA concurrent with an increase of DNA replication. The addition of hydroxyurea, an inhibitor of DNA synthesis, or removal of phytohormones from the medium inhibit this mRNA induction but allow a basal level of expression. Constructs containing a GUS reporter gene under the control of H3 and H4 histone promoters have been introduced into tobacco. *Arabidopsis* (H4A748, H4A777) and maize (H3C4, H4C14, H4C7) promoters showed a high level of expression in meristematic tissues of transgenic tobacco. In non-proliferative tissues, these promoters had a low but significant level of expression. Protoplasts prepared from transgenic tobacco plants expressing GUS under control of H4A748 *Arabidopsis* promoter and H3C4 maize promoter were cultivated under different conditions affecting the DNA synthesis. Analysis of H4A748-GUS and H3C4-GUS activity revealed the existence of (i) a basal replication-independent activity in the absence of any DNA replication and (ii) a replication-dependent activity induced simultaneously to the DNA replication. In order to monitor expression in a monocot, *Arabidopsis* and maize histone promoters have been introduced into rice and the initial findings will be discussed. (Supported by Rhone-Poulenc Agrochimie and CNRS)

553 FUNCTIONAL ANALYSIS OF PLANT HISTONE GENE PROMOTERS

Marc LEPETIT*, Martine EHLING, Nicole CHAUBET, and Claude GIGOT IBMP-CNRS 12 rue du General Zimmer 67084 STRASBOURG FRANCE.*Present adress: Biology Department Texas A&M University College Station TX 77843-3258 USA

We have shown that histone gene promoters from maize and *Arabidopsis* have (i) a basal constitutive replication-independent activity and (ii) a replication dependent activity induced when DNA synthesis occurs (see other poster *Lepetit* et al.). Transient expression studies in tobacco protoplasts with constructs containing a GUS reporter gene under the control of Arabidopsis and maize histone gene promoters have been conducted. In these expenments 35S/CAT gene has been used as an internal standard . Transcription occurs within the first 24h of culture in the absence of DNA replication and is insensitive to inhibition of DNA synthesis. This transient expression reflects the replication-independent activity of the histone gene promoters. Deletion derivatives of the maize H4C7 promoter have been tested in transient expression studies in tobacco protoplasts. It was possible to localize the region within the promoter responsible for replication-independent activity. A region of 45 base pairs was shown to be responsible for this replication independent activity. Substitution of three base pairs within this region drastically reduces the activity of H4C7 promoter. Tobacco has been transformed with these constructs and transgenic plants are currently undergoing analysis. (Supported by Rhone-Poulenc Agrochimie and CNRS)

554 CHARACTERIZATION OF THE ROOT-SPECIFIC GENE TobRB7.

Christopher G. Taylor, Yan Li, Ming-De Deng, Gregoria N. Acedo, and Mark A. Conkling. North Carolina State University, Department of Genetics, Raleigh, NC 27695-7614, USA

TobRB7 is a tobacco root-specific cDNA. TobRB7 mRNA is expressed at the root meristem, immature central cylinder, and at the sites of lateral root initiation but cannot be detected in leaves, stems, or shoot meristems. Two genes, TobRB7-5A and TobRB7-18C, encode TobRB7 proteins that are 250 amino acids long and are identical with the exception of two amino acids. Amino acid similarities of the TobRB7 proteins to an evolutionarily conserved family of membrane channel proteins (including the mammalian MIP26, the bacterial glpF, and the soybean nodulin Nod26 proteins) imply that TobRB7 may function as a membrane channel. The 5' flanking sequences of the two genes exhibit >80% nucleic acid identity for the proximal 800 nucleotides. The promoter of the TobRB7-5A gene has been analyzed by deletion analyses. Both positive and negative regulatory DNA elements responsible for root-specific expression have been identified. These reside within the promoter regions conserved for the two genes. The regulatory elements correlate with DNA sequences that bind root nuclear proteins in gel retardation assays.

555 LOCALIZATION OF H3 HISTONE mRNAs IN PARSLEY SEEDLING.

Sheng-Cheng Wu, Per Gregersen and Klaus Hahlbrock.
Max-Planck Institut, 5000 Koln 30, Germany.

The structural analysis of several histone H3 genes isolated from parsley (Petroselinum cripum) reveals that they are of plant characteristics known to be very different from those of animals'. The expression of these genes was studied in cell suspension and in 18-day-old seedlings of parsley. The concentration of H3 mRNAs in cell suspension reaches its maximum 2 days after subculture in fresh medium and is consistent with the highest level of cell division. In parsley seedlings, H3 mRNAs are mostly accumulated in young roots and emerging primary leaf, but rarely detected in older leaves, cotyledons and hypocotyl. Using in situ hybridization technique, we further examined the distribution pattern of H3 mRNAs at cellular level. As expected, abundant hybridization signals are localized in the cell proliferation zones of both shoot and root apical meristems. Strong signals were also found in the emerging leaf primordium where cell division continued. The hybridization signals distributed in all three cell-dividing zones are not homogeneous, but "patch-patterned". The patched signals may possibly result from the asynchronized cell division during plant development. These results allow us to believe that the structural differences between plant and animal histone genes may not have an impact on the basic expression mode of histone genes, rather, they could probably reflect differences in regulatory mechanism.

556 INTRODUCTION AND EXPRESSION OF THE GUS-GENE IN TULIP

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Tulip (Tulipa gesneriana L.) is the most important flowerbulb in Holland. It is a monocot species (Liliaceae) with a long generation cycle, due to a 5 year juvenile period. To accelerate breeding, efforts were made to establish a transformation procedure for this crop. We studied shoot regeneration, Agrobacterium infection and particle bombardment. Adventitious shoot formation can be obtained from both bulbscale explants and young flower stems. The latter procedure was applied to several cultivars and a number of related species. It was shown that the fourteen genotypes tested all reacted quite well in this regeneration system giving rise to approximately ten shoots per explant. To investigate the sensitivity for Agrobacterium transformation, flower stem explants were infected with wild-type strains and disarmed derivatives of A. tumefaciens strains Ach5, C58 and A281 and with A. rhizogenes strain LBA 9402. All strains were provided with the binary plasmid p35SGUSintron, harbouring the NPT II gene and the intron-containing GUS gene. Using a histochemical reaction, blue spots indicative for glucuronidase expression were observed 10 days after infection. So, Agrobacterium is able to tranfer genes into this monocot. Compared to Agrobacterium, the introduction of genes by using the particle delivery system (DuPont) was also very effective. A plasmid with the GUS-gene under control of the CAMV35S promoter combined with the Adh intron or under control of the TR2' promoter was used to study transient expression in segments of young flower stems. The large number of blue spots showed that both promoters were effective in GUS gene expression in tulip.

557 A MYB-RELATED GENE OF ARABIDOPSIS CONTROLS THE DETERMINATION A SINGLE CELL TYPE.

M. David Marks, Jeff Esch, and David Oppenheimer. School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588-0118.

We are using the development of the trichomes on Arabidopsis as a model for studying the control of the initiation and maturation of individual plant cell types. The trichomes provide an excellent model because 1) their development occurs on the surface of the plant, 2) since Arabidopsis trichomes are composed of single cells, the developmental pattern is relatively simple, and 3) many mutations that effect trichome formation have been identified. Our goal is to isolate and characterize the genes defined by the trichome mutations. We have isolated the GL1 gene, which is required for trichome initiation, by a T-DNA tagging approach (1,2). Sequence analysis has revealed that the gene is a member of the myb class of transcriptional activators (of which the maize C1 is also a member). Using a GUS reporter gene we have found that the gene is only transiently expressed in very young leaf primordia, before the initiation of differentiation of trichomes is apparent. Using the same reporter gene construct, we have also found that the GL1 gene product is not required for its own regulation. By placing the GL1 coding sequence under control of the CaMV 35 S promoter we are able to manipulate the pattern of trichomes on the leaf. Finally, we have found that Arabidopsis contains a myb-related gene family. We are attempting to identify other members that control the determination of other cell types.

1) Marks and Feldmann, 1989. Plant Cell 1:1043-1050.
2) Herman and Marks. 1989. Plant Cell 1:1051-1055.

POSTER ABSTRACTS

558 INVESTIGATING THE MOLECULAR BASIS OF THE *SLENDER* MUTATION IN BARLEY. Petra H.D.Schünmann, Kim S.Turk and Helen J.Ougham. AFRC-IGER, Welsh Plant Breeding Station, Aberystwyth, Dyfed, SY23 3EB, Wales, U.K.

The developmental mutant, *slender* barley, results from an alteration in a single nuclear gene. Plants homozygous for the variant allele exhibit a leaf extension rate of up to four times that of the normal plant and consequently are attenuated and fragile. In addition, normal low temperature growth constraints are removed, enabling the mutant plant to grow at sub-zero temperatures. The *slender* plant thus provides an ideal system for analysis of the metabolic limitations of growth. Using differential hybridisation, a number of clones have been isolated from a wild-type cDNA library (λgt10) which show reduced expression in the basal region of the *slender* plant. These are being further characterised. To supplement this work, the developmental stages along the lengths of *slender* and wild-type seedlings have been compared to identify physiologically equivalent zones. To isolate *slender*-specific transcripts, i.e. those genes whose expression may be repressed in wild-type barley by the normal product at the *slender* locus, a second library, prepared from *slender* cDNA, is being screened. By such approaches we hope to identify and characterise both the *slender* gene and those loci whose expression is modulated by the *slender* gene product.

559 Control of DNA replication by cytokinins in the shoot meristem of mustard. A. Jacqmard, C. Houssa and G. Bernier. University of Liege, Department of Botany, B-4000 Liege, Belgium.

Although cytokinins are important promoters of plant cell division *in vitro* and *in vivo*, their mode of action remains unknown. Our study indicates that they promote cell division in shoot meristems of intact plants partly by controlling the DNA replication process.

A single application of benzyladenine (BA) 4.5 10^{-5}M on the apical meristem of vegetative plants of *Sinapis alba*, the mustard, was able to cause increases of both mitotic and DNA synthetic indices (Bernier *et al.*, 1977; unpubl. results). Using the same experimental system and the DNA fibre autoradiography method (Van't Hof, 1976), we found that, in response to BA application, the replicon size was 2-fold shortened indicating an activation of latent origins and the degree of synchrony of replicon family activation was 2.5-fold increased. These combined effects caused a 3.5-fold shortening of the S-phase duration. We hypothesize that one of the proteins involved in the initiation of DNA replication is a target for cytokinins (Supported by Concerted Research Actions 88/93-129 and F.R.F.C. 2.4507-87).

Bernier, G., Kinet, J.-M., Jacqmard, A., Baverange, A. & Bodson, M. (1977). Plant Physiol., 60, 282-285. Van't Hof, J. (1976). Exp. Cell Res., 103, 395-403.

560 MOLECULAR APPROACHES TO TOMATO DEVELOPMENT. K. Theres J. Brandstadter, S. Knapp, Y. Larondelle, R. Meissner, T. Roßbach, M. Roßberg, R. Simon, E. Tillmann and P.Starlinger. Institut fur Genetik, Universitat zu Koln, D-5000 Koln 41, F.R.G.

In higher plants the process of organogenesis and differentiation is thought to be initiated in the apical meristems by the onset of specific gene expression patterns. With the goal of studying genes involved in this process, we are trying to isolate genes which are exclusively or preferentially expressed in the shoot apical meristem of the tomato plant (*Lycopersicon esculentum*) by differential cDNA hybridisation. So far three genes have been isolated which show a preferential expression in the tomato shoot apex. Sequence analysis revealed that two of these genes (AT1 and AT2) show a similarity to the tomato proteinase inhibitor II and a potato gene which is similar to the soybean proteinase inhibitor CII (Bowman-Birk), respectively. The third gene does not show a similarity to any other gene in the data banks. Presently these genes are studied further by RNA in situ hybridisation and antisense experiments.

In a second approach we want to use a transposon tagging system to isolate developmentally important genes. For this reason we have introduced the maize transposable elements *Ac* and *Ds* into tomato. Nine T-DNA insertions, harbouring a transposable element each, have been mapped on the tomato chromosomes up to now using the Inverted Polymerase Chain Reaction (IPCR) technique to obtain a probe and subsequent RFLP analysis. Presently we are using some of these transgenic tomato lines for the first tagging experiments.

561 GENE EXPRESSION PRIOR TO ROOT AND SHOOT MERISTEM FORMATION IN TOBACCO THIN CELL-LAYER EXPLANTS Debra Mohnen, Teresa A. Gruber, and Karen Liljebjelke. Complex Carbohydrate Research Center & Dept. of Biochemistry, Univ. of Georgia, Athens, Georgia 30602.

Thin cell-layer explants (TCLs) from flower branches of tobacco can be induced to form root, vegetative shoot, or flower meristems by culture on Linsmaier and Skoog media containing specific concentrations of auxin and cytokinin (1). The types of meristems formed in TCLs cultured on defined media can be regulated by addition of cell wall-derived α-1,4 oligogalacturonides (DP of 10-14) to the media (2,3). The goal of this study was to identify genes that could serve as markers of pre-meristem pattern formation in TCLs undergoing root and shoot organogenesis. Histological analysis of TCLs grown for 0, 3, 6, 9, 12, and 14 days on root-inducing and shoot-inducing media revealed that root and shoot meristems could be observed after 14 days of culture. Differential screening of cDNA libraries made from mRNA of TCLs grown for 12 days on shoot-inducing medium (TCL-SIM) or root-inducing medium (TCL-RIM) (4) identified 11 non-cross-hybridizing groups of clones: four groups hybridized preferentially to TCL-SIM cDNA and 7 groups hybridized preferentially to TCL-RIM cDNA. Partial sequence analysis of representative genes from 10 of these groups showed that three groups have sequence similarity to previously identified genes: histone H3, α1 elongation factor, and a potato tuber protein, p322. Northern analysis of the temporal expression of these genes using total RNA isolated from TCLs cultured for 0, 3, 6, 9, 12, and 15 days on five different media (root, vegetative shoot, flower, transition, and transition plus oligogalacturonides) will be presented, as will results of current efforts to analyze the spatial expression of these genes during TCL organogenesis by *in situ* hybridization of TCL sections. 1. Mohnen *et al.*, (1990) *Development* 108:191-201. 2. Eberhard *et al.*, (1989) *The Plant Cell* 1:747-755. 3. Marfa *et al.*, (1991) *J. Cell Biochem.* 15A:151. 4. Gruber *et al.*, (1989) *Genetics* 122:s27. Supported in part by DOE DE-FG09-87ER13810 as part of the USDA/DOE/NSF Plant Science Centers program and DE-FG09-85ER13425

562 CONSERVATION OF AN ESSENTIAL EUKARYOTIC REPLICATION FACTOR IN BRASSICA NAPUS Nancy A. Markley And Maurice M. Moloney, Department Of Biological Sciences, University Of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada, T2N 1N4.

The Proliferating Cell Nuclear Antigen (PCNA) is an essential replication factor in eukaryotic cells. In animals, PCNA functions as a part of a molecular mechanism to coordinate the sequential initiation of lagging and leading strand synthesis by Polymerase-α and -δ, respectively. Furthermore, PCNA specifically increases the processivity of Pol-δ, allowing for efficient replication by the leading strand polymerase. A 1004 bp cDNA clone was isolated from a *B. napus* (cv. Westar) apical cDNA library by hybridization to a heterologous probe specific to rice PCNA. Sequence analysis shows that the *B.napus* PCNA shares approximately 84% amino acid homology with the human PCNA when conservative substitutions are included. Northern blot analysis indicates that the expression of PCNA mRNA is correlated with the proliferative state of cells/tissues. Using a bacterial expression vector, the PCNA cDNA was expressed in *E.coli* as a 33.6 kDa polypeptide. A broad specificity monoclonal antibody raised to the rabbit PCNA cross-reacted with this plant polypeptide. The existence of PCNA in plants raises a number of important questions about plant cell DNA replication, in particular, the existence and potential role of pol-δ in plants. We are currently testing the ability of the *B napus* PCNA to interact with the mammalian pol-δ *in vitro*, by measuring processive DNA synthesis on a poly (dA)/oligo(dT) synthetic template. This experiment will establish whether the plant PCNA is functionally equivalent to the mammalian PCNA *in vitro*, as was demonstrated for the human and yeast PCNA. Further studies include transient expression of antisense RNA in protoplasts, *in situ* hybridization experiments, and the isolation of the *B napus* PCNA genomic clone. It is anticipated that these studies will help elucidate the molecular mechanisms for DNA replication in higher plants.

563 MOLECULAR CLONING AND DEVELOPMENTAL EXPRESSION OF CYTOPLASMIC RIBOSOMAL PROTEIN S24 FROM *BRASSICA NAPUS*. Peta C. Bonham-Smith, Tammy L. Oancia, Maurice M. Moloney, Department of Biological Sciences, University of Calgary, Calgary T2N 1N4, Alberta, Canada.

We have isolated two cDNA clones which appear to encode the 40S ribosomal subunit protein S24 from *Brassica napus* (oilseed rape). The open reading frame in both clones contains 390 bases encoding a deduced polypeptide sequence of 130 amino acids (100% homology between clones) with 80% sequence identity to the corresponding S24 polypeptide of yeast. Both the yeast and rapeseed proteins have a net positive charge of +9 however, the rapeseed S24 protein has a molecular mass of 14778 Da compared to 14762 Da for the yeast protein. The rapeseed ribosomal protein S24 is encoded by a small multi-gene family with at least two actively transcribed members. A single transcript of approximately 1-1.2 kb, corresponding to ribosomal protein S24, is most abundant in actively dividing tissues such as apical meristem and young internode and less abundant in root, including the root meristem, and bud tissue. No transcript was detected in young (0.1-0.5 cm diameter) leaves, mature leaves or mature stem.

POSTER ABSTRACTS

564 APPLICATION OF FLOW CYTOMETRY TO CELL-CYCLE ANALYSIS IN SHOOT MERISTEMS. A. Jacqmard. University of Liege, Department of Botany, B-4000 Liege, Belgium.

Flow cytometry (FCM) has become increasingly popular for cell-cycle analysis with animal cell systems (Gray and Darzynkiewicz, 1987). The major advantages of this technique over conventional microscopy are its rapidity, accuracy and sensitivity. A disadvantage in the case of plant tissues is the requirement for single-cell suspensions : either the tissues have to be converted into a protoplast suspension or the nuclei must be released by mechanical chopping of the tissues. We have developed a procedure for a high-yield isolation of protoplasts from shoot meristem tissues allowing an accurate FCM analysis with a few micrograms of tissues. These protoplasts are small, undifferentiated, with little pigment and small vacuoles. We shall present DNA distributions in the shoot meristem of two species : Sinapis alba, a dicot, and Lolium temulentum, a monocot. The DNA distributions were resolved into 3 different phases (G1, S and G2 M) and the fraction of protoplasts in each phase was estimated by the "Sum of broadened rectangles" method (Dean, 1985). Possible applications (multiparameter analysis, cell-cycle duration) will be discussed.

Gray, J.W. & Darzynkiewicz, Z. (1987). Techniques in cell cycle analysis. Humana Press, Clifton, New Jersey.
Dean, P.N. (1985). In "Flow-Cytometry : Instrumentation and Data Analysis" M.A. Van Dilla, P.N. Dean, O.D. Laerum & M.R. Melamed, Eds, pp 195-221, Analytical Cytology Series, Academic Press.

565 ONSET OF DNA SYNTHESIS IN ROOT MERISTEMS DURING GERMINATION OF ALLIUM CEPA SEEDS. M. Beatrice Bitonti, A. Chiappetta, Anna M. Innocenti. University of Calabria, Department of Ecology,87030 Arcavacata di Rende (Cs), Italy.

In the present work we evaluated the progression of the quiescent meristem cells to cycling in embryo roots of Allium cepa, starting from the onset of DNA synthesis during seed germination.
The results,obtained by autoradiographic methods after ^{3}H-Thymidine feeding,show that after a 84 hr period of water imbibition the DNA synthesis is not resumed in the embryonic root meristems of ungerminated seeds.
When seedling roots are considered we have evidence that the root protrusion is only acomplished by cell elongation and the early DNA synthesis after imbibition is clearly related to differentiation process of metaxylem cellular line. Experiments are in progress to evaluate, by cytopnotometric analyses, the nuclear DNA content in the maturing metaxylem cells.

567 MORPHOLOGY AND ANATOMY OF VEGETATIVE SHOOT APICAL MERISTEM T-DNA INSERTIONAL MUTANTS OF ARABIDOPSIS. Fred Behringer, Joseph Callos and June Medford. Pennsylvania State University, Biology Department, University Park, PA 16802

We are studying *Arabidopsis* mutants which display altered growth of the apical meristem to gain an understanding of how the shoot meristem maintains itself as a formative region and yet continually differentiates at its margins to produce the shoot and leaves. The mutants were selected from T-DNA mutagenized seedlings. Three distinctive mutants segregate 3 normal :1 mutant from selfed heterozygous plants and 3/4 of the progeny, including all mutants, are kanamycin resistant (conferred by the T-DNA). These data suggest that the mutations are a result of T-DNA insertion at a single locus generating a recessive allele. Chromosomal regions adjacent to the T-DNAs have been isolated from all three mutants by plasmid rescue. Progress on isolation and characterization of the genes will be reported. Scanning electron micrographs of the mutants indicate that the genetic lesions produce abnormal expansion of the shoot meristem, disorganized leaf initiation and improper cell expansion. Light microscopic examination of the apical meristems reveals changes in meristem organization. These descriptive studies will contribute to understanding the functions of the tagged genes and how the meristem integrates signals in development.

568 VEGETATIVE DEVELOPMENT OF THE SHOOT APICAL MERISTEM IN *ARABIDOPSIS* CHARACTERIZED BY SCANNING ELECTRON MICROSCOPY. Joseph Callos, Fred Behringer, and June Medford. Pennsylvania State University, Biology Department, University Park, PA 16802

We are studying vegetative meristems in *Arabidopsis* to define landmark events that mark distinct stages in development. These events will be used to compare development in wild-type plants to that in three potential T-DNA insertional mutants affecting meristem functions. The seedlings emerge from the soil at 3.5 days with the first two leaf primordia already initiated from a rectangular shaped meristem. The first two primordia are situated 180° with respect to each other. Stipules form at the flanks of the primordia and they overtop the meristem as they develop. The third primordium becomes distinct at 6 days and is initiated 90° with respect to the first pair. Between 6-7 days the meristem begins to bulge becoming rounder, and the fourth primordium is initiated 90° with respect to the third. Primordia five through eight are initiated in a spiral phyllotaxy from a meristem that is distinctly dome shaped. The plants then undergo a transition to the inflorescent state. Scanning electron micrographs of the mutants indicate that the genetic lesions produce abnormal expansion of the shoot meristem, disorganized leaf initiation and improper cell expansion.

566 TRICHOME DEVELOPMENT IN *Arabidopsis*: CLONING OF *ttg*. Amanda R. Walker and John C. Gray. Botany School, University of Cambridge, Cambridge CB2 3EA, UK.

Trichome or leaf hair development in *Arabidopsis thaliana* provides an ideal system for the study of cell differentiation. Trichomes in this species are single cells with three branches on the upper leaf surface. Mutations at a number of loci affecting trichome number and shape have been described. We are concentrating on the *ttg* locus which affects trichome number and several other apparently independent pathways. *ttg* mutants are glabrous, lack anthocyanins in the testa (seed coat) and plant body, have an altered testa structure and do not excrete seed mucilage. To clone the locus we have been mapping the RFLP markers from chromosome 5 with recombinants between *ttg* and *ms1* generated by Jeff Esch and David Marks. Only one RFLP (21503) maps between these loci which are 6 centimorgans apart. Recombinants are being generated between *ttg* and proximal markers *ga3* and *ch5* so that the proximal RFLPs (4556 and 6843) can be accurately mapped in relation to *ttg*. The RFLPs will be used to initiate a chromosome walk in collaboration with Zoe Wilson and Bernie Mulligan.

569 CONSTRUCTION AND SCREENING OF CDNA LIBRARY OF CAULIFLOWER VEGETATIVE MERISTEM. Bibo Xu and June Medford. Pennsylvania State University, Department of Biology, University Park, Pennsylvania 16802

We are interested in molecular events in shoot meristems. We first took advantage of fortuitous amplification of meristem in a cauliflower head. Several genes, "meri" and their promoters are being studied. In addition, a library specifically to dome of vegetative plants was constructed. This library was made from a group of ~800 cells utilizing PCR for its production. Total RNA was extracted from domes of plants two to three weeks after germination. 3 µg of total RNA was used in cDNA synthesis. A synthesized 19mer adaptor, which carries an EcoRI and a SalI sites, was ligated to both ends of the cDNA. The ligated mixture was amplified with PCR using one strand of the 19mer adaptor as primer. The amplified cDNA was then ligated to λ phage arms and subsequently packaged, which gave rise to a library of 10^{6} clones. We will describe our analysis for developmentally important genes.

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570 PCR AMPLIFIED cDNA LIBRARIES FROM SUGAR BEET (*BETA VULGARIS* L.) APICAL TISSUE. John R. Bray[1], Keith J. Edwards[2], Ian Jepson[2], Gareth I. Jenkins[1] and Wolfgang Schuch[2] (1: Departments of Biochemistry and Botany, University of Glasgow, Glasgow G12 8QQ, Scotland, UK. 2: Plant Biotechnology Group, ICI seeds, Jealotts Hill, Bracknell, Berks RG12 6EY, UK). We are investigating the regulation of flowering in sugar beet (*Beta vulgans* L.). We are attempting to clone genes expressed in the shoot apical meristem since the early events in floral evocation and morphogenesis take place in this tissue. Because of the very small amount of tissue available we have made use of the polymerase chain reaction (PCR) to amplify cDNA prepared from small amounts of RNA prior to cloning. Initially, sugar beet leaf RNA was used to produce a PCR amplified library as there was no limit on the amount of material available. We succeeded in producing a PCR amplified library from small amounts of leaf cDNA which was amplified at least 1000 fold prior to cloning. Screening of this library with a maize *cab* gene probe showed that the proportion of *cab* genes represented in this library was of the right order of magnitude based on the abundance of *cab* transcripts in leaf tissue. Probes for other genes also give predicted results. Following the same protocol, a PCR amplified cDNA library was produced using apical RNA from a small number of sugar beet plants. Characterisation and screening of this library is now in progress.

571 THE STUDY OF ROOT DEVELOPMENT USING T-DNA TAGGED MUTANTS OF ARABIDOPSIS THALIANA. Shirley A. Coomber[1], Philip Benfey[2] and Ken A. Feldmann[3] 1. King's College (London), Campden Hill Road, London, W8 7AH, UK, 2. Rockerfeller University, 1230 York Avenue, New York, New York 10021-6399, USA, 3. University of Arizona, Building 36, Tucson, Arizona 85721, USA.

Traditionally the study of root development has been limited to physiological analysis of environmental and hormonal effects on root growth. One of the drawbacks of looking for mutants in root structure has been the difficulty of screening large numbers of plants from genetically well characterized species such as maize and petunia. However the small size of *Arabidopsis thaliana* allows the visual inspection of roots of 50-100 seedlings grown vertically on a single 10 cm square agar plate. *Agrobacterium*-mediated transformation of higher plants involves the integration of T-DNA (Transfered DNA) at random into the plant genome. This phenomenon has been used to produce insertional mutants in *Arabidopsis* using an unusual seed infection technique which circumvents problems of somaclonal variation associated with more traditional tissue culture transformation protocols (Feldmann and Marks, 1987, Mol. Gen. Genet. 208:1-9). 8100 individual transformants produced by this method were visually screened for lines showing abnormal root development. Preliminary results showing the spectrum of mutants obtained will be presented.

572 TISSUE AND STAGE-SPECIFIC EXPRESSION OF A VEGETATIVE LECTIN IN PEA. Michael S. Dobres, Jhang Ho Pak, Dipanwita Maiti, Laurel Belmont and Albert List. Department of Bioscience, Drexel University, Philadelphia, PA 19104

The seeds of leguminous plants contain high levels of carbohydrate binding proteins called lectins. These lectins are typically synthesised in cotyledons during the mid-maturation stage of seed development and are localized within protein bodies or storage vacuoles. In contrast, immunologically related non-seed legume lectins have been localized in root tips and shoots of several different legumes. Although, the molecular properties of many lectins have been well characterized, the biological role (s) of these proteins during plant development is obscure. We are investigating the function of a bud-specific lectin (*Blecrn*) in pea *Pisum sativum*.L. (var Alaska). The *Blectin* gene (*Blec*) appears to encode a vegetative homologue of the pea seed-lectin. The function of this vegetative lectin is unknown. *Blec* mRNA accumulates in apical buds and is either absent or present at very low levels in the expanded leaves below the bud (Dobres and Thompson, 1989, Plant Physiology 89: 833-838). *In situ* hybridization reveals that *Blec* mRNA accumulates in meristematic epidermal tissue of all developing organs throughout the life-cycle of the plant (leaves, roots, petals ovary *etc.*). At the very least, *Blec* appears to play some role in defining the outermost layers of developing organs. Curiously, however, it is not highly expressed in the protodermal tissue of the apical-dome, but is first detectable in the epidermis of the second set of leaf primordia below the apical dome. We are currently pursuing experiments aimed at further elucidating its function in plant development.

573 MT-DNA OF JUVENILE, ADULT AND REJUVENATED SEQUOIA SEMPERVIRENS. Li-Chun Huang, Shaw-Fen Liu and Suwenza Lius. Academia Sinica, Institute of Botany, Nankang, Taipei, Taiwan

The developmental phase of shoot apical meristems of woody perennials can be reversed in a graded series by a sequence of repeated grafts onto juvenile rootstocks in vitro. The required number of repetitions depends on the desired extent of morphogenetic change and the species. Restoration of readily observable and measurable growth vigor and 100% rooting in shoot cuttings is achieved after a sequence of 4 repeated grafts for Sequoia sempervirens and 7 grafts for Citrus reticulata and C. sinensis. Two-dimensional PAGE revealed distinguishable leaf proteins between adult and juvenile or rejuvenated shoots. Since work on lower organisms had suggested a role of the cytoplasm in maturation, this investigation has been directed at mt-DNA as being possibly involved in phase change of plants. Actively growing adult and juvenile Sequoia shoots contained double-stranded circular mt-DNA of comparable sizes, but yielding different restriction fragments. Rejuvenation by grafting resulted in diminishment of fragments (3.2 and 3.5 kb) typically seen in adult shoots and appearance of those (3.6 and 4.0 kb) associated with juvenile shoots. The prominent fragments were cloned via plasmid PBR322 in E. coli. Bioassays are being developed to examine the fragments for maturing and rejuvenating effects.

574 FLOW CYTOMETRIC ANALYSIS OF CHANGES IN CELL CYCLE ASSOCIATED WITH NUTRIENT DEPRIVATION AND DIFFERENTIATION CAPACITY IN *Nicotiana tabacum* THIN CELL LAYER EXPLANTS. Stephen G. Ernst. University of Nebraska, Department of Forestry, Fisheries and Wildlife, Lincoln, NE 68583.

Flow cytometry (FC) is being used to monitor changes in the cell cycle associated with various *in vitro* manipulations of *Nicotiana tabacum* cv. Samsun thin cell layer explants. The goal of these experiments is to determine if changes in cell cycle can be monitored by FC in explants comprised of heterogeneous cell types, and for which cellular competence associated with shoot induction is manipulated by the timing of application of exogenous growth regulators.

There are at least two "sets" of cells, based on ploidy, that are cycling in the tetraploid *N. tabacum* cv. Samsun thin cell layer explants. One set corresponds to a normal 2n/4n group of cells cycling from G1 to G2, where n=24 and the 2n DNA content is approximately 9.0 pg, based on a chicken red blood cell standard. The second set corresponds to a 4n/8n group of cells cycling from A1 to A2 (A=alternate ploidy level), where n=24. The two sets of cell types appear to be equally distributed in the epidermal and subepidermal cell layers, but a greater proportion of the cells in the epidermal layer are non-cycling.

Thin cell layer explants which are determined for shoot organogenesis, but which have not yet begun to differentiate, were deprived of sucrose for various intervals and then restored to sucrose containing medium. Three days on sucrose-free medium was sufficient to remove the majority of cells from G2 and A2 (only 1.6 and 5.5-percent, respectively), and accumulate in G1, A1 and the respective S phases. After six days on sucrose-free medium, the cells began to cycle once again, with G1/G2/S and A1/A2/S levels returning to normal levels. Experiments with bromodeoxyuridine-labeling will also be discussed in relation to the above.

575 ISOLATION AND CHARACTERISATION OF DNA REPLICATION ORIGINS FROM PEA (PISUM SATIVUM). Nanette D. Davies, John A. Bryant, Paul N. Fitchett, Department of Biological Sciences, University of Exeter, EX4 4QG, U.K.; Jack Van't Hof, Brooknaven National Laboratory, Upton, N.Y., 11943, U.S.A.

We have employed cell synchrony procedures combined with BND-cellulose chromatography of replicating DNA in two different approaches aimed at isolation of DNA replication origins. Firstly, we have used probes for an extensively amplified set of genes, those coding for rRNA, for which the time of replication in S-phase is known (1). The establishment of replication forks at the start of S-phase has been located to a particular 209 bp tract of DNA in the non-transcribed spacer of the rRNA genes. This is known as ori-r-9(2). The function of ori-r-9 as an origin has been confirmed by 2-D-gel analysis of the replicating structures which are initiated at this point. Secondly, we have cloned the DNA which replicates right at the beginning of S-phase. Eight early-S clones have been obtained, and together with ori-r-9, have been sequenced. Comparison of the sequences of the early-S clones and of ori-r-9 with each other, with yeast ars elements/replication origins and with replication origins from animals reveals several striking features of similarity in sequence organisation. The function of the sequences motifs common to the various types of replication origin is being investigated.

(1) Van't Hof, J., Lamm, S.S. & Bjerknes, C.A. (1987). Plant Mol. Biol. 9, 77-86.
(2) Hernandez, P., Bjerknes, C.A., Lamm, S.S. & Van't Hof, J. (1988). Plant Mol. Biol. 10, 413-422.

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576 A POSSIBLE PLANT HOMOLOGUE OF A DNA REPLICATION GENE FROM *Schizosaccharomyces pombe*. Mike Lyne, John Bryant, & Stephen Aves. University of Exeter, Dept. of Biological Sciences, Exeter, EX4 6QG, U.K.

The fission yeast *Schizosaccharomyces pombe* is widely used as a simple model to study the eukaryotic cell cycle. Many mutants can be isolated from *S. pombe* which are conditionally deficient in the cell division cycle (Nasmyth & Nurse, 1981). One of these mutations, *cdc23*, causes a greatly decreased rate of DNA replication and blocks in S-phase. Complementation studies of this mutant with a *S. pombe* gene bank brought about partial rescue of the mutant phenotype. A 1.8 kb DNA fragment was found to be sufficient for rescue when present on a multicopy plasmid. Molecular and genetic techniques showed the 1.8 kb fragment to be an extragenic suppressor of *cdc23*. The gene was named *suc23* (suppressor of *cdc23*). From protein sequence comparisons the predicted product of *suc23* shows strong homology with Ca^{2+}/calmodulin-dependent protein kinases (implicating it as a possible regulatory protein). The transcription of this gene was found to be cell cycle regulated, peaking in S-phase (Aves *et al.*, 1991). When probing with *suc23*, a single-copy sequence was detected in human genomic DNA under conditions of low stringency (S. Ball, personal communication). Experiments are currently in progress to identify *suc23* homologues in various plant species.

Ref: Nasmyth K. & Nurse, P. (1981) *Molec. Gen. Genet.* 182:119-124
Aves S.J., Cooper L. & Tongue N. (1991) *J. Gen. Micro.* (submitted).

577 DNA-BINDING ACIVITY FROM THE DNA REPLICASE COMPLEX FROM PEA (*PISUM SATIVUM*) SHOOT MERISTEMS. J.Al-Rashdi, S.K.Burton, and J.A.Bryant.
University of Exeter,Dept of Biological Sciences, Exeter, England Ex4 4QG.

The replicative DNA polymerase-primase is extracted from meristematic cells as a large ($1.25x10^6$ Kdal) multi-enzyme complex. In addition to polymerase-primase, the complex contains endodeoxyribonuclease, single-strand-specific endodeoxyribonuclease, ribonuclease-H, topoisomerase 1, protein kinase and a DNA binding activity (1). The DNA binding activity is assayed by gel mobility shifts using endonuclease-restricted plasmid and pea DNA. The study of the biochemistry and intraction between binding activity and DNA includes the use of different types of the DNA sequence as substrates e.g pHE1.5, a recombinant plasmid containing a plant DNA replication origin (2), use of competitor DNA's e.g Poly (dI).(dC), and DNA footprint analyses. Fractionation of the replication complex is achieved by electrophoresis in SDS-PAGE. The separated polypeptides are blotted onto nitrocellulose filters and challenged with ^{32}P labelled DNA, either *Hin*dIII-digested pea DNA or *Hin*dIII linearized pHE1.5. Both DNA's are bound strongly at a position on the filter corresponding to a polypeptide molecular weight of 42Kdal; there is also a low level of binding exhibited by three higher molecular weight polypeptides (ca 60 to 118 Kdal). We ascribe the binding activity at 118Kdal to DNA polymerase. The 42Kdal polypeptide, which exhibits the strongest binding activity, is now being purified from the complex by DNA-cellulose and hydroxy-apatite chromatography. The 42Kdal polypeptide's DNA sequence recognition will be compared to that of the whole complex. We are also studying the intraction between DNA and the binding activity by electron microscopy and have been able to visualise plasmid DNA to which protein complexes are bound. We thank J. Van't Hof for supplying pHE1.5.

Ref 1: Bryant, J.A., Fitchett, P.N., Hughes, S.C. and Sibson, D.R. (1991). J. Exp. Bot. *in press*.
2: Hernandez, P., Bjerknes, C.A., Lamm, S.S., Van't Hof, J. (1988). Plant Molecular Biology 10; 413-422.

578 PROTEIN KINASES, TOPOISOMERASES AND DNA REPLICATION IN ROOT AND SHOOT MERISTEMS OF PEA. Donato Chiatante, Paola Brusa, Department of Biology, University of Milan, Via Celoria 26, 20133 Milan, Italy; John Bryant, Paul Fitchett, Department of Biological Sciences, University of Exeter, Exeter, EX4 4QG, U.K.

There is increasing evidence that protein phosphorylation is directly involved in DNA replication, in addition to its role in overall control of the cell cycle. In an accompanying poster (Lyne, Aves and Bryant) we show that a cell cycle gene involved in DNA replication codes for a protein kinase which is clearly distinct from the $p34^{cdc2}$ type protein kinase involved in overall cell cycle control. The target protein for the S-phase protein kinase is not yet known, but there is increasing evidence that it may be topoisomerase I. We have prepared whole nuclei from pea root meristem cells during the period in which DNA replication activity is resumed during germination; we have solubilized the more tightly bound proteins from the nuclei with 0.5M NaCl. These proteins include protein kinase and topoisomerase I and a protein which is recognised by an antibody to the PSTAIRE sequence of the $p34^{cdc2}$ protein kinase. In subsequent purification of the nuclear extracts, the kinase is very closely associated with topoisomerase I. Further, when the nuclear proteins are probed with antibodies to human topoisomerase I, the antibodies identify topoisomerase I as a major substrate for phosphorylation. Other, as yet unidentified polypeptides are also phosphorylated. We have also prepared DNA replication complexes from pea shoot meristems. The complexes are only very loosely bound into the nucleus but remain associated together through an extensive purification procedure. In addition to DNA polymerase-primase, the complexes contain an array of other enzymes involved in DNA replication, including a distinct subpopulation of topoisomerase I and protein kinase. In assays with exogenous substrates, the kinase phosphorylates casein much more strongly than histone H_1. It also phosphorylates several polypeptides in the replication complex, identification of which is in progress.

579 BARLEY RIBOSOMAL PROTEIN L17 GENES - STRUCTURE AND EXPRESSION. Lene H. Madsen and Kirsten Gausing. University of Aarhus, Department of Molecular Biology, DK-8000-Aarhus C, Denmark.

Barley has two genes encoding ribosomal proteins homologous to yeast YL17 and L22 from archaebacteria, eubacteria and chloroplasts. Both genes are active as shown by the isolation of corresponding cDNA clones, and the derived proteins are called HvL17-1 and HvL17-2 (1). The HvL17-1 and part of the HvL17-2 gene have now been isolated. The HvL17-1 gene contains 6 introns of very variable length (40-1500 bp), and the exon/intron organization of the HvL17-2 gene appears identical. High levels of ribosomal protein mRNA was detected in the zone of meristematic and young, developing cells in barley leaves (1). Significant amounts of L17 mRNA is stored in the desiccated barley embryo, but within a few hours of imbibition new primary transcripts are detectable (intron specific probes), and within the next 8-10 hours a 3-fold increase of L17 mRNA per total RNA is seen.

(1) Madsen, L.H., Kreiberg, J.D. and Gausing K. (1991) Curr Genet. *in press.*

580 DIFFERENTIAL GENE EXPRESSION DURING GERMINATION AND CYTOKININ-MEDIATED BUD INDUCTION IN *PICEA ABIES* (NORWAY SPRUCE). Annika Sundås, Karolina Tandre and Peter Engström. Department of Physiological Botany, Uppsala University, Box 540, S-751 21 Uppsala, Sweden.

A pulse-treatment of isolated embryos of *Picea abies* with cytokinin suppresses the normal germinative development and induces a synchronous *de novo* formation of adventitious buds from subepidermal cells. Chloroplast maturation and greening is delayed and cell elongation is suppressed as a result of the treatment. To analyse the patterns of gene expression associated with germination and bud induction we have isolated cDNA clones that are differentially expressed in bud induced and in vitro germinating embryos

A Northern blot analysis identified two main categories of clones that were differentially expressed during normal seedling development; germination abundant and seedling abundant clones. Subclasses showing enhanced or suppressed expression in the bud induced embryos were found among the germination abundant clones and a subclass showing suppressed expression in bud induced embryos were found among the seedling abundant clones.

The clones has been further characterized by DNA sequencing and *in situ* hybridization.

Among the germination abundant cytokinin enhanced clones a histone H2A clone has been identified and the expression of this clone correlates with cell division activity.

In the seedling abundant category we have identified clones that correspond to genes coding for chloroplast related proteins as well as an α-tubulin clone which show suppressed expression in the bud induced embryos. Among the seedling abundant clones we also identified a polyubiquitin clone which is not affected by the bud induction treatment.

We conclude that a major shift in gene expression during seedling development is associated with the transition from cell division to cell elongation and differentiation at the end of germination. This transition is suppressed by the cytokinin treatment.

581 MOLECULAR ANALYSIS OF FLORAL INITIATION IN *LOLIUM TEMULENTUM*. M.T. Zagotta[1], L.T. Evans[2], R.W. King[2], and D.R. Meeks-Wagner[1].
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[2]CSIRO Division of Plant Industry, Canberra, ACT 2601, Australia

The transition from vegetative to reproductive development results in the initiation of new patterns of organogenesis at the shoot apical meristem of flowering plants. Physiological and morphological observations of the shoot apex have demonstrated that an early event in this transition is the suppression of the vegetative program, including the cessation of leaf primordia formation and differentiation. We have begun an investigation of the molecular mechanisms that orchestrate the process of floral initiation/vegetative suppression in the qualitative long-day monocot, *Lolium temulentum* cv. Ceres. The precise photoperiodic control of floral initiation in Ceres plants has allowed us to perform a detailed temporal analysis of gene expression during the transition to flowering. By differential screening of a *Lolium* vegetative apex cDNA library, we have identified a set of independent cDNA clones that represent apex-specific genes whose expression is significantly decreased upon floral initiation. The transcript levels revealed by these apex-specific clones are high in vegetative apices and greatly reduced in apices harvested eight days post-induction, at which time spikelet primordia have formed but glume primordia have not yet initiated. We present here the initial characterization of the tissue expression patterns of the genes represented by these cDNA clones in vegetative and florally-induced plants. Further studies will focus on the regulation of these genes in response to floral initiation/vegetative suppression and their potential function in maintaining the vegetative pattern of morphogenesis.

POSTER ABSTRACTS

582 CHARACTERIZATION OF ACTIN GENES OF *STRIGA ASIATICA*. S.J. Wolf and M.P. Timko. Department of Biology, University of Virginia, Charlottesville, VA 22901

Striga asiatica, commonly called witchweed, is a root parasite of important agronomic crops such as corn and sorghum. *Striga* attaches to its host root by means of a specialized root organ called a haustorium. We have previously reported the development of a novel system for the study of haustorial formation in *Striga asiatica* which used *in vitro* root culture (Plant Science 73:233-242). Cultured roots form haustoria following exposure to 2,6 dimethoxy-*p*-benzoquinone (2,6 DMBQ), a haustorial induction factor recently isolated from host root extracts. Haustorial development from the root meristem is a rapid process with morphological effects seen in 4 - 6 hours following exposure to 2,6-DMBQ. Upon induction, root elongation stops abruptly, rapid cellular differentiation within the root tip is initiated, a localized expansion of cortical cells occurs, and surface haustorial hairs develop. Within 18 - 24 hours the stages of haustorial development outside of the host are completed. It is believed that cytoskeletal actins play a role in cellular processes such as cytoplasmic streaming, root hair elongation, and cell division. Actin proteins are encoded by small multigene families in several plant species. In addition, tissue-specific function or regulation of the different plant actin genes has been proposed. Southern blot analysis with a heterologous probe has suggested that at least five actin genes exist within the *Striga* genome. As a step toward studying tissue-specific expression of actin during *Striga* haustorial development we have used the DNA polymerase chain reaction to isolate partial cDNA and genomic clones encoding several different actin genes. Studies of the characterization of *Striga* actin genes and their expression during haustorial development will be presented.

583 REGULATION OF GENE EXPRESSION DURING HAUSTORIAL DEVELOPMENT IN WITCHWEED (*STRIGA ASIATICA*) Christa S. Florea and Michael P. Timko. University of Virginia, Department of Biology, Charlottesville, Virginia 22901 USA

Striga asiatica (Witchweed) is a noxious parasite of commercially important grasses world-wide. *Striga* attaches to and penetrates its host plant root through the development of a specialized multicellular organ termed a haustorium. Haustorial formation is initiated in elongating radicles by the perception of a host-derived signal (2,6-dimethoxy benzoquinone and related molecules). Following signal perception there is a cessation of radicle elongation, increased cell division resulting in radial expansion, and the elaboration of haustorial hairs. The development of a mature haustoria is completed within 18 - 24 hrs. We are currently investigating the extent to which differential gene expression is involved in controlling these developmental processes and in particular the role played by cytoskeletal proteins (e.g., tubulins) in haustorial formation. Southern blot analysis of total genomic DNA suggests that there are approximately 6-9 genes encoding β-tubulins in *Striga*. A *Striga* EMBL4 genomic library was screened with an *Arabidopsis* cDNA probe encoding a highly conserved region in plant tubulins. A number of hybridizing phage were isolated and the structure of the genes contained on these phage are being characterized. In addition, monoclonal antibodies to highly conserved domains in animal tubulins have been used to study the accumulation of β-tubulin isoforms in developing haustoria. Expression of some β-tubulin related antigens were detected in developing haustoria that were either poorly represented, or not present in uninduced radicles. Studies toward elucidating the basis for the cell-type/developmental specific expression of these proteins are in progress.

584 ANALYSIS OF TWO MUTATIONS WHICH ALTER THE FATE OF CELLS IN THE MAIZE LEAF. John Fowler, Anne Sylvester and Michael Freeling. University of California, Berkeley, Department of Plant Biology, Berkeley, CA 94720

The ligular region of the maize leaf occurs at the junction of its two major organ-components, the blade and the sheath. The boundary between these two morphologically distinct tissues is defined by the ligule, a small fringe of tissue on the inner surface of the leaf, and the auricle, a thickened area which lies just above the ligule. We have focused on characterizing mutants which affect the morphology of this region in order to understand the genes which are involved in regulating the development of the maize leaf. Two such mutations are *Liguleless3* and *Liguleless4*, which eliminate or displace the ligule and auricle in specific domains of the leaf. Morphological characterization of mutant plants shows that elimination of the ligule is accompanied by transformation of cells which normally adopt a blade cell fate into ones which instead adopt a sheath cell fate and that the mutants act early during the development of the leaf primordia. Genetic analysis of the mutations indicates that the alleles act semidominantly, and that interactions with most of the other mutations which affect this region are additive. These results have led us to hypothesize that both *Lg3* and *Lg4* cause the ectopic expression of factors which are involved in defining the fate of cells in the leaf. The similarity of the phenotypes of these two mutations to that of the *Kn1* mutant suggested that these genes might belong to a family of regulatory genes with similar protein motifs. The *Kn1* gene has recently been shown to contain a homeobox sequence (Vollbrecht, et. al., *Nature* 1991). Probing Southern blots at low stringency with probes made from cloned maize homeobox sequences have revealed several bands which are other potential homeobox-containing genes. Using segregating populations of *Lg3* and *Lg4*, we have shown that both mutations are closely linked to homeobox sequences and therefore may be mutations in these homeobox genes. Further evidence supporting this conclusion will be presented. J.F. is a Howard Hughes Medical Institute Predoctoral Fellow.

585 p34^{cdc2} HISTONE H1 KINASE IN PEA. Heidi Feiler, Betty Prewett and Thomas Jacobs, Department of Plant Biology, University of Illinois, Urbana, Illinois 61801.

Macroscopic form and growth dynamics in higher plants are directly influenced by cell division patterns in meristems. However, little is known about the molecular basis for mitotic regulation in plant cells. We have selected *Pisum sativum*, the garden pea, for an analysis of the ubiquitous *cdc2*-centered cell regulatory system, originally identified in fission and budding yeasts. A monoclonal antibody (MAb-J4) raised against *Schizosaccharomyces pombe* p34 recognizes a polymorphic signal at 34 kDa in pea protein extracts, comparable to the phosphorylated isoforms of mammalian p34. Non-denatured protein extracts of mitotic *Pisum sativum* (pea) tissues were fractionated by gel filtration, electrophoretically separated under denaturing conditions and immunoblotted. p34 cross-reactive material was apparent across the elution profile, indicating that p34 exists as both a monomer and as part of a high molecular mass complex in pea. Histone H1 kinase (H1K) activity was largely confined to higher molecular mass fractions, those carrying the least phosphorylated form of pea p34. As in other systems, p13-Sepharose precipitates the multiple forms of pea p34, including the active H1K. Meristematic tissues (epicotyl hook and root tip) are enriched in the active H1K form of p34 when compared to mature root, stem or leaf tissues. We also report the cloning of a portion of the pea homologue of *cdc2* (*Pscdc2*) using a novel immunological method to verify clones generated by polymerase chain reaction. DNA sequence analysis reveals perfect conservation of the hallmark "PSTAIR" peptide segment found in all *cdc2* gene products analyzed to date. Southern blot analysis indicates that the *cdc2* gene exists as a single copy in the pea genome. Multiple faint bands observed under less stringent washing conditions may indicate a family of *cdc2*-related genes in pea. Northern blot analysis of *Pscdc2* expression in various pea tissues shows that the 1.35 kb transcript is enriched in mitotic tissues.

586 ACTIVATION OF FLAVONOID BIOSYNTHESIS IN ROOTS OF *V.SATIVA* SSP. *NIGRA* PLANTS BY INOCULATION WITH *RHIZOBIUM LEGUMINOSARUM* BIOVAR VICIAE. Kees Recourt, Leon A. Mur[1], Arjen J. van Tunen[1], Anton A.N. van Brussel Ben J.J. Lugtenberg and Jan W. Kijne. Department of Plant Molecular Biology, Leiden University, Nonnensteeg 3, 2311 VJ Leiden, and [1]Free University, Department of Genetics, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

Flavonoids released by roots of *Vicia sativa* ssp *nigra* (*V.sativa*) activate nodulation genes of *Rhizobium leguminosarum* biovar viciae (*R.l.* viciae). Recently, it has been shown inoculation with infective *R.l* viciae bactena stimulate the release of 8 *nod* gene–inducing flavanones and chalcones (1).

In this study we showed that the additional release of flavonoids is due to an increased flavonoid biosynthesis in roots of *V.sativa*. It appeared that inoculation of *V.sativa* roots with infective *R.l.* viciae bactena, increases the activity of at least two flavonoid biosynthesis enzymes; phenylalanine ammonia–lyase (PAL) and eriodictyol methyltransferase (EMT). By using northern blot hybridization, we showed that *V.sativa* roots contain a chalcone synthase (CHS) gene which is homologous to a CHS gene of *Petunia hybrida*. Upon inoculation with infective *R.l.* viciae bactena, it appeared that the expression of this CHS gene increases. In conclusion, the results suggest that infective *R.l.* viciae bacteria enhance the activity and/or biosynthesis of flavonoid–related enzymes in roots of *Vicia sativa* subsp. *nigra*.

1. Recourt K, Schripsema J, van Brussel AAN, Lugtenberg BJJ and Kijne JW: Inoculation of *Vicia sativa* subsp. nigra roots with *Rhizobium leguminosarum* biovar viciae results in release of *nod* gene activating flavanones and chalcones. Plant Mol Biol (in press).

587 MOLECULAR AND CELLULAR ANALYSIS OF MERISTEM INITIATION. Marta J. Laskowski and Ian M. Sussex. University of California, Department of Plant Biology, Berkeley, CA 94720.

The aim of our research is to determine how groups of cells become organized as meristems, and, in particular, to determine the sequence of events associated with the transition from non-dividing, partially differentiated cells into actively dividing meristems. We have chosen to use the formation of lateral roots in radish (*Raphanus sativus*) as a model for meristem formation. In radish, the formation of lateral roots can be induced by applying auxin to cultured seedlings. The new primordia originate from pericycle cells that are located next to the protoxylem poles. Although the number of lateral roots formed on a given centimeter of parent root increases as a function of time in auxin, even a ten minute incubation in 90 µmol IAA is sufficient to induce the subsequent formation of lateral roots. Preliminary results indicate that a significant increase in cell division activity does not take place until approx. 4 hours later. To determine what, if any, changes in gene expression accompany meristem initiation, we are making a subtracted cDNA library that will be enriched for clones expressed after a brief incubation in auxin. We are specifically interested in those mRNAs whose expression is induced prior to the initiation of cell division.

POSTER ABSTRACTS

588 MOLECULAR ANATOMY OF THE SHOOT APICAL MERISTEM.
Andrew Fleming, George Owttrim and Cris Kuhlemeier
Institute of Plant Physiology, University of Berne, Altenbergrain 21, CH-3013 Berne, Switzerland

The shoot apical meristem is a highly organized tissue that in a precisely determined spatial and temporal order (i) perpetuates itself and (ii) produces leaf primordia. Over the past two centuries numerous theories have attempted to describe and explain the phenomena of apical growth, differentiation and leaf pattern formation. We would like to understand the underlying molecular-genetic mechanisms. In order to define different cell types within the shoot apex we use gene probes as specific molecular markers. We carefully dissected 200 tomato apical meristems and from the resulting tissue (approx. 2 mg) constructed a cDNA library. Reasoning that many genes with interesting expression patterns and functions might not be expressed exclusively in the apex, we tested random clones from the library by Northern blotting and in situ hybridization. The first gene analyzed in this way, HC13, is highly expressed in the adaxial cells of leaf primordia and may be a good marker for primordia development. Additionally, we are determining the expression pattern of genes coding for proteins with known biochemical function. A gene for a lipid transfer protein is constitutively expressed in the meristem, but in primordia and young leaves, expression gradually becomes restricted to the epidermis. Two divergent genes for translation initiation factor eIF-4A are highly expressed in the apex; possible cell-specific expression within the apex is under study.

589 COMPARATIVE STUDY ON SOMATIC EMBRYOGENESIS AND ORGANOGENESIS IN RICE. D.H.Ling and A.Komamine
South China Institute of Botany Guangzhou, China

The characters and the induce condition between organogenesis (O) and embryogenesis (E) of three varieties of indica rice were compared in agar and suspension culture. E callus possessed the structure of distinct layer. The embryoid consisted of coleoptile, coleorhiza and scutellum. The 'O' type callus did not possess the structure of differentiation like E-callus. For the frequency of plant regeneration (PR), the duration of keeping the ability of PR and the number of regenerated plant per callus in E callus were higher, longer and more than that in O-callus respectively. The O-type callus was friable in structure and easier for establishing a fine suspension culture which lost the ability of PR in liquid condition very fast. On the contrary, for establishing a fine suspension culture from E callus was more difficult and spent longer time than that from O-callus. 2,4-D and kinetin could independently induce 'O' and commonly induce 'E' in primary and subsequent culture of rice somatic cells. The regenerated plants of rice could be obtained by the pass way of both 'O' and 'E'. So the conditions of inducing 'O' and 'E' and the characters of 'O' and 'E' in rice were not so specialization like carrot and tobacco. In this experiment we observed the exchanges of 'O' and 'E'-callus each other and O type and E type callus occuring mixturely.

590 THE Ec PROTEIN OF MATURE WHEAT EMBRYOS: THE FIRST PROVEN ZINC METALLOTHIONEIN FOUND IN A HIGHER PLANT. Ichiro Kawashima, Mitsuo Chino and Byron G. Lane. University of Toronto, Department of Biochemistry and University of Tokyo, Department of Agricultural Chemistry

A 'full length' cDNA library was prepared from the bulk mRNA of mature wheat embryos. The library was screened with two sets of mixed ^{32}P-labeled oligonucleotide probes. The sequences of the probes were chosen to correspond to parts of cDNAs that could encode the partial polypeptide sequence that was previously determined by Edman degradation of the Zn-containing Ec protein [Lane *et al., Biochem. Cell Biol.* 65, 1001-1005 (1987)]. The two oligonucleotide probes that were employed corresponded to amino-acid residues 2-8 (Cys-Asn-Asp-Lys-Cys-Gly-Cys) and 35-41 (Cys-Gly-Cys-Gly-Glu-His-Cys). With allowance for a G/C preference in the third positions of anticodons in other wheat-embryo mRNAs, one probe encoded amino-acid sequence 2-8: (5')TGCAACGACAAGTGC(T)GGG(A,T,C)TG(3'), whereas the other probe was complementary to the sequence that encodes amino acids 35-41: (5')CAGTGCTCGCCG(A)CAG(A,T,C)CCG(A)CA(3'). Eleven positive signals were detected in a screen of 10^5 plaques in the cDNA library. The DNA inserts from all positive plaques were sequenced. All encode 5'-untranslated regions, 3'-untranslated regions, and open-reading frames (81 amino acids) very similar to the polypeptide sequence previously determined for the Ec protein. The amino acids of all cDNA-encoded polypeptide sequences correspond to at least 56 of the 59 amino acids in the polypeptide sequence determined by Edman degradation of the Ec protein. In every case, the full polypeptide sequences correspond, unmistakably, to those expected of class II metallothioneins. Two different amino-acid sequences, and three different mRNA sequences are encoded in the eleven Ec-Zn metallothionein cDNAs ; the latter three probably correspond to the A, B and D genomes in hexaploid wheat. A ^{32}P-labeled cDNA has been used to isolate a single genomic clone by screening 5 X 10^5 plaques in a wheat genomic library (a generous gift of Dr. Michael Murray, Agngenetics, Madison, WI, U.S.A.).

591 IN VITRO MANIPULATION OF CORN MORPHOGENESIS FOR TRANSFORMATION.
Heng Zhong, C. Srinivasan and Mariam B. Sticklen. Departments of Crop and Soil Sciences and Entomology, Michigan State University, East Lansing, MI 48824-1311. A rapid, large scale corn regeneration system was developed through somatic embryogenesis and axillary and adventitious bud formation from shoot tips of corn (*Zea mays* L.). Shoot tip explants from aseptically-germinated corn seeds and from the precociously-germinated immature zygotic embryos regenerated 20 to 50 shoots per shoot tip within four weeks. More than 500 shoot primordia were regenerated from a single seedling shoot tip within nine weeks of sowing seeds. High frequency somatic embryogenesis was directly induced from multiple shoot cultures or induced from embryogenic calli proliferated from both adventitious shoot cultures and direct-embryo forming cultures. High regenerative competence was observed in each of the 20 corn genotypes tested. By simple manipulation of culture media, corn plants were regenerated from shoot tips, young tassels, and ears at high frequency. The morphogenetic pathways of corn plant can now be controlled *in vitro* to interconvert shoot, tassel and ear from one to another. In addition, multiple ears containing developed ovaries and silks have been induced to form *in vitro* from cultured axillary buds. Experimental control of regeneration, redifferentiation and conversion systems may become important in developmental biology research. This system is readily amenable for genetic transformation of corn using electroporation and Biolistic^{TM} microprojectile bombardment. All meristematically active explant sources (immature tassels, ears, and shoot tips) were used for transformation with several genes using the Biolistic^{TM} microprojectile bombardment and electroporation, and hundreds of putatively transformed corn plants have been regenerated. Transient expression of the reporter gene Gus driven by rice actin regulatory elements has been confirmed in plant. Work is in progress to confirm the stable integration and expression of foreign genes in the corn genome using these techniques.

592 EFFICIENT SOMATIC EMBRYOGENESIS IN SOYBEAN VIA SOMATIC EMBRYO CYCLING. Patricia J. Moore, Wennuan Liu, Glenn B. Collins, Dept. of Agronomy, University of Kentucky, Lexington, KY 40546.

Soybean [*Glycine Max* (L.) Merrill] is the second largest cash crop in the U.S. and is the most important source of oil and high protein meal. Scientists have been working to improve soybean traits such as oil composition and disease resistance. Genetic engineering techniques can be used to improve traits of economically important species, as well as allow scientists to study basic cellular and developmental processes. Manipulation of plants through genetic engineering requires not only the ability to introduce foreign DNA into plant cells but also requires the ability to regenerate plants through tissue culture from transformed cells. Regeneration of soybean (*Glycine max*) has been accomplished through somatic embryogenesis induced on zygotic cotyledon explants by high auxin treatment. Here we describe the establishment of an efficient system for somatic embryogenesis via somatic embryo cycling. This system has dramatically increased both the frequency and number of embryos per responding explant. Our results show that the most important factors for efficient somatic embryogenesis are explant tissue and auxin type. Genotype differences are also observed. Histological studies to determine the origin of the somatic embryos are underway. By increasing the number of somatic embryos induced, we have increased the number of target cells available for transformation. We intend to use this system in the development of an efficient, coupled transformation-regeneration system for *Glycine max*.

593 EMBRYONIC MUTANTS OF ARABIDOPSIS THALIANA ISOLATED FOLLOWING AGROBACTERIUM-MEDIATED SEED TRANSFORMATION. Deena Errampalli, Linda Castle, Leigh Mickelson, Alan Rowlan, Brenda Stewart, Amy Tomlinson, and David Meinke. Oklahoma State University, Department of Botany, Stillwater, OK 74078. Siliques from 2900 transgenic families of Arabidopsis produced by Ken Feldmann following seed transformation with Agrobacterium tumefaciens were screened for aborted seeds. Over 75 families were shown in subsequent generations to be segregating for recessive embryonic mutations similar to those isolated following chemical mutagenesis. Two fusca mutants that accumulated anthocyanin in cotyledons were also found. Mutant families were subsequently analyzed for segregation ratios, patterns of developmental arrest, distribution of aborted seeds in heterozygous siliques, response in culture, nopaline production, ratio of kan^r:kan^s progeny, frequency of kan^r wild-type plants, and patterns of T-DNA insertion. Roughly 30% of these mutants are blocked at the globular stage of embryogenesis; another 30-40% reach a later stage of development, and the remainder are blocked early in development. Interesting phenotypes include the presence of highly abnormal suspensors, embryos with altered patterns of symmetry, mutant seeds with twin embryos, and bloated embryos with altered cell morphology. A putative female gametophytic mutant that produces 50% rather than 25% aborted seeds following self-pollination has also been identified. Previous studies with 18 mutants produced in a similar manner (Errampalli et al., Plant Cell 3: 149-157, 1991) suggest that approximately half of these mutants are tagged with T-DNA. We are currently using the approaches outlined above to identify tagged mutants with interesting patterns of abnormal development that merit further analysis at the molecular level. The long-term goal is to determine the functions of specific housekeeping and regulatory genes during plant embryogenesis.

POSTER ABSTRACTS

594 **MOLECULAR CHARACTERIZATION OF T-DNA TAGGED GENES ESSENTIAL FOR ARABIDOPSIS EMBRYO DEVELOPMENT.**

Linda A. Castle, Leigh A. Mickelson and David W. Meinke. Department of Botany, Oklahoma State University, Stillwater, OK 74078

A large number of *Arabidopsis* genes are essential for normal embryo development. Many different embryonic mutations induced by EMS, X-rays and T-DNA insertion have been identified. These mutations result in phenotypes ranging from zygotic lethals to arrested or distorted globular, heart, linear and mature embryos. Sixteen embryonic (*emb*) genes have been mapped covering all five chromosomes (Patton et al., MGG, in press). Several families segregating for embryonic mutations following *Agrobacterium tumefaciens* infection of seeds have been analyzed at the molecular level for T-DNA insertions consistent with gene tagging (Errampalli et al., Plant Cell 3:149-157, 1991). Two putatively tagged genes, *emb76* and *emb78*, have been well-characterized genetically and are now being further characterized at the molecular level. Embryos homozygous for *emb76* arrest at the globular stage and have abnormally large suspensors. Embryos homozygous for *emb78* accumulate excessive anthocyanin in the cotyledons and fail to survive after germination. T-DNA insertion patterns in both families have been analyzed by hybridization with right border, left border and internal T-DNA probes. Analysis of the complex insertion pattern in *emb76* indicated that right border inverse PCR would not allow plant junction fragments to be isolated. Plant sequences flanking the T-DNA inserts have been isolated by right border plasmid rescue following sucrose density fractionation of *Eco*RI digested genomic DNA from heterozygous plants. Several unusual T-DNA rearrangement products were also isolated. Genomic and cDNA libraries prepared from immature siliques and mature seeds are being used to isolate the wild-type alleles. Data will be presented on T-DNA insertion patterns, gene isolation, sequence analysis and potential roles of these genes in embryogenesis. This work was supported by NSF grant DCB-8905137.

595 EXPRESSION OF FOREIGN GENES IN TRANSGENIC YELLOW-POPLAR PLANTS.

Dayton Wilde[1], Richard Meagher[2], and Scott Merkle[1]. [1]School of Forest Resources and [2]Department of Genetics, University of Georgia, Athens, Georgia 30602.

Yellow-poplar (Liriodendron tulipifera) is a hardwood forest tree species that can be regenerated from suspension culture by somatic embryogenesis. Synchronization of development is enhanced by initiating somatic embryogenesis with a fraction containing small clusters (<140 μm diameter) of meristematic cells. Plasmid DNA with genes encoding GUS and NPT II was introduced into these cells by microprojectile bombardment. Embryogenic suspension cultures were started with the resulting kanamycin-resistant microcalli and transgenic plants were regenerated under constant antibiotic selection. An ELISA for NPT II and a fluorometric assay for GUS showed that the expression of both enzymes varied by less than four-fold among independently transformed callus lines. A histochemical assay for GUS revealed a heterogeneous pattern of staining with the substrate X-Gluc among cell clusters grown in liquid selection medium. A similar pattern of X-Gluc staining has been observed in maize callus stably transformed by plasmids carrying the GUS gene and selectable markers. In transformed yellow-poplar suspensions, expression of GUS and NPT II was detected by quantitative assays both in cell clusters that had reacted positively (blue) and negatively (white) with X-Gluc. Factors other than gene expression must, in some cases, be responsible for negative results in the histochemical assay for GUS in cultured cells (e.g. antioxidants). Somatic embryos induced from transformed cell cultures were found to be uniformly GUS$^+$ by histochemical analysis. All transgenic plants sampled expressed the two marker genes in both root and shoot tissues.

596 SOMATIC EMBRYOGENESIS IN CHICKPEA (CICER ARIETINUM L.)

J.Divakar Rao and G.M.Reddy. Department of Genetic, Osmania University, Hyderabad-500 007. India.

A system for somatic embryogenesis from cotyledons of chickpea (Cicer arietinum L.) was developed. Different combinations of medica, culture conditions were evaluated to optimize the frequency of somatic embryos. Callus was induced on Gamborg's basal medium supplemented with 3% sucrose and different concentrations of 2,4-Dichlorophenoxy acetic acid, but 2 mg/l was found to be optimum for production of embryogenic callus. Mature embryos developed on callus in the presence of 2,4-D (1.0 mg/l). These globular embryos differentiated into mature embryos when transferred to medium lacking growth regulators. Somatic embryos transferred B_5 media supplemented with BAP, however produced shoots. The results would be discussed in the light of recent and relevant literature.

597 REGENERATION IN JUTE SPECIES AND GLYOXALASE-I ENZYME ACTIVITY.

Zeba I. Seraj: Asha B. Sarker; Rakho H. Sarker and Ahmed S. Islam.
1. Department of Biochemistry, University of Dhaka, Dhaka-1000 Bangladesh. 2: Department of Botany, University of Dhaka.

Glyoxalase-I activity of jute callus induced from hypocotyl segments in MS media containing BAP, tyr and nordehydroguarietic acid (NDGA) is significantly lower than that of callus induced in the same media without NDGA and in MS media containing 2,4-D but no NDGA. We achieved multiple regeneration only from calli showing minimum glyoxalase-I activity when the calli was subcultured either one or two times in MS media without hormones. Esterase enzyme patterns for the embryogenic and non-embryogenic jute calli are also different. Cellular morphology of the embryogenic calli show the presence of many starch granules.

598 SECRETED PROTEINS IN PLANT EMBRYOGENESIS.

A.J. de Jong, F.A. van Engelen, M.V. Hartog, P. Sterk, E.A. Meijer, J. Cordewener[1], A. van Kammen & S.C. de Vries. Department of Molecular Biology, Agricultural University Wageningen, Dreijenlaan 3, 6708 HA Wageningen, The Netherlands; [1] Centre of Plant Breeding Research, Mansholtlaan 15, 6708 PA Wageningen, The Netherlands

Somatic embryogenesis in carrot is accompanied by the phytohormone - controlled expression of secreted extracellular matrix glycoproteins (De Vries et al., 1988, Genes & Dev. 2, 462-476). Some of these proteins are associated with the cell walls of non-embryogenic suspension cells (van Engelen et al., 1991, Plant Physiol., in press), while others are exclusively synthesized by embryogenic cells (Sterk et al., 1991, Plant Cell, submitted). By using the glycosylation inhibitor tunicamycin, arrested somatic embryogenesis could be rescued by a secreted 38 kDa cationic peroxidase (Cordewener et al., 1991, Planta, in press), while arrested embryo development in the mutant cell line ts11 (LoSchiavo et al., 1990, Mol. Gen. Genet. 223, 385-393) could be rescued by a single secreted 32 kDa medium protein. These results demonstrate that several secreted glycoproteins are indispensable for correct somatic embryo development. Employing *in situ* localization of mRNAs encoding an embryo-secreted lipid transfer protein (LTP) an identical gene expression pattern was found in somatic and zygotic embryos, indicating that proteins secreted by somatic embryos are also involved in zygotic embryogenesis. Based on morphological examination of arrested and rescued somatic embryos, the biochemical and molecular identification of several secreted proteins and the expression pattern of the encoding genes, we propose a function for secreted proteins in the formation and maintenance of the embryo epidermis by promoting the restriction of epidermal cell expansion. Extracellular matrix proteins which have an effect on embryo development may therefore represent primary target genes for plant growth regulators that affect embryo development.

599 MOLECULAR GENETICS OF OIL BODY MEMBRANE PROTEINS IN BRASSICA NAPUS AND ARABIDOPSIS

C.Batchelder, G.Hübner, J.S.Keddie and D.J.Murphy. The Cambridge Laboratory, Institute of Plant Science Research, John Innes Centre, Colney, Norwich, NR4 7UJ, UK.

Triacylglycerol, the major storage lipid of plants, accumulates as oil bodies in several plant tissues including seed mesocarp, endosperm and cotyledonary cells. In their mature form, oil bodies are surrounded by a protein-lipid coat. A special class of oil-body membrane proteins has been characterised in many oilseeds including important Crucifers, such as rapeseed (*Brassica napus*) and *Arabidopsis thaliana*. Termed oleosins, these proteins constitute approximately 20% of total seed protein and it is thought that they function in protecting and stabilizing the oil body and possibly as receptors for lipase binding. *Arabidopsis* and rapeseed oleosin genomic clones have been obtained by screening genomic libraries with a 5' fragment of the rapeseed oleosin cDNA. Sequence data have been compiled and will be presented.

The aim of this investigation is to elucidate the role of oleosins in seed oil deposition and mobilisation by transforming rapeseed with an antisense construct and cause down-regulation of oleosin gene expression. An antisense approach provides a way of targeting specifically the gene of interest without interfering in other processes. Part of the rapeseed oleosin cDNA will be cloned into a binary vector in both normal and reverse orientations, so that both sense and antisense effects can be studied.

POSTER ABSTRACTS

600 IN VITRO MODEL SYSTEM FOR INVESTIGATION OF DNA DOUBLE STRANDED BREAKES REPARATION BASED ON USING OF HIGHER PLANTS PROTEIN EXTRACTS. LA Sitailo, VD Naumenko, IF Kanevsky, II Bubryk. Institute of Cell Biology and Genetic Engineering, Ukrainian Academy of Sciences, Acad. Lebedeva Str. 1, Kiev-GSP-22, 252650, USSR.

It has been established that the in vitro model system for investigation of DNA double stranded breakes (dsb) reparation is based on using higher plants protein extracts. The system is based on searching the possibility of homologous recombination between two linear pUC-19 DNA fragments. As a result of recombination processes gene lacZ is restored in their structure and recombinants with blue phenotype appear on the media with IPTG and x-Gal.

Dsb in plasmid DNA has been provoked using EcoRI and Bgl I restriction enzymes. The possibility versions of recombination in proposed system by means of substrate ligation and nonhomologous recombination is discussed.

601 **CHARACTERIZATION OF MAIZE HMG-COA REDUCTASE.** Kathryn B. Moore[1], Wendy Woodward[1], Al B. Agellon[1] and Karen K. Oishi[1,2]. University of Arizona, Department of Molecular and Cellular Biology[1] and Department of Plant Sciences[2], Tucson, Arizona 85721. The isoprenoid biosynthetic pathway in plants supplies the major metabolites for the synthesis of vast array of compounds including carotenoids, cytokines, abscisic acid and gibberellic acids. 3-hydroxy3-methylglutaryl Coenzyme A reductase (HMGR), a multi-gene family in dicots, catalyzes the rate-limiting step in this pathway, and has been shown to be differentially regulated during development. We are interested in characterizing the developmental expression of HMGR during maize embryogenesis. Using a tomato cDNA probe homologous to the conserved catalytic domain, we have determined that HMGR is also a multigene family in maize. Southern blot analysis of maize inbred lines *W22* and *1S2P* as well as the recombinant inbred lines *CM37*, *T232*, *CO159*, and *TX303* using the enzymes *BglI*, *BglII*, *EcoRI*, *HaeII*, *HindIII*, *SacI*, *SacII*, *SalI*, *XbaI* revealed very little polymorphism in the restriction fragment patterns, suggesting that the maize HMGR genes are highly conserved. We have isolated genomic clones and are mapping these genes on the maize genome. We have also characterized the activity of HMGR in 4-5 day old shoots and roots as well as 24 hour imbibed embryos.

602 DIFFERENTIATION OF EMBRYOIDS IN SAFFLOWER, Carthamus tinctorius, P.Seeta and S.Y.Anwar, Dept. of Genetics, Osmania University, Hyderabad-500 007, India.

Somatic embryos were developed in cv. Mangira from the cotyledon derived calli on MS medium supplemented with 2 mg/l 2,4-D and 15% v/v coconut milk. The embryoids differentiated into plantlets on MS basal medium.

603 ISOLATION AND CHARACTERIZATION OF THREE HOMEODOMAIN CONTAINING GENES INVOLVED IN FRUIT DEVELOPMENT IN TOMATOES. Neelima R. Sinha and Sarah Hake. University of California, Berkeley and Plant Gene Expression Center, USDA, Albany, CA 94710.

The first reported homeodomain containing gene, Knotted (Kn1), was recently isolated from maize. Using the Kn1 homeodomain as a probe to a 10-12 days post-fertilization fruit cDNA library from tomato we have isolated three homeodomain containing cDNAs. Sequence analysis has revealed that the homeodomains of these genes are very similar to that reported for Kn1, indicating that these genes most likely function as transcriptional factors. The genes are all low to single copy based on Southern hybridizations and show different expression patterns with one common theme - they are all most highly expressed in pre- and post-fertilization gynoecia. We are in a process of mapping these genes to chromosomal locations and looking at expression patterns in tissues to further elucidate the functions of these genes.

604 SOMACLONAL VARIATIONS IN TISSUE CULTURE OF SCILLA SCILLOIDES COMPLEX. Jae-Wook Bang and Hae-Woon Choi. Chungnam National University, Department of Biology, Daejeon, Korea

Chromosome complements of Scilla scilloides complex are composed of two kinds of genomes, A(x=8) and B(x=9). Seven cytogenetic types of genome, AA, BB, ABB, AABB, ABBB, AABBB and AAABBB were found in the natural populations of S. scilloides complex in Korea. To determine the somaclonal variations depending on the cytogenetic types in tissue culture, whole plants were regenerated through somatic embryogenesis from the calli induced from the bulb segments of AA, BB and AABB genome plants, respectively. Callus and shoot were induced on the MS solid medium containing 2mg/l of 2,4-D, 2mg/l of NAA, and 2mg/l of BAP. Calli were initiated in 10 days of culturing. Actively growing shoots were placed on the hormone free MS medium for rooting. It took for 6-7 weeks to get a whole plantlets regenerated from the bulb segment cultures. Calli and regenerants were analysed cytogenetically. Chromosomal variations in numbers and structures such as aneuploids, chromsomal breakage, and dicentric chromosomes were observed in the cells of calli and regenerants. The frequencies of chromosomal variations regenerants were lower than those in the callus cellls. Phenotypic variations in the growth of rate of callus, leaf numbers and the morphologies in shoot and root depending on the cytogenetic types of genome were found. Variation in isoenzyme pattern were also detected in the regenerants. It is suggested that tetraploid(AABB) is more stable than diploids(AA or BB) in tissue culture of S. scilloides complex

605 PLANT REGENERATION FROM EMBRYOGENIC CALLI OF TRITICALE. G. Padmaja and G. M. Reddy, Osmania University, Hyderabad-500. 007, A.P., INDIA.

Calli derived from mature embryos exhibited embryogenesis on LS medium supplemented with 2 mg/l 2,4-D. Somatic embryos were observed on the surface of the soft friable calli at the end of 6-8 weeks. Development of somatic embryos was observed on LS medium with lower levels of 2,4-D (1.0 mg/l). Germination of somatic embryoids was achieved with a frequency of 21.82% on LS Basal medium and on LS medium with 0.1 mg/l Kinetin and 1% mannitol, it increased to 34.63%.

POSTER ABSTRACTS

606 HORMONES INFLUENCE TO THE PROCESS OF DIFFERENTIATIO: IN CALLUS TISSUE OF COTTON. G.B.Nasyrova, L.N.Gryglna, N.P.Cherkasnina, S.A.Dzhatayev, L.G.Holmuratov and A.Abdukarimov. A.S.Sadykov's Institute of Bioorganic Chemistry, Academy of Sciences Uzbek SSR, Tashkent, USSR.

Process of callus formation and differentiation in tissue culture of cotton cv.S-4727, S-4880 and line L-22 of G.hirsutum L. and cv.2906 of G.arboreum L. were studied. Callus tissue was induced from hypocotyls upper pieces of 5 days old sterile seedlings. Mediums for induction and subculturing contained salts and vitamines according to MS, but differed by hormones combinations and quantities: different combinations of NAA and kinetin and 2,4-D and kinetin were used. Intensivity of callus formation and differentiation in callus tissue of tracheids and meristemoids for every sort and line was very specific and was determined by hormones combinations and concentrations.

607 CHARACTERIZATION OF TRANSGENIC PAPAYA PLANTS. MM Fitch[1], RM Manshardt[1], D Gonsalves[2], JL Slightom[3], JC Sanford[4]. [1]Dept. of Horticulture, University of Hawaii, Honolulu, HI; [2]Dept. of Plant Pathology, Cornell University, Geneva, NY; [3]The Upjohn Company, Kalamazoo, MI; [4]Dept. of Horticultural Sciences, Cornell University, Geneva, NY.

Stable transformation of papaya has been achieved via particle bombardment and with *Agrobacterium tumefaciens*. A chimeric gene for the coat protein of papaya ringspot virus (PRV) flanked by the selection gene *kan* and the reporter gene *gusA* on a binary cosmid vector derived from pGA482 was transformed into papaya. Our objectives were 1) to develop a papaya transformation system and 2) to produce papaya cultivars with genetically engineered virus resistance. Three types of tissues were treated. Embryogenic zygotic embryos pre-treated with 2,4-D yielded 2.3 times more transgenic isolates than did cultures of embryogenic callus when transformed with the particle gun. Cultures prepared from freshly explanted hypocotyl sections did not produce transgenic plants. Plant regeneration was greatest in the zygotic embryo cultures. Sixty-five percent of the zygotic embryo isolates from particle bombardment regenerated plants, whereas only 20% of the embryogenic callus isolates regenerated plants. Regeneration from embryogenic callus may be inhibited by long-term (6 mo.) culture with 2,4-D prior to bombardment. All of the plants were positive for NPTII sequences amplified with PCR. About one third of the plant isolates expressed GUS and all GUS-positive isolates except one were positive for the coat protein gene of PRV in PCR assays. Southern hybridizations of genomic DNA probed with PRV sequences confirmed the PCR data. Fragmentation of plasmid DNA may have occurred with particle bombardment. Coat protein could not be detected by ELISA. Fewer transgenic isolates were recovered following *Agrobacterium*-mediated transformation, but, unlike particle-gun-derived isolates, all isolates apparently contain the full complement of genes targeted to the plant cells. Plants of a single transgenic line tested for virus resistance in the greenhouse have shown resistance.

608 PRIMARY AND SECUNDARY SOMATIC EMBRYOGENESIS IN CASSAVA. C. Raemakers (1/2), H. Amati (1), G. Staritsky (2), E. Jacobsen (1) and R. Visser (1). Department of Plant Breeding (1) and Tropical Crop Science (2), Agricultural University Wageningen; P.O Box 386, 6700 AJ Wageningen, Netherlands.

A procedure has been established to obtain routinely somatic embryos and plants from cassava. Four stages can be discerned: stage 1: induction of embryos, stage 2: germination of embryos, stage 3: shoot development of germinated embryos (GE) and stage 4: rooting of plants. With thirteen of the fifteen tested clones originating from Africa, South America and Indonesia GE and plants were obtained.
Leaf lobes of greenhouse grown plants gave the best results, but the efficiency of regeneration depended on the growing conditions of the plants. For example M. Col 22 formed GE on 5-80 % of lobes of greenhouse grown plants whereas 10-30 % GE were formed on lobes of in-vitro grown plants.
Between 50-90 % of the primary somatic embryos formed new (secundary) embryos. Evan after 8 cycles (stage 1——>stage 2——>stage 1 etc.) the GE retained their full embryogenic capacity and were able to develope in morphological normal cassava shoots.
Experiments directed at improving the multiplication factor between the different cycles are performed with the final goal to obtain (single cell originating) embryogenic cultures.

609 BASAL DELETIONS IN EMBRYONIC PATTERN MUTANTS OF *ARABIDOPSIS*. T. Berleth & G. Jürgens. Institut f. Genetik, Universität München; Maria-Ward-Str. 1a; D-8000 München 19; FRG

A number of *Arabidopsis* mutants show severe pattern alterations in the basal half of the seedling which reflect deviations from wild type development early in embryogenesis. Interestingly, complete deletions of root structures are usually associated with an almost complete reduction of the hypocotyl. In spite of overall similarities these mutants fall into two major phenotypic groups: *möwe* and *monopteros*.

In *möwe* mutants the root and most of the hypocotyl up to the anthocyan ring is deleted. Two cotyledons and the epicotyl are always present and usually normal. All *möwe* mutants so far tested complement each other. By contrast, twelve mutants of the *monopteros* phenotype are alleles of one gene, also called *monopteros* (*mp*). In the mutant seedlings root and hypocotyl are replaced by a basal hook; two cotyledons can be formed giving the seedling a *mowe*-like appearance. In all *mp* lines, however, there is a considerable proportion of mutant seedlings which show various degrees of cotyledon fusion. In the latter case the entire seedling architecture is altered and the epicotyl is deleted. A chromosomal walk to clone the *monopteros* gene has been initiated.

610 DIFFERENTIAL GENE EXPRESSION IN CARROT SOMATIC EMBRYOS IS ACCOMPLISHED THROUGH TRANSLATIONAL CONTROL. Nestor Apuya and J. Lynn Zimmerman, University of Maryland, Department of Biology, Baltimore, MD 21228.

We have isolated two genes whose expression is enhanced in the globular stage of somatic embryogenesis of carrot. These two genes encode a translation Elongation Factor (EF-1α) and the β-subunit of the mitochondrial ATP synthase (ATP-2) in carrot, and were identified by screening a cDNA library constructed from polysomal mRNA from globular embryos. A comparison of polysomal mRNA from callus cells, globular embryos and plantlet stage embryos reveals that the abundance of the EF-1α mRNA in globular embryo polysomes is 2X greater than in polysomes of callus cells and 20X greater than in polysomes of plantlet stage embryos. A parallel analysis of the ATP-2 gene reveals that this mRNA is 3X greater in polysomes of globular embryos than in callus polysomes and 30X greater than in plantlet polysomes. At the same time, however, the abundance of either mRNA is equivalent in the total RNA from callus cells, globular and plantlet stage embryos. These results reveal that the developing globular embryo is capable of selectively translating specific mRNAs presumably required for or important in early embryogenesis, and that these mRNAs would never be identified by differential screening using total RNA. Moreover, they highlight the potential significance of translational level control in directing the important developmental program of plant embryogenesis.

611 Structure, function & subcellular targeting of oleosins during embryogenesis in oilseeds.

D.J.Murphy, I.Cummins, Li Ming, M.J.Hills, G.Hubner, J.S.Keddie, J.Ross. Cambridge Laboratory, Institute for Plant Science Research, John Innes Centre, Colney Lane, Norwich, NR4 7UJ, Norfolk.

Oleosins are a class of low molecular weight hydrophobic proteins found in the oil-bearing tissues of plants. To date, some 12 oleosins have been sequenced. In all cases, the proteins are made up of a highly conserved 70 residue central hydrophobic domain, flanked by polar C- and N- terminal domains which are variable both in length and sequence composition. The secondary structure of rapeseed oleosins has been analyzed using circular dichroism and Fourier-transform infra-red spectroscopy. It is concluded that the proteins are largely made up of an unusual hydrophobic ß-strand structure, with relatively little α-helical content. A structural model is proposed whereby the oleosin *in vivo* is embedded in storage oil droplets by means of a 70-residue hydrophobic ß-strand domain while the polar terminal regions are aligned at the oil-water interface. This model is supported by data from antibody/proteolysis studies. Data will also be presented to support a novel method for the subcellular targeting of oleosins, via the endoplasmic reticulum, to oil bodies.

POSTER ABSTRACTS

612 ISOLATION OF AN *ARABIDOPSIS WNT-1* HOMOLOG. Ian Brady,[1] Tom Brady[1], J.H.N. Schel[2] and N.C.A. de Ruijter[2] (1) Hamilton College, Department of Biology, Clinton, NY 13323 (2). Agricultural University, Department of Plant Cytology and Morphology, Wageningen, The Netherlands.

A putative *Arabidopsis thaliana* gene (*Awnt-1*) has been isolated from an *Arabidopsis* genomic DNA library. Despite the diversity of eukaryotes, there is considerable evidence of highly conserved DNA regions. The *wnt-1* family of genes exhibits a high degree of sequence homology and seems to be involved in embryonic development in a number of species. In mouse, *wnt-1* and *wnt-3* expression is found in the developing neural tube; in *Drosophila*, *Dwnt-1 wingless* is essential for segmentation; and in *Xenopus*, *Xwnt-1* is first detected at the early neurula stage. Using mouse *wnt-1* and *wnt-3* and *Dwnt-1* cDNAs (R. Nusse, Netherlands Cancer Institute), we probed for homologous sequences in plants which, we hypothesized, might also exhibit a role in early development. Accordingly, we hybridized digoxigenin labelled sense and anti-sense *wnt-3* and *Dwnt-1* RNA to EcoR1 digested maize and *Arabidopsis* DNA. Under moderate stringency conditions, both probes hybridized to 3 bands of maize and 2 of *Arabidopsis*. We extracted RNA from leaves, stems, fruits, embryos, etc. from both maize and *Arabidopsis* and hybridized dig-labelled anti-sense RNA to slot blots of the RNAs. We detected hybridization to leaves and roots of *Arabidopsis* and to stems and 2mm embryos of maize. An initial screening of an *Arabidopsis* genomic DNA library with the *wnt* probes detected one strongly hybridizing phage with a 13kb insert. We are currently rescreening the library for other possible homologs prior to sub cloning and sequencing to determine the degree of homology to the *wnt* family.

613 TISSUE AND DEVELOPMENTAL PATTERNS OF DNA METHYLATION IN THE CARROT *Dc-8* AND rRNA GENES. Shujun Chang, Ronald J. Newton, Jane Magill[2], and Clint Magill[3], Texas A&M University, Departments of Forest Science, Biochemistry and Biophysics[2] and Plant Pathology and Microbiology[3], College Station, TX 77843

Differences in methylation of carrot DNA were revealed using four pairs of restriction endonuclease isoschizomers to digest DNA isolated from roots, leaves and embryogenic callus. Probes for the *Dc-8* locus, (an ABA responsive gene usually expressed only in embryonic tissues) and the 5.8s rDNA genes were used on Southern blots to detect differential methylation. No methylation at GCGC sites (Cfo I/Hha I) was found in any tissue. Extensive methylation was found at GATC (Sau 3A/Mbo I) and CC(A/T)GG (EcoR II/BstN I) sequences with both probes in all tissues. However, evidence for allele-specific and tissue-specific methylation of the inner C in CCGG sites in root and leaf but not callus DNA was revealed by differences in the sizes of fragments produced following Hpa II and Msp I digestion.

614 MOLECULAR ANALYSIS OF AN AUXIN-INDUCIBLE EMBRYO MARKER PROTEIN FROM PEA. H.-J.Jacobsen[1], Susanne Stirn[2], Albert J.Robertson[3] and Sandra Altherr[1]. [1]Universität Bonn, Institut f. Genetik, FRG, [2]Universität Hamburg,Institut f. Allgemeine Botanik,FRG, [3]University of Saskatchewan, Crop Develop. Centre, Saskatoon, Canada

In many plant species, auxins have been found to trigger the transition of competent somatic cells to somatic embryos, while in later stages auxins inhibit embryo development. In pea it is possible to study the induction of somatic embryo formation *ab initio*, since the transfer of competent tissues to the auxin-containing induction medium is clearly defined. Using a differential hybridoma screening, we selected monoclonal antibodies against embryo-specific proteins. One of the antibodies recognizes a soluble 50 kD-protein expressed in early somatic embryogenesis of pea and some other mono- and dicot plants. This protein is also found in meristematic plant tissues. The pI of the protein is 5.8-6.0, and it can be isolated from nuclei. The protein is not glycosylated, and represents neither actin or tubulin nor is it a typical storage protein. Its expression is strongly enhanced within 2-6 hours in freshly isolated pea protoplasts treated with embryo-inducing concentrations of 2,4-D or Picloram.

615 CELL CYCLE CONTROL AND INDUCTION OF EMBRYOGENESIS IN HORMONE SHOCKED SOMATIC CELLS. Dénes Dudits, János Györgyey, László Bögre, Tamás Kapros, Kinga Németh, Ivan Stefanov, László Bakó. Institute of Plant Physiology, Biological Research Center, 6701 Szeged, HUNGARY

Synchronized cells and culture systems for induction of somatic embryogenesis are used to identify component of cell cycle control in auxin-induced reactivation of cell division. Using immunological detection methods we provide evidences for the existence of maturation promoting factor (MPF) as a complex between cdc2 and cyclin in alfalfa cells. The histone H1 kinase activity of the complex is variable during cell cycle. We also describe differential expression of histone H3 variants, cdc2 and other kinase genes during synchronous cell division and development of somatic embryos. Based on 3'end specific probes the Northern analysis indicated S phase specific expression for H3c-1 gene. The other H3c-11 is expressed in all cell types and the level of expression is influenced by auxin treatment or protoplast isolation procedure. Both of the histone H3 genes are expressed in somatic embryos. The expression of cdc2 genes is also under transcriptional control. Auxin treatment can cause a fluctuation in amount of cdc2 transcripts. With the help of the described molecular markers we propose a general concept about the central role of auxin stimulated signal transduction and induction of cell division in transition of somatic cell into embryogenic ones.

616 EMBRYO- AND LIGHT-SPECIFIC REGULATORY ELEMENTS OF A CAB GENE OF SOYBEAN. Cynthia S. LeVesque and Linda L. Walling. University of California, Department of Botany and Plant Sciences, Riverside, CA 92521

In soybean, the chlorophyll A/B binding protein gene, Cab3, encodes 33% of the Cab mRNA in leaves and 47% of the Cab mRNA in developing embryos. The expression of Cab3 in these organs is primarily regulated at the level of transcription. To identify leaf- and embryo- specific DNA-protein interactions, nuclear proteins were isolated from leaves, roots, 35 DAF embryos, and 85 DAF embryos and utilized in gel mobility shift assays with overlapping subclones of the 5' flanking region of Cab3 These experiments have identified several embryo-specific, as well as leaf- and root-specific complexes. Complexes are specific for each tissue and strictly correlate with the transcriptional activity of Cab3. Several fragments form unique complexes with each of the nuclear protein extracts suggesting that regulation of this gene is an intricate process. Methylation interference assays are being employed to further delineate the cis-acting elements involved. In addition, iodination and UV crosslinking experiments are being attempted to identify the corresponding trans-acting elements.

617 Transformation of indica rice (IR54) protoplasts. Jianying Peng, Halina Kononowicz, Thomas K. Hodges. Purdue University, Department of Botany and Plant Pathology, West Lafayette, Indiana 47907, U.S.A. We have established a system to genetically engineer indica rice protoplasts *via* PEG-mediated transformation. In order to obtain transgenic plants, protoplasts isolated from suspension cells of an indica rice variety IR54 were co-transformed by polyethylene glycol (PEG) treatment with two plasmids, one is pKAN containing the *neo* gene and the other pPUR containing the *gusA* gene. Protoplast cultures were grown on medium with feeder cells before being transferred to selection medium supplemented with G-418. Transformed calli were readily recovered at the end of 2 to 3 weeks in selection medium. After being transferred to regeneration medium, calli resistant to G-418 regenerated plants in several separate experiments. These plants have grown to maturity in a growth chamber. Southern blot analysis of DNA isolated from leaves of putative transgenic plants verified the presence of the transferred *neo* and *gusA* genes in the plant genome. Inheritance of the introduced foreign gene was obtained in progeny through backcrossing the R0 plants to untransformed plants. Study of gene expression showed that CaMV 35S-GUS gene was active in all the organs examined, with highest expression in roots and the lowest in leaves. Results from histochemical analysis for GUS activity in different tissues will be presented.

POSTER ABSTRACTS

618 SPATIAL AND TEMPORAL EXPRESSION PATTERNS DIRECTED BY THE T-DNA GENE 5 PROMOTER DURING SOMATIC EMBRYOGENESIS IN CARROT

Jim Mattsson, Chumpol Borkird and Peter Engström
Dept. of Physiol. Botany, University of Uppsala Box 540, 75121 Uppsala, SWEDEN

We have analyzed the patterns of expression of a β-glucuronidase (GUS) gene fused to the promoter of the Agrobactenum tumefaciens T-DNA gene 5 during embryogenesis in carrot, Daucus carota, L.. Gene expression was monitored by a histochemical assay of β-glucuronidase activity. The gene 5 promoter, although of bacterial origin, conferred expression to the marker gene in all stages of embryo development. The patterns of expression, however, differed between embryos in different stages of development. In the globular stage expression was confined to the basal part of the embryo, suggesting that the promoter is sensitive to regulatory functions active in the primary establishment of polarity in the radially symmetric globular embryo. In the heart and torpedo stages of development GUS expression was high in the entire embryonic axis, but not in the cotyledons. During germination expression was reduced in the elongating hypocotyl and radicle, and high levels of expression were detected only in the regions surrounding the shoot and root menstems. This pattern suggests that the promoter responds to factors that promote transcription in relation to active cell division.

619 PLANT REGENERATION AND TRANSFORMATION WITH PROTOPLASTS OF TWO JAPONICA RICE VARIETIES SUSCEPTIBLE TO BARLEY YELLOW DWARF VIRUS.

Fujiang Wen, Jianying Peng, Richard M. Lister, Jeffrey R. Vincent, Brian A. Larkins* and Thomas K. Hodges. Purdue University, Department of Botany and Plant Pathology, West Lafayette, Indiana 47907, and * University of Arizona, Department of Plant Sciences, Tucson, Arizona 85721 U.S.A.

We are interested in obtaining resistance to barley yellow dwarf viruses (BYDV) in cereals, by transformation with cDNAs encoding the viral coat protein. To this end we have regenerated plants from protoplast cultures of two susceptible japonica rice varieties, Radon and Baldo, and established an efficient transformation system for them *via* direct DNA uptake. Embryogenic calli developed from immature embryos were used to establish cell suspensions. Protoplasts isolated from these suspensions were cultured in Kao medium containing IR 52 or IR 45 rice cell suspensions as nursing cells. The protoplasts grew vigorously and developed into embryogenic calli within 2-3 weeks. Plants were regenerated from 21-35% of the calli within 1-3 weeks after transferring to regeneration medium. Each of three plasmid constructs, pPUR, pAct-GUS, and pAciGUS, all containing the *gusA* gene but driven by the CaMV 35S, rice actin, or maize *Adh*-1 promoters, respectively, were used together with pKAN (containing the *neo* gene) for co-transformations of protoplasts of Baldo rice by PEG-mediated DNA uptake. After selection on media containing the antibiotic G-418, about 21% of the surviving calli showed GUS activity. Fluorometric assays revealed that all constructs expressed *gusA* in the transformed calli, with pAct-GUS expression being highest and pPUR lowest. Protoplasts of both Radon and Baldo have been used in co-transformations using cDNAs representing BYDV coat proteins and pKAN. One hundred and fifty Radon plants have been regenerated from G-418-resistant calli. Screening for the coat protein cDNA in these plants is in progress.

620 EFFICIENT PLANT REGENERATION AND TRANSFORMATION OF CREEPING BENTGRASS (*AGROSTIS PALUSTRIS* HUDS.).

Heng Zhong, Mark G. Bolyard, C. Srinivasan and Mariam B. Sticklen. Departments of Crop and Soil Sciences and Entomology, Michigan State University, East Lansing, MI 48824-1311. We have established a efficient plant regeneration system via somatic embryogenesis in an economically important turfgrass, *Agrostis palustris* Huds. The effects of 2,4-D or dicamba with or without BA on callus initiation, growth, and embryogenesis from mature caryopses were evaluated. In the presence of 30 μM dicamba, all concentrations of BA tested significantly enhanced both embryogenic callus formation and embryogenic efficiency. The highest frequency of embryogenic callus formation and embryogenesis was achieved on medium containing 30 μM dicamba and 2.2 μM BA. Over 80% of the somatic embryos matured and formed plantlets after transfer to half-strength MS basal medium. We used this regeneration system to transform turfgrass using microprojectile bombardment. Embryogenic callus was bombarded with the plasmid pAct1-F (McElroy et al. 1990, Plant Cell, 2: 163-171) containing the Gus gene driven by rice regulatory elements. Histochemical analyses revealed regions of GUS activity in 19 out of 22 bombarded embryogenic calli, but not in controls. The presence of Gus gene was demonstrated in 5 out of 15 plants tested by Southern blot hybridization of polymerase chain reaction (PCR) amplified DNA from leaf and stem samples. Southern analyses of genomic DNA showed the presence of positive signals in 3 out of the 5 plants tested. Detection of GUS RNA using reverse transcriptase, Replinase™ and PCR revealed GUS gene transcription in 1 out of 5 plants tested. Western analysis of this transformed plant revealed a cross-reacting band when using an antibody to Gus, indicating expression of b-glucuronidase. This transgenic plant was acclimated to the greenhouse within three months of culture initiation. Plasmids containing a *Bacillus thuringiensis* (BT) toxin gene specific for lepidopteran pest resistance, an EPSP synthase gene for glyphosate resistance, or an ALS gene for chlosulfuron resistance have also been introduced into turfgrass. Work is in progress to confirm the integration and expression of these foreign genes in regenerated plants.

621 GENE TRANSFER AND DEVELOPMENT OF TRANSGENIC PLANTS OF RICE

Koulin Ge, Jinshui Yang, Ming Lao, Yunzhu Wang, Bei Wang, C.C.Tan
Fudan University, Shanghai 200433, P. R. China

Two suspension cell lines of rice were used as recipient systems for gene transfer of pBI121 containing report genes GUS and NPT-II, in partially digestion by enzymes. We found that blue cell was not observed in protoplast electroporation and considerable protoplasts smashed when the voltage was adjusted to higher 250 v/cm; however, partially digested small cell groups could torelate and the percentage of blue cells reached up to 0.30 at the 2250 v/cm. Some transformant calli were screened out by selective medium N_6 with 100mg/l Kanamycin. NPT-II dot autoradiograph assay was carried out for detection of NPT-II enzyme activity, in transformant calli of H124-14, which proved that the NPT-II gene had been transcripted and translated successfully. Southern blot of transformant calli of H124-4 with GUS probe showed that hybridized bands were obvious. A lots of transformant calli of H124-4 selected on selective medium were transferred onto N_6 medium added with ZT 3 or 5 mg/l, NAA 0.01 mg/l, sucrose 2 or 3% as differentiation medium and 38 plants were regenerated after culture for 130 days, from which one was albino and 40% are transgenic plants. Furthermore, we have also worked on some experiments about novel gene transfer system by means of electrophoresis pulse with lower voltages and embryo-callus as recipient. Some results were positive and exciting.

622 ANALYSIS OF BASIC PROTEINS FROM BARLEY ENDOSPERM

Ragnar Flengsrud, Institute of Biotechnological Sciences, Section of Chemistry, Agricultural University of Norway, P.O. box 40, N-1432 ÅS-NLH, Norway.

Proteins from barley aleurone and starchy endosperm were separated by 2-dimensional electrophoresis in the NEPHGE system. Seeds were from the barley cultivars Bomi and Carlsberg II and from the induced Carlsberg II mutant Risø 5678 line, and in addition, from two lines which both carry the Risø 5678 mutant allele. Tissue-specific proteins were detected in both tissues of all lines studied. Two of the major proteins in starchy endosperm of Bomi and of one of the mutant carrier lines were found in less amounts in the aleurone and could not be detected at all in neither of the tissues of the other lines. The separated basic proteins were blotted onto membranes for sequencing and the line-specific proteins showed hydrophobic properties. Parts of the N-terminal sequence have been obtained for one of the two proteins as well as for some additional proteins. This work is now in progress.

623 *ARABIDOPSIS* MUTANTS THAT DOWN REGULATE THE EXPRESSION OF A SOYBEAN SEED STORAGE PROTEIN GENE.

Satoshi Naito, Koji Goto, Masami Yokota Hirai*, Hirokazu Tsukaya and Yoshifumi Komeda. Molecular Genetics Research Laboratory and *Faculty of Agriculture, The University of Tokyo, Tokyo 113, Japan.

A β-glucuronidase (GUS) fusion was constructed using a 0.9kb 5' region of the gene encoding the α' subunit of β-conglycinin, a 7S seed storage protein of soybean, and was introduced into *Arabidopsis*. GUS activity in the seeds of the transgenic *Arabidopsis* was very high while the activity was at background level in leaves and roots. The GUS protein is so stable that cotyledons in 10-day-old seedlings retained ca 10% of the GUS activity found in the mature seeds. Thus, by assaying GUS activity in one cotyledon of a seedling, the expression of GUS in that seed can be estimated. This provides a means of isolating *Arabidopsis* mutants that do not express GUS in the seeds. The transgenic *Arabidopsis* seeds were mutagenized and M2 seedlings were screened for low GUS activity in the cotyledons. After screening 17,000 M2 seedlings, we obtained about 40 mutants with low GUS activity. By making test crosses to parental transgenic strains and to wild type (nontransgenic) strain, we identified 18 mutants that down regulate GUS activity in the seed *in trans*. The level of GUS mRNA in immature seeds of these mutants paralleled residual GUS activity in the seeds, indicating that these mutations affect the accumulation of GUS mRNA. Preliminary results showed that when the mutants were crossed with another transgenic *Arabidopsis* line carrying an intact α' gene, accumulation of the α' subunit segregated in the F2 progeny, suggesting that the mutation affects activity of the α' subunit promoter.

POSTER ABSTRACTS

624 ANALYSIS OF mRNA ACCUMULATION IN PRECOCIOUSLY GERMINATING *BRASSICA NAPUS* EMBRYOS. Sherryl R. Bisgrove[1], Martha L. Crouch[1], F. Rudolf Turner[1], and Donna E. Fernandez[2]. [1]Dept. of Biology, Indiana University, Bloomington, IN 47405, [2]Dept. of Botany, University of Wisconsin, Madison, WI 53706.

Although embryogeny and germination are normally separated temporally by a period of developmental arrest, precociously germinating embryos exhibit features of both stages. In *Brassica napus*, immature embryos of various ages can be excised from developing seeds and will precociously germinate when cultured on basal medium. Embryos of all ages produce a radicle and initiate growth at the shoot apex; however, young embryos (38 DPA) produce a series of secondary cotyledons, older embryos (47 DPA) produce a mixture of secondary cotyledons and leaves and nearly mature embryos (60 DPA) produce leaves. Since younger embryos retain more embryonic characteristics than older embryos, it seems possible to turn off or on parts of each program independently. We are examining the extent of overlap at the molecular level by culturing embryos of different ages and monitoring the levels of mRNAs characteristic of either embryo maturation (storage protein) or germination (Harada *et al.* 1988, Mol. Gen. Genet. 212: 466-473). We have isolated mRNAs from different organs at various times after the initiation of germination. The analysis of these mRNAs will allow us to determine whether the programs are expressed in the same pattern throughout the embryo or in different ways in different organs. These data should provide insight into the action of factors involved in regulating the "switch" between embryogeny and germination. (Supported by NSF DCB-8616198).

627 BREEDING NEW VARIETY OF SWEET CORN WITH BACK CROSS TRANSFORMATION METHOD. Huang Bingsheng, The Research Institute of Agricultural Science of Huayin, 223000.

Sweet corn is being developed rapidly in China. We have transformed our country's fine inbred line and synthetic variety into ones having gene of sh_2, su_1 and se. It is a useful approach to create new type hybrid of sweet corn by means of combing the new inbred lines. Such as extra sweet corn Yangtian No.1 (sh_2synthetic variety), Miyu No.4 (sh_2hybrid), Huaition No.8 and Huaition No.10 (hybrid with gene of se) and Huaition N0.6 (su_1hybrid). These hybrid and synthetic variety have greater adaptability, higher yield, resistance against the leaf deseases and better quality. Which have been put into production in China. In the practical transformating hybrid with gene of se, we have explored a new technique to identify hybrid with the background of gene of se. It is suggested that a new breeding method using gene of se in breeding comes into being.

625 ARTIFICIAL SEEDS MADE FROM POTATO TRANSFORMED WITH THE THAUMATIN GENE. Meizhu Yang, Mulian Den and Zhangliang Chen. National Laborory of Protein Engineering and Plant Genetic Engineering, Biology Department, Peking University, Beijing 100871, China

The patato (Solanum tuberosum, cultivars 85T-14-3 and 86-2) tuber and in vitro-grown microtuber discs was transformed with Agrobacterium containing the gene encoding thaumatin, a sweetening protein in its Ti-plasmid. The transgenic potato were obtained on the medium containing 100mg/l kanamycin and the expression of the gene was detected with Southern and Northern blots. The transformation efficiency reaches as high as 60% for microtuber discs and 75% for tuber discs. In order to seek an alternative and convenient way to introduce transgenic potato plants to the field in long distance, we have applied the new techniques of artificial seed to the transgenic potato plant. The somatic embryoes of transgenic potato were capsulized by artificial seed coat. The germination rate was 100% on the sterilized medium and 96% in the soil. We obtained about 91% of normal plants from these germinated seeds and the expression of thaumatin gene was detected in most of these potato plants. This has provided a practical way for application of transgenic plants such as virus-resistance in agriculture.

628 CHARACTERIZATION OF THE MAIZE *Vp7* GENE. Karen K. Oishi. University of Arizona, Department of Molecular and Cellular Biology, Department of Plant Sciences, Tucson, Arizona 85721. The maize *Vp7* gene is involved in the cyclization of the carotenoid, lycopene to the beta- and alpha-carotene in the maize carotenoid/abscisic acid biosynthetic pathway. Biochemical analysis of *vp7* mutant seeds has shown a preferential accumulation of lycopene and deficiency of abscisic acid in the mature embryo (1). In order to investigate the regulation of expression of genes involved in the biosynthesis of carotenoid/abscisic acid during embryogenesis, the *Vp7* gene was isolated by transposon-tagging cloning with the maize transposon, *Mutator*-1. Twelve mutant alleles of *vp7* isolated from *Mutator* lines have been found to contain 2 insertions; *Mu*1 and 2.3 kpb, 1 deletion: 0.7 kbp and 4 RFLP markers which segregate with the mutant allele. The *Vp7* gene is present as a single copy gene encoding a 2.9 kbp transcript. The VP7 transcript is present in 28 DAP kernels, young seedling shoots and roots, and seedling leaves. A VP7 cDNA clone will be isolated from a cDNA library constructed from young seedling shoots and characterized by sequence analysis.

(1) S.J. Neill, R. Horgan and A.D. Parry (1986) Planta 169:87-96.

626 BARLEY α-AMYLASE INHIBITOR LOCALIZATION IN DEVELOPING AND GERMINATING KERNELS. Sharon M. Gubbels[1], Alexander W. MacGregor[3], Michael J. Sumner[2] and Robert D. Hill[1]. University of Manitoba, Depts. of Plant Science[1] and Botany[2], and Canadian Grain Commission[3], Winnipeg, Manitoba, Canada R3T 2N2

The biological role of a proteinacious α-amylase inhibitor, which inhibits both barley α-amylase and bacterial subtilisin *in vitro*, was investigated in developing and germinating barley (*Hordeum vulgare* cv. Bonanza) kernels by comparing the location of the inhibitor to that of both α-amylase and starch granules, using immunocytochemistry and polarizing microscopy. α-Amylase inhibitor and starch granules, but not α-amylase, were found in endosperm, scutellum, and coleoptile of developing kernels. α-amylase inhibitor, which was dispersed in the scutellar parenchyma cells of mature, uninbibed kernels, becomes associated with the starch granules during the first 3 days of imbibition (DI). α-Amylase inhibitor also associates with starch granules in both coleoptile and root at this stage. α-Amylase was detected in direct association with starch granules in the scutellar parenchyma after 2 DI and in the coleoptile parenchyma after 4 DI. α-Amylase and α-amylase inhibitor are abundant in the endosperm adjacent to the scutellar epithelium and the adjoining aleurone. The location of both proteins moves towards the central and distal portions of the grain as imbibition continues. These results indicate that the α-amylase inhibitor protein is closely associated with α-amylase during germination and is generally found in the kernel tissue containing starch granules. It suggests that, in vivo, the function of α-amylase inhibitor is to attenuate starch granule degradation by binding α-amylase.

629 CHARACTERIZATION OF b-32, A NOVEL RIBOSOME-INACTIVATING PROTEIN OF MAIZE. Hank W. Bass and Rebecca S. Boston, Dept. of Botany, North Carolina State University, Raleigh, NC 27695

The maize b-32 protein is a 32 kDa cytosolic albumin that maps to chromosome 8, accumulates in the endosperm of developing kernels, and is under the control of the regulatory gene *Opaque-2*. By sequence analysis and *in vitro* assays, we have determined that b-32 is a ribosome-inactivating protein (RIP). RIPs make up a large and widely distributed class of plant cytotoxic proteins that irreversibly inactivate eukaryotic ribosomes by depurination of a specific 28S rRNA adenine residue that is essential for translational elongation. Several different RIPs, such as ricin, gelonin, and α-trichosanthin have been tested for use as cell-specific immuno-toxins as well as anti-viral and insecticidal agents. Using *in vitro* assays, we have identified two distinct RIP activities in developing kernels. RIP activity associated with b-32 can be distinguished by an acidic pI that allows binding to anion exchange resins. The other RIP activity in kernels can be separated by its binding to cation exchange columns, a characteristic of all previously reported RIPs. Work is in progress to examine b-32 gene expression in relation to biological RIP activity in normal and mutant kernels of maize.

POSTER ABSTRACTS

630 CHARACTERIZATION OF FUNCTIONAL DOMAINS OF b-32. Julie E. Krawetz and Rebecca S. Boston. North Carolina State University, Department of Botany, Raleigh, North Carolina 27695

The maize b-32 protein has a molecular weight of approximately 32 kDa and is under the control of the *Opaque 2* locus. Sequence analysis has identified regions of amino acid conservation between b-32 and the well characterized ribosome-inactivating proteins (RIPs) Ricin-A from castorbean and RIP30 from barley seed. Included in this conservation are two RNA binding domains, and a region identified as the active site cleft of RicinA by X-ray crystallography (Ready, et al, Proteins 3:53). *In vitro* RIP assays have revealed the presence of RIP activity in the b-32 kernel protein. We have used an expression vector to produce b-32 in *E. coli*. Work is in progress to identify domains important for RIP activity *in vitro* and *in vivo*.

631 CHARACTERIZATION OF MATURATION Mat-1 GENE EXPRESSION IN SOYBEAN SEED. Chwen-Fang Lee, Robert W. Rinne, and Alan L. Kriz. University of Illinois, Department of Agronomy, Urbana, Illinois 61801

The soybean maturation-specific Mat-1 gene is expressed at high levels during late embryogenesis and is induced by seed desiccation. Previous studies showed that precocious desiccation can switch a soybean seed from a development program to a maturation program. We used a Mat-1 cDNA probe to investigate gene expression in normally developing seed and in seeds subjected to precocious drying treatments. During normal seed development, Mat-1 transcripts are first detected at 50 DAF and are present at high levels from 65 to 85 DAF. High levels of Mat-1 expression may, however, be induced in seeds harvested at 35 DAF and subjected to a drying treatment for 3-14 days. Mat-1 transcripts are embryo-specific, and Mat-1 transcripts are present in both axis and cotyledon. ABA does not induce Mat-1 expression in normally developing seeds, but Mat-1 expression is induced in germinating seeds treated with ABA. ABA also supresses germination and allows Mat-1 transcripts to persist, at reduced levels in mature seeds. The Mat-1 gene not only provides a convenient marker for analysis of seed maturation and desiccation processes, but may also play a vital role in these processes.

632 CHARACTERISATION OF DIFFERENTIALLY-EXPRESSED GENE FAMILIES IN SEEDS OF TOMATO (Lycopersicon esculentum var Ailsa Craig). C.J. Hutchings, K.J. Harrison, E. Thomas, E. Zaki and T.A. Brown.

Gene expression studies in tomato seeds have identified seed-specific transcripts for a vicilin-like storage protein, a 2S storage protein (homologous to castor bean and Brazil nut proteins) and a protein which has homology with wheat and rice lectins. Other seed-abundant transcripts include proteinase inhibitors (homologous to the potato I family) and a reverse transcriptase sequence, Tle 1, that is expressed differentially during seed development. Nucleotide sequences, together with expression profiles and transcript data, will be presented for these gene families.

633 CHARACTERIZATION OF A cDNA EXPRESSED DURING IMBIBITION / GERMINATION OF BARLEY SEEDS. Gregory R. Heck and T-H David Ho Washington University, Dept. of Biology, Box 1137, St. Louis, MO, 63130, USA

A cDNA clone, pclRP1, was obtained from a library made with poly(A)+ RNA isolated from mature, imbibing embryos of Hordeum vulgare cv. Himalaya. This clone was distinguished by its abundant expression which rapidly peaks from almost undetectable quantities within 12 hours (h) of moisture exposure. Crude dissections of the germinating embryo reveal the highest levels of expression in the scutellum, followed by several fold lower levels in the coleoptile and ensheathed leaves, and an absence in emerging radicles. A low but detectable quantity of IRP1 mRNA is then maintained in all green portions of the barley plant examined. A similar pulse of expression can be seen when 30 h imbibed embryos are re-dried and imbibed again. Further studies revealed even higher expression of the IRP1 mRNA in the aleurone layer upon imbibition which is subsequently suppressed when the aleurone layer is treated with gibberellic acid (GA_3). Immunoprecipitations of *in vivo* labeled proteins and western analysis done with polyclonal antibodies raised against TrpE-IRP1 fusion protein corroborate the observations at the mRNA level. The greatest rate of synthesis for IRP1, relative to total protein, occurs between 1.5 and 6 h of embryo imbibition, although IRP1 continues to accumulate in the scutellum to 48 h. The 2.7 kb pclRP1 cDNA has been sequenced and encodes a deduced translation product of 82 kD. IRP1 synthesized *in vitro* from cRNA migrates identically in electrophoretic separations compared to immunoprecipitated *in vivo* labeled IRP1. IRP1 does not have homology to sequences in available data bases. Irp1 appears to be single copy gene and has been mapped to chromosome 3 with the aid of wheat-barley addition lines. A genomic clone containing the entire coding sequence of Irp1 plus several kb of 5' and 3' sequence has been isolated and is currently under investigation. Irp1 homologues have been detected in other members of the Gramineae under high stringency hybridizations and may indicate an important conserved role for IRP1 in the early events of imbibition/germination of the cereals

634 CHARACTERIZATION OF cDNAs ENCODING WHEAT CM PROTEINS - HETEROLOGOUS EXPRESSION IN E. coli. Philippe Joudrier, Valerie Lullien, Remi Alary and Marie-Françoise Gautier. Laboratoire de Technologie des Céréales, INRA, 34060 Montpellier, France.

The large cereal trypsin/ α -amylase inhibitor family includes the CM proteins that are components of the wheat tetrameric α-amylase inhibitor. In Triticum aestivum, CM proteins are a group of at least five components (CM1, CM2, CM3, CM16 and CM17) whereas in Triticum durum only three components (CM2, CM3 and CM16) have been identified. We have characterized cDNA clones encoding the three Triticum durum CM proteins and two Triticum aestivum CM proteins (CM16 and CM 17). Comparisons of the primary structure of CM proteins underline a high homology and the conservation of the number and position of cysteine residues. In addition, no changes have been observed between Triticum durum and Triticum aestivum identical CM protein subunits. Assuming that the two species diverged about 8000 years ago, this strongly suggests that, besides their involvement in insect defense mechanisms, CM proteins could play another function in wheats. In developing Triticum durum endosperm the highest level of CM mRNAs were detected at mid-maturation suggesting that the three CM proteins are coordinately and specifically expressed in seeds. In order to study the structure of CM proteins, we used E. coli as an heterologous host to produce the CM 16 protein. The recombinant protein is found in inclusion bodies. conditions of solubilisation and renaturation of the CM 16 protein are in progress.

635 CHARACTERIZATION OF AN *ARABIDOPSIS* OLEOSIN. Gijs J.H. van Rooijen, Linda I. Terning, William R. Scowcroft, and Maurice M. Moloney. Dept of Biological Sciences, 2500 University Drive N.W. Calgary, Alberta, Canada T2N 1N4

Most plant seeds store triacylglycerols in sub-cellular organelles called oil-bodies. Oil-bodies isolated from different seeds have a similar diameter (0.5 -1 mm) and are surrounded by a monolayer of phospholipids into which polypeptides are embedded. The function of these proteins is still not clear, but it has been suggested that it might be structural and/or as a lipase attachment site. We are interested in a particular class of oilbody proteins called oleosins. Oleosins are the most abundant oil-body proteins and have a typical secondary structure: A hydrophylic N-terminal, a hydrophobic central domain, and an amphipathic C-terminal domain. It is possible that these different domains perform different functions in the developing seed. Oleosins are seed specific and regulated by phytohormones and osmotic stress. Using the polymerase chain reaction (PCR) we have cloned a rapeseed oleosin gene (OB800), encoding a part of a 19 kDa protein. OB800 was used as a probe to clone the equivalent of this gene in *Arabidopsis* by screening a genomic library.We will present the sequence of an 1797 bp insert which includes the complete coding region and 861 nucleotides of the 5' untranslated region. The rapeseed and *Arabidopsis* sequences were aligned and show that the central and 3' end of these genes are very homologous. The *Arabidopsis* DNA coding sequence is interrupted by an intron of 240 bp which contains seed specific enhancer like sequences. We fused a reporter sequence to the coding region of the oleosin and experiments are now in progress to determine the importance of the N-terminal, central and C-terminal domains of the oleosin for transport and targeting to the oil-body. The coding region of the oleosin will be altered in the three different domains and reintroduced in *Arabidopsis* by *Agrobacterium* mediated transformation.The effect of these mutations will be studied doing sub-cellular fractionation and immunological detection of the reporter peptide.

POSTER ABSTRACTS

636 CHANGES IN DNA CONTENTS AND PROTEIN PATTERNS DURING TOMATO SEED GERMINATION. Raoul J. Bino, Lieke Kraak, Jaap G. van Pijlen, Mary van Koppenhagen, Henny Askamp and Jan Bergervoet.
Centre for Plant Breeding and Reproduction Research, P.O. Box 32, 6700 AA Wageningen, The Netherlands

Relative nuclear DNA contents in tissues of tomato seeds and seedlings were measured with a flow cytometer. Embryo's in dry tomato seeds contained nuclei with a relative DNA content at the 2C level, apparently the cells were arrested at the G_1 stage of the nuclear DNA replication cycle. During imbibition in water sharp 4C signals arise, reflecting the replication of chromosomal material. DNA synthesis occurred principally in the 'root tip' tissue of the embryo. After four days of imbibition (i.e. shortly before the start of visible germination), the percentage of 4C nuclei raised to more than 70% in cells of the root region. No significant changes in DNA contents were observed in endospermic cells of the seeds. DNA contents were also analyzed in tomato seeds which were imbibed for 7 days at -0.5 MPa PEG-6000 and subsequently rehydrated. These (non-germinated) primed seeds showed a high 4C signal in root cells. The rate at which the 4C signal in primed seeds arised was slower than that during imbibition in water. Apparently, imbibition of tomato seeds was accompanied with the onset of replicative DNA synthesis. DNA replication, however, was not associated with germination as was shown in the primed seeds. Using two-dimensional SDS-PAGE, protein profiles were analyzed in the 'root tip' tissue of the embryo at stages before and after the start of DNA replication. The observed changes in DNA contents and protein patterns will be correlated with tomato seed germination performance and seedling quality.

637 CLONING OF A SOYBEAN CDNA CODING FOR PHYTOENE DESATURASE. Glenn E. Bartley, Paul V. Viitanen and Pablo A. Scolnik.
Du Pont Experimental Station, Wilmington, DE 19880-0402.

Carotenoid biosynthesis is important to all photosynthetic and many non-photosynthetic organisms. These C_{40} isoprenyl pigments serve as photoprotectants and assist in light harvesting for photosynthesis. Carotenoids owe their yellow, orange, and red pigmentation to a polyene chain called a chromophore. Phytoene, the first carotenoid in the biosynthetic pathway, is colorless due to a short chromophore of only three conjugated double bonds. Two successive desaturations of phytoene result in the formation of ζ-carotene, a yellow carotenoid and an intermediate in the pathway to lycopene, β-carotene, and other carotenoids.

Here we report the molecular isolation of a cDNA clone which, based on a complementation assay using the photosynthetic bacterium Rhodobacter capsulatus, codes for an enzyme that catalyzes the two desaturation reactions that convert phytoene to ζ-carotene. This cDNA contains an open reading frame with the capacity to code for a 572-residue protein Pdsl, of predicted M_r 63,851. Comparison of the resulting primary protein sequence to sequences of fungal and bacterial carotenoid desaturases revealed homology in several areas of the proteins, including a dinucleotide-binding motif which could mediate binding to FAD. In vitro chloroplast import experiments confirmed import and processing of the precursor to a mature form of the protein. In addition, hybridization of the cDNA to Glycine max and Glycine soja genomic blots indicated that this gene is a member of a low copy number family. Using restriction fragment length polymorphisms between these two species, one locus was genetically mapped.

638 CONSTRUCTION OF ALEURONE TISSUE SPECIFIC cDNA LIBRARY FROM A SINGLE RICE GRAIN USING POLYMERASE CHAIN REACTION AND DETECTION OF ALEURONE SPECIFIC GENE EXPRESSION. Kunisuke Tanaka and Yoshinori Sugiyama.
Department of Biochemistry, Kyoto Prefectural University
Shimogamo, Kyoto 606, JAPAN.

To detect aleurone tissue specific gene expression, we constructed a cDNA library for the aleurone layer from a single developing rice grain. Materials and Methods: RNA was obtained from a sheet of manually peeled aleurone layer from one thirds of a single rice grain. After homogenization, phenol extraction, and ethanol precipitation, poly(A)$^+$ RNA was prepared using oligotex-dT30(TAKARA), for reversetranscription into single strand cDNAs using oligo(dT) as the primer. This cDNA was used as the templet for the polymerase chain reaction(PCR) after oligo(dG) tail was introduced at the 3' end. 25 cycles PCR was done at 94℃ 2 min, 60℃ 2min, 72℃ 4.5min using oligo(dC) and oligo(dT) as the primers. The resulting cDNAs were ligated into Bluescript SK$^+$ after cDNAs shorter than 200bp were removed by a Sephacryl S400 spin column. Result: A cDNA library consisted of about 10^6 individual clones were obtained. Several aleurone tissue specific sequences were detected. Among them, 400bp sequence of highly homologous to barley germine polypeptide was detected showing very high specificity to aleurone tissue.

639 DIFFERENTIAL GENE EXPRESSION DURING IMBIBITION OF DORMANT AND NONDORMANT *AVENA FATUA* EMBRYOS. William E. Dyer, Plant and Soil Science Department, Montana State University, Bozeman, MT 59717

Seed dormancy, a unique form of plant developmental arrest, allows germination to be distributed temporally and spatially. The physiology of seed dormancy has been well characterized in *Avena fatua* but the molecular mechanisms controlling dormancy maintenance and release are not understood in this or other species. To begin characterization of biochemical events associated with dormancy maintenance and release, soluble embryonic proteins were isolated from dormant and nondormant (after-ripened) *A. fatua* 'AN265' caryopses from the same seed lot after 1, 3, 6, and 12 hours of imbibition. Protein patterns on two-dimensional silver-stained gels from dry embryos or embryos imbibed 1 hour were identical. However, after 6 hours of imbibition, dormant embryos contained two distinct polypeptides not visible in nondormant embryos. By 12 hours, germination-specific proteins were visible in nondormant embryos. Poly(A$^+$) mRNA was isolated from embryos imbibed under similar conditions, translated *in vitro* in a rabbit reticulocyte system, and the products separated on two-dimensional gels. No differences were detected between dormant and nondormant embryos that were dry or imbibed 1 hour. However, after 3 hours of imbibition dormant embryos contained low levels of a message encoding a 38 kD protein that was not visible in nondormant embryos. As judged by protein levels, the mRNA was present by 3 hours after the start of imbibition, and gradually declined over the next 9 hours. The results support the idea that dormancy is maintained in imbibed seeds by differential gene expression. Differential screening of subtraction libraries is underway.

640 DIFFERENTIAL REGULATION OF TWO TIGHTLY LINKED CATALASE GENES OF THE CASTOR BEAN. Masaharu Suzuki, [1]Tsukaho Hattori and Tadashi Asahi. Scool of Agriculture, Nagova University, Nagova 464, Japan and [1]Center for Molecular Biology and Genetics, Mie University, Tsu 514, Japan

Immunoblot analysis with anti-(sweet potato catalase) antibody detects several tissue-specific isoforms of catalase subunits in the castor bean, suggesting that the castor bean enzyme is encoded by a small multigene family. Using the sweet potato cDNA clone, we have cloned two catalase genes (CAT1 and CAT3), which were found to be tightly linked on a chromosome with a distance of 2.4Kb. RNA blot hybridization analysis using gene specific probes for CAT1 and CAT3 revealed that the expression of the both genes dramatically increased in endosperm and cotyledon tissues after two days of germination, when active biogenesis of glyoxisomes occurs. Expression of CAT3 gene was, however, much higher in hypocotyl and roots, where expression of CAT1 gene was hardly detectable. Organ specificity of CAT1 and CAT3 expression well matched the appearance of the organ specific isoforms of the catalase subunits. These results suggested that CAT1 encodes the catalase subunit specific for tissues having glyoxisomes, whereas CAT3 encodes the subunit present in both tissues which have glyoxisomes and unspecialized microbodies. To investigate the regulatory mechanisms for the organ specific and germination activated expression of these two genes, we have produced transgenic tobacco plants carrying various length of CAT and CAT3 promoters fused to GUS reporter gene. Temporal and spacial espression pattern of these transgenes in the developing germinating seed tissues are currently being analyzed.

641 DNA ENDOREDUPLICATION PATTERNS IN DEVELOPING MAIZE ENDOSPERM ARE CONTROLLED BY A MATERNAL EFFECT. G. L. Yerk[1], R. V. Kowles[3], R. L. Phillips[1], L. Zhang[2], and F. Srienc[2]. University of Minnesota, Dept. of Agronomy and Plant Genetics[1] and Bioprocessing and Technology Institute[2] St. Paul, MN 55108 and St. Mary's College, Dept. of Biology[3], Winona, MN 55987.

Nuclei preparations of whole endosperms from maize inbreds, their reciprocal hybrids, F_2 and F_3 progeny stained with Mithramycin A were evaluated using the flow cytometer. Number of rounds of endoreduplication, percentage of nuclei per round, average DNA content per nucleus, and cell number were obtained for each preparation. Flow cytometric measurements indicate DNA increase in maize endosperm is due to endoreduplication of nuclear DNA. The maximum number of rounds of endoreduplication varies from 3 to 7 depending on the line examined. Examination of graphs of nuclei number *vs.* DNA content generated by the flow cytometer reveal that hybrid progeny have the same endoreduplication pattern as the female parent used in the hybrid. Examination of F_2 progeny reveal no difference in endoreduplication pattern among individuals within an F_2 family and no difference between patterns of F_2 progeny generated from reciprocal F_1 hybrids. F_3 progeny segregated for their endoreduplication patterns. This finding coupled with the previous data indicate that a maternal effect is responsible for the observed patterns, ie. the genotype of the maternal parent's nuclear genome controls the DNA endoreduplication pattern of the progeny.

POSTER ABSTRACTS

642 DNA SEQUENCE ANALYSIS OF A LUPIN L-ASPARAGINASE GENE: POSSIBLE REGULATORY PROMOTER ELEMENTS. Eva Vincze, James Dickson, Murray Grant, Laura Smith, Karen Rodber, Leesa Lochhead, Richard Macknight[1], Kevin Farnden[1] and Paul Reynolds. DSIR Fruit and Trees, Palmerston North, New Zealand and [1]Biochemistry Department, University of Otago, Dunedin, New Zealand.

L-asparaginase is a key enzyme involved in asparagine utilisation in lupin. The enzyme hydolyses the amide group of asparagine to yield aspartate and ammonia.

A developing seed L-asparaginase cDNA clone was used as a probe to isolate an asparaginase genomic clone from a Lupinus angustifolius genomic library. The 3.2kb of DNA sequenced consisted of 1136bp of promoter and upstream regulatory region, four exons and three introns. A 1.1kb fragment from the 5' flanking region was fused with B-glucuronidase (GUS) reporter gene. The chimeric gene conferred high expression in developing seeds and low expression in roots and expanding leaves in transgenic tobacco. The pattern of GUS expression in tobacco and that of L-asparaginase activity in lupin was strikingly similar.

Analysis of the 5' regulatory region revealed consensus sequences for the TATA box, CAAT boxes, embryo and seed specificity and, interestingly, for nodule/root specificity. These sequences are being investigated by deletion analysis in transgenic plants. Gel mobility retardation assays and DNase footprinting are being utilised to monitor DNA-protein interactions.

643 EXPRESSION OF HSP70 GENE FAMILY MEMBERS DURING SEED DEVELOPMENT Amy DeRocher[1], Lisa Lauzon[2], and Elizabeth Vierling[2,1] University of Arizona, Departments of [1]Molecular and Cellular Biology and [2]Biochemistry; Tucson, Arizona 85721.

HSP70s are molecular chaperones that are believed to associate with proteins and facilitate their proper folding or transport across membranes. We have cloned and sequenced two cytoplasmic HSP70s from *Pisum sativum* : HSP70 which is strongly heat regulated in vegetative tissues, and HSC70 which is constitutively expressed in most tissues. Analysis of HSP70 and HSC70 mRNA and protein in maturing pea seeds indicates that these genes are also temporally regulated during seed development. Pea seeds were grown under controlled conditions (16hr, 18°C days and 8 hr, 14°C nights), well below heat stress induction temperatures. Under these conditions HSC70 mRNA and protein are abundant in both vegetative and embryonic tissues until the mid-maturation phase of development but decline in the cotyledons during later stages of seed development. In contrast, HSP70 mRNA and protein are expressed in the cotyledons and axis throughout the maturation phase of development, peaking in the dry seeds. HSP70 and HSC70 cDNAs have been sequenced and their derived amino acid sequences determined. Pea HSP70 and HSC70 encode 648 and 647 amino acid proteins, respectively, that are 86.1% identical at the amino acid level. The two pea proteins are 89.7% identical through the amino terminal 407 amino acid ATP binding domain, but only 80.0% identical through the rest of the proteins. This includes a divergent 15 amino acid segment near the carboxy terminus which contains only one identical residue and five conservative replacements. We are raising antisera against this peptide and against a conserved domain in the amino terminal end of HSP70. These antisera will be used to study further the expression of HSP70 family members in specific tissues during seed development. The differential regulation of HSP70 and HSC70 during heat stress and development implies that there may be differences in the function of these two chaperones.

644 EXPRESSION OF A NOVEL LIPID TRANSFER PROTEIN GENE IN ALEURONE CELLS OF DEVELOPING BARLEY GRAINS.

R.B. Aalen, R. Kalla, A. Lönneborg, C. Linnestad, S. Klemsdal and O.-A. Olsen. Plant Molecular Biology Lab NLVF, P.O.B 51 N-1432 Ås-NLH, NORWAY.

Aleurone cells of barley are characterized by their conspicuous components - aleurone grains and lipid bodies (spherosomes). During the differentiation and maturation stages of development vacuoles mature to aleurone grains, and storage lipids are deposited in the proliferating spherosomes. 33 days after pollination the total lipid content of the aleurone cells accounts for 20% of the cell mass. The expression pattern of an aleurone specific transcript, represented by the cDNA clone B11E, coincide with spherosome proliferation. Interestingly, B11E encodes a protein with 78% identity to a 7 kDa Lipid Transfer Protein (LTP) from wheat grains. The novel barley LTP is a 102 aa preprotein including a postulated 35 aa N-terminal extension with all the characteristics of a signal peptide. LTP gene products have been characterised from several developing plant tissues such as the coleoptile of maize seedlings and the tapetum of tobacco anthers. Transgenic tobacco plants expressing the transcript encoded by the B11E cDNA show developmental disturbances. Experiments with the aim to define subcellular site(s) of action of the novel LTP in the developing grain and transgenic plants are underway.

645 AN ENDOPEPTIDASE FROM SENESCING BEAN PODS: NUCLEOTIDE SEQUENCE OF CLONED cDNA. T. Minamikawa, T. Tanaka and D. Yamauchi. Tokyo Metropolitan Univ., Dept. of Biol., Tokyo, 192-03 Japan

In Phaseolus vulgaris, the nitrogenous nutrients from leaves are, at least in part, first stored in pods in the form of protein, which is then translocated as amino acids to developing seeds. Endopeptidase activity increases as pods are senescing, and is thought to be responsible for protein mobilization in the pods. Ion-exchange chromatography of enzyme extracts from pods collected at 22 to 27 days after flowering revealed three forms of endopeptidase. One of the enzymes, designated EP-C1, was purified as a 33 kDa polypeptide. By using antibodies raised against EP-C1, a cDNA clone was isolated from a cDNA expression library of the pods. While the length of EP-C1 mRNA was estimated to be 1.4 kb, the insert of the clone was about 0.7 kb in length. Therefore, using this cDNA as a probe, another cDNA clone (λPPP130) containing about 1.3 kb insert was isolated from the same library, and the inserted cDNA was sequenced. The cDNA contains a 1083 bp translated region, lacking initial ATP codon, followed by a 3' untranslated region which includes a putative polyadenylation signal. The deduced amino acid sequence of the cDNA has a high homology with that of a cDNA for the cysteine-endopeptidase from cotyledons of germinating Vigna mungo seeds (H. Akasofu et al. 1989. Nucl Acids Res 17: 6733). By using the antibodies and the cDNA, changes in amounts of EP-C1 and its mRNA in pods during development of fruits were estimated, and the results suggested that synthesis of EP-C1 is regulated at the transcriptional level. EP-C1 was immunologically detected also in cotyledons and axes of seedlings and stems at a senescing stage.

646 EFFECT OF THE EMBRYO AXIS ON CATALASE GENE EXPRESSION IN THE ENDOSPERM OF CASTOR BEAN FOLLOWING IMBIBITION. Robert T. Mullen and David J. Gifford. University of Alberta, Department of Botany, Edmonton, Alberta, Canada, T6G 2E9

In germinated castor bean (Ricinus communis L. cv. Hale) seeds, the catabolism of stored lipids generates products that are utilized as primary energy sources for the growth of young seedlings. Following mature seed imbibition, and correlating with the rates of fat catabolism, glyoxylate cycle enzymes such as catalase undergo characteristic patterns of increase and subsequent decline in activity. Sequestered in the glyoxysomes, catalase is a developmentally regulated enzyme that provides an excellent marker to investigate the mechanisms controlling gene expression. Catalase requires the presence of the embryo axis for the attainment of high levels of activity in cell free extracts. Two dimensional electrophoresis indicates differences in the de novo protein synthesis of germinating endosperm, plus and minus the embryo axis. To analyze the molecular control mechanisms involved, catalase has been purified using hydroxylapatite ion exchange, gel filtration, chromatofocusing, and gel electrophoresis. Denaturing and non-denaturing isoelectic focusing of catalase on polyacrylamide gels indicate multiple isoform patterns characteristic of those reported from other plant sources. Antibodies raised to purified castor bean catalase are being used to examine embryo axis control of gene expression by quantifying changes in catalase protein during and post germination.

647 FUNCTIONAL ANALYSIS OF VP1. Leonard Rosenkrans, Vimla Vasil, Indra K. Vasil and Donald R. McCarty. Vegetable Crops Dept. University of Florida, Gainesville, FL. 32611.

The VP1 protein controls expression of a number of genes which are required for normal processes involved with kernel maturation as well as anthocyanin accumulation in the aleurone. Previous work in this laboratory has demonstrated that VP1 regulates expression of two genes which represent examples of the two processes just mentioned: *C1*, which is itself a transcriptional regulator of structural genes in the anthocyanin biosynthetic pathway, and Em, a member of the LEA (late embryogenesis abundant) class of seed proteins. VP1 been shown to exhibit the characteristics of a transcriptional activator, although no sequence similarities to known transcription factors have been found. To address the functional significance of VP1 protein sequences, a series of terminal and internal deletion mutants were made in the VP1 protein and tested for ability to activate Em and *C1* GUS reporter constructs in maize protoplasts. The results of these experiments help to define functional domains in VP1.

POSTER ABSTRACTS

648 Histochemical localisation of L-asparaginase expression in transgenic tobacco. Murray Grant and Michael W. Bevan, Cambridge Laboratory, John Innes Centre for Plant Science Research, Colney Lane, Norwich, Norfolk, UK.

Legume seed development is highly regulated, with the synthesis of seed storage proteins subject to developmental, organ specific and environmental controls. The developing seed pod acts as a sink for nitrogen, which is transported in plants predominantly as amides. In lupin over 80% of the fixed nitrogen is transported as asparagine and about 80% of that entering the developing pod is metabolised (Atkins et al., 1975). Asparaginase catalysed deamidation is probably the major method of remobilising nitrogen from transported asparagine. During pod filling, lupin L-asparaginase (ASNase) activity increases 10 fold and its mRNA accumulation precedes the detection of conglutin precursors (Gayler et al., 1989).

The ASNase gene from *Lupinus angustifolius* has been cloned (Lough et al., 1991). Here we present the analysis of regulatory sequences of the lupin ASNase gene. A construct containing a translational fusion of -1163 to +82 of the ASNase gene with the reporter gene B-glucuronidase (GUS) was used to study the induction and histochemical localisation of GUS during the development of tobacco seeds.

Our results indicate that GUS activation is temporally and spatially controlled in a complex manner during the seed maturation process. This induction precedes tobacco seed storage protein deposition (Chen et al., 1988).

Histochemical localisation of GUS activity in developing anthers and during the development of F1 seedlings will also be presented.

649 Identification and Molecular Characterisation of two Different Groups of cDNA clones encoding ADP-glucose Pyrophosphorylase in Potato Tubers G. Chauhan and M.W. Bevan, Cambridge Laboratory, John Innes Centre for Plant Science Research, Colney Lane, Norwich, Norfolk, UK.

Synthesis of ADP-glucose from ATP and glucose-1-P is catalyzed by ADP-glucose pyrophosphorylase. This represents one of the main regulatory steps in the biosynthesis of starch. In maize, ADP-glucose pyrophosphorylase is a heterotetramer of subunits encoded by two distinct genes; shrunken-2 and brittle-2.

cDNAs encoding for corresponding shrunken and brittle subunits in Desiree potato tubers have been isolated and sequenced. Examination of the deduced amino acid sequence reveals a substantial amount of homology to known ADP-glucose pyrophosphorylase subunits of plants and bacteria, including regions involved in substrate binding and activator binding.

Both sets of cDNA's are incomplete. Thus, Rapid Amplification of CDNA Ends (RACE) has been carried out to isolate the 5' regions of both sets of cDNA's. At present the PCR product is in the process of being characterised.

Southern blot analysis shows that the genes have a low copy number in potato. Northern analysis reveals the presence of abundant transcripts of both subunits in both developing tubers and leaves.

At present, cDNA's from subunits are being used as probes to isolate ADP-glucose pyrophosphorylase clones from a genomic potato library. The aim is to characterise regions of the promoter which may confer sucrose inducibility.

650 **IDENTIFICATION OF GENES INVOLVED IN THE REGULATION OF TOBACCO SEED MATURATION** Bernd Reiss, Josefa-Maria Balsalobre, Christiane Plum, and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, D 5000 Köln 30, F.R.G.

Seed storage proteins accumulate to high levels during development of the seed. The expression of these proteins is tissue specific and developmentally coordinated. Two different approaches are used to identify genes participating in the control of the expression of such proteins. The first one is based on T-DNA mutagenesis. *Agrobacterium tumefaciens* inserts part of the Ti-plasmid, the T-DNA, into the chromosome, a process which has been shown to cause mutations. We have modified this basic principle to allow the identification of regulatory genes by gene activation. A selectable marker gene was combined with a seed-specific promoter and transgenic plants were generated. These plants were transformed with the activating vector. Insertions which tag a regulatory gene lead to the activation of the selectable marker and therefore can be identified easily. Several plant lines containing putatively tagged genes were obtained. These lines are analysed currently. In the second approach, a detailed cis-analysis of a seed specific promoter was performed and proteins binding specifically to regulatory regions identified. This information will be used to identify the corresponding genes by direct expression cloning.

651 Identification of Genes Induced in Leaves of Potato Plants Around the Time of Tuberisation.

S.Jackson & U.Sonnewald, IGF, Berlin, Germany.

It is known from grafting experiments that the tuberisation signal originates in the leaves of potato plants when the environmental conditions are correct. This signal has yet to be identified. This project aims to clone genes in the leaf that are involved in the transduction pathway from the environmental stimuli to the tuberisation signal which is transported to the stolons and induces them to form tubers. Poly A+ RNA was isolated from leaves of potato plants induced, and non-induced, to tuberise. These were used in a differential screen of a leaf cDNA library made from induced potato plants. Five clones have been identified that are induced in the leaves of tuberising potato plants. The sequence and expression pattern of these clones are analysed.

652 IDENTIFICATION OF A POTENTIAL MUTANT IN *ARABIDOPSIS THALIANA* WITH ABNORMAL OVULE DEVELOPMENT. Leonore Reiser and Robert Fischer. Department of Plant Biology, University of California, Berkeley, CA 94720.

The ovule contains two genetically distinct tissues, the megagametophyte and sporophyte, that develop and interact in a complex fashion during sexual reproduction and seed development in higher plants. To begin to dissect genetically how ovules function, we have sought to analyze mutations that disrupt ovular development in *Arabidopsis thaliana*. To this end we screened the collection of 7,000 T-DNA mutagenized *Arabidopsis thaliana*. lines (kindly provided by the DuPont Corporation) for female sterile plants. Sterile plants were identified by the failure of the siliques to elongate to a mature size after pollination and then used in reciprocal crosses to determine if sterility was due to defects in the anthers or gynoecium. One potential mutant *fms*1, displays normal flower morphology and makes abundant functional pollen, but is completely sterile. Preliminary histological analysis of *fms*1 shows ovules with an abnormal morphology which appear to lack functional embryo sacs. A more thorough developmental analysis to determine the exact nature of the defect is in progress. DNA blot analysis indicates that two T-DNAs have been inserted into the genome of *fms*1. More extensive DNA-blot analysis of individuals in a segregating population is underway to determine which T-DNA is responsible for the female sterile phenotype.

653 IMMUNOLOGICAL STUDIES OF ALLANTOINASE EXPRESSION IN SOYBEAN SEEDS AND SEEDLINGS. Jennifer Van Es, Judy Lindell, and Mary Alice Webb. Purdue University, Department of Botany and Plant Pathology, West Lafayette, Indiana 47907

Ureide biogenesis, a specialized form of nitrogen assimilation, occurs transiently during seed germination and seedling growth in soybean and may be important in mobilizing seed reserves early in seedling development. Allantoinase catalyzes the final reaction in ureide biogenesis, the conversion of allantoin to allantoic acid. Analysis of allantoinase activity over the first two weeks of seedling development revealed distinct patterns of activity in cotyledons, epicotyl, and root/hypocotyl regions. Polyclonal antibodies produced against allantoinase purified from soybean seeds have been used to probe Western blots and tissue prints to study patterns of allantoinase expression and distribution in developing seedlings. Western blot analysis of cotyledonary extracts from ungerminated seed and germinating seeds one day post-imbibition revealed an immunoreactive band of approximately 30 kD, corresponding to the subunit size previously determined for seed allantoinase. Probes of extracts from cotyledons of developing seedlings up to 14 days post-imbibition revealed different patterns of immunoreactive bands, suggesting that either different isozymes were expressed or the enzyme was modified in some way at later stages of seedling development. Tissue prints probed with anti-allantoinase antibodies showed that the enzyme was evenly distributed throughout the cotyledons in ungerminated seed and germinating seeds one-day after imbibition. In contrast, uneven patterns of distribution were observed in cotyledons of developing seedlings with an apparent concentration of allantoinase in the cotyledonary epidermis in older seedlings. These patterns were distinct from the distribution of total protein observed in tissue prints of comparable stages stained with India ink. Continuing studies will be aimed at developing molecular probes to study allantoinase expression at mRNA levels for comparison with our immunological studies.

POSTER ABSTRACTS

654 ISOLATION OF A PLEIOTROPIC *ARABIDOPSIS THALIANA* MUTATION (mat-1) THAT AFFECTS BOTH THE DEVELOPMENT OF THE SEED COAT AND THE ROOT SYSTEM. Dirk Valvekens and Marc Van Montagu. Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

We have isolated a pleiotropic mutation (mat-1) that both affects the structure of the seed coat and the development of the *Arabidopsis thaliana* root system. Using scanning electron and light microscopy we examined the structure of the *Arabidopsis* mutant and wild-type seed coats. Several aspects of the early development of the seed coat are discussed as well as its behavior in early germination. Also the development of the wild-type and mutant root system was studied. The mutation is currently being mapped in order to allow future isolation of the different alleles of the mat-1 locus.

655 ISOLATION AND CHARACTERIZATION OF AN OAT ENDOSPERM (1-3, 1-4)-β-GLUCANASE cDNA CLONE. Debra J. Martin, Song J. Yun, Howard W. Rines*, Burle G. Gengenbach and David A. Somers. University of Minnesota and USDA-ARS*, Department of Agronomy and Plant Genetics, St. Paul, MN 55108

Hydrolysis of endosperm cell wall β-glucans by β-glucanases is necessary during barley seed germination to allow α-amylase access to the starchy endosperm cells for conversion of starch into maltose (Annu. Rev. Plant. Mol. Biol. 40:305). Additionally, oat coleoptile cell elongation and cell wall loosening are associated with turnover of mixed-linkage β-glucans by β-glucanases (Plant Physiol. Biochem. 27:61). We recently detected high (1-3, 1-4)-β-glucanase (EC 3.2.1.73) activity in early stages of developing oat (Avena sativa) endosperms. This activity decreased rapidly as the seed matured. In an effort to characterize the enzyme and determine its role in oat grain development, several cDNA clones have been isolated from a cDNA library made from 10-14 days after flowering endosperm. One truncated endosperm cDNA clone was identical to the 300 bp 3' region of an oat leaf β-glucanase cDNA clone (S. Yun, this meeting) suggesting that, as reported in barley (Mol. Gen. Genet. 224:437), at least one β-glucanase isoenzyme is expressed in both the endosperm and leaf. Another truncated endosperm cDNA clone shared >80% identity with the oat leaf β-glucanase cDNA clone. Development and tissue-specific expression characteristics of the β-glucanases genes will be presented.

656 ISOLATION AND CHARACTERIZATION OF AN OAT (1-3, 1-4)-β-GLUCANASE GENE EXPRESSED DURING LEAF AND KERNEL DEVELOPMENT. Song J. Yun, Debra J. Martin, Howard W. Rines*, Burle G. Gengenbach, and David A Somers. University of Minnesota and USDA-ARS*, Department of Agronomy and Plant Genetics.

(1-3, 1-4)-β-glucanase (E.C. 3.2.1.73) hydrolysis of cell wall (1-3, 1-4)-β-glucans accompanies cell growth and degradation of endosperm cell walls during cereal grain germination (Ann. Rev. Plant Mol. Biol. 40:305). Oat (*Avena sativa*) leaves, roots, immature kernels, and kernels germinated for 96 hr contain (1-3, 1-4)-β-glucanase activity. To investigate the potential role of (1-3, 1-4)-β-glucanases in oat leaf and kernel development, we isolated a (1-3, 1-4)-β-glucanase clone from an etiolated leaf cDNA library of oat. This library was screened with a 30-mer oligonucleotide probe whose sequence was based on a highly conserved amino acid sequence present near the mature polypeptide amino terminus of barley (1-3) and (1-3, 1-4)-β-glucanases. The 1.5 kb oat leaf cDNA clone contained sequences for a putative 28 amino acid signal peptide plus the mature polypeptide and a 3' untranslated region. The putative signal peptide exhibited >92% residue identity to barley (1-3, 1-4)-β-glucanases at both DNA and amino acid sequence levels and had a hydrophilic amino-terminal region and a hydrophobic core that becomes more hydrophilic toward its carboxy-terminus. It also contained a Val-Glu-Ser sequence that precedes the signal peptide cleavage site reported in both barley and wheat (1-3, 1-4)-β-glucanases (Mol. Gen. Genet. 224:437). The first 200 bp sequence of the amino terminus of the mature polypeptide shares about 80% residue identity with two barley (1-3, 1-4)-β-glucanase sequences and about 61% residue identity with that of a barley (1-3)-β-glucanase (Plant Mol. Biol. 13:31). Preliminary genomic Southern blots probed with the cDNA clone indicated that oat (1-3, 1-4)-β-glucanase genes are low or single copy genes and members of a small gene family. Developmental expression patterns of the gene are currently being investigated.

657 LINKAGE MAPS IN PEA. T. H. Noel Ellis, Roger P. Hellens, Lynda Turner and David Lee. John Innes Institute, Colney Lane, Norwich, England, U.K.

We have compiled a linkage map, comprising about 150 markers, from the analysis of the segregation of RFLPs in recombinant inbred populations of pea. This map contains at least one feature reflecting a difference in the linkage order between two of the parents. This map has been compared to similar maps derived from other crosses, using the same probe set. The relationship between these maps, and with previously published maps based on classical or isozyme markers, will be presented. The significance of the observed differences will be discussed in the context of the estimated genetic distances between the parental lines.

658 **LOSS OF DNA SEQUENCE INTEGRITY FOLLOWING LONG-TERM SEED STORAGE AND ARTIFICIAL AGING.**
Joe Kamalay, Patty Sweeney and Raman Tejwani Agron.Dept.,Ohio State Univ., Columbus, OH 43210

We are investigating changes in the integrity of DNA sequences following seed aging. Damage to the genetic material has been implicated in the proposed mechanisms for aging. We feel that plant seeds represent ideal organisms to investigate the effects of aging on DNA. Total genomic DNAs were taken from deteriorated soybean seeds: (1) stored at (-10°C) since 1958; (2) freshly harvested seeds exposed to 100% relative humidity/40°C for 24, 48, and 72 hours (artificial aging). Denaturing agarose gel electrophoresis revealed extensive single strand breaks in DNA from aged seeds. Artificially aged seeds showed lower levels of damage, but detectable levels of damage. In vitro assays for the number of DNA pol I primer sites in non-denatured DNA confirmed these observations and allowed a preliminary estimate of the extent of strand scissions. Differences in sequence deterioration of specific regions within the soy genome are currently under investigation. When restricted DNAs were separated under denaturing conditions, genomic blots showed that individual DNA fragments within the aged seeds were highly degraded. We have also made direct measurements of the relative condition of individual genes by the specialized application of the polymerase chain reaction. We have discovered that the double-strands of DNA in dry soybean seeds are "nicked" after long-term storage and to a lesser extent, following artificial aging. The naturally or artificially aged seeds can repair the damage associated with aging, but about 50% of them cannot germinate. We intend to determine if the damage to these molecules is random or non-random.

659 mRNAs STORED IN SUNFLOWER DRY SEED ACCUMULATE IN VEGETATIVE TISSUES IN RESPONSE TO DIFFERENT STRESS. Concepcion Almoguera and Juan Jordano Instituto de Recursos Naturales y Agrobiologia, C.S.I.C Apartado 1052, 41012 Sevilla, SPAIN

We have cloned cDNAs corresponding to mRNAs accumulated in sunflower dry seed. Four different clones studied show response to both developmental and environmental cues. mRNAs hybridizing to cloned cDNAs accumulate late in embryogenesis and in young seedling, in response to ABA, osmotic stress, and both to low- and high-temperature stress. Sequence similarities identify two of the studied clone as plant small heat-shock proteins.

POSTER ABSTRACTS

660 MOLECULAR CHARACTERIZATIONS OF MATURATION GENES (MAT) DURING SOYBEAN SEED EMBRYOGENESIS. Yau-Jan Chyan, Lila O. Vodkin, Alan L. Kriz and Robert O. Rinne. University of Illinois, Department of Agronomy, Urbana, IL 61801

The shift from germination potential to successful seedling growth requires expression of several distinct gene families during soybean seed development Precocious desiccation and ABA treatment have been shown to stimulate the expression of a gene family which encodes a class of proteins that accumulate to high level during the late embryogenesis stages. We have identified two cDNA clones, pMat1 and pMat9, which are expressed at the onset of desiccation and accumulated during the late maturation stage in soybean seed. DNA sequence analysis showed that these two mRNAs are highly conserved at the 5' and 3' translated regions (about 90% homology), but the expression patterns of these genes are different during both the normal developmental stages and precocious desiccation. The open reading frames of Mat genes show some similarity to those of rice rab and cotton lea genes. Possible mechanisms of gene regulation and the flunctions of these gene products will be addressed.

661 MOLECULAR AND STRUCTURAL STUDIES OF SOYBEAN OIL BODY ASSEMBLY. Deborah Loer, Andrzej Kalinski, Jane Weisemann, and Eliot Herman. USDA/ARS, Plant Mol. Biol. Lab., Beltsville, MD 20705-2350

Soybean seed oil accumulates in the cytoplasm as droplets of triglycerides bounded by a monolayer of phospholipids in which a few distinct polypeptides are embedded. There is little information on the genetics, biochemistry, and cell biology of these oil storage organelles (oil bodies). The quantity of oil body membrane protein expressed is directly related to the quantity of reserve oil accumulation. Therefore, we are using these proteins as *in situ* probes to study the regulation, assembly, and mobilization of oil bodies. We have isolated the major oil body protein of soybean, the 24 kDa oleosin. The isolated protein was used to elicit polyclonal antibodies that were then used to conduct biochemical and structural studies on the assembly and mobilization of oil bodies. To begin a molecular approach, we isolated and characterized two cDNA clones for 24 kDa soy oleosin. Nucleic acid sequence analysis shows a full-length open reading frame encoding a polypeptide of 23,366 Da. The two cDNA clones differ in both their nucleic acid sequence and predicted amino acid sequence, indicating that soybean oleosin can be expressed as at least two isoforms. The deduced protein structure of soybean oleosin indicates that it has a relatively amphipathic aminoterminal region, a hydrophilic carboxyterminal region, and a hydrophobic central region that has extensive homology with the hydrophobic regions of oleosins characterized from other plants. However, there is little homology between 24 kDa soy oleosin and maize or carrot oleosin in the hydrophilic regions of the molecule. Experiments using a 24 kDA oleosin cDNA clone to determine the mechanism by which oil body proteins become associated with the incipient oil storage organelle will be discussed.

662 MOLECULAR STUDIES ON CLIMATE-INDUCED DORMANCY IN BARLEY. J. van Beckum;R. Schuurink;N. Sedee;R. van der Meulen; M. Schipper;M. Wang and F. Heidekamp.Center for Phytotechnology RUL-TNO Dept. of Molecular Plant Biotechnology, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

Impaired germination caused by dormant barley seeds can cause severe problems in the malting process. Unfortunatly some of the good malting barley varieties which are currently in use show high levels of post-harvest dormancy. We are interested in elucidation of the molecular mechanism wich mediate post harvest dormancy in barley. As a first step towards a better understanding of the molecular mechanisms of dormancy, malting barley varieties were grown in phytotrons under different climatic conditions which lead to the harvest of mature seeds with high and low degrees of dormancy. mRNA was extracted from developing seeds and used to construct cDNA libraries. Screening of these libraries lead to the selection of a number of clones of which the expression seems to be condition-related. Characterization of these clones is in progress.We also studied the effect of ABA on gene expression in dormant and non-dormant mature seeds. Therefore, aleuron layers were isolated and incubated with ABA and the expression of the RAB gene was studied. Preliminary results show that these aleuron layers have a different expression level of the RAB gene and a different sensitivity to ABA. Further analysis is in progress and results of the 2 approaches will be discussed.

663 MUTATIONS AFFECTING EXPRESSION OF THE *Viviparous-1* DEVELOPMENTAL GENE OF MAIZE. Christian B. Carson and Donald R. McCarty. Vegetable Crops Dept. University of Florida, Gainesville, FL. 32611.

We will present recent discoveries regarding expression of the maize *Vp1* gene based on studies from a large collection (20) of independent *vp1* mutant alleles. We know that *Vp1* expression is a cell autonomous and tissue specific rendering target tissues ABA insensitive. Null alleles, many expressing no detectable message, cause viviparv and inhibit anthocyanin accumulation in maize embryo and endosperm development. Alternatively, the phenotypes of 2 alleles (*vp1*-mum3 and *vp1*-mum5) result from *Mu1* transposon insertion and are regulated by suppression, exhibiting various degrees of vivipary and pigment formation, from phenotypically null to fully suppressed, WT appearing kernels. *Mu1* is situated 5' to the gene in these alleles, presumably replacing the core promoter with a functional alternative that expresses mRNA encoding a full sized protein, the amount depending on the level of suppression. Another class of *vp1* alleles (4 examples) prevent anthocyanin accumulation, but can induce developmental arrest. At least 2 of these (*vp1*-Mc and *vp1*-C) express C-terminal truncated VP1 protein, indicating distinct functional domains encoded by *Vp1*. The function of the mutant product has been evaluated by expression in maize protoplasts. Presently, VP1 specific immunoprecipitation techniques are allowing studies of protein expression and interactions with abscisic acid.

664 NUCLEAR PROTEINASES AND THEIR INHIBITORS IN THE PROCESS OF WHEAT SEED GERMINATION. Evelina A. Ivanova and Gyulnar Vafina. Institute of Biology, Acad. Sci. Urals Branch, USSR, Ufa, 450054

The basic structural and functional elements - nucleoplasm, chromatin and nuclear matrix, are isolated from purified cellular nuclei of wheat seedling tissues on different levels of physiological processes. A correlation between proteinases and their inhibitors is studied by affinity chromatography. Immunoreactivity of the structural and functional nuclear elements is investigated with the help of antibodies to indolylacetic acid. A high content of proteolytic and inhibitor complexes and a high sensitivity to auxin, a growth hormone of a nuclear matrix, are observed in the process of unfolding of ontogenetic programmes in the early stages of wheat seedling differentiated growth, namely, the period of a maximum coleoptile growth.

665 ORGANIZATION AND EXPRESSION OF GENES CODING FOR BARLEY SEED PEROXIDASES. Søren K. Rasmussen, Anette Johansson and Bodil Theilade. Risø National Laboratory, Plant Biology Section, DK-4000 Roskilde, Denmark.

We have isolated a full-length cDNA (1,2) for barley peroxidase BP 1 and a genomic clone for peroxidase BP 2. The enzymes, including peroxidase BP 3, were purified from resting barley grains (3). The isoelectric point is 8.5 for BP 1 and >10 for BP 2 and BP 3. One third of BP 1 is non-glycosylated and BP 1 has an open N-terminus. The amino acid sequence of BP 1 and BP 2 are 73% identical, but shows less than 50% identity to all other known plant peroxidases. Codon usage is strongly biased toward a C or G in third position indicating that these genes are highly expressed. BP 1 and BP 2 both have a signal peptide and they are apparently C-terminally processed. In contrast to other peroxidase genes, the BP 2 gene is intronless. Analyses of RNA and protein from aleuron, endosperm and embryo tissue shows high levels of BP 1 in the endosperm only. Time course analysis of RNA from endosperm showed BP 1 transcript 11 to 27 days after flowering (daf) and maximum was detected at 15 daf. A second transcript appears 13 daf. RFLP-mapping shows that BP 1 is linked to Est 4 (9.9 cM) on barley chromosome 3.

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POSTER ABSTRACTS

666 PROMOTER ELEMENTS OF GENES TRANSCRIBED IN DEVELOPING BARLEY ALEURONE CELLS CONTAIN COMMON SEQUENCE MOTIFS AND A myb-PROTEIN BINDING SITE. Lönneborg A., Kalla R, Linnestad C., Klemsdal S.K, Aalen R., Potter R. and Olsen O.-A. Plant Molecular Biology Lab NLVF, P.O.Box 51, N-1432-Ås, Norway.

Cereal endosperms, including barley, consist of only two tissues, the aleurone layer and the starchy endosperm (see this poster session). In order to study the regulatory circuitry underlying aleurone cell differentiation, we have isolated cDNAs for transcripts present in aleurone layer, but not in the starchy endosperm of immature grains. One group, including aleurone specific transcripts, is represented by the B11E (Ltp1) and B12A (Ltp2) cDNAs, both encoding novel LTPs, while B22E is a representative of a second group including transcripts present also in other tissues. Genes encoding the Ltp1, Ltp2 and B22E transcripts have been isolated and sequenced. Particle bombardments using the B22E promoter fused to the GUS and the maize regulatory "R" marker genes show promoter activity in developing barley and maize aleurone cells, respectively. Similar experiments using the Lpt1 and Lpt2 promoters will be discussed. Sequence comparisons of the promoter region of the three genes identifies several common putative regulatory motifs. One of the genes contains a myb-protein binding site. Screening of an expression library from young aleurone cells for clones encoding proteins binding to the putative regulatory promoter elements of the isolated aleurone genes is underway.

667 THE RATE OF GENE TRANSCRIPTION INCREASES TENFOLD DURING THE SYNCYTIAL STAGE OF BARLEY ENDOSPERM DEVELOPMENT. Odd-Arne Olsen, Marie Bosnes and Fredrik Weidemann. Plant Molecular Biology Lab NLVF, P.O.Box. 51, N-1432-AAs, Norway.

In analogy with animal embryo systems, barley endosperm development may be viewed as resulting from the activation, in a hierarchial order, of several distinct gene sets. As a minimum, three such gene sets may be postulated, specifying cell wall biogenesis, starchy endosperm and aleurone cell formation, respectively. Based on microscopy studies of wild type and sex-mutant endosperms we divide endosperm development into four stages, each marking major events in the differentiation proces. At each stage, the isolation of endosperm tissue for molecular studies is described. During the Syncytial stage (I) a syncytium, resulting from the repeated divisions of the primary triploid endosperm nucleus, lines the walls of the embryosac (IA). Preceeding cellularization, the syncytium becomes vacuolarized (IB). Puls-labelling experiments using 3H-Uridine demonstrate that the rate of transcription in endosperm nuclei increases tenfold during stage I. During the Cellularization stage (II), a uniform cellular endosperm is formed. Initiating the Differentiation stage (III) the peripheral cells of the cellular endosperm becomes highly vacuolar, marking the onset of aleurone cell formation. Cessation of starchy endosperm and aleurone cell mitosis, respectively, mark the end of stage IIIA and IIIB. Main events during the Maturation stage (IV) are accumulation of storage products. We are currently studying gene promoters specifying transcription in the aleurone layer during stage III and IV (see this poster session).

668 REGULATION OF GENE EXPRESSION IN EMBRYOS OF MAIZE *VIVIPAROUS* MUTANTS BY ABA. Renato Paiva and Alan L. Kriz. University of Illinois. Department of Agronomy, Urbana, IL 61801

The maize *viviparous* mutants are characterized by precocious germination prior to the onset of seed desiccation. Each of these mutants is defective in ABA metabolism: embryos homozygous for the *vp1* mutation contain normal ABA levels but are insensitive to this phytohormone: embryos homozygous for the other mutants contain reduced ABA levels and are apparently defective in ABA synthesis. To gain an understanding of ABA-regulated processes, we investigated the expression patterns of specific genes in embryos of *viviparous* mutants. Our results indicate that these mutants do not fully switch in gene expression from a pattern of embryo development to one of germination. Since most of these mutants are deficient in ABA biosynthesis, we also looked at the effects of ABA on gene expression in developmentally-staged embryos. This study suggests that for the induction of *Globulin* genes by ABA, a funcuonal *Vp1* gene product is required. The expression pattern of embryo-specific genes and the effect of ABA on their expression during the process of precocious germination will be discussed.

669 ROLE OF LIPIDS IN SOME PROCESSES OF MAIZE GRAIN DEVELOPMENT. Shtemenko N.I., Zamorueva L.F., Kravzova N.V. Biology Institute of Dniepropetrovsk State University

Composition of lipids and fatty acid content in anatomic parts and subcellular particles of maize grain of different types were investigated. It was shown, that more quantity of unsaturated and higher fatty acids in tissues coincided with less ability to release free amino acids, that was connected with physiological state of a tissue, i.e. with penetrability of plant membranes. These phenomena were shown to take place in seed-coats of maize and was verifield on large collection of maize grain differ in resistance to some deseases. Fatty acid composition of protein bodies of different types of maize grain had some differences and that may have some cover functions in protection of reserve proteins from proteolysis. On the forth day of seedling, when process of zeins destruction started and appearence of large quantity of free amino acids took place in a endosperm, the lipid complex of protein bodies was shown to rebuild in direction of formation of low molecular weight fatty acids. Some analogous phenomena of rebuilding of lipids and changing of free amino acids composition are investigating in the cases of pesticide treatment of plants and are shown to be widely-distributed.

670 SEED MAJOR ALBUMIN GENES INFLUENCE THE EXPRESSION OF SEED LECTIN IN PEA. Rosa Rao and Patrizia Spigno. University of Naples, Dept of Plant Genetics. Portici, Italy

Pea major albumins (MA) are present exclusively in the embryo where they accounts for approximately 15-40% of total cotyledonary proteins. They are formed by one or two polypeptides with molecular weights between 25 and 24 kD which are coded by a single genetic locus. The MA gene copy number, however, is quite variable in different genotypes beeing estimated from three to eight copies per haploid genome; it influences the MA accumulation during seed development and in the mature seed suggesting that gene copies is one of the factor controlling seed albumin synthesis. Mutants lacking MA protein as a consequence of structural deletions at the MA locus show reduced levels of the lectin, a protein of about 20 kD molecular weight, in the mature seed; this low amount of the lectin protein, is inherited by F1 hybrids obtained by reciprocal crosses of the mutant lines with normal lines. Futhermore these mutants show a reduced level of expression of the lectin gene during the seed development thus suggesting a possible implication of the MA locus on the synthesis and deposition of pea seed lectin.

671 SEED PROTEIN GENE PROMOTERS FROM FABA BEAN AND OAT: SPECIFICITIES OF EXPRESSION IN SPACE AND TIME IN TRANSGENIC TOBACCO SEEDS. Reinhard Panitz, Helmut Bäumlein, Ute Heim, Roland Schubert, Winfriede Weschke and Ulrich Wobus

Institute of Genetics and Crop Plant Research, 0-4325 Gatersleben, Germany.

Seed protein genes are expressed in a highly regulated fashion during seed development. According to the general view, these genes become transcriptionally activated after a large number of embryonal cells have been formed, i.e., during the cell expansion stage of development. We have isolated and characterized genes coding for a B-type legumin (LeB4), a 50kD vicilin (Vic1), an abundant mRNA of unknown function (Usp30.1), all from *Vicia faba*, and a legumin-like globulin from *Avena sativa* (Asglo5). The genes were studied by *in situ* hybridisation or using gus fusions. Their promoters govern different expression patterns in transgenic tobacco seeds and show strong activity already during the earliest developmental stages studied, the late globular embryo (shown for LeB4 and Asglo5). The appearance of the respective proteins is presently studied by immunological methods in the same tissue sections as used for *in situ* hybridisation. Promoter deletion studies of genes LeB4 and Usp30.1 have defined *cis*-elements responsible for qualitative (space and time) and quantitative aspects of expression.

POSTER ABSTRACTS

672 SOMES GENES REGULATED BY OXIDATIVE STRESS ARE ALSO EXPRESSED DURING AGING IN SOYBEAN SEEDS.
Xavier Gidrol[1], Norbert Dégousée and Abdelmajid Noubhani. Station de Physiologie Végétale, INRA BP 81, 33883 Villenave d'Ornon Cedex, France. 1. Current adress: Harvard School of Public Health, Laboratory of Toxicology, 665 Huntington Av., Boston, MA 02115.
As a first step toward understanding the molecular mechanisms of seed aging, we present here a biochemical analysis of genes expressed during both oxidative stress and the aging process. When soybean seeds were exposed to hydrogene peroxide, or an atmosphere enriched with singlet oxygene, or acceleratedly aged at 42°C and 100% relative humidity, several common proteins were induced. As demonstrated by Matters and Scandalios (1986), superoxide dismutase is induced by oxidative stress. Similarly a dramatic increase in SOD activity was observed during seed aging, while catalase activity remained very low. Further analyses demonstrated that the activity increase resulted from an induction of Cu, Zn SOD. These results suggest that seed aging could be regarded as an oxidative process.

673 STRUCTURE AND EXPRESSION OF THE GENES ENCODING SEED PROTEINS OF CANAVALIA GLADIATA Daisuke Yamauchi and Takao Minamikawa. Tokyo Metropolitan Univ., Dept. of Biol., Tokyo, 192-03 Japan.
Major seed proteins of C. gladiata are canavalin, a 7S globulin, and concanavalin A (Con A), a seed lectin. We selected essentially full-length cDNAs for these two seed proteins from a library by immunological screening. The canavalin cDNA encodes a polypeptide of 445 amino acid residues, and the sequence shows a high homology with those of other 7S seed globulins. The Con A cDNA encodes a polypeptide of 290 amino acid residues. The sequences of Con A from C. gladiata and C. ensiformis (D. Carrington et al. 1985. Nature 313: 64-57) are highly homologous with each other, and hence it is assumed that Con A from C. gladiata undergoes the same posttranslational processing as Con A from C. ensiformis including the transpeptidation of the precursor polypeptide. We estimated levels of canavalin and Con A mRNAs at successive stages of the seed development using the cDNA probes, and results indicated that the two mRNA levels are differently regulated. We also isolated the genes encoding both of the seed proteins and determined their nucleotide sequences alone with the 5' and 3' flanking regions. The gene for canavalin is consisted of six exons and five introns. The 5' upstream region includes 'vicilin' box that have been found in the 5' upstream regions of other 7S seed globulin genes. The gene for Con A contains no introns. The 5' upstream beyond a consensus TATA box of the Con A gene showed a low homology with other seed lectin genes hitherto reported. No typical CAAT box was found in the upstream of the initiation site of the gene. The 5' upstream region of both genes include AT-rich sequences.

674 THE 5'-CATGCAT-3' SEQUENCE MODULATES THE ACTIVITY OF GLYCININ GENES. Luiz Orlando de Oliveira, Jean-Marc Lelievre and Niels C. Nielsen. USDA Agricultural Research Service, Agronomy Department, Purdue University, West Lafayette, IN 47907
The sequence 5'-CATGCAT-3' is found upstream from a number of genes expressed in seeds of legumes and has been identified as a potential regulatory sequence. To explore this possibility, upstream flanking regions of a Gy2 glycinin gene of soybean were fused to the reporter gene β-glucoronidase (GUS) and introduced into tobacco by use of a binary vector. Two types of constructions and their controls were formed. One construction contained 361 bp of upstream flanking Gy2 DNA, 59 bp of untranslated leader sequence and 7-bp (+56) of Gy2 coding region. This fragment was fused in frame to the GUS reporter element. The 7 bp in the 5'-CATGCAT-3' element contained within the legumin box (-89 to -124) was intact in the controls and deleted in the experimental fusion gene. The other type of constructions were identical except that there were 2.3 kbp of upstream flanking DNA rather than 361 bp. Although β-glucoronidase activity was present when the 5'-CATGCAT-3' element in the fusion genes were destroyed, the activity was at least an order of magnitude lower than for the controls. Thus, even though it has not yet been possible to demonstrate the binding of nuclear proteins to this element, its absence causes a profound apparent down-regulation in expression from the fusion gene.

675 WHEAT ENDOSPERM NUCLEAR PROTEINS INTERACT WITH AN α/β-GLIADIN PROMOTER. Robert Luis Vellanoweth and Thomas W. Okita. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340
The promoter region of the wheat α/β-gliadin seed storage protein gene was analyzed for interactions with nuclear proteins from developing wheat seeds. Six different complexes were detected within the first 165 bp upstream of the transcriptional start site. One of the binding proteins (CABP) mapped to a conserved CA-rich element while another (PalBP) mapped to an adjacent, palindromic sequence. Using synthetic oligonucleotides in competition experiments, 3 of the remaining complexes were found to result from interactions at a 29 bp sequence homologous to a wheat HMW glutenin seed storage protein gene enhancer element. The last complex was formed by a relatively non-specific AT binding protein. With the exception of PalBP, all the binding activities were coordinately expressed during the development of the wheat seed and coincided with the pattern of accumulation of the gliadin mRNA: a fairly rapid increase from 3 days after flowering to 15 DAF followed by a general decrease. PalBP activity, on the other hand, first appeared at mid stage and was present at a constant level throughout later stages of development. Evidence will be presented suggesting that binding of PalBP is interfered both by a soluble factor and through a steric competition of its binding site with CABP. The results suggest potential roles for the various factors in modulating the expression of the gliadin genes.

676 WHEAT EMBRYONIC mRNA WHICH DISAPPEAR WITH THE LOSS OF DORMANCY. Naoto Kawakami, Chisato Kawabata and Kazuhiko Noda. Yokohama City University, Kihara Institute for Biological Research, Mutsukawa, Minami-ku, Yokohama 232, Japan
The embryo responsiveness (in germination) to 10 uM abscisic acid (ABA) of dormant wheat (Triticum aestivum line Kitakei-1354) seeds and nondormant (cv. Chihoku) seeds are compared. Embryos of Chihoku lost their responsiveness during seed maturation, while those of Kitakei-1354 responded to ABA even at harvest time and lost their responsiveness gradually during after-ripening period. Inbibition at low temperature below 15°C can break dormancy of Kitakei-1354 seeds. The embryos isolated from Kitakei-1354 seeds treated at low temperature lost responsiveness as observed in mature Chihoku and after-ripened Kitakei-1354 seeds. These results suggest that the responsiveness of embryos to ABA is a main factor of wheat seed dormancy. By analyzing the translation products of poly(A)⁺RNA prepared from embryos, we have found mRNAs for four polypeptides which decreased or disappeared during seed maturation in Chihoku, but did not change at level in Kitakei-1354. To characterize these mRNAs, embryos of dormant and after-ripened Kitakei-1354 seeds were treated with ABA. The levels of three out of the four mRNAs were maintained in the embryos of dormant seeds but decreased in the embryos of after-ripened seeds. One of these three mRNAs disappeared during inbibition of whole seed at low temperature. This mRNA, which decreased along with the loss of dormancy, appear to be related to maintaining dormancy. cDNA cloning of the transcript is in progress.

677 ALTERED PERICARP CELL DIFFERENTIATION IN TOMATO PLANTS EXPRESSING A CHIMERIC *ipt* GENE IN FRUIT. Belinda Martineau, Raymond E. Sheehy and Catherine M. Houck. Calgene, Inc., 1920 Fifth Street, Davis, California 95616. The cytokinin class of plant growth regulators is associated with various aspects of plant growth and development, perhaps foremost among them the promotion of cell division and the delay of senescence processes. Historically, perturbing endogenous cytokinin levels through exogenous application has been used in attempts to discern its effects on specific plant tissues and organs. We are interested in the role cytokinins play in tomato fruit development. To avoid problems with exogenous application, we opted to increase cytokinin levels *in planta*. The *ipt* gene from *A. tumefaciens*, encoding isopentenyl transferase (IPT), was therefore used to construct a chimeric gene for gene transfer experiments. The IPT enzyme catalyzes the conversion of isopentenyl pyrophosphate and AMP to isopentenyl AMP which, in turn, serves as a precursor for other active cytokinins in plants. The regulatory sequences from a fruit-specifically expressed gene, 2A11 (Pear *et al.* 1989 *Plant Molecular Biology* 13:639-651), were used in the chimeric gene construct to restrict *ipt* gene expression. Fruit from half of the transformed plants exhibited a dramatic phenotype reminiscent of the physiological tomato disorder "blotchy ripening" in which islands of firm, green pericarp tissue exist in the midst of otherwise soft, red ripe fruit. The phenotype was inherited in a Mendelian fashion. We have documented expression of the chimeric *ipt* gene by measurement of both steady-state RNA levels and zeatin riboside levels in fruit and leaves. We have also examined the accumulation of mRNA encoding the cell-wall degrading enzyme, polygalacturonase, in the various regions of "blotchy" fruit. Our results indicate that the developmental process of fruit ripening, a senescence-related phenomenon, has been disrupted in these transformed plants. In green islands fully expanded, chloroplast-containing cells with intact cell walls have not differentiated into mature, lycopene-rich cells with walls in which the middle lamella has been degraded. We interpret this effect on fruit cell differentiation as being consistant with delaying senescence, an attribute long associated with elevated levels of cytokinin in plants.

POSTER ABSTRACTS

678 INHIBITION OF PECTIN ESTERASE BIOSYNTHESIS IN TRANSGENIC TOMATO

Colin R. Bird, John A. Ray, Wolfgang Schuch, Christopher J. Smith[1], Colin F. Watson[1], Lisa Hall[1], Don Grierson[1], Greg Tucker[2], Duncan Robertson[2], Angus Murray[3], Graeme Hobson[3]. ICI Seeds, Plant Biotechnology Section, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, U.K.; [1]Departmenet of Physiology and Environmental Science and [2]Department of Applied Biochemistry and Food Science, University of Nottingham, Faculty of Agriculture and Food Sciences, Sutton Bonington, Loughborough, LE12 5RD. U.K.: Horticulture Research International, Worthing Road, Littlehampton, West Sussex, BN17 6LP, U.K.

Pectin esterase (PE) catalyses the de-methylation of cell wall pectin in tomato fruit by removal of the methyl group from the C6 position of galacturonic acid residues. However, the role of pectin de-methylation in cell wall development has not been established. Transgenic tomato plants expressing PE antisense RNA have been generated in order to study the function of PE. Tomato fruit with less than 10% of normal levels of PE have been identified. Progeny homozygous for the PE antisense gene have been grown in large scale trials for identification of changes in the characteristics of these fruit. No major changes in fruit morphology have been observed in these plants. However, changes in cell wall composition and the activities of individual PE isozymes have been observed. Significant improvements in the quality of processed tomatoes have been achieved using fruit with reduced PE activity.

679 INHIBITION OF CAROTENOID BIOSYNTHESIS IN TRANSGENIC TOMATOES

Colin R. Bird, John A. Ray, Jonathon D. Fletcher, Jeremy M. Boniwell, Alison S. Bird, Chantal Teulieres, Ian Blain[1], Peter M. Bramley[1] and Wolfgang Schuch. ICI Seeds, Plant Biotechnology Section, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, U.K. [1] Department of Biochemistry, Royal Holloway and Bedford New College, University of London, Egham, Surrey TW20 0EX, U.K.

Transgenic tomato plants expressing antisense RNA to a ripening related gene (pTOM5) had yellow ripening fruit and pale coloured flowers. The yellow fruit colour has been correlated with a severe reduction in the level of the pTOM5 gene mRNA during fruit ripening. The level of carotenoids in ripening fruit from selected transgenic plants showing yellow fruit was reduced by more than 97%. In addition, the carotenoid lycopene which is primarily responsible for the red colour of ripening fruit was reduced to undetectable levels (<0.1 %). These data indicate that the pTOM5 gene product plays a crucial role in tomato fruit carotenoid biosynthesis. Further biochemical analysis has clarified which enzyme involved in carotenoid biosynthesis is inhibited in these plants.

680 RIPENING-RELATED UPSTREAM REGION OF THE AVOCADO CYTOCHROME P-450 GENE

Kristin R. Bozak, An-Chi Lin, & Rolf E. Christoffersen, Biological Sciences Department, University of California, Santa Barbara, CA 93106 A ripening-related cytochrome P-450 cDNA from avocado (Bozak et al.(1990) *PNAS* 87:3904-3908) was used to screen a genomic library of avocado, resulting in the identification of two classes of hybridizing clones. Southern blot and sequence analysis of one class (designated *cyp*71A1) indicates it is the cytochrome P-450 gene represented in the cDNA. The second class appears to represent a different cytochrome P-450 family member for which no cDNAs have been identified. In order to identify ethylene/ripening-related control elements of the *cyp*71A1 gene, we are analyzing transient expression of promoter/reporter gene fusions electroporated into avocado fruit protoplasts. A 1.6 Kb region immediately upstream of the *cyp*71A1 gene was fused to the reporter gene ß-glucuronidase (GUS) and electroporated into protoplasts derived from mesocarp tissue. We have shown that the *cyp*71A1 promoter/GUS fusion is expressed in protoplasts from ripe fruit at a level approximately 1/5 that of an efficiently expressed control gene (35S CaMV promoter/GUS fusion). We are currently investigating the ethylene dependence of the *cyp*71A1 promoter/GUS expression using ethylene antagonists and biosynthetic inhibitors. Further, we are constructing 5' deletions of the upstream region in order to localize important control elements of the *cyp*71A1 gene.

681 GALACTOSE-INDUCIBLE EXPRESSION OF AN AVOCADO CYTOCHROME P-450 (CYP71A1) IN YEAST

Kristin R. Bozak[1], Daniel P. O'Keefe[2], and Rolf E. Christoffersen[1]. [1]Biological Sciences Department, University of California, Santa Barbara, CA 93106. [2]Du Pont Co., Central Research Dept., Wilmington DE 19880 Sequence analysis of cDNA clones encoding a cytochrome P-450 mRNA that accumulates during ripening of avocado fruit revealed a polypeptide that was designated CYP71A1 (Bozak et al. (1990) *PNAS* 87:3904-3908). Using antiserum generated to a P-450 polypeptide isolated from ripe avocado tissue and characterized as an N-demethylase (O'Keefe and Leto (1989) *Plant Phys.* 89:1141-1149), we have shown that P-450 antigen is undetectable in unripe tissue, and increases with ripening. This increase in antigen correlates with an increase in the activities of two cytochrome P-450 enzymes: *p*-chloro-N-methylaniline (pCMA) N-demethylase and *t*-cinnamic acid hydroxylase. In order to determine which, if either, of these activities is derived from CYP71A1, we have used a heterologous galactose-inducible system to express CYP71A1 in yeast cells. Both immunological and spectroscopic assays indicate the presence of a galactose-inducible CYP71A1 polypeptide in transformed yeast. Microsomal fractions derived from these cells exhibit *p*CMA N-demethylase activity. As *p*CMA is a xenobiotic, the *in vivo* substrate of CYP71A1 is presently unknown. The substrate specificity of CYP71A1 is currently under investigation.

682 ISOLATION OF GENES INVOLVED IN LEAF SENESCENCE.

Vicky Buchanan-Wollaston, Department of Biological Sciences, Wye College, University of London, Ashford, Kent, TN25 5AH. U.K.

During its life span a leaf undergoes at least 3 stages of development. The first stage is a period of rapid growth when the leaf is a net importer of nutrients. Later, the leaf becomes an asset to the plant and provides a source of photosynthate to the rest of the plant. Finally, environmental or developmental signals induce the leaf to start the process of senescence. During this phase the nutrients that have been built up in the leaf are mobilised and transported to developing parts of the plant. The biochemistry and physiology of the processes involved in this important phase in plant development have been the subject of much study but so far little is known at the molecular level. Knowledge of the processes that control leaf senescence should allow manipulations to be made to the rate of senescence in crop plants which has the potential of increasing the yields of grain crops and improving the protein value in forage crops. Genes involved in leaf senescence are being identified in *Arabidopsis* by isolation of mutants. Mutants that may be defective in protein mobilisation or chlorophyll breakdown have been identified and further analysis and charactersation of these will be discussed. Also, genes involved in leaf senescence in *Brassica napus* are being isolated by cDNA cloning using RNA produced in senescing leaves and identification of senescence related genes by hybridisation and subtraction with RNA from non senescing leaves. Progress in this approach to the isolation of genes that are specifically induced during leaf senescence will be presented.

683 RIPENING-RELATED UPSTREAM REGION OF THE CELLULASE GENE (*cel*1) FROM AVOCADO.

Laura G. Cass & Rolf E.Christoffersen, Biological Sciences Department, University of California, Santa Barbara, CA 93106. We have previously characterized the *cel*1 gene from avocado that is responsible for ripening-associated cellulase mRNA (Cass et al., 1990 MGG 223:76-86). In order to identify ripening-related control elements of the *cel*1 gene, we are analyzing transient expression of promoter/reporter gene fusions electroporated into avocado fruit protoplasts. To demonstrate the transcriptional/translational competence of protoplasts derived from both unripe and ripe mesocarp, a control promoter (35S CaMV) fused to the chloramphenicol acetyl transferase (CAT) gene was introduced into both cell types. The CaMV promoter gives equal levels of CAT expression irrespective of the stage of fruit ripening. In contrast, the 1.5 kb-upstream sequence of *cel*1 fused to the CAT gene is expressed at least 10-fold lower in protoplasts derived from unripe fruit as compared to protoplasts from ripe fruit. To localize the upstream region responsible for ripening-regulated expression, a series of 5' deletions of the *cel*1 promoter were tested in the ripe-derived protoplasts. Deletions from -1500 to -660 give rise to similar levels of activity in the transient expression assay. Further deletion of the upstream sequence to -470 reduces the activity of the gene by at least 50-fold indicating an important *cis*-acting element between -660 and -470 nucleotides from the *cel*1 transcription start site. Together, these results suggest that factors necessary for expression of the *cel*1 gene are limiting in unripe protoplasts and present in the ripe protoplasts. In order to investigate the ethylene dependency of *cel*1 gene expression in this protoplast system, the effects of ethylene antagonists and biosynthetic inhibitors are currently being measured.

POSTER ABSTRACTS

684 REGULATION OF GENE EXPRESSION BY ETHYLENE DURING TOMATO (*Lycopersicon esculentum*) FRUIT RIPENING. Jill Deikman, Julie Montgomery, Linda Margossian, Lola Peñarrubia and Robert Fischer. Department of Plant Biology, University of California, Berkeley, CA 94720.

The plant hormone ethylene plays an important role in controlling fruit ripening. The onset of ripening of many fruits is initiated by increased levels of ethylene that, in turn, activates gene expression. To study this process we have cloned mRNAs that accumulate during fruit ripening and in unripe fruit exposed to exogenous ethylene. Genes that encode two of these mRNAs (E4 and E8) have been studied in detail and are rapidly transcriptionally activated by ethylene. We are currently studying E4 and E8 gene function by analyzing fruit from transgenic tomato plants bearing their respective antisense genes. To begin to understand how ethylene regulates E4 and E8 gene transcription we have mutagenized their 5'-flanking regions and have analyzed expression of these mutated genes in both stably transformed plants and in a micro-projectile transient assay system. These experiments indicate that short, discrete DNA sequences are responsible for transmitting the ethylene signal. We are currently analyzing nuclear proteins that interact with these regulatory DNA sequences.

685 PURIFICATION AND CLONING OF THE β-SUBUNIT OF TOMATO FRUIT PG1
Dean DellaPenna and Lian Sheng Zheng
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The activity of the pectin-hydrolyzing cell wall enzyme polygalacturonase (PG) increases dramatically during the ripening of many fruits. In tomato PG activity is due to three closely related, posttranslationally-derived isoforms, PG1, PG2A and PG2B. PG1, appears first during ripening and is a heterodimer containing one catalytic PG2 polypeptide associated with an ancillary 38 KDa cell wall glycoprotein, the β-subunit. Previous results have indicated that PG1 may be the active form of the enzyme *in vivo* with regard to pectin degradation suggesting that the β-subunit plays an important role in regulating pectin degradation during fruit ripening. In order to further our understanding of the regulation of PG biosynthesis and pectin degradation during ripening we have purified PG1 and isolated the β-subunit by FPLC chromatography. N-terminal sequence has been obtained and used to make degenerate oligonucleotides for cDNA library screening. Progress on biochemical analysis and cloning of the β-subunit of PG1 will be reported. Supported by NSF Grant DCB-90-18154.

686 COMPARISON OF THE ACTIVE-SITE AMINO ACID SEQUENCES OF 1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE: ANALYSIS BY POLYMERASE CHAIN REACTION. Jian Guo Dong and Shang Fa Yang, Mann Lab, Dept. of Vegetable Crops, Univ. of California, Davis, CA 95616.

1-Aminocyclopropane-1-carboxylate (ACC) synthase which converts S-adenosylmethionine to ACC performs as the rate-limiting step in ethylene biosynthesis. We have isolated a ripening-related ACC-synthase cDNA clone from apple fruit. Comparison of the deduced amino acid sequence of the apple enzyme with the reported sequences from tomato and winter squash revealed seven highly conserved regions, including the previously-identified active-site center (^{260}YSLSKDLGLPGFRVG^{274}). To compare the active-site center of ACC synthase with those of other species, polymerase chain reaction (PCR)-based amplification of RNA was employed. Based on two of the conserved regions which precede and follow the active-site center respectively, two mixed oligonucleotide primers were used as PCR primers. Total RNA isolated from fruits of avocado, pear and peach was reverse-transcribed into first strand cDNA and amplified by PCR with *Taq* polymerase. While no product was amplified from mRNAs isolated from unripe fruits, a 320-bp sequence was amplified from the mRNA of ripe fruits. The deduced amino acid sequence of each product contained the active-site region, indicating the specific amplification by PCR of ACC-synthase mRNA. The active-site center of avocado and pear contained L-266. When genomic DNA isolated from peach was used as a template for PCR, two sequences were amplified. One contained L-266 and the other contained M-266. Analysis of mRNA isolated from different ripening stages of peach fruit revealed that only the sequence containing M-266 increased during ripening. Comparison of the amino acid sequences of the active-site center deduced from PCR fragments indicates that Leucine-266-containing sequences are more homologous to each other than to the M-266 containing ones. Likewise, the M-266 containing sequences are more homologous to each other than to the L-266 sequences. The above results indicate that ACC synthase may largely be classified into two types; L-type and M-type. These two classes of enzyme represent different gene products and are isoenzymes.

687 GENE REGULATION DURING FRUIT RIPENING: CLONING AND CHARACTERIZATION OF TOMATO FRUIT VACUOLAR INVERTASE cDNA AND GENOMIC SEQUENCES. K. Elliott, W. Butler, C. Dickinson, Y. Konno, T. E. Mirkov and L. Fitzmaurice. SIBIA, P.O. Box 85200, San Diego, CA 92186.

Fruit of the wild tomato species, Lycopersicon pimpinellifolium, has a higher soluble solids content than that of the cultivated species, L. esculentum. Invertase, which catalyzes the hydrolysis of sucrose into glucose and fructose (soluble solids), is active in the wild species at earlier stages of fruit development and shows a higher level of activity later in fruit development. In order to explore the relationship between invertase gene expression and soluble solids content, we isolated invertase cDNA and genomic clones from L. esculentum cv. UC82 and L. pimpinellifolium libraries. The cDNA libraries were prepared using poly(A)$^+$ RNAs from pink fruit and screened with antibodies directed against purified tomato fruit vacuolar invertase. The genomic libraries were screened with a full-length L. esculentum cv. UC82 invertase cDNA clone. Analysis of total RNA isolated from various stages of fruit development reveals that, in L. pimpinellifolium, invertase mRNA appears in green fruit and is present at high levels in orange and red fruit. However, in L. esculentum cv. UC82, invertase mRNA does not appear until the pink stage of fruit development and is present at high levels only in red fruit. Sequence analysis reveals minor differences between the exons, introns and regulatory regions of the two species. These differences may account for the differences in invertase gene expression during fruit development; alternatively, a trans-acting factor may be involved.

688 CLONING AND EXPRESSION OF LIPOXYGENASE IN RIPENING TOMATO FRUIT. B.J. Ferrie, C.G. Bowsher, S.J. Rothstein and J.E. Thompson. University of Guelph, Dept. of Molecular Biology and Genetics, Guelph, Ontario, Canada.

The precise physiological role of plant lipoxygenases has not been defined, although they are thought to be involved in fruit ripening and abscission, senescence and resistance to plant pathogens. We are investigating the role and expression of lipoxygenases in ripening tomato fruit. This fruit contains membrane-associated and soluble forms of the enzyme which have the same molecular mass (~100kD), but are distinguishable by their isoelectric points and enzyme kinetics. The membrane-associated form has been purified and degenerate oligonucleotides determined from the amino acid sequences were used together with PCR to clone a 800 bp fragment of a lipoxygenase gene. The fragment is ~64% homologous to a cDNA of soluble pea lipoxygenase. Also, this pea cDNA was used to probe a cDNA library made from mature green tomato fruit. A partial-length clone was isolated and sequenced and found to have ~65% homology with the pea sequence that possibly codes for the soluble isoform. The cDNA clones coding for both lipoxygenase isozymes will be used to determine the pattern of regulation in these genes.

689 MAPPING AND CHARACTERIZATION OF FRUIT RIPENING LOCI OF TOMATO. James Giovannoni, Martin Ganal, Gregory Martin, Rod Wing and Steven Tanksley. Cornell University, Department of Plant Breeding and Biometry, Ithaca, New York 14853.

Fruit ripening represents a tightly regulated and coordinated developmental program unique to plants. Several single locus mutations exerting dramatic effects on the ability of tomato fruit to ripen normally have been identified previously, although none of the corresponding ripening genes have yet been cloned. In particular, the *rin* and *nor* mutations result in nearly complete blockage of the ripening process and thus represent key regulatory steps of tomato fruit development. Toward the ultimate goal of isolation of fruit ripening genes via map-based cloning techniques, we have localized ripening loci on a high-density RFLP map of tomato. In addition, a yeast artificial chromosome (YAC) library of tomato to be utilized in subsequent genomic walking experiments is currently under construction. Techniques for simplified high resolution RFLP mapping and tightly linked probe isolation employing large mapping populations and pooling of segregating individuals will be discussed.

POSTER ABSTRACTS

690 THE *rin* TOMATO MUTANT AS A TOOL TO STUDY GENE EXPRESSION DURING FRUIT RIPENING. Julie E. Gray, Steve Picton and Don Grierson. University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, U.K.

rin (ripening inhibitor) mutant tomatoes have impaired fruit ripening and do not soften, turn red or synthesize ethylene, sugars and other flavour components normally. We have studied the molecular biology of fruit ripening using a cDNA library of 2000 clones containing 19 classes of ripening-related clones. Several mRNAs have been shown to be present at reduced levels in *rin* mutant fruit. Southern analysis shows that the genes for each of these ripening-related mRNAs are apparently normal in the *rin* genome suggesting that the *rin* mutation alters the expression of some ripening genes. A combination of sequence analysis and antisense technology has led to the identification of a number of the ripening-related mRNAs from this original cDNA library, including mRNAs involved in cell wall degradation (polygalacturonase and pectinesterase), ethylene biosynthesis (ethylene forming enzyme) and carotenoid biosynthesis (phytoene synthase). We are currently using *rin* fruit mRNA to screen a new tomato fruit cDNA library (approximately 11 million clones) to identify and characterise further genes involved in the regulation of fruit ripening.

691 POLYGALACTURONASE EXPRESSION IS IMPAIRED BY HEAT STORAGE OF RIPENING TOMATO FRUITS. Varda Kagan-Zur*, Denise M. Tieman, Yossi Mizrahi* and Avtar K. Handa. Purdue University, Horticulture Department, W. Lafayette, IN 47907.

We have investigated effects of high temperature storage on polygalacturonase (PG) expression in ripening tomato fruits at the mRNA, protein and activity levels. Storage of breaker and turning stage fruits at 34°C caused disappearance of PG mRNA while transfer to 20°C partly restored PG mRNA accumulation. However, a decrease in the ability of fruits to accumulate PG mRNA upon transfer to 20°C was observed with increasing length of storage at 34°C. PG activity present in turning stage tomatoes at the onstart of storage at 34°C persisted at least up to 14 d and increased during subsequent storage at 20°C in fruits which were stored for less than 6 d at 34°C. Fruits stored at 34°C for more than 6 d did show some accumulation in PG mRNA but not in PG activity upon transfer to 20°C. PG protein levels are being quantified using immunoblotting to determine the basis of this discrepancy. Collectively, our data suggest that high temperature impairs expression of PG at the transcriptional level in ripening tomato fruits and the degree of recovery of PG expression is dependent upon the length of storage at high temperature.

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692 MOLECULAR CLONING AND CHARACTERIZATION OF TOMATO FRUIT INVERTASE. Ellen Klann, Serge Yelle, Roger Chetelat, Joseph DeVerna[1] & Alan Bennett. Mann Laboratory, Department of Vegetable Crops, University of California, & [1]Campbell's Institute for Research and Technology, Davis CA 95616.

A wild tomato relative, *Lycopersicon chmielewskii*, has a high level of total soluble sugar and unlike *Lycopersicon esculentum*, accumulates sucrose rather than hexose. Genetic and biochemical analysis of crosses between the two species indicates that the trait is controlled by a single recessive gene and is associated with low levels of fruit acid invertase. To test whether the invertase gene controls the trait of sucrose accumulation and contributes to high total soluble sugar content we have isolated a cDNA clone encoding tomato fruit acid invertase. Invertase was purified from ripe tomato fruit, cleaved with cyanogen bromide and the amino termini sequenced. Oligonucleotides corresponding to these protein sequences were used to identify a clone from a ripe tomato fruit cDNA library whose predicted amino acid sequence is very similar to carrot extracellular invertase. The cDNA is 1950 base pairs long, encoding the entire mature protein, but missing the translation initiation codon and a predicted signal sequence. The 5' end of the cDNA was subsequently isolated by RACE PCR. Genomic Southern analysis at moderate stringency suggests that the gene is present as a single copy in the tomato genome. Analysis of mRNA levels indicate that invertase mRNA levels increase dramatically during fruit ripening. Analysis of mRNA levels from developing fruit of *Lycopersicon esculentum*, *Lycopersicon chmielewskii* and a BC_2F_3 population will be presented, as well mapping of the sucrose accumulation trait and the invertase gene to their chromosomal location.

693 CELLULASE (ENDO-β-1,4-GLUCANASE) GENE EXPRESSION IN WILDTYPE AND TRANSGENIC TOMATO FRUIT. Coralie C. Lashbrook[1], James J. Giovannoni[2], Jamie Stott[2], Eric A. Holst[1], Robert L. Fischer[2] and Alan B. Bennett[1],Mann Laboratory, Department of Vegetable Crops, University of California, Davis, CA. 95616 and [2] Plant Biology Department, University of California, Berkeley, CA. 94720.

The degradation of cell wall polymers by specific cell wall hydrolases during fruit ripening is thought to be required for tomato fruit softening. Polygalacturonase-mediated pectin degradation is now known to be neither necessary nor sufficient for tomato fruit softening. Hemicellulose degradation by plant 'cellulases' may in fact contribute to tomato fruit softening. We have cloned the cDNAs of two related members of the tomato fruit cellulase gene family. TCI 1 and TCI 2 are distinct gene products with significant sequence differences and exhibit different mRNA expression patterns during ripening. We are currently analyzing tomato plants transfected with antisense genes corresponding to TCI 1 and have identified transformants in which the level of TCI 1 sense mRNA is significantly decreased. We are evaluating the relative capacities of protein extracts from wildtype and transgenic fruit to direct the degradation of carboxymethylcellulose to establish whether TCI 1 may contribute to the dramatic rise in carboxymethylcellulase activity long chronicled in fruit ripening literature.

694 ISOLATION OF cDNA CLONES FOR CHROMOPLAST-SPECIFIC PROTEINS. Susan D. Lawrence, Kenneth Cline, and Gloria A. Moore. Fruit Crops Dept., University of Florida, Gainesville, FL 32611

The transition from chloroplast to chromoplast in ripening tomato fruit involves the degradation of the photosynthetic apparatus, the breakdown of starch reserves, and the accumulation of carotenoids. We are interested in genes for proteins that mediate these processes, especially those involved in the carotenoid biosynthetic pathway. Most or all of these proteins are likely to be encoded in the nucleus, synthesized in the cytosol, and targeted to chromoplasts. In support of this is our observation that a set of ripening enhanced in vitro translation products are imported into plastids in vitro. Our approach has been to isolate cDNA clones whose corresponding mRNA is enhanced during ripening. These clones are used to obtain the corresponding mRNA by hybrid selection or in vitro transcription. Translation products are used in an in vitro chloroplast protein import assay to identify those clones that encode plastid targeted proteins. This approach has already identified one clone for a chromoplast specific protein.

695 ANALYSIS OF THE DEFENSE RESPONSE OF WILD-TYPE AND RIPENING-MUTANT TOMATO FRUIT TO PATHOGEN ATTACK
Raffaele Romano* and Dean Della Penna**
*Centro di Studio per il Miglioramento Genetico degli Ortaggi-CNR- Via Universita 133 Portici, 80055 ITALY
**Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

During the course of ripening most fruit becomes increasingly susceptible to a number of pathogens to which they had previously been resistant. Although this phenomenon has been recognized for over 50 years, the biochemical and molecular basis of this altered susceptibility has not been well studied. We are utilizing the ripening tomato fruit and the ripe fruit pathogen *Colletotrichum coccoides*, the causative agent of anthracnose, as a model host-pathogen system to examine the host defense response of ripening fruit to pathogen attack. The effect of pathogen inoculation on the several well studied plant defense responses is being anaalyzed. The timing and intensity of HRGP production, induction of PAL and CHS expression, phytoalexin biosynthesis and production of defense-related hydrolytic enzymes in pathogen challenged unripe and ripe tomato fruit will be reported.

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696 A RIPENING-RELATED cDNA FROM APPLE. G.S. Ross[1], H.L. Bray[2], M.L. Knighton[1] and M. Lay Yee[1]. [1]DSIR Fruit & Trees, DSIR, Private Bag Auckland, NEW ZEALAND. [2]Department of Cellular and Molecular Biology, University of Auckland, Private Bag, Auckland, NEW ZEALAND

We report the isolation of a ripening-related apple cDNA which may be involved in ethylene production. Poly A$^+$ RNA was extracted from ripe apple fruit (*Malus domestica* Borkh cv. Golden delicious) and a cDNA library constructed. The library was screened with pTOM13, a tomato cDNA clone thought to code for an enzyme involved in ethylene biosynthesis in that fruit. An apple cDNA clone (pAP4) was isolated and is being sequenced. Initial information indicates that the 1.2 kb pAP4 insert is full length and has considerable homology with the tomato clone. In apple cortical tissue, expression of pAP4-complementary RNA increased with ethylene production by the fruit. Expression was enhanced in ethylene-treated fruit and in wounded fruit, but was not observed in leaves either before or after ethylene treatment or wounding.

697 Characterization of cDNAs encoding a prenyl-transferase and fruit-ripening related proteins in *Capsicum annuum*. Rodolphe SCHANTZ , Susanne ROMER, Jacques-Henry WEIL, Marcel KUNTZ. *Bilal CAMARA. Institut de Biologie Moléculaire des Plantes du CNRS, Strasbourg, FRANCE and *Université de Bordeaux, FRANCE.

The ripening of bell pepper is linked to a chloroplast-chromoplast transition. During this process, chlorophyll catabolism as well as massive carotenoids accumulation occurs. The first steps of carotenoid biosynthesis are catalysed by operationally soluble enzymes localized in the plastid stroma and encoded by the nuclear genome. The availability of antibodies raised against the main enzymes of phytoene synthesis allowed us to screen an expression library from bell pepper ripening fruits. Using antibodies directed against geranyl-geranyl pyrophosphate synthase, we have isolated a cDNA whose deduced peptide sequence shows homology to prenyl-transferases from procaryotic sources, especially in a highly conserved domain corresponding probably to the pyrophosphate-binding site. The level of the corresponding mRNA during chromoplast differentiation shows a sharp peak in orange fruits.

Among other positive clones, a cDNA which is strictly fruit ripening-specific has been isolated and studied. This cDNA encodes a 35 kD polypeptide. At the N-terminal, the sequence corresponds to a potential transit peptide, while the putative mature protein is highly acidic. The function of this protein is under investigation.

698 TOMATO ALCOHOL DEHYDROGENASE 2 IS A RIPENING-RELATED PROTEIN. Ai-Ru Sun-Chen and Theodore Chase, Jr. Rutgers University/NJAES, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903-0231

Tomato (Lycopersicon esculentum) possesses two alcohol dehydrogenases (ADHs). ADH1 is specific to pollen and seeds, increasing during ripening of fruit; it has not been shown to be induced by anaerobic stress. ADH2 was described as induced by anaerobiosis in roots and other tissues; it also appears briefly in seeds during ripening. It is the ADH present in pericarp and its activity there increases 3-4 fold during ripening (from mature green to red ripe). Messenger RNA for this enzyme increases 12-20 fold during the same period. The response of the enzyme during ethylene induction of ripening appears to be indirect, since there is little or no response of mRNA or activity during the first day after ethylene treatment, the main increase coming 4-7 days after treatment. ADH2 mRNA and activity are rapidly (within 1 hr) induced by anaerobic treatment of fruit, to levels much higher than reached during ripening in air. Pyruvate decarboxylase, the prior enzyme in the anaerobic fermentative pathway, is similarly induced during anaerobiosis, but not during ripening (peak levels at breaker stage, declining thereafter), indicating that ADH2 induction during ripening is separately controlled from induction by anaerobiosis.

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699 ANTISENSE RNA INHIBITION OF PECTINMETHYL-ESTERASE EXPRESSION IN TRANSGENIC TOMATOES. Denise M. Tieman, Robert W. Harriman, G. Ramamohan, and Avtar K. Handa. Purdue University, Department of Horticulture, W. Lafayette, IN 47907.

Demethoxylation of pectin by pectinmethylesterase (PME) is believed to allow pectins in the cell wall to be further degraded by polygalacturonase. However the exact role of PME in overall cell wall degradation and in tomato fruit ripening remains to be elucidated. To determine the role of PME in these processes we have introduced a portion of a PME genomic clone into tomato in the sense and antisense orientations under the contol of the CaMV 35S promoter. Several plants containing the antisense construct show significantly reduced levels of PME gene expression in their fruits at the activity, protein and mRNA levels when compared to control fruits or fruits expressing the sense RNA strand. High levels of antisense RNA can be detected in leaves of transgenic plants. The introduced genes segregate in a normal Mendellian fashion, and plants homozygous for the antisense gene have been identified. We are characterizing fruits expressing antisense RNA for changes in softening behavior, degree of methylation of pectin, and length of the pectin polymers found in the cell walls.

700 ALTERATION OF CARBOHYDRATE METABOLISM IN TRANSGENIC RICE. Nicola M. Ayres, Dave Andrews and William D. Park. Texas A&M University, Department of Biochemistry, College Station, TX 77843

Rice plants can accumulate more than a ton per acre of nonstructural carbohydrate in the leaf sheath and culm before heading. Not only does this provide a portion of the starch stored in grain, it provides a biological buffer to prevent grain abortion and leaf death when sink demand exceeds current photosynthetic rate. By altering the timing and levels of carbohydrate accumulation, we should be able to influence yield. To do this we have isolated a series of tissue and developmental stage-specific rice clones and have developed a rapid and efficient microspore derived protoplast transformation and regeneration system for rice. Protoplasts were transformed using polyethylene glycol and were selected with hygromycin. Plants regenerated from protoplasts of these cultures have a high frequency of diploidization, typically about 80%. Experiments are in progress to alter the level of key enzymes involved in regulating glycolysis in rice plants by introducing genes from bacteria and preliminary data indicates that this results in alterations in the the levels of the corresponding metabolites.

701 ANALYSIS OF THE IN VIVO FUNCTION OF THE SO-CALLED LIPID TRANSFER PROTEIN IN ARABIDOPSIS THALIANA Ursula Hecht, Sharon Thoma, Jose Botella, Chris Somerville

Plant lipid transfer proteins (LTPs) mediate intermembrane transfer of lipids *in vitro*, but there is so far no direct evidence of their function *in vivo*. In fact, there are indications that suggest that LTP is not involved in transfer of phospholipids between intracellular membranes. Using a cDNA probe for the *Arabidopsis* LTP gene the genomic clone of *Arabidopsis* was isolated. It was sequenced including an upstream region of 1 kb. To analyze the *in vivo* role of LTP we have produced transgenic plants containing three different antisense constructs in which the *Arabidopsis* LTP cDNA was placed in reverse orientation behind the CaMV 35S promotor. Several plants were isolated which contained very low levels of LTP compared to wild type plants. These plants are being analyzed for their lipid composition and their cellular morphology.

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702 ANALYSIS OF TWO LINKED GENES CODING FOR THE ACYL CARRIER PROTEIN (ACP) FROM *ARABIDOPSIS THALIANA* (*columbia*). Scott R. Baerson, Colleen Jacks, and Gayle K. Lamppa. The University of Chicago, Department of Molecular Genetics and Cell Biology, Chicago, Illinois 60637.

The acyl carrier protein (ACP) plays a pivotal role in the *de novo* synthesis of fatty acids. During fatty acid biosynthesis, nascent chains are covalently bound to a phosphopantetheine prosthetic group attached at serine 38 of the protein. Two linked genes, A1 and A2, coding for nearly identical isoforms of the acyl carrier protein were isolated from an *A. thaliana* (*columbia*) genomic library and sequenced. The amino acids deduced from the nucleotide sequence of the two genes indicate they encode distinct transit peptides, but the mature proteins are the same except for residue 79. Both genes are predicted to contain three introns in similar positions, although they differ in sequence and length. The introns interrupt regions coding for a) the transit peptide, b) the junction of the transit peptide and mature protein, and c) the highly conserved domain surrounding serine 38 to which the phosphopantetheine is attached. Primer extension analyses indicate that at least A1 is active in young *Arabidopsis* plants. Tobacco leaf explants were transformed with an *A. tumefaciens* strain carrying a chimeric gene comprised of approximately 1.0 kb of the 5' flanking sequence from the A1 gene transcriptionally fused to the ß-glucuronidase (GUS) coding region, and kanamycin resistant calli were tested for GUS activity. Our results indicate that the A1 gene 5' flanking region, which contains a unique thymidine-rich sequence motif, is capable of directing high levels of GUS expression in tobacco. Experiments conducted in parallel using a CAMV 35S promoter/GUS fusion construct resulted in approximately 2 to 4-fold higher levels of GUS activity. Experiments are in progress to determine the pattern of tissue specificity conferred by the acyl carrier protein A1 gene promoter in transgenic plants.

703 TISSUE-SPECIFIC EXPRESSION OF THE MAIZE *WAXY* GENE. Erica Unger, Brad Roth and Ben Bowen. Department of Biotechnology Research, Pioneer Hi-Bred Intl, Johnston, IA 50131.

The *Waxy* locus in maize regulates the formation of amylose in the endosperm by encoding the gene for starch granule-bound ADP-glucosyl:starch transferase. The expression of the *Waxy* protein and mRNA is limited to developing endosperm and pollen. Chimeric genes containing a 1.0 kb fragment isolated from the region 5' to the *Waxy* transcription unit fused to the coding regions of either firefly luciferase or bacterial ß-glucuronidase (GUS) have been constructed. Reporter gene expression has been observed at levels 25-fold greater than background in A636-derived endosperm callus suspension cultures 24-hours following microprojectile bombardment. In contrast, non-regenerable Black Mexican Sweet corn suspension cells, and maize coleoptile tissue do not support luciferase expression driven by the *Waxy* promoter following similar treatments. A series of promoter deletions has been constructed and the transient activity determined in the endosperm suspension system, with the goal of identifying endosperm-specific expression signals and the associated trans-acting factors. Additionally, the same set of promoter deletions is being tested in transgenic tobacco, where differential expression in endosperm cells and pollen will allow sequences which are involved in the tissue-specific expression of the *Waxy* gene to be identified.

704 CLONING OF A cDNA FOR AN EPIDERMIS-SPECIFIC mRNA FROM SENECIO ODORIS. Jaeho Pyee and P. E. Kolattukudy. Ohio State Biotechnology Center and Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210.

The epidermal cells synthesize the complex cuticular components that protect plants. Since nothing is known about the genes that code for the enzymes involved in the biosynthesis of the cuticle, we have initiated studies aimed at isolating genes specifically expressed in the epidermis and identifying their specific functions. Poly(A)$^+$RNAs isolated separately from both epidermal peels and internal tissues of Senecio odoris leaves were used to construct total and subtracted cDNA libraries. Differential screening of the libraries provided several putative positive clones. One of them, epi 91-23, has been further characterized. Northern blot analysis of tissue-specific RNAs probed with the subcloned cDNA demonstrates that the corresponding gene is expressed exclusively in the epidermis and produces a message of approximately 2.1 kb. The nucleotide sequence of the near full-length cDNA clone and amino acid sequence derived from the open reading frame failed to show homology with any gene in the current GenBank and Swiss-Prot data bases. The cDNA is now being used to isolate a corresponding genomic clone and its upstream promoter region which will be used to analyze the spatial regulation of gene expression.

705 CLONING AND SEQUENCING OF TWO DIFFERENTIALLY EXPRESSED SUCROSE SYNTHASE GENES FROM POTATO. Hongyong Fu, Jie Du and William D. Park. Texas A & M University, Department of Biochemistry and Biophysics, College Station, TX 77843

By sequencing PCR amplified cDNAs and examining the restriction maps of a series of genomic clones isolated from a lambda library, we identified two different classes of genes for sucrose synthase in potato (*S. tuberosum*). The T type genes are expressed more abundantly in tubers, whereas the V type are expressed at higher levels in roots, stems, and leaves. Clones with long 5' sequences for each type have been completely sequenced. The general structure of both genes is the same, with 14 exons and a particularly long leader intron, although most of the introns in the T type clone are longer than in the V type. The coding regions of the two clones are 86% homologous at the nucleic acid level and the amino acid homology of the deduced protein is 92%. Both genes are slightly more homologous to shrunken than sus from maize, but they show higher homology to the Nodulin-100 gene isolated from soybean. The 5' and 3' untranslated regions, introns, and promoter regions of the two classes of potato sucrose synthase genes are highly divergent, but they still share many small regions with high homology near the TATA box, transcription start site and polyadenylation site, as well as at several sites in the 5' flanking region. Results of analysis of transgenic potato plants containing GUS constructs for both classes of genes will be presented.

706 CHARACTERIZATION OF POTATO HMG CoA REDUCTASE GENES AND STUDIES OF EXPRESSION BY *IN SITU* HYBRIDIZATION.

R. W. Roxby*, F. Garcia-Garcia§, K. Hahlbrock§ and D. Davis*. *Dept. of Biochemistry, University of Maine, Orono, Maine 04469-0131 USA and §Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG.

Primers based on sequences of HMG CoA Reductase which are conserved in all known genes were used in PCR amplification of genomic and cDNA sequences of potato, cv. Datura. Amplification products of cDNA sequences include segments of at least two genes and products of two sizes were amplified from genomic DNA. The deduced protein sequence of one of the cDNA derived sequences is highly homologous to known HMG CoA reductases. The other cDNA derived sequence exhibits low homology to known sequences. *In situ* hybridization was carried out on various tissues of normal, helathy plants and on leaves infected by *Phytophthora infestans*. Noteworthy in healthy plants was the association, in some tissues, of the labelled probes with plastids. No induction of corresponding mRNAs was observable in infected leaves however.

707 EXPRESSION AND MOLECULAR CHARACTERIZATION OF A MATURE CASTOR STEAROYL-ACP DESATURASE IN E. COLI, AND ISOLATION AND CHARACTERIZATION OF HOMOLOGOUS ARABIDOPSIS GENOMIC CLONES. John Shanklin and Chris Somerville, Michigan State University Department of Energy Plant Research Laboratory, East Lansing, MI 48824.

The Stearoyl-ACP desaturase is responsible for converting stearoyl-ACP (18:0-ACP) to the mono-unsaturated oleoyl-ACP (18:1-ACP). Recently we reported the purification of the enzyme from avocado mesocarp, and the cloning of cDNAs from castor and cucumber (PNAS 88:2510-2514). We have engineered a construct lacking the transit peptide, and expressed it in a pET expression system in E. coli. The desaturase accumulates to 20-30 percent of the total soluble protein in these cells, and forms functional dimers. We have purified the desaturase to homogeneity using DEAE-sephacel and size exclusion chromatography. Preliminary analysis using atomic absorption spectroscopy reveal the presence of several moles of Fe per dimer. Experiments are in progress to determine the exact stoichiometry. Qualitative analysis revealed the presence of labile sulfur, consistent with the model that the desaturase posesses an Fe-S center. Visible spectra of the purified desaturase is similar those of Fe-S proteins with a 280/320 nm specific absorbtion ratio of approximately 0.07. EPR, resonance Ramen spectroscopy, and FTIR are currently being used to elucidate the structure of this center. We have also isolated several Arabidopsis genomic clones and are in the process of characterizing them with the goal of constructing promotor-reporter gene fusions. Data on the progress of these experiments will be presented.

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708 EXPRESSION OF SOYBEAN SEED LIPOXYGENASE 2 IN TRANSGENIC TOBACCO TISSUE. Wenlian Deng, Scott W. Grayburn, Thomas R. Hamilton-Kemp*, Art Hunt, Glenn B. Collins and David F. Hildebrand. University of Kentucky, Dept. of Agronomy & *Horticulture, Lexington, KY 40546

In order to assess the possible involvement of lipoxygenase (LOX) in plant growth, development, and/or defense, we increased the expression of LOX in tobacco tissue by over expression of the soybean seed LOX2 gene. The LOX2 cDNA was manipulated by replacing its 5'-untranslated sequence with the translational enhancer of the alfalfa mosaic virus (AMV) and subcloning into a plant expression vector, 3' to a duplicated 35S promoter. The AMV-LOX2 construct was transferred into tobacco using Agrobacterium tumefaciens A281. The LOX2 was expressed in both transgenic tobacco calli and plant leaves at levels upto 0.1-0.2% of total extracted protein. The introduced LOX2 affected fatty acid oxidative metabolism as evidenced by a 50-400% greater production of C_6-aldehydes. The impact on C6-aldehyde formation was greater than the effect on production of fatty acid hydroperoxides. This result is consistent with other studies, indicating a greater relative propensity of soybean seed LOX2 in generating C6-aldehyde than other characterized LOX isozymes.

709 EXPRESSION OF TISSUE SPECIFIC AND CONSTITUTIVE ACP ISOFORMS IN *ARABIDOPSIS*. John B. Ohlrogge, Alenka Hlouŝek-Radojčić, David Shintani and Dusty Post-Beittenmiller. Michigan State University, Department of Botany and Plant Pathology, East Lansing MI. 44824

We have characterized the occurance and expression of multiple acyl carrier protein (ACP) isoforms in *Arabidopsis*. Immunoblot analysis of ACPs from *Arabidopsis* tissues separated by native PAGE revealed a complex pattern of multiple ACP isoforms. All tissues examined (leaves, roots and seeds) expressed at least three forms of ACP. The immunoblot identifications of ACP bands were confirmed by acylation of ACP extracts with *E. coli* acyl-ACP synthetase. A full length cDNA clone has been isolated which has 70% identity with a previously characterized *Arabidopsis* genomic ACP clone (Post-Beittenmiller et al, *Nucl.Acid.Res.* 17, 1777, 1989). Based on RNA blot analysis and RNase protection studies, the cDNA clone represents an ACP which is expressed in leaves, seeds, and roots. Using PCR, we subcloned the mature coding sequences of the two available ACP clones into *E. coli* expression vectors. The *E. coli* expressed proteins comigrate on PAGE gels with ACP isoforms which are expressed in leaves, seeds, and roots. Thus, the two clones appear to represent constitutively expressed *Arabidopsis* ACPs. In addition to these two ACP isoforms, protein blots indicate seed, leaf and root each express two or more tissue-specific isoforms. We have purified the most abundant leaf-specific isoform and the sequence of its 25 N-terminal amino acids indicates it represents a distinct, previously uncharacterized *Arabidopsis* ACP sequence.

710 GENES FOR THE LARGE AND SMALL SUBUNIT OF BARLEY ENDOSPERM ADP-GLUCOSE PYROPHOSPHORYLASE. P. Villand, E. Lüthi, L.A. Kleczkowski, A. Lønneborg & O-A Olsen, Plant Molecular Biology Laboratory, NLVF, 1432 Ås-NLH, Norway.

Two cDNA clones, most probably encoding fragments of the large and the small subunit of ADP-glucose pyrophosphorylase (AGP), were isolated from total RNA of barley endosperm by polymerase chain reaction (PCR). Sets of degenerate primers, based on conserved regions of the previously published amino acid sequences, were used for synthesis and amplification of cDNA. Based on the derived amino acid sequences, one of the clones was 94 and 70% identical to WE:AGA7 and Sh2, cDNAs encoding the large subunit of wheat and maize endosperm AGP, respectively. The other clone was 91 and 70% identical to Rsc6 and Bt2, cDNAs for the small subunit of rice and maize endosperm AGP, respectively. The two clones isolated from the barley showed 50% identity on the amino acid sequence level. The cDNA clones were used as probes for the screening of a barley genomic library. From 2 X 10^6 lambda clones, 18 clones hybridized to the small subunit cDNA, and 5 clones hybridized to the large subunit cDNA. Hybridization and PCR analysis allowed each of these two groups to be dividied into two subgroups, indicating small gene families. Sequence analysis of two of the λ-clones has revealed sequence identities to the large and the small subunit cDNAs. Analysis of the λ-clone for the small subunit also uncovered similar, but not identical, intron organization as found for a genomic clone for the subunit of rice endosperm AGP. More details on structure and organization of ADP-glucose pyrophosphorylase genes in barley will be presented.

711 GENE EXPRESSION AT THE WX LOCUS IN RICE (OLYZA SATIVA). Hiro-Yuki Hirano and Yoshio Sano. National Institute of Genetics, Mishima 411, Japan

The wx locus controls amylose synthesis in the rice endosperm. We have cloned the wx^+ gene from rice and determined the nucleotide sequence of its coding region. The gene was composed of a number of exons and introns and the homologies in deduced amino acid sequence of the mature products of the wx^+ gene (wx^+ protein) among rice, maize and barley were 86-88%. Gene expression of the wx locus was analyzed with reference to the tissue specificity, the development of seeds and the response to the environmental change. The wx+ gene was expressed specifically in only the endosperm and pollen, although the amount of the wx^+ protein in pollen was about 2% of that in endosperm. The wx^+ gene was active at the early stage and middle stages but almost inactive at the late stage of seed development. During active transcription of the gene, the wx^+ protein appears to accumulate linearly. The level of the wx^+ protein in plants grown at semi-low temperature (18°C) during seed maturation was higher than that of plants grown at normal temperature (28°C). The endosperm grown at 18°C contained elevated level of amylose content. It is suggested that the low quality of rice grown at low temperature in the northern area of Japan is due to the higher amylose content, which is caused by the enhancement of wx^+ gene expression.

712 ISOLATION AND CHARACTERIZATION OF GRANULE BOUND STARCH SYNTHASE GENES FROM WHEAT. Che-Hong Chen, Paul Gross, Larry Fang, Leslie Walker, Steve Mejza, Jim Wong and Marcella Lillis. Sogetal, Inc., Hayward, California 94545

The Wx loci of maize, barley, and several other plants have been shown to code for granule-bound starch synthase (GBSS), an enzyme important for the production of amylose in the seed. Using PCR primers homologous to other cereal GBSS genes, we have amplified several GBSS gene fragments from wheat (*Triticum aestivum*). With the amplified fragments as probes, we have cloned several partial GBSS cDNAs from a wheat endosperm library and two different genomic clones from a wheat genomic library. Our Southern analysis indicates that there may be three GBSS genes in hexaploid wheat. The coding sequence of the wheat gene sequenced to date is 94% identical to the known sequence for the GBSS gene from barley. Chimeric constructs which fuse the putative promoter and 5' regulatory region from this wheat GBSS gene with a reporter gene have been analyzed for transient expression in a variety of wheat tissues, including endosperm, microspore, and suspension culture. Results of these expression experiments are reported here.

713 ISOLATION OF GENES INVOLVED IN STARCH SYNTHESIS

Hiroaki Shimada(1), Tadashi Baba(2), Midori Ishikawa(1), Tsutomu Kawasaki(1), Satoru Okumura(1), Yuichi Tada(3)
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Plant starch is synthesized mainly by starch synthase, which makes amylose, and branching enzyme which produces the α 1-6 branching linkages characteristic of amylopectin. The waxy gene encodes a major starch synthesizing enzyme which is localized in the endosperm starch granules. Based upon known waxy sequences from maize and barley, PCR primers were prepared and PCRs using rice genomic DNA were carried out to amplify a specific fragment (1.0 kb). Sequence analysis showed this fragment to be a rice waxy sequence since its nucleotide sequence was highly homologous to those of other plants. Genes for branching enzyme have been cloned from E. coli and a cyanobacteria Synecococcus species. PCR primers were synthesized based upon conserved amino acid sequences, and a 0.6 kb fragment was specifically amplified from rice genomic DNA. The amino acid sequence predicted from its nucleotide sequence exhibits more than 80% homology with those from E. coli and cyanobacteria. This suggests that the rice genome also contains a sequence homologous to the bacterial genes. However, a large divergence is evident between this sequence and the branching enzyme I gene expressed in plant endosperm.

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714 INCREASED STARCH AND DRY MATTER DEPOSITION IN TRANSGENIC RUSSET BURBANK POTATO TUBERS. David M. Stark, Gerard F. Barry, Yvonne M. Muskopf, Kurt P. Timmerman, and Ganesh M. Kishore. The Monsanto Company, 700 Chesterfield Village Pkwy., St. Louis, MO 63198. Dry matter content is a critical determinant of the value of potato tubers, especially to food processors. Starch is the major contributor to dry matter in tubers and we targeted enhancement of starch biosynthesis as a means of increasing dry matter content. Transgenic Russet Burbank potato plants have been generated which express a gene involved in starch biosynthesis. Starch content of transgenic tubers is increased significantly, with a concomitant increase in specific gravity and a higher dry matter content. On average, these tubers contain 23.9% higher levels of dry matter relative to controls, with some lines containing nearly 50% higher levels. Iodine staining of tuber cross-sections reveals that the starch is evenly distributed and, unlike in control tubers, also accumulates in the vascular tissue and to a high level in the pith. Details concerning gene expression and the resultant effects on starch biosynthesis will be presented.

715 *Kas* GENES ENCODE β-KETOACYL-ACP SYNTHASES - KEY ENZYMES OF FATTY ACID SYNTHESIS. Sakari Kauppinen, Mads Siggaard-Andersen, Margit Wissenbach and Penny von Wettstein-Knowles. Carlsberg Laboratory, Department of Physiology, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark and Institute of Genetics, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K, Denmark. Fatty acid synthesis is an iterated process in which C_2-units from malonyl-CoA are added to a growing acyl chain by fatty acid synthetase. Key components of this plastid localized enzyme complex are β-ketoacyl-ACP synthases I and II which catalyze condensation reactions. Synthase I is most active with C_{2-14} substrates and is highly sensitive to the antibiotic cerulenin, while synthase II utilizes C_{10-16} substrates and is far less sensitive. ^{3}H-cerulenin tags three highly homologous dimeric stromal proteins (α_2, $\alpha\beta$, β_2) from barley chloroplasts. Fifteen *Kas12* cDNAs encoding the β_2-protein have been isolated from barley leaf cDNA libraries using a polymerase chain reaction generated product as a probe. Sequencing of a full-length cDNA revealed an open reading frame of 462 residues including a 35 amino acid transit peptide not present in the mature protein. A genomic clone for the single copy *Kas12* gene and its promoter has been characterized and the gene mapped to barley chromosome 2 using Chinese Spring wheat-Betzes barley addition lines. The coding regions for the mature barley β-polypeptide and the homologous *fabB* encoded β-ketoacyl-ACP synthase I of E. coli have been expressed as C-terminal fusions with the glutathione-S-transferase of S. japonicum. Polyclonal antibodies have been raised to the purified fusion proteins. Analyses of the antibodies and their potential use in isolating other β-ketoacyl-ACP synthases will be presented, as will the results of using ^{3}H-cerulenin to tag sensitive condensing enzymes in other plants.

716 MOLECULAR BIOLOGY OF STARCH SYNTHESIS IN WHEAT Charles Ainsworth and Joanna Clark, Department of Biological Sciences, Wye College (University of London), Ashford, Kent, TN25 5AH. U.K. cDNA libraries made from RNA isolated from wheat endosperm harvested during grain filling (cv.Chinese Spring) have been screened with heterologous probes for ADP-glucose pyrophosphorylase and the *waxy* locus. A full-length (2.2 kb) *waxy* locus cDNA clone and full-length clones for both subunits of ADPG pyrophosphorylase have been isolated. Sequence comparison of the wheat *waxy* locus clones from maize, barley and rice shows that the coding region is highly conserved. The wheat cDNA clone encodes a protein of 615 amino acids. Hybrid release translation using this clone yields a protein of apparent MW 67 kDal. The mature protein, solubilised from starch grains, has an apparent MW of 60 kDal. N-terminal sequencing of the mature protein shows that a 70 amino peptide is cleaved from the precursor protein at the alanine residue 71. Southern analysis using deletion lines of Chinese Spring shows that the *waxy* locus in wheat is organised as a triplicate set of single copy genes on the short arms of chromosomes 7A and 7D and the long arm of chromosome 4A. The ADPG pyrophosphorylase clones encode the small (51,000 molecular weight) and large (54,000 molecular weight) subunits of the enzyme as shown by hybrid select translation and sequence analysis. Both clones show a high degree of homology at the amino acid and nucleotide levels with their maize counterparts, the *Brittle-2* and *Shrunken-2* loci. *Waxy* locus and ADPG pyrophosphorylase transcripts accumulate to high levels during mid grain filling which is consistent with enzyme activities and rates of starch synthesis. Data on the metabolic regulation of the genes will be presented.

717 MOLECULAR CLONING OF cDNAs CODING FOR MAIZE AND RICE BRANCHING ENZYMES. T. Baba, K. Mizuno, K. Kimura, H. Shimada, and Y. Arai. Univ. of Tsukuba, Inst. of Appl. Biochem., Japan.

Plant branching enzyme is a key enzyme in starch biosynthesis. The enzyme has been reported to be present in two forms in maize kernels, branching enzymes I and II. To facilitate further studies of this enzyme, we have identified cDNA clones encoding branching enzyme I from maize and rice cDNA libraries in λgt11. The nucleotide sequence of the overlapping cDNA inserts indicates that maize branching enzyme I is initially synthesized as a precursor protein with a 64-residue leader peptide at the N-terminus. Rice branching enzyme I also has a 60-residue sequence as the N-terminal extension. These leader sequences are rich in basic and hydroxylic amino acids and thereby appear to be transit peptides for the amyloplast. The deduced amino acid sequence of the maize mature enzyme is highly conserved in the rice protein. However, these plant branching enzymes show only a low degree of sequence similarity with bacterial branching enzyme. Since another branching enzyme gene, which codes for a polypeptide structurally similar to bacterial enzyme, is located in the genomes of maize and rice, branching enzyme I may be functionally distinguishable from bacterial enzyme. Northern blot analysis of total RNAs, using the maize branching enzyme I cDNA as a probe, demonstrates that the gene for this enzyme is expressed abundantly in the early stages of endosperm development, and expression continues through endosperm maturation.

718 MOLECULAR GENETIC ANALYSIS OF FATTY ACID ELONGATION IN SEEDS OF ARABIDOPSIS. Ljerka Kunst and Edward Underhill. NRC Plant Biotech. Institute, Saskatoon, S7N 0W9, Canada We have isolated a collection of six mutants of Arabidopsis thaliana with reduced levels of very long chain fatty acids (VLCFAs) in their seed storage lipids. The mutants were identified by direct fatty acid analysis of 2,000 mutagenized seed samples using gas chromatography. All the mutations are heritable, affect nuclear genes and cause major changes in elongation of 18:1 to 20:1 acyl groups. Genetic complementation studies have shown that these mutants belong to three different complementation groups, indicating that the elongation process is controlled by several different loci. We have analyzed one line, AC56, in detail. In this mutant, a single co-dominant nuclear mutation at the FAE1 locus (Fatty Acid Elongation #1) results in a deficiency in acyl chain elongation from 18:1 to 20:1, 20:1 to 22:1 and 18:0 to 22:0. This observation suggests that the product of the FAE1 gene is shared by three different elongation systems, involved in both saturated and monounsaturated VLCFA synthesis. In A. thaliana it is possible to clone genes identified by a mutation using the technique of chromosome walking. As a first step towards cloning the FAE1 gene by chromosome walking, we have mapped the fae1 mutation to chromosome four on the A. thaliana genetic map. We are currently attempting to determine the exact position of the FAE1 locus relative to the restriction fragment length polymorphism markers.

719 MOLECULAR CLONING AND CHARACTERIZATION OF ß-KETOACYL-ACP SYNTHASE GENES FROM *RICINUS COMMUNIS*. Andrée Genez, David McCarter, Janet Nelsen, Janice L. Bleibaum, Gregory A. Thompson, Vic C. Knauf and David Stalker. Calgene, Inc., 1920 Fifth Street,Davis, California 95616.

ß-Ketoacyl-ACP synthases catalyze the condensation reaction of fatty acid synthesis required to form fatty acids for membranes and storage lipids in plants. We have purified a fraction from developing castor endosperm which contains a synthase activity capable of elongating 10:0-ACP. SDS-PAGE analysis of the purified preparation showed two bands at 46 kDa and 50 kDa. cDNA clones for each protein species were isolated from a castor endosperm cDNA library, and the deduced protein sequences of each showed that they encoded distinct but homologous polypeptides. Each polypeptide contained an active site motif observed for ß-ketoacyl-ACP synthases from other organisms. Genomic Southern analysis suggests that each cDNA likely represents a single gene in castor. Northern analysis indicates that the message for the 50 kDa polypeptide is more abundant than is that for the 46 dKa species; both messages are expressed early in embryo development with maximum levels around 20-25 days post-anthesis. Expression of the two genes coincides with synthase activity and with storage lipid synthesis in developing castor endosperm.

POSTER ABSTRACTS

720 MOLECULAR CLONING AND CHARACTERIZATION OF ACETYL-CoA CARBOXYLASE AND OTHER BIOTIN ENZYMES OF PLANTS. Basil J. Nikolau[1] and Eve Syrkin Wurtele[2]. Iowa State University, [1]Dept. Biochemistry & Biophysics and [2]Dept. Botany, Ames, IA 50011.

Biotin-containing enzymes catalyze key reactions in lipogenesis, gluconeogenesis and amino acid metabolism. Due to the importance of these enzymes in metabolism they have been extensively characterized in animals and microorganisms. In stark contrast, the metabolic function, structure and regulation of biotin enzymes in plants is poorly understood. Our laboratories are pursuing research to fill this void in our knowledge of these enzymes. The six biotin-containing polypeptides present in plants have M_rs of 210,000, 140,000, 73,000, 50,000, 39,000 and 34,000. The best characterized plant biotin enzyme is acetyl-CoA carboxylase which catalyzes the first and probably rate-limiting reaction of fatty acid biosynthesis. Two forms of this enzyme have been identified in plants, one with a large biotin-containing subunit (210 kDa), and a second with a smaller biotin-containing subunit (50 kDa). These two isozymes may enable plants to differentially regulate the supply of malonyl-CoA for the synthesis of a number of phytochemicals, including fatty acids, flavonoids and very long-chain fatty acids. We have isolated cDNA and genomic clones of the 50 kDa acetyl-CoA carboxylase of carrot and are using them to characterize the function of this enzyme. In addition we have characterized a cDNA clone from tomato that codes for the 73 kDa biotin-containing polypeptide which we have determined to represent the biotin subunit of 3-methylcrotonyl-CoA carboxylase. 3-Methylcrotonyl-CoA carboxylase catalyzes a reaction in leucine oxidation. A third cDNA clone has been isolated from soybean that codes for a biotin enzyme. The sequence of this clone shows similarity to the animal propionyl-CoA carboxylases and yeast pyruvate carboxylase, both of which are biotin enzymes. Experiments are being conducted to directly identify the enzymatic function of the protein product coded by the soybean cDNA clone.

721 MOLECULAR BIOLOGY OF LIPID TRANSFER PROTEINS.
Jean-Claude Kader. Physiologie Cellulaire & Moléculaire (URA CNRS 1180). Université Paris 6 , 4 place Jussieu, Paris, France

Lipid transfer proteins (LTP), detected in all plants studied up to now, are characterized by their ability to facilitate *in vitro* movements of phospholipids between membranes (2). In addition, LTPs are able to bind fatty acids or their CoA esters (1). LTPs purified from various plants have common structural properties : a similar number of residues (91 to 93), a high homology of several domains including a central polar zone and an identical number of cysteine residues (eight) placed at conserved positions (1). The isolation of cDNA clones corresponding to maize LTP, obtained in our laboratory in a cooperative program with M. Delseny (Perpignan, France) and P. Puigdomenech (Barcelona, Spain) (3) as well as the characterization of cDNAs coding for LTP from spinach (4) and barley (5) , indicated that a signal peptide could be involved in the synthesis of these proteins . This was confirmed by the finding that maize LTP is synthesized as a precursor form on membrane-bound polysomes (6) and that the synthesis of spinach LTP involves a secretory pathway (4). In the light of these observations, the *in vivo* role of LTP, supposed to be involved in the intracellular movements of lipids for membrane biogenesis, has to be reexplored. Future investigations will consider the cell localization of LTPs and the expression of their genes in order to determine their physiological roles in plant cells.

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722 MOLECULAR ANALYSIS OF CAROTENOID BIOSYNTHESIS Ins Pecker, Daniel Chamovitz, and Joseph Hirschberg, Department of Genetics, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.

In order to study the regulation of carotenoid biosynthesis we have cloned the gene (*pds*) that encodes phytoene desaturase (PDS) - the key enzyme in β-carotene biosynthesis, from cyanobactena, algae and plants. The deduced amino acid sequence of PDS in the cyanobactenum *Synechococcus* PCC7942 indicates a polypeptide with neutral hydrophobicity of 474 amino acid residues. A short amino acid sequence that is homologous to a conserved motif in the binding site for NAD(H) and NADP(H) was identified, suggesting that these dinucleotides serve as cofactors in phytoene desaturation. Several norflurazon and flundone-resistant strains of *Synechococcus* PCC7942 were isolated. Point mutations in *pds* that lead to amino acid substitutions in PDS were found to confer herbicide resistance in these mutants. Alteration in the katalytic kinetics of the enzyme in the mutants, which results in reduction of carotenoid synthesis *in-vivo*, demonstrates that PDS is a rate limiting step in the pathway. Using the cyanobactenal gene as a molecular probe, we have obtained cDNA clones of *pds* from the carotene over-producing alga *Dunaliella bardawil* and from tomato. The deduced amino-acid sequence of PDS from tomato is 65% identical (79% similar) to the cyanobacterial PDS and is highly conserved with the *Dunaliella* PDS. The residues which are mutated in herbicide-resistant strains of cyanobactena, are conserved in algae and plants, as well as the dinucleotide binding motif in the amino terminus. The open reading frames of *pds* from *Dunaliella* and tomato extend upstream (111 codons in tomato) to the conserved initiation codon of *pds* in cyanobactena . This region is inferred to function as a transit peptide for targeting the enzyme to the inner side of of the plastid's envelope. A single locus for *pds* was found in tomato and was mapped to chromosome 3, close to, or even overlapping the *r* locus which is involved in carotenoid biosynthesis during fruit ripening. Our results pave the way for manipulating carotenogenesis and application of herbicide resistance by means of genetic engineering.

723 MODULATION OF STEAROYL-ACP DESATURASE LEVELS IN TRANSGENIC RAPESEED. Jean C. Kridl, Deborah S. Knutzon, William B. Johnson, Gregory A. Thompson, Sharon E. Radke, Joann C. Turner, and Vic C. Knauf. Calgene, Inc. 1920 Fifth St., Davis, California 95616

Triacylglycerols (TAGs) in low-erucic acid rapeseed are composed of predominantly sixteen and eighteen carbon saturated and unsaturated fatty acids esterified to a glycerol backbone. Unsaturated fatty acids in TAGs of most plants derive exclusively from stearate via delta-9 desaturation to oleate in plastids. Therefore, the activity of stearoyl-ACP desaturase, the enzyme which catalyzes this step, is potentially a controlling factor in determining stearate levels in vegetable oils. We have isolated cDNA clones encoding stearoyl-ACP desaturase from developing seeds of safflower, castor, and *Brassica campestris*. The safflower desaturase has been introduced into *B. napus* plants in chimeric constructs with seed-specific promoters from acyl carrier protein (ACP), napin and the constitutive CaMV 35S promoter. Antisense chimeric constructs with the *Brassica* desaturase and the same seed-specific promoters have been introduced into both *B. campestris* var. Tobin and several *B. napus* varieties. The effect of modulation of stearoyl-ACP desaturase levels on plant fatty acid synthesis and TAG accumulation will be described.

724 PROBING THE REGULATION OF FATTY ACID SYNTHESIS: IN VIVO LEVELS OF ACYL-ACYL CARRIER PROTEIN IN DEVELOPING OILSEEDS. Jan G. Jaworski[1], Dusty Post-Beittenmiller[2], and John B. Ohlrogge[2], 1. Miami University, Chemistry Department, Oxford, OH 45056; 2. Michigan State University, Department of Botany and Plant Pathology, E. Lansing, MI 48824.

Acyl-acyl carrier proteins (ACPs) are intermediates of fatty acid synthesis which can be used to probe sites of regulation in this pathway. Acyl-ACPs from seeds of spinach and castor were separated by urea-PAGE and measured using techniques described previously (J. Biol. Chem. 266:1858-1865 (1991)). Seeds of both spinach and castor have a single major isoform of ACP which greatly simplifies the pattern of acyl-ACP intermediates. In both seeds, the level of malonyl-ACP is similar to or greater than the level of acetyl-ACP, and furthermore, these two species were the major acyl-ACPs observed. This pattern was unlike the pattern observed in leaf, in which the level of malonyl-ACP was much lower than that of acetyl-ACP. The high levels of malonyl-ACP suggested that regulation of fatty acid synthesis in seed may be different from regulation in leaf. To further examine the question of regulation in seed, acyl-ACPs were measured in seeds at different stages of development. Because the rate of fatty acid synthesis is believed to change during development, we expected a change in the pattern of acyl-ACP levels which might be suggestive of a site of regulation. Surprisingly, the pattern of acyl-ACPs were remarkably similar over most stages of development, during which time the levels of both acetyl- and malonyl-ACP remained high. This was in sharp contrast to leaf tissue, in which acetyl-ACP levels rapidly change 5-fold in concert with the different rates of fatty acid synthesis in light and dark tissue. At the final stages of seed maturity, the long chain acyl-ACP pools disappeared but acetyl-ACP remained high.

725 REGULATION OF THE EXPRESSION OF THE STARCH PHOSPHORYLASE GENE IN POTATO. Benoit St-Pierre, Charles Bertrand, Mario Cappadocia[1] and Normand Brisson. Department of Biochemistry and [1]Biology, Université de Montréal, Montréal, Canada.

The enzyme starch phosphorylase catalyzes the reversible phosphorolysis of α-1,4 glucans in plants. The enzyme is abundant at all stages of development in potato tubers but is found in low levels in leaves and roots. It is also expressed, in parallel with the accumulation of starch, in the petioles of induced leaf cuttings from which the axillary bud has been removed. We are currently studying the cis-acting elements involved in the regulation of the expression of the starch phosphorylase gene. A large DNA fragment corresponding to the 5' flanking region of the gene was fused to the *E. coli uidA* (β-glucuronidase, GUS) reporter gene and transgenic plants were produced by *Agrobacterium*-mediated transformation. In axenic culture, GUS activity was high in stems, petioles and microtubers but low in leaf blades. Incubating leaves on 300 mM sucrose did not induce GUS activity. In greenhouse-grown plants, the GUS activity was high in tubers, stolons, and under certain conditions in petioles and stems, but low in leaf blades and roots. GUS activity was localized mainly in starch-containing parenchyma cells by histochemical assays. A series of 5' deletion constructs of the starch phosphorylase gene have been produced and the effect of these deletions on the expression of the gene in transgenic potato plants will be discussed.

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726 REGULATION OF GLYCOLYTIC GENES DURING THE ANAEROBIC STRESS RESPONSE Philip M. Kelley, Shailesh K. Lal, Scott Johnson, Jill Hyslop, and E. Barry Simpson, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588-0118

Flooded roots experience an energy crisis and must switch from efficient aerobic production of energy to the much less efficient anaerobic fermentation. The key enzyme in this switch is pyruvate decarboxylase (PDC). Initially during aerobic treatment roots metabolize pyruvate to lactic acid. As the pH in the cell drops, the enzyme PDC is activated and the cell switches to a mixed fermentation where ethanol is the major product. This changes allows the plant to survive for significant periods (72 hours) of anaerobiosis, avoiding toxic acidosis. Our laboratory has accumulated data that suggest that two types of post-translational modification of glycolytic enzymes may play an important role in the dramatic changes of cellular metabolism occurring in the switch from aerobic to anaerobic metabolism. Proteolytic processing and phosphorylation of glycolytic enzymes may mediate the dramatic shift in metabolism and gene expression in flood stressed plants. Our work currently focuses on the proteolytic cleavage and possible activation of specific glycolytic enzymes. We are also studying a cAMP dependent protein kinase associated with this flood stress. We believe that this protein may be an important regulatory protein in effecting the biochemical and genetic responses elaborated by the plant as it seeks to adapt to the loss of oxygen caused by flooding.

727 RFLP MAPPING AND CHROMOSOME WALKING TO THE *ARABIDOPSIS THALIANA FAE1* LOCUS. [1]Bertrand Lemieux, [2]Brian Hauge and [3]Christopher Somerville [1]Department of Biology, York University, Toronto M3J-1P3, [2]Department of Molecular Biology, Massachussets General Hospital, Boston, 02114 and [3]MSU/DOE Plant Research Laboratory, E. Lansing, 48824.

After analyzing the seed fatty acid composition of 3,000 *Arabidopsis thaliana* M3 seed families, we identified 7 true breeding mutant lines with altered fatty acid profiles. We believe that one of these lines, which is deficient in the elongation of 18:1 to 20:1 (*fae1* locus), encodes the structural gene for the oleyl-CoA elongase. The mutant allele of this locus exhibits a co-dominant inheritance pattern relative to the wild-type allele, thus indicating that the level of the FAE1 gene product regulates the amount of 18:1 elongation. Our RFLP mapping indicates that the *fae1* locus is located 0.9 centi-Morgan (cM) from Goodman RFLP clone 3088 and 3.6 cM from Goodman clone 8300. We have used RFLP clones 3088, 8300 and pG11 (located on the bottom arm of chromosome 4) to isolate 6 clones of 120 to 220 kbp from a Yeast Artificial Chromosome (YAC) walking library of *Arabidopsis*. The homology between the YAC clones and the RFLP clones was confirmed by probing Southern blots of CHEF gels of the YAC clones. As a result of our RFLP mapping we have isolated lines which are recombinant in the 4.5 cM region between RFLP clones 3088 and 8300; both of which flank the *fae1* locus. These recombinant lines will enable us identify recombination breakpoints within a cosmid clone containing the FAE1 gene. By chromosome walking from these RFLP markers, we are assembling a contig map of this region of the *Arabidopsis* genome. We will identify open reading frames within this cosmid by using it as a probe against a tissue specific cDNA banks of *Arabidopsis thaliana*. This work was supported by a grant from NSERC to B. Lemieux and by grants from the NSF, DOE and USDA to C.R. Somerville.

728 REGULATED EXPRESSION OF CHIMAERIC THIOESTERASE GENES IN TRANSGENIC B.NAPUS SEEDS. Richard Safford, Marilyn T. Moran, Jacqueline de Silva, Susan J. Robinson, Antoni R. Slabas, Robert Shields and Stephen G. Hughes. Cell Sciences, Unilever Research, Colworth House, Sharnbrook, Bedford, MK44 1LQ

Advances in gene transfer technology for oil seed crops have raised the possibility of producing upgraded oils in planta. Optimism for achieving modified oil composition without loss of seed viability or yield is based on (i) the wide diversity of fatty acid compositions occurring naturally in plant oils (ii) synthesis of storage oil is a seed-specific, developmentally regulated process. As such, the isolation of promoter elements from seed-expressed fatty acid biosynthetic genes will allow expression of introduced genes to be confined to seed tissue, thus avoiding disruption of membrane synthesis throughout the plant (iii) mutation breeding studies have shown that single gene changes can produce significantly altered oil compositions. As part of our programme aimed at modifying B.napus oil composition we describe the isolation and functional characterisation of a seed specific promoter from a B.napus acyl carrier protein (ACP) gene. Use of the promoter to express chimaeric thioesterase genes, intended to modulate fatty acid chain length, in transgenic rapeseed will be presented.

729 SITE-DIRECTED EXPRESSION OF A PROTEIN A:ACP-I FUSION GENE IN TRANSGENIC PLANTS. Chengbin Xiang and Dan J. Guerra, University of Idaho, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843.

Acyl carrier protein (ACP) is the central cofactor and co-substrate for at least 12 reactions of *de novo* fatty acid synthesis (FAS) and lipid metabolism in higher plants, and is considered as potential site of regulation for FAS. Recently a protein A:ACP-I fusion was prepared to probe the mechanism of this regulation. It was demonstrated that purified protein A:ACP-I *in vitro* does not down-regulate *de novo* FAS in contrast to native holo ACP-I. This suggested that the mutant fusion protein could be used as an instrument to examine *in vivo* regulatory mechanisms. In order to determine if this phenomenon holds true *in vivo*, a transgenic approach was employed to express the fusion gene in plants. CamV 35s promoter was used for constitutive expression, while the α'subunit of ß-conglycinin promoter/enhancer was used to drive seed-specific expression. By causing the specific expression of this fusion gene we hope to contrast its impact on membrane lipid and triacylglycerol biosynthesis. To target the fusion gene product into plastids where it functions, a 240 bp sequence coding for the transit peptide of soybean Rubisco pre-ss was used. These constructs were introduced into binary vectors and subsequently used to transform plants via *Agrobacterium*-medicated gene transfer. Transgenic tobacco plants have been obtained. We are currently screening these transgenic plants. Preliminary results on the impact of the fusion protein on the *in vivo* regulation of FAS will be presented. We have also transformed *Arabidopsis* via gene replacement strategies and expect to report on the impact of homologous recombination of the fusion protein with constitutive ACP-I.

730 ANALYSIS OF PATATIN REGULATORY ELEMENTS IN TOMATO. Jie Du, Hongyong Fu, Qian Gao, Gregory May, Brian Taylor, and William Park, Texas A&M University, Dpts. of Biochemistry and Biology, College Station, TX 77843

To examine how "tuber-specific" genes could have evolved from components in nontuberizing species, we characterized patatin *cis*- and *trans*-acting factors in tomato. Tomato has what appears to be a normal Class-II patatin gene which is expressed in roots and also has all of the *trans*-acting factors necessary for sucrose induction of a potato Class-I patatin gene. However, the endogenous patatin genes in tomato are not sucrose inducible. The reason for this is that the only region in tomato homologous with the Class-I patatin promoter is not attached to a patatin structural gene. We isolated this Class-I homologous region from the tomato variety VFN8. Other than three short insertions, it is greater than 90% homologous to the 33 and 37 bp repeats in the potato class-I patatin promoter and some of their flanking sequences. The Class-I homologous region in tomato is adjacent to a series of potential open reading frames which appear to be expressed in a wide range of tissues. The Class-I homologous region from tomato has sucrose inducible enhancer activity very similar to that seen in the corresponding region of potato. However, as would be expected since it does not contain the patatin sequences between -87 and -40 that we have found to be critical for "tuber-specific" expression, it is normally expressed at high levels both in tubers and in the vascular tissue of leaves. These data suggest that not only does the Class-I patatin gene contain two distinct regulatory domains, they are on different chromosomes in tomato and may be evolutionarily distinct.

731 THE ABERRANT DISTRIBUTION OF ZEINS IN PROTEIN BODIES OF THE MAIZE ENDOSPERM MUTANT *floury-2*. Craig R. Lending and Brian A. Larkins. Department of Biological Sciences, State University of New York College at Brockport, Brockport, NY 14420 and Department of Plant Sciences, University of Arizona, Tucson, AZ 85721.

We have examined the structure and development of protein bodies in endosperm that contains the maize mutation *floury-2*. The distribution of α-, β- and γ-zeins within protein bodies has been examined by immunocytochemical techniques at both the light and electron microscopic level. A comparison of the distribution of the various zeins with those observed in normal endosperm shows striking deviation from the typical protein body structure [Lending and Larkins, 1989, *The Plant Cell* 1:1011]. Protein bodies in normal endosperm are spherical and contain a central core of α-zeins surrounded by a peripheral shell of β- and γ-zeins. In contrast, the protein bodies within *floury-2* endosperm are irregular and the zeins are disorganized; patches of β- and γ-zeins occur within irregularly lobed clusters of α-zein within the lumen of the rough endoplasmic reticulum. The implications of this aberrant distribution will be discussed, both with respect to protein body development and kernel characteristics.

POSTER ABSTRACTS

732 ANALYSIS OF WHEAT SEED STORAGE PROTEIN GENE PROMOTERS IN A TRANSIENT ASSAY. Ann E. Blechl, Gale F. Lorens[1] and Frank C. Greene. Western Regional Research Center, Agricultural Research Service, USDA, Albany, California 95710. [1]Present address: Agronomy Department, University of Florida, Gainesville, Florida 32611.

We are utilizing a suspension cell line derived from developing maize endosperm as a transient assay system for promoter function of genes expressed in cereal endosperm tissue. Protoplasts derived from these cells are electroporated in the presence of plasmids containing hybrid genes consisting of test promoters transcriptionally fused to either the Chloramphenicol Acetyltransferase (CAT) or luciferase coding sequences. After systematic optimization of each step of the assay protocol, levels of CAT or luciferase expression driven by maize *ADHI* promoter and first intron are 1000x those of promoterless control plasmids. We have tested the relative strength of several wheat seed storage protein gene promoters in this system. Using luciferase as the reporter, an α-gliadin gene region extending from 2800 to 45 bp upstream of the start codon supported expression levels about 2x background. Regions from the High Molecular Weight (HMW) Glutenin Subunit genes *Glu-1Dx5* and *Glu-1Dy10* extending from -434 to -5 bp and -450 to -24 bp (relative to their translational start codons), respectively, were assayed using the CAT reporter. These promoters support equal levels of expression, 30-40 times a promoterless CAT control. Constructs including 2800 and 1400 bp of Dy10 5' flanking regions are no more active than the -450 construct. Small deletions from the 5' end of the -434 Dx5 promoter significantly lower its expression. The precise sequences missing in these deletions are being determined and will be presented. The results of these analyses will define the *cis*-acting elements necessary for expression the HMW Glutenin genes in this maize endosperm-derived suspension cell line.

733 BEAN SEED STORAGE PROTEIN ß-PHASEOLIN WAS EXPRESSED IN THE ENDOSPERMS OF TRANSGENIC RICE PLANTS. Zhenwei Zheng, Shouhua Xiao, and Norimoto Murai. Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803

To improve nutritional quality of rice seed protein, bean 7S storage protein ß-phaseolin and pea 11S storage protein legumin genes were used to transform rice. Both genomic and cDNA coding sequences of the genes were fused to a native rice storage protein glutelin (Gt-1) gene promoter (a 1.8 kbp or 5.0 kbp fragment) or to a bean phaseolin gene promoter. After protoplast transformation, mature transformed plants were regenerated and selfed for F1 progeny. ELISA analysis of the endosperms of transformed seeds indicated a wide variation of phaseolin expression level among transgenic plants tested. The highest quantity of phaseolin expressed was 2.1% of the total seed protein in the endosperm of mature rice seeds among seven transgenic rice plants when a chimeric phaseolin gene was expressed under control of 1.8 kbp glutelin gene promoter. Preliminary results showed that phaseolin genomic coding sequence was as effective as its cDNA counterpart to express phaseolin in monocotyledonous rice. Higher expression level should be expected in homozygous F1 seeds of the primary transformants or in seeds containing a chimeric phaseolin gene expressed under control of 5.0 kbp glutelin gene promoter. We are testing phaseolin expression in embryos of rice seeds transformed with a chimeric phaseolin gene under control of the bean phaseolin promoter. We are also studying the overall efficiency of bean protein expression in rice, i.e. intron processing, glycosylation, endoproteolitic cleavage, disulfide bond formation and accumulation of bean storage protein in the vacuolar protein bodies of rice endosperms.

734 BEYOND Bt ENDOTOXIN: HIGHER PLANT GENES WHICH ENHANCE INSECT RESISTANCE IN TRANSGENIC PLANTS. Vaughan A. Hilder, Angharad M.R. Gatehouse, John A. Gatehouse and Donald Boulter, University of Durham, Department of Biological Sciences, Durham DH1 3LE, U.K.

Plant genetic engineering makes the transfer of genes from any original source to crop plants feasible. The strategy of the Durham Group is to use protein insect control mechanisms evolved in plants themselves for introduction into transgenic crops. The first successful example of this approach uses the transfer of a trypsin inhibitor gene (CpTI) from cowpeas to tobacco, thereby improving resistance of the transgenic tobacco plants to insect predators. Similar protection to that demonstrated in the growth-room has now been confirmed in the field. Transgenic plants show small differences in a number of morphological characteristics, as compared to controls, but since these fall within the range of variability of control plants they should be readily restorable to normal. On its own a single protective mechanism of this type gives only partial control and combinations of several different genes will therefore be required. Fortunately, a wide spectrum of plant protein protectants exists in Nature, including several different types of protease inhibitors, amylase inhibitors and lectins. Amongst these are proteins with relatively high insect toxicity but low mammalian toxicity. Furthermore, when transgenic tobacco plants containing both a pea lectin and the CpTI gene were challenged with Heliothis, they showed additive protection over and above that when either gene alone was present. Another characteristic of natural protein protectants is that they give more general protection. For example, CpTI has been shown to be active against some members of the Lepidoptera, Coleoptera and Orthoptera. This approach is now becoming well established with the demonstration of enhanced insect protection in transgenic plants expressing several proteins other than CpTI, both by us and other groups; our data on snowdrop lectin will be presented. Whilst chemical pesticides may be more effective at lower concentrations than these natural protein protectants, the latter by comparison are more plant, ecological and user-friendly.

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735 *CIS*-ELEMENTS IN THE SPORAMIN GENE PROMOTER REQUIRED FOR EXPRESSION IN TRANSGENIC TOBACCO.

Atsushi Morikami and Kenzo Nakamura. Laboratory of Biochemistry, School of Agriculture, Nagoya University, Chikusa, Nagoya 46401, Japan.

Genes encoding sporamin, the tuberous root storage protein of sweet potato, are expressed at high-level in stems of plantlets cultured on sucrose-containing medium. The chimeric gene containing 1-kb 5' promoter region of a sporamin gene, fused to the reporter β-glucuronidase(GUS) gene expressed GUS activity predominantly in stems of transgenic tobacco plants. Histochemical analysis of transgenic tobacco sections stained with X-gluc demonstrated that reporter gene expressed strongly in internal phloem of the vascular system and the base of axillary bud, and weakly in pith parenchyma. Deletions from the 5' of the sporamin gene promoter revealed that a region from -305 to -237 contains *cis*-regulatory element(s) is necessary for expression of reporter gene both in the internal phloem and at the base of axillary bud [(1)]. Fusion of either one of the following three regions; -970 to -306, -305 to -237, or -192 to -94, to the -86 fragment of the promoter containing TATA-box gave a expression in pith parenchyma. These results suggest that multiple *cis* regulatory elements in the 5'-upstream of this sporamin gene can confer the expression in pith parenchyma.

(1) Ohta et al. Mol. Gen. Genet. 225: 369-378 (1991)

736 A COMMON NUCLEAR FACTOR SP8BF BINDS TO THE 5'-UPSTREAM REGIONS OF THREE DIFFERENT GENES CODING FOR MAJOR TUBEROUS ROOT PROTEINS OF SWEET POTATO. Sumie Ishiguro and Kenzo Nakamura. Lab. of Biochem., Sch. of Agric., Nagoya University, Chikusa-ku, Nagoya 464-01, Japan

Sporamin and β-amylase are two major proteins of tuberous roots of sweet potato, and expression of genes coding for sporamin and β-amylase is induced concomitantly in leaf-petiole cuttings by exogenous supply of sucrose or polygalacturonic acid. We have used DNase I footprinting assay to characterize nuclear factors that bind to the 5'-upstream regions of gSPO-A1, gSPO-B1 and gβ-Amy genes that encode A-type sporamin, B-type sporamin and the subunit of β-amylase, respectively. Nuclear extracts from sucrose-treated petioles protected a region around -155 of gSPO-A1 and a region around -880 of gβ-Amy from DNase I digestion on both strands. These two protected regions contained an identical eight nucleotide sequence ACTGTGTA, designated SP8a, in opposite directions with respect to transcription start sites. Gel mobility shift assay with SP8a oligonucleotide and competition experiments indicated that a common factor SP8BF binds to the SP8a sequence in gSPO-A1 and gβ-Amy. Binding of SP8BF to the SP8a-oligo was abolished by mutation within the SP8a sequence. Fragments of the 5'-upstream region of gSPO-B1 also competed for the binding of SP8BF to the SP8a-oligo, and DNase I footprinting assay revealed three binding sites for SP8BF in the 5'-upstream region of gSPO-B1. These three sites in gSPO-B1 contained an identical seven nucleotide sequence of TACTATT, designated SP8b, which shared 4 nucleotides at identical positions with the SP8a sequence. The inverted repeats of the SP8b sequence was also present at one protected site in the 5'-upstream region of gβ-Amy. The SP8BF activity was present not only in sucrose treated petioles but also in tuberous roots and non-treated, fresh petioles of the sweet potato. Furthermore, the activity was also detected in stems of tobacco plantlets, suggesting that the SP8BF is an ubiquitous factor. In addition to the SP8BF, we also detected several other factors which bind to the 5'-upstream region of gβ-Amy.

737 CHYMOTRYPSIN INHIBITOR GENES IN WINGED BEAN (Psophocarpus tetragonolobus (L.) DC.): ANALYSIS OF THEIR SPECIFIC EXPRESSION. Yoshiki Habu, Yoichi Sakata, Katsuhiro Umemoto and Takeshi Ohno. Hokkaido University, Department of Agriculturalchemistry, Sapporo, Japan.

Winged bean seeds contain several kinds of Kunitz-type chymotrypsin inhibitor (WCI). WCI proteins accumulated during seed maturation and their mRNAs were detected transiently in mid-maturation stage seeds. In addition, winged bean has a tuberous root as another propagative organ in which WCI proteins and their mRNAs also accumulated. To reveal the mechanism by which the expression of WCI genes are regulated during differentiation of embryos or tuberous roots, we analyzed the structure of WCI gene family and the functions of their regulatory regions. WCIs were encoded by small multigene family. We cloned seven WCI genes which hybridized strongly with the cDNA encoding WCI-3, the major WCI in seeds. Four genes (WCI-2, 3a, 3b and x) were presumed to encode functional inhibitor proteins and the others were to be pseudogenes. Analyses of transgenic tobacco plant containing WCI-promoters fused to GUS (beta-glucuronidase) structural gene are now in progress. So far, the 1.2Kb region upstream of WCI-2 coding sequence was shown to confer seed specific expression in tobacco plant. Nuclear factor(s) binding to the upstream region of WCI-3a gene was detected in nuclear extract prepared from mid-maturation stage seeds of winged bean. Its precise binding specificity will be described.

POSTER ABSTRACTS

738 CLONING OF 35 KDa LYSINE-RICH SEED STORAGE PROTEIN GENE FROM AMARANTHUS. Anjana Raina and Asis Datta,* Jawaharlal Nehru University, Molecular Biology Laboratory, New Delhi-110 067, INDIA.

Seeds are the major source of protein for most of the world's population but most often they are deficient in one or the other essential aminoacid. In order to improve the nutritional quality of seeds and to study the regulation of seed storage protein gene we have purified a protein of 35 KDa from seeds of Amarantnus. It has a high lysine content. Polyclonal antibodies were raised against this protein in rabbits which were furtner affinity purified.

A cDNA library was made in λgt11 and screened with antibody. A total of 6 recombinant plaques were picked up and plaque purified. Clones were confirmed by hybrid selected- translation and immunoprecipitation; largest clone nas an insert of 1.2 Kb. Northern blot analysis using the cDNA clone as a probe also gave a single band of 1.2 kb indicating the presence of a full length cDNA clone. RNA level was also determined at various developmental stages and correlated to protein synthesis pattern.

739 CHARACTERIZATION OF THE GAMMA-ZEIN LOCUS IN QUALITY PROTEIN MAIZE (QPM). Mauricio A. Lopes, Koichi Takasaki & Brian A. Larkins, University of Arizona, Department of Plant Sciences, Tucson, AZ 85721.

Quality Protein Maize (QPM) genotypes are *opaque-2 (o2)* mutants that carry genetic factors responsible for modification of a soft and opaque endosperm to a hard and translucent phenotype. Because the components of this genetic system and its mode of action are unknown, further development and future usefulness of QPM may depend upon deciphering the genetic, biochemical and molecular mechanisms acting in modified genotypes. Endosperm modification in QPM is associated with increases in the content of the 27 kD gamma-zein, a storage protein that appears to play an important role in protein body formation. Using the Polymerase Chain Reaction (PCR), we cloned and sequenced the gamma-zein locus from a QPM variety. Unlike most of the commonly used imbred lines that have only one gene encoding this protein, QPM genotypes normally have two genes. RFLP's resulting from sequence variations within the coding regions of these genes allowed us to study the inheritance at this locus in combinations of modified vs. unmodified genotypes. Extraction of DNA and storage proteins from individual kernels allowed us to relate allelic variations in segregating progenies with patterns of protein accumulation in the endosperm.

740 CLONING OF A GENE ENCODING SEED STORAGE PROTEIN OF LEUCAENA LEUCOCEPHALA. Feng Liu, Zhi-Qiang Pan, Nai-Sui Pan and Zhang-Liang Chen, National Laboratory of Protein Engineering and Plant Genetic Engineering, Peking University, Beijing 100871, China

Leucaena leucocephala (Mimosaceae), a tropical plant, has been a very important forage for livestocks in tropical area. The leaves and seeds contain proteins up to 30% and 33% of dry weight respectively. We have been interested in studying these proteins and regulation of the seed storage protein. Total DNA from leaves of L. leucocephala was isolated and digested partialy with Sau3A. Using bacterophage lambda EMBL3 as a cloning vector, we have constructed the genomic library with $3.5X10^6$. The library was screened with a gene encoding the α'-subunit of the soybean 7S storage protein. Four positive clones were obtained and three of them were chosen for further analysis. We have now nearly finished the full-length sequence of the gene, and revealed about 60% homology between genes encoding storage protein of L. leucocephala and soybean. The work is progress for studying the regulation of gene expression in the leucocephala plant.

741 CUSTOM POLYMERASE CHAIN REACTION ENGINEERING PLANT EXPRESSION CASSETTES USING CLONED AND GENOMIC DNAS. Roger F. Drong and Jerry L. Slightom, Molecular Biology Unit-7242, The Upjohn Co. Kalamazoo, MI 49007

The combination of polymerase chain reaction amplification and custom-synthesized oligodeoxyribonucleotide primers is a very potent method to modify DNA so that many complex genetic engineering steps can be simplified. We refer to this procedure as custom polymerase chain reaction (CPCR) engineering. We have used CPCR engineering to amplify specific regions of either cloned or genomic plant DNAs and to place useful restriction enzyme sites adjacent to the amplified regions. By using this technique, we have engineered several seed protein gene expression cassettes: a seed specific cassette consisting of the *Phaseolus vulgaris* phaseolin gene promoter (pvP) and 3'- flanking DNA region (pvA) to obtain the cassette pvPAexp, and a seed specific cassette that also includes the phaseolin signal peptide (SP) and translation termination codon (T) to obtain the cassette pvPSPTAexp. The power and versatility of the CPCR engineering technique was further demonstrated by engineering the promoter and 5'-flanking DNA region of a gene directly from total genomic DNA, specifically the seed storage protein gene that encodes the α'-subunit of β-conglycinin of *Glycine max*. Each seed specific expression cassette has been engineered so that the 5' and 3' segments are linked by an internal *NcoI* site into which modified (CPCR-engineered) gene coding sequences that have been flanked with *NcoI* sites can be inserted. Functionality of the phaseolin expression cassette, pUC18pvPSPTAexp, is being tested after cloning a synthetic and CPCR-engineered DNA region that encodes a major portion of the Brazil nut (*Bertholletia excelsa*) 2S albumin seed storage protein gene.

742 CLONING AND CHARACTERIZATION OF COIXIN GENES. Adilson Leite[1], Laura M.M. Ottoboni[1], Maria Luisa N.P. Targon[1], José A. Yunes[1], Silvia R. Turcinelli[1] and Paulo Arruda[1,2]. [1]Centro de Biologia Molecular e Engenharia Genética and [2]Departamento de Genética, IB, Universidade Estadual de Campinas, 13081, Campinas, SP, Brasil

Coixins are the major storage proteins of *Coix lacryma-jobi* L. seeds. They are synthesized by membrane-bound polyrribossomes and assembled into protein bodies. Coixins can be grouped into α- and γ-coixins according to its solubility. Alpha-coixins are composed by four size classes of 27 (C1), 25 (C2), 17 (C4) and 15 (C5) kDa, while γ-coixin (C3) is composed by only one polypeptide. cDNA and genomic clones representing C2 and C4 α-coixins and C3 γ-coixins were isolated from cDNA and genomic libraries, and their sequences were determined. The C2 α-coixin clones encodes a 27 kDa protein consisted of a signal peptide and 10 tandem repeats of 20 amino acid blocks separated by QQLL loops. This protein is highly homologous to the 22 kDa α-zein from maize and the 22 kDa α-kafirin from sorghum. The C4 α-coixin clones encodes a 18.3 kDa polypeptide characterized by high contents of methionine (11.6%) and cysteine (5.2%). This protein is homologous to the 17 kDa β-zein. The C3 γ-coixin clones encodes a protein containing a cysteine rich domain followed by tandem repeats formed by units of Pro-Pro-Pro-Val-His-Leu. This protein is highly homologous to the maize and sorghum γ-prolamins. The results will be discussed in the context of the structure and evolutionary relationship between the prolamin genes of *Coix*, maize and sorgnum.

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743 CLONING OF A cDNA ENCODING A PUTATIVE CYSTEINE PROTEASE FROM GERMINATING MAIZE (*Zea mays* L.) SEEDS. Everaldo G. de Barros & Brian A. Larkins, University of Arizona, Department of Plant Sciences, Tucson, AZ 85721.

Proteins A_1-A_4 are cysteine proteases that appear to be responsible for the first steps of zein degradation during germination of maize seeds. To isolate and study the gene(s) encoding these proteases, we constructed a cDNA expression library in lambda phage and screened it with polyclonal antibodies to A_1-A_4. However, no immunopositives were detected. As an alternative approach, we screened the same library with antibodies against EP-B, a cysteine protease from the aleurone of germinating barley seeds, because these antibodies strongly reacted with A_1-A_4. However, this procedure was also unsuccessful. One possible explanation for these results is that the potential chimeric proteases made were active and, consequently, deleterious for the host cells. As a third approach to isolate the gene(s), we screened the library with a heterologous probe, a cDNA encoding EP-B, from germinating barley seeds. After two rounds of screening, five positives were isolated. The longest clone (pMCP10A) showing significant homology to the barley cDNA was sequenced and characterized. It encodes a putative cysteine protease of 25 kD and part of its pro-sequence (62 amino acids). Transcripts hybridizing to pMCP10A are present in the endosperm between 2 and 6 DAG, but not at 0 DAG. The number of genes corresponding to pMCP10A is no larger than 5. Experiments are underway to demonstrate if pMCP10A indeed encodes A_1-A_4.

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744 CLONING AND CHARACTERIZATION OF A GENOMIC CLONE OF ARCELIN 1 FROM *PHASEOLUS*. Janice L. Anthony and Timothy C. Hall. Texas A&M University, College Station, TX 77843-3258

A size-selected genomic library was constructed in λZap II from the *Phaseolus vulgaris* line SG3. SG3 seeds express high levels of arcelin, a protein closely related to bean lectin, and low levels of phaseolin, the major seed storage protein in beans. The SG3 line contains arcelin in the Sanilac genetic background. The library was screened with an arcelin PCR product obtained from SG3 genomic DNA. Of six clones obtained, the deduced amino acid sequence of one (Z5) matched the N-terminal sequence obtained from microsequencing of the mature arcelin protein. The Z5 clone was used to probe genomic blots of arc+ and arc- wild type bean lines, the cultivated variety Sanilac (arc-), and SG3 (arc+). Although the lectin profiles for wild type lines and cultivated lines were disparate, the lectin profiles for arcelin-expressing lines were nearly identical, strongly suggesting linkage of arcelin and lectin genes. Novel phaseolin bands were present in arc+ lines probed for phaseolin, indicating concommitant introduction of new phaseolin-related sequences with arcelin. Arcelin mRNA was developmentally regulated and was first seen 15 days postanthesis. The presence of arcelin correlated with a decreased phaseolin mRNA level.

745 *CIS*-ACTING TRANSCRIPTIONAL REGULATORY ELEMENTS FROM SOYBEAN β-CONGLYCININ GENES. Philip A. Lessard, François Bernier, Randy D. Allen, Toru Fujiwara, Brett Lu and Roger N. Beachy. Washington University, Department of Biology, St. Louis, Missouri 63130. Genes encoding β-conglycinins, the 7S seed storage proteins in soybean, are expressed only in seeds during middle to late stages of development. Much of the regulation of these genes occurs at the level of transcription. Individual genes that encode either the α' or β subunits of β-conglycinin are themselves differentially regulated with respect to their timing and level of mRNA accumulation as well as their response to the phytohormone abscisic acid (ABA). A seed-specific transcriptional enhancer has been identified within the α' subunit gene, and numerous mutations have been generated to delineate sequences within this enhancer that control its level of activity as well as its tissue specificity. One region within this enhancer that is essential for its activity contains a highly conserved "vicilin box" sequence as well as a binding site for SEF4, a DNA binding factor found in soybean seeds. Sequences from the α' subunit gene upstream of this enhancer have also been examined for possible regulatory function. Quantitative *cis*-acting elements have also been identified upstream of the β subunit gene coding sequence. In cultured cotyledons, increases in the concentration of exogenous ABA result in increased accumulation of β subunit mRNA; expression of the α' subunit gene is unaffected. ABA responsive elements (ABREs) have been tentatively identified within the β subunit gene. These elements share little homology with the ABREs described by other groups [Marcotte et al., (1989) Plant Cell, 1:969; Mundy et al., (1990) PNAS 87:406].

746 DETERMINATION OF REGULATORY ELEMENTS ON THE PROMOTER OF CRUCIFERIN GENE BnC1. André M. Nantel, Pierre Bilodeau, Diane Beauseigle, Christiane Cantin-Nantel and Guy Bellemare. Département de Biochimie, Faculté des Sciences et Génie, Université Laval, Québec, PQ, Canada, G1K-7P4.

Cruciferin is the main seed storage protein in *Brassica napus*. It is encoded by three gene subfamilies totalling 9-12 members. Under our growth conditions, cruciferin genes are transcribed in maturing embryos between 21 and 31 days post-flowering. In order to understand the transcriptionnal regulation of tissue-specific genes in *B. napus*, we are defining by *in vitro* and *in vivo* techniques the position and function of regulatory sequences in the promoter of the most expressed cruciferin gene, BnC1. By gel retardation and footprinting assays, we determined that sequences of the BnC1 promoter between -842 and +87 are recognized by numerous trans-acting factors and that binding of these nuclear proteins is affected by varying concentrations of KCl, $ZnCl_2$ or ABA and by embryo development. We are also making use of transgenic plants to characterize our promoter *in vivo*. Promoter fragments with 5' deletion points at -1897, -1254, -842, -617, -499, -377 and -186 were fused with a GUS reporter gene and used in an *Agrobacterium*-mediated transformation of *Nicotiana benthamiana*. Results from these transformations will be presented.

747 DNA-BINDING PROTEIN ACTIVITIES WERE ASSOCIATED WITH *CIS*-REGULATORY ELEMENTS REQUIRED FOR DEVELOPMENTAL CONTROL OF BEAN SEED STORAGE PROTEIN PHASEOLIN. Yasushi Kawagoe and Norimoto Murai. Dept. Plant Path./Crop Physiol., Louisiana State University, Baton Rouge, LA 70803

The 5'-deletion analysis of the β-phaseolin upstream sequence uncovered the existence of several *cis*-acting regulatory elements required for developmental control of bean seed storage protein phaseolin. These includes a negative (-295/-424) and a positive (-227/-294) element for phaseolin expression in seeds, and a silencer element (-106/-294) to repress phaseolin expression in stem and root. DNA-binding activities of crude nuclear proteins from bean cotyledons, bean leaves, and tobacco seeds were compared by gel-retardation assay, exonuclease III protection assay, and competition assay with synthetic oligonucleotides. Gel-retardation assay with bean cotyledon nuclear extracts demonstrated one, one, four, two, and one major retarded bands when DNA fragments -14/-64, -65/-105, -106/-226, -227/-294, and -295/-391, respectively, were used as radiolabeled DNA probes. Consistent with gel retardation assay, exonuclease III protection assay also indicated the existence of multiple sites of DNA/protein interaction. Exonuclease III-protected nucleotides -351/-352, -277/-296, and -156/-201 were detected in the region containing the negative, positive, and silencer element, respectively. Competition with synthetic oligonucleotides showed that at least one nuclear protein binds to the -342/-384, -269/ -290 and -170/ -205 region. Among other exonuclease III-protected sites, the target sequence CANNTG of MyoD family (helix-loop-helix) were found at -97, -156/ -164 and -245. It is possible that a member of HLH family may play a role in the phaseolin gene regulation.

748 DETECTION OF PHASEOLIN mRNA IN DEVELOPING BEAN SEEDS BY *IN SITU* HYBRIDIZATION TO DIGOXIGENIN LABELLED PROBES Tom Brady1, J. Brady1, A.A.M. van Lammeren, J.H.N. Schel (1)Hamilton College, Clinton, NY 13323; Agricultural University , Dept. Plant Cytology and Morphology, Wageningen, The Netherlands.

Phaseolin, the major storage protein in the French bean *Phaseolus vulgaris*, accumulates rapidly in the developing embryo and represents more that 50% of the protein in the mature seed. At some stages in development, ß phaseolin mRNA accounts for more than 40% of the total cotyledonary RNA. To determine the pattern of phaseolin mRNA accumulation during development, sense and anti-sense digoxigenin labelled (dig-UTP Boehringer) probes synthesized from the EcoR1/ BamH1 fragment of the phaseolin gene were hybridized to glutaraldehyde and/or formaldehyde fixed 10µ sections of developing bean seeds. Fixation, embedding, *in situ* and post-hybridization washings were performed essentially according to Cox and Goldberg (1988). Hybridization was detected with anti-dig/alkaline phosphatase (Boehringer) resulting in a bright blue signal. Phaseolin message can be detected in isolated cells of the cotyledons in 8mm seeds. By 11 mm the message is distributed throughout the cytoplasm of cotyledonary cells but is not observed in the wall, amyloplasts or in vascular or epidermal cells. As the seed develops the message is found in the nonvascular tissue of both the radicle and the plumule but at a much reduced level from that in the cotyledons. By 20 mm, when the seed is still green and has reached its maximum length before dehydration, the level of signal detected in the cotyledons has already started to decline. With the onset of dehydration, a decrease in seed length and the first appearance of anthocyanins in the seed coat (17mm), the message has virtually disappeared from cells in the central two thirds of the cotyledon: although it is still present in the embryonic axis. By the time the seed is fully dehydrated and a deep purple (15 mm) the message can no longer be detected.

749 EXPRESSION OF THE ß-SUBUNIT OF CONGLYCININ IN CULTURED SOYBEANS IS REGULATED AT MANY LEVELS

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Soybean seeds that are cultured in liquid media synthesize storage proteins as if they were still developing on the plant. However, when methionine is added to the medium, the ß-subunit of ß-conglycinin is not produced. Functional mRNA's which code for the ß-subunit are not detectable by in vitro transcription after three days. Run-on transcription experiments with isolated nuclei indicate that transcription is reduced by at most 25% in the presence of methionine. The rate of degradation of ß-mRNA is doubled in the presence of methionine according to pulse-chase labelling experiments and experiments measuring the approach to steady state levels of mRNA. Changes in mRNA stability have led us to apply RNase H mapping to detect differences in the extent of polyadenylation. In order to account for the rapid disappearance of functional ß-mRNA in the presence of methionine, we suspect that expression of the gene encoding the ß-subunit of conglycinin is regulated by some other as yet unidentified process as well as transcriptional control and mRNA stability.

POSTER ABSTRACTS

750 EXPRESSION OF NAPIN IN BRASSICA NAPUS MICROSPORES INDUCED FOR EMBRYOGENESIS. K. Boutilier, C. Nozzolillo and B.L. Miki* University of Ottawa, Department of Biology, Ottawa, Ontario, K1N 6N5. *Plant Research Centre, Agriculture Canada, Ottawa, Ontario, K1A 0C6.

Isolated microspores from Brassica napus can be induced to form haploid embryos in vitro by culturing them for four days at 32.5°C. We have detected the very strong, but transient expression of genes encoding the seed storage protein napin in microspores immediately after the treatment used to induce embryogenesis. This response was not seen in somatic tissues and appeared to be restricted to populations of cells with embryogenic potential. The expression of other genes monitored in this study, including the Brassica napus 12S seed storage protein cruciferin, was not induced by this treatment. In order to determine which specific napin genes are expressed at this time a cDNA library was constructed with poly(A)$^+$ mRNA from microspores induced for embryogenesis. Preliminary sequence analysis of the 17 napin cDNA clones isolated from the induced microspore library indicates that the majority these clones may correspond to a new, highly similar class of napin genes that can be organized into one large group consisting of five smaller subgroups.

751 THE GENE FAMILY OF MANNOSE BINDING LECTINS IN AMARYLLIDACEAE SPECIES. Els J.M. Van Damme and Willy J. Peumans, Catholic University of Leuven, Laboratory for Phytopathology and Plant Protection, Willem de Croylaar 42, 3001 Leuven, Belgium.

A new class of plant lectins with exclusive specificity towards mannose was isolated from different species of the family Amaryllidaceae. Besides their unique specificity these lectins differ from all other known plant lectins in that they occur in almost all tissues at high concentration (up to 10 % of the total protein) and that their accumulation is developmentally regulated. In addition, all Amaryllidaceae lectins are extremely complex mixtures of isolectins.

Screening of cDNA libraries constructed using polyA RNA isolated from Galanthus nivalis, Narcissus cv. Fortune and Hippeastrum hybr. resulted in the isolation of several lectin cDNA clones for each species. Although the sequence data show that different lectin clones of the same species definitely differ in their nucleotide sequence and deduced amino acid sequence, the lectin clones from the three Amaryllidaceae species are markedly homologous. Evidently, Amaryllidaceae lectins are encoded by gene families which have been conserved from an evolutionary point of view.

752 GENETIC CONTROL OF CHAPERONE-LIKE PROTEINS INVOLVED IN ZEIN ACCUMULATION. A. Marocco[1], S. Cerioli[1], M. Motto[2], R. Thompson[3] and F. Salamini[3]

1. Istituto Genetica, Università Cattolica S. Cuore, Piacenza, Italy
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Three independent maize mutations, f12, De-B30 and Mc which reduce zein level in the endosperm, also control the level of a polypeptide bound to protein bodies (b-70). This protein is separable into two isoforms (b-70I and b-70II) which are overexpressed in f12; only b-70I is present in De-B30 and Mc. Both b-70I and II resemble heat shock proteins (HSP70) in that they bind ATP, cross react with anti-HSP70 antibodies and have N-terminal sequence homology to chaperone-like heat shock proteins. A third protein, b-70III, also binds ATP and reacts with the same antibody, providing evidence for the presence in endosperm extracts of a cytosolic chaperone-like protein. b-70III level is not altered by the mutations studied here. The repression effect of the three mutations on zein accumulation may be mediated by the alteration of a zein transport or zein assembly process involving b-70I and b-70II.

753 EFFECT FACTORS ON PROTOPLAST REGENERATION OF CEREALS CROPS. Xiang-hui Li. Institute of Genetics Academia Sinica. Beijing 100101,China

Plant regeneration of cereals have been breaking the back of tough job. A. Plants regeneration from crop protoplasts including major cereals: Oryza sativa L.(indica, japonica subspecies rice),Triticum aestivum(winter Spring and half winter wheat), Trititrigia, Zea mays L.(corn and forege corn), Setaria italica(L) Beauv, Sorghum vulgare Pers.and Hordoum vulgare L. B. Effect factors on regeneration: 1. Establishment of somatic embryogentic callus are influenced by factors: Genotype, source of explant, regulation of 2,4,D, N and C source in culture media, culture condition, screening of embryogenic calli in subculture. 2. Factors of somatic embryo development in protoplasts: Environment of protoplast culture:Regulation of important components in culture media, such as NH_4^+,NO_3^-, phytohormones, osmatic press and protoplast density. 3. Effective and simple systems for protoplast culture were established.

754 ENDOGENOUS CEREAL α-AMYLASE INHIBITORS. Robert J. Henry,* Ian C. Haak and Paul S. Brennan. Queensland Wheat Research Institute, Toowoomba 4350. *Present address: Queensland Agricultural Biotechnology Centre, University of Queensland, St Lucia 4072, Australia.

Many seed storage proteins may have other biological roles. The bifunctional α-amylase/subtilisin inhibitor (BASI) of cereals is a relatively abundant protein in the endosperm. The inhibitor may function to regulate the hydrolysis of starch by α-amylases during germination. The protein from barley is an inhibitor of the group 2 α-amylase isoenzymes from cereals. In *in vitro* experiments α-amylase 2 from wheat is inhibited best by the barley inhibitor and less effectively by the wheat inhibitor. The inhibitor from rye has an intermediate effect. PCR has been used to amplify the coding region of genes from several cereal species. These genes show a high degree of homology. The BASI gene is located on chromosome 2. The addition line in which chromosome 2 of Betzes barley has been added to Chinese Spring wheat is being used to attempt a transfer of the gene from barley to wheat. Callus from immature embryos of the addition line is being cultured with the aim of the inducing homeologous interstitial translocation of the gene from barley chromosome 2 to wheat. Species specific PCR is being developed to screen regenerated plants. The presence of this gene may help to protect wheat from damage caused by pre-harvest sprouting.

755 GAMMA-ZEIN CONTENT IS RELATED TO ENDOSPERM MODIFICATION IN QUALITY PROTEIN MAIZE (QPM). Mauricio A. Lopes & Brian A. Larkins. University of Arizona, Department of Plant Sciences, Tucson, AZ 85721.

Opaque-2 (*o2*) is a mutation in one of the regulatory loci that controls storage protein gene transcription in maize (*Zea mays*, L.) kernels. It results in a significant reduction in the prolamine content of the endosperm, which becomes chalky and soft. Quality Protein Maize (QPM) genotypes are modified, hard-endosperm, *o2* mutants that carry genetic factors responsible for increased synthesis of the gamma-zein protein (mol wt 27 kD). The genes encoding this storage protein are not under regulatory control of the *O2* locus. We used a combination of genetic and biochemical analyses to investigate the relationships between endosperm modification and gamma-zein content in QPM. The use of unmodified and modified genotypes allowed us to demonstrate, by reciprocal crosses and F2 segregating progenies, that degree of vitreousness and increased synthesis of gamma-zein in modified endosperms are both dosage-dependent and directly correlated. Additionally, we found that these same genetic factors are active in other soft-endosperm mutants such as *floury-2* (*fl2*), and *floury-2;opaque-2* (*fl2;o2*), as well as in normal inbred lines. Although endosperm modification appears to be the result of a genetically complex system, its primary effect appears to be on the synthesis of the gamma-zein protein. Our studies suggest that there may be a relationship between the high content of this protein and increased kernel vitreousness in QPM.

POSTER ABSTRACTS

756 HOW CORRESPOND LEGUMIN SUBUNITS TO THE 12S GLOBULIN GENE FAMILY OF VICIA FABA? Christian Horstmann, Bernhard Schlesier, Albrecht Otto, Susanne Kostka and Klaus Müntz. Institut für Genetik und Kulturpflanzenforschung, Gatersleben O-4325, and Institut für Molekularbiologie, Berlin-Buch O-1115, Federal Republic of Germany

12S globulins of the field bean (Vicia faba L.), called legumin (MW 356 kDa), comprise 6 similar subunits (MW appr. 60 kDa) each composed of an appr. 40 kDa alpha- and an appr. 20 kDa beta-polypeptide, both linked by an SS-bridge. Legumin subunits are synthesized as precursors composed of the alpha- and beta-chain in peptide linkage and correspond to members of the 12S globulin multi-gene family. Two classes of legumin subunits are distinguished: A - containing Met residues and B - free of Met residues. Only genes encoding B-type subunits have so far been described (Heim et al. PMB (1989) 13, 653). Two leguminA-specific subunit sequences were derived from cDNA clones. HPLC-based subunit fractionation, peptide mapping and partial amino acid sequence determination were applied to identify subunits corresponding to the known field bean 12S globulin-specific DNA-clones. Correspondence was elucidated between 6 major legumin subunits and 6 DNA clones representing 4 groups of genes. In addition one clone corresponding to the minor legumin subunits with MW appr. 75 to 80 kDa is known from the work of Heim et al. (PMB (1989) 13, 653).

757 ISOLATION OF DNA FROM SOME THAI RICE VARIETIES AND cDNA FROM AROMATIC RICE. D.Varasundharosoth, L.Meksongsee, P.Harinasut and Y. Chiemchaisri. Kasetsart University, Department of Biochemistry, Bangkok, Thailand.

As part of a research work on rice storage protein genes for genetic manipulation to increase protein content of the popular aromatic rice KDML 105, seed proteins were fractionated from some Thai rice varieties to identify high protein varieties. The samples included the local red varieties GS 12052, GS 11012, GS 10244, GS 2683 together with KDML 105 since a variety of red rice has been reported to be high in protein content. The red variety GS 2683 showed the highest protein and glutelin contents. SDS-PAGE of the protein fractions revealed that the major protein subunits have the following sizes; the globulin to be 57 KD, prolamin as 16, 13 & 10 KD and glutelin as 23 KD. DNA was isolated from the above varieties as well as from some other recommended varieties i.e. RD 10, KPM 148, KTH 17 and NHS 4. Agarose gel electrophoresis of the DNAs separated these varieties into two groups of slightly differing band mobilities. Restriction fragment patterns of double digested DNA minipreparations will be presented. cDNA library was initiated by preparing poly A mRNA from KDML 105 and cDNA synthesis was attempted.

758 ISOLATION AND CHARACTERIZATION OF THE GAMMA-ZEIN GENES FROM QUALITY PROTEIN MAIZE (QPM). Koichi Takasaki, Mauricio A. Lopes & Brian A. Larkins.

University of Arizona, Department of Plant Sciences, Tucson AZ 85721.

Quality Protein Maize (QPM) are genetically modified *opaque-2* mutants with hard and vitreous endosperm. The most characteristic biochemical feature of QPM endosperms is the elevated amount of the 27 kD gamma-zein, a storage protein that appears to play important roles in protein body formation and endosperm structure. Highly modified genotypes contain two to three times the normal level of this protein in the endosperm, and its amount is proportional to the degree of modification in partially modified endosperms. Using the Polymerase Chain Reaction (PCR) we isolated the coding and promoter regions of the gamma-zein from a QPM variety. Unlike most of the commonly cultivated genotypes, this variety contains two genes encoding this protein. Comparisons with other published sequences revealed high degree of similarity between the genes from QPM and other normal or non-modified opaque genotypes, suggesting that overexpression at this locus is caused by factors other than cis modifications of the regions we analysed.

759 LECTIN GENE ORGANIZATION IN BEAN SEEDS. Angelo Santino, M. Gloria Daminati, Alessandro Vitale, Andrea Allavena* and Roberto Bollini. Istituto Biosintesi Vegetali, CNR, via Bassini 15, 20133 Milano and *Istituto Sperimentale per l'Orticoltura, MAF, via Paullese 28, 20075 Montanaso L., Italy.

In bean seeds the proteins belonging to the lectin family are the most abundant antibiosis factors against insect predation. Members of this family so far identified have been grouped into four classes: i) the abundant phytohemagglutinin (PHA), ii) the minor mitogenic lectin expressed in both PHA containing and PHA null genotypes (Pinto-lectin), iii) arcelin and iv) α-amylase inhibitor (αAI). Each class is inherited as a single gene and presence is dominant to absence, but the number of genes expressing the different lectins has not been determined. It has been demonstrated that PHA and arcelin genes are closely linked, in genotypes where both proteins are present. We show here that arcelin types 1 and 2 are immunologically related to αAI while arcelin types 3 to 5 are not, and that presence of αAI is alternative to that of arcelin types 1 or 2. Furthermore, by Western and Southern blot analysis of BC6F5 lines, obtained by introgression of the PHA null character in a PHA containing cultivar, we also show that PHA, Pinto-lectin and αAI genes are closely linked. These data further support the hypotesis that lectin genes might have been originated by duplication and divergence of an ancestral gene and that their encoded proteins may have developed new functions in plant defence. Supported in part by the PFs "Biotecnologie e biostrumentazione" and "RAISA" of the CNR.

760 PURIFICATION AND CHARACTERIZATION OF THE PROTEASE RESPONSIBLE FOR THE POST-TRANSLATIONAL PROCESSING OF THE 11S SEED STORAGE PROTEINS OF SOYBEAN. M. Paul Scott, Rudolf Jung and Niels C. Nielsen. USDA Agricultural Research Service and Agronomy Department, Purdue University, West Lafayette, IN 47907.

Precursors of 11S seed storage globulins undergo a post-translational proteolytic processing step at a highly conserved site. In soybeans this event permits the assembly of glycinin from trimers into hexamers. We have identified the activity responsible for this processing event in extracts of immature soybean seeds. Preparations of the enzyme hydrolyze octapeptides containing the cleavage site of glycinin. Using this substrate in assays, we purified the processing enzyme about 500-fold and characterized its activity. It is a glycosylated sulfhydryl protease with a molecular weight in solution of 40 kDa, and has an acidic pH optimum. Microsequence determinations on products from digestion by the purified protease revealed that soybean proglycinin and prolegumin from field bean were both cleaved at the proper asparagine-glycine peptide bond.

761 PURIFICATION OF LYSINE-RICH PROTEIN AND DETERMINATION OF ITS GENOMIC DNA. Yuxiang Jing, Bolin Liu and Ting-Yun Kuang. Institute of Botany, Academia Sinica, Beijing 100044 PR China. Screening and isolating lysine-rich protein gene is the first step to upgrade the quality of wheat and rice proteins by biotechnique. We collected 10 species of plant seeds from 6 families including Betulaceae, Cruciferae, Juglandaceae, Leguminasae, Pedaliaceae and Pinaceae. Amino acid composition assay showed that 4 species of them contain more than 5.5%lysine in total seed proteins. A Chinese bean from Leguminosae which contains 7.9% lysine in total seed proteins was chosen as experimental material. 5 different procedures were used to solubilize proteins from the seeds. One seed extract sample containing 8.4% lysine was characterized on 1 mm 2-D (IEF/SDS) gel. 22 kinds of proteins were purified by preparative electrophoresis on 3 mm 2-D (SDS/IEF) gels and the amino acid compositions of the proteins were analyzed, in which a 12 kD protein contains 10.5% lysine. Its N-terminal sequence of 18 amino acid residues was determined and the corresponding oligodeoxynucleoride probe was synthesized for screening lysine-rich gene. Genomic DNA from seedlings was isolated and digested with Bam HI, Eco RI and Hind III. The homologous DNA fragments were obtained with southern blot. We aim at cloning the cDNA(s) encoding lysine-rich protein, fusing it with recipient tissue-specific promoter and transferring the chimeric gene into cereal crops. (We thank Prof. Pei-Sung Tong for encouragement and helpful suggestions.)

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762 REGULATORY GENES AFFECTING ZEIN DEPOSITION IN MAIZE ENDOSPERM. Mario Motto, Massimo Maddaloni, Hans Hartings, Carlotta Balconi, Eduardo Rizzi, A. Marocco, Francesco Salamini[1], Stephan Lohmer[1], Richard Thompson[1]. Istituto Sperimentale Cerealicoltura, Bergamo, Italy; 1) Max-Planck-Institut, Köln, Germany

In maize, the synthesis and deposition of zeins is affected by several dominant and recessive mutants. We have investigated the effect of three dominant mutations f12, De-B30, and Mc which reduce zein level in the endosperm. They also control the level of a polypeptide bound to protein bodies (b-70) which is separable into two isoforms by 2D-gel. It is shown that both b-70s resemble heat shock proteins in that they bind ATP, cross react with HSP antibodies and has N-terminal sequence homology to chaperon-like HSP70. The negative effect of the three mutations on zein accumulation may be due to an alteration of zein transport or zein assembly system to which b-70s partecipate. Out of the recessive mutants o2, o6 and o7, the locus O2 controls the genes encoding the 22 kD zeins and an abundant albumine-like protein, termed b-32. The O2 protein contains a leucine zipper domain characteristics of some mammalian and fungal activation factors. It is shown that the promoter b-32 gene is activated in vivo in the presence of the O2 gene product and that the information necessary for this activation resides in a 440 bp DNA fragment containing five O2 binding sites. Two of these sites are embeddes in copies of the "endosperm box", a motif thought to be involved in endosperm-specific expression, which is also represented in 22 kD zein promoters. The O2 protein is also shown to be capable of binding in vitro and activating in vivo, its own promoter. The regulation of zein synthesis in endosperm cells by amino acid supply will be also presented and discussed.

763 THE SEQUENCE SPECIFICITY OF THE MATURATION PROTEASE FOR 11S PROGLOBULINS. Rudolf Jung, M. Paul Scott and Niels C. Nielsen. USDA Agricultural Research Service, Agronomy Department, Purdue University, West Lafayette, IN 47907

Precursors of the 11S seed storage proteins undergo post-translational proteolytic processing at a asparagine-glycine bond that has been conserved during evolution. This cleavage is required for assembly of trimers of proglobulin subunits into hexamers of mature subunits. In addition to a strict conservation of amino acids at the cleavage site, the physical properties of the 7 amino acids immediately COOH-terminal to the cleavage site are also well conserved. To study the specificity of the protease that carries out this proteolytic step, the appropriate region of cDNAs that encode 11S storage globulins were modified by site directed mutagenesis. By transcribing and translating these mutant cDNAs in vitro, proglobulins were produced that could be used as substrates for the purified maturation protease. Preliminary results indicated that either deletion of the P1 asparagine, or substitution of glutamine for the asparagine, eliminated the ability of the protease to cleave the proglobulins. Conservative changes in the 7 amino acids COOH-terminal to the cleavage site seemed not to hinder cleavage. A more systematic evaluation is in progress, and those results will be presented.

764 ISOLATION OF A KUNITZ PROTEASE INHIBITOR-LIKE PROTEIN FROM SEEDS AND ROOTS OF WHITE CLOVER. C.L. Diaz[1)], J.C. Stam[2)], B.J.J. Lugtenberg[1,2)] and J.W. Kijne[1,*)]. 1)Center for Phytotechnology and 2)Department of Plant Molecular Biology, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands.

Protein extracts from seeds of 5 different white clover (Trifolium repens L.) cultivars each contained a 21 kDa-polypeptide which is cross-reactive with anti-pea-lectin (Psl) antibodies, but is different from clover lectin (trifolin A). Seeds of cultivar "Dutch clover" were used to isolate and characterize this cross-reactive material (Psl-CRM) using FPLC-ion-exchange chromatography, FPLC-gel filtration, and isoelectric focusing. The molecular mass of Psl-CRM is 21 kDa, as judged from SDS-PAGE and gel filtration. The isoelectric point of this protein is 6.2. Amino acids 1 to 40 of the N-terminal region of this protein showed 35 to 43% sequence homology to proteins of the Kunitz protease inhibitor family, the highest homology being found to the main storage proteins of sweet potato (sporamin A and B). Psl-CRM was also present in extracts either from young white clover roots or from isolated root hairs, and appeared to be localized (indirect immunofluorescence) on the tips of growing root hairs. This pattern of localization is similar to those of Psl in pea and trifolin A in clover, and suggests that synthesis of lectins and this lectin-related protein has a common regulatory basis.

765 MUTATIONS IN THE fusC GENE OF ARABIDOPSIS PREVENT CRUCIFERIN ACCUMULATION IN SEEDS. Andreas J.Müller and Christian Horstmann, Institute of Genetics and Crop Plant Research, D-O-4325 Gatersleben, Germany.

Chemical mutagenesis of Arabidopsis thaliana resulted in several mutations that led to unscheduled anthocyanin synthesis during late embryogenesis but did not impair postgermination development. These mutants were found to be allelic and thus to define one gene (fusC). The seeds of fusC mutants which could be germinated prematurely lacked desiccation tolerance and turned shrunkenand non-viable after drying. SDS-PAGE of proteins isolated from immature and mature seeds revealed that the fusC embryos did not accumulate the cruciferin (12 S) proteins which in mature wildtype embryos comprise about 50% of total protein und represent the major class of storage proteins. In contrast, the mutation did not prevent the accumulation of the 2S storage proteins but reduced their amount. The mutant seeds had also a reduced level of triacylglycerol with only slightly deviating fatty acid composition. Experiments has been initiated to test the hypothesis that the fusC^{+} gene code for a trans-acting protein that regulates the expression of several seed-specific genes.

766 MOLECULAR ENGINEERING OF THE MAIZE GLOBULIN-1 GENE. Yunxia Wang and Alan L. Kriz. University of Illinois, Department of Agronomy, Urbana, IL 61801

Improvement of maize grain protein quality by increasing levels of the essential amino acids lysine and tryptophan may best be achieved through genetic engineering approaches. Maize globulins, the major proteins of the embryo, are ideally suited to modification by in vitro manipulation: the total globulin fraction of the grain (2-5% of total grain protein) is encoded by only two genes (Glb1 and Glb2) and, unlike zeins, these proteins already contain a significant amount of lysine. In addition, natural variation in globulin components, with respect to type and quality, is tolerated by the seed. A series of modified versions of Glb1 has been generated by site-directed mutagenesis. The number of lysine residue in the GLB1 protein has been increased by 50% from 22 to 31, and tryptophan residues have been increased from 3 to 7. The modified gene constructs have been characterized by in vitro translation of synthetic mRNAs and by expression as fusion protein in E. coli. Additional in vivo assays in heterologous systems are being conducted to further charactenze the modified Glb1 gene constructs.

767 MICROGETEROGENEITY OF MAIZE PROLAMINES. Plamenetskaya I.U., Institute of Maize, Dneiropetrovsk, USSR.

The endosperm of maize seed contains a group of prolamine storage proteins called zeins. These proteins constitute more then 50% of the total protein in the mature seed. Zein displays much heterogeneity when separated by electrophoresis or isoelectric focusing in polyacrylamide gels. So these methods are useful for genetic and taxonomic studies of maize. Besides, electrophoretic separation of zeins is very convenient way to determine genetic purity and hybridization level in maize seed stocks. The purpose of this work was to show microheterogeneity of prolamines connected with glycosylation of protein chains. Zeins were extracted from mature kernels by 70% ethanol, separated by the acid polyacrylamide gel electrophoresis with 8 molar urea and transfered from slab gels onto the nitrocellulose sheets by electrophoretic semi-dry method. Electroblotting was performed in acid buffer with 6 molar urea and ethanol. A detailed characterization of glycosylation sites were carried out by sequential incubation of the sheets with lectins and peroxidase. Such lectins as: WGA (lectin of wheat germ), RCA (lectin of Ricinum communis) and LAL (lectin of Labernum anagyroides) were used in this work. Visualization of the peroxidase was done with 4-chloro-1-naphthol. All selected lectins interacted with zein proteins. WGA revealed (d-glcNAc)-(d-glcNAc)-chains in zein patterns, RCA - terminal d-galactose, LAL - terminal or core l-fucose.

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768 · MOLECULAR CLONING OF COMPLEMENTARY DNAs ENCODING CRYSTALLOID POLYPEPTIDES OF WHITE SPRUCE AND DOUGLAS FIR. Isabel Leal and Santosh Misra. University of Victoria, Department of Biochemistry and Microbiology, Victoria, B.C. V8W 3P6

We are presently characterizing the expression of storage proteins and desiccation proteins in zygotic and somatic embryos of white spruce. The storage protein mRNAs corresponding to the crystalloid proteins were identified by *in vitro* translation of mRNAs followed by SDS-PAGE analysis of immunoprecipitated polypeptides. The major polypeptides have approximate MW's of 65, 50, 21, and 18 kDa. The activity of crystalloid mRNAs was high during the early stages of embryogenesis and declined during later stages of maturity. Mature seed and seedlings lack detectable mRNAs for these proteins. A parallel analysis of somatic embryos *in vitro* demonstrated that the storage proteins synthesized in somatic embryos are very similar to those of zygotic embryos and can be used as molecular markers to follow the process of somatic embryogenesis *in vitro*. In order to investigate the developmental regulation of storage protein genes, we have constructed a cDNA library from an early-maturation stage poly(A)+ mRNA. Several clones obtained from the cDNA library by immunoscreening are currently being analyzed. In addition, a radish nuclear gene which codes for a desiccation protein has been used in dot-blot hybridization studies and shown to cross-hybridize with the stages of embryonic development of white spruce.

769 MUTATIONS IN THE SEF BINDING SEQUENCES OF A β-CONGLYCININ PROMOTER AND EXPRESSION IN TRANSGENIC TOBACCO PLANTS. Toru Fujiwara and Roger Beachy, Department of Biology, Washington University, St. Louis, Missouri, 63130 Expression of the α' subunit gene of β-conglycinin, the 7S seed storage protein of soybean, is tissue- and temporal- specific in soybean and in transgenic plants. A DNA fragment (-257 to -79) of the promoter region of the α' subunit gene has a seed specific enhancer activity on the CaMV 35S promoter. Several factors present in soybean embryo (SEF1-4) that interact with the promoter of the α' subunit gene have been identified. Among the factors, SEF3 and SEF4 bind to the DNA fragment which has the enhancer activity. Mutations were made in the recognition sequences of the SEF3 and the SEF4 as well as in CATGCAT sequences (core sequence of the legumin box) in order to identify seed specific trans acting factor(s). The binding of the SEF3 and SEF4 to the DNA fragment is impaired by mutations in each recognition sequence. Promoters with various mutations were fused to the GUS gene and then linked to the complete α' subunit gene in the transformation plasmid which serves as an internal control for position effects. Enhancer fragments with various mutations were inserted into the EcoRV site of the CaMV 35S promoter and fused to the GUS gene. Reconstituted genes were introduced into tobacco plants by *Agrobactenun* mediated transformation. Expression of these constructs in transgenic plants indicated that *in vitro* binding of SEF3 and/or SEF4 does not accurately predict the *in vivo* activity of the *cis* sequence.

770 NEW ELECTROPHORETIC TECHNIQUE FOR HYDROPHOBIC PROTEIN AS PROLAMINES SEPARATION

Grishko A.V., Savchenko M.P., Kovalchuk T.M.
Institute of Maize, Dnepropetrovsk, USSR

New electrophoretic technique allied hydrophobic chromatography is describe for protein separation. The procedure can be used to separate protein in accordanse of their hydrophobic aminoacid contents. Hydrophobic proteins are separated in process of migration of charged protein-ionic detergent complex in nonionic detergent gradient. The nonionic detergents as SDS are competing with the ionic detergents as TRITON X-100 and NP-40 for hydrophobic sites in proteins. The charge of proteins is decreasing or changing onto opposite during its migration in nonionic detergent gradient. The procedure as describe was applied to separation that hydrophobic protein as wheat gliadin and maize zein on number of component. After electrophoresis the proteins are appeared as sharp and compressed bands allied isoelectrophocusing. Obtained electrophoregrams are reflected protein microgeterogeneity connected with different content of hydrophobic aminoacids. These spectrum are reflected variety differences of sample too.

771 PHOTOPERIOD CONTROL OF POPLAR BARK STORAGE PROTEIN mRNA ACCUMULATION. Gary D. Coleman, Tony H.H. Chen, Leslie H. Fuchigami. Department of Horticulture, 2042 Cordley Hall, Oregon State University, Corvallis, OR 97331

Bark storage proteins accumulate in the bark of many woody plants during autumn and winter. In poplar, the accumulation of the 32 kD bark storage protein is controlled by photoperiod. We have isolated a near full length cDNA encoding for the poplar 32 kD bark storage protein and determined its nucleotide sequence. The derived amino acid sequence shows that poplar bark storage protein is rich in serine, leucine and lysine and is a basic protein (pI 8.0). Poplar bark storage protein is similar to the poplar wound induced cDNAs clone 4 and clone 16 (Parson et al. 1989). DNA gel blot analysis suggests that poplar bark storage protein is encoded by a multigene family of about five genes. Poplar plants grown in long days contained low levels of mRNA for the bark storage protein. Exposure to short days results in an increase in bark storage protein mRNA within 7 days. After 21 days of short day exposure, high levels of mRNA were detected. The accumulation of bark storage protein mRNA in response to short days was also observed in plants exposed to natural shortening daylenths. Our results indicate that the accumulation of poplar bark storage protein mRNA is controlled by photoperiod. This finding will provide a useful system for investigating photoperiodism in woody plants.

772 THE PCR CLONING OF CANAVALIN AND CONCANAVALIN B FROM THE JACK BEAN PLANT *Canavalia ensiformis*. J.D. Ng, J.E. Setsuda, T.-P. Ko and A. McPherson. Department of Biochemistry, University of California, Riverside CA 92521

Canavalin and concanavalin B are two of the three dominant reserve proteins of the Jack Bean, *Canavalia ensiformis*. Canavalin, a trimeric molecule of Mr=150,000, typifies the classical vicilin storage proteins, and its detailed structure is now known by x-ray crystallography. Concanavalin B is a monomeric protein of Mr=31,500 whose structure is now similarly known by diffraction methods to be an αβ-barrel, a structural motif observed to be common to many enzymes. In pursuit of furthering our structure/function investigation of canavalin, the full length cDNA for canavalin was obtained by the method of the Polymerase Chain Reaction (PCR). The internal deduced amino acid sequence repeat observed in canavalin, as well as in other vicilin class reserve proteins, is reflected as a structural repeat of two homologous domains in the canavalin monomer. These repeats suggest that canavalin as well as other vicilin proteins may have derived from a single domain ancestral gene which has undergone internal duplication. The PCR cDNA of canavalin was further subcloned into a T7RNA polymerase expression vector and expressed in *E. coli*. A similar approach using PCR and conventional library screening has been used to obtain the nucleotide and its deduced amino acid sequence of concanavalin B. The canavalin and concanavalin B nucleotide and amino acid sequences reported here in conjunction with the proteins' three-dimensional x-ray structures allow us to identify appropriate regions of the molecules useful for site-specific mutagenesis. Ultimately these studies are directed toward the nutritional enhancement of important legume seed crops.

773 Role of the amino and carboxy terminal regions in the structure and folding of wheat high molecular weight glutenin subunits. Noam Shaul,* Jody D. Steffen-Campbell,** Olin D. Anderson,** Frank C. Greene** and Gad Galili*
*Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel, and **U.S.D.A. Western Regional Research Center 800 Buchanan street, Albany, California 94710. U.S.A.

Among the various cereal crops, the flour of bread wheat has the unique capability of forming leavened bread. This superiority is related mainly to a class of storage proteins called high molecular weight glutenin subunits (HMW-GS) which are assembled into large, disulfide bonded aggregates. Quality variations in bread making among wheat cultivars is correlated most clearly with allelic variation of HMW-GS genes, and especially those encoded by the D genome. Our laboratory is interested in the structure, folding and the assembly process of the HMW-GS. In order to study this, the bacterium *Escherichia coli* was utilized to synthesize proteins encoded by four naturally occuring wheat HMW-GS gene alleles of the *Glu-1D* locus, as well as from four bipartite chimeras of these genes. The *Escherichia coli* HMW-GS protein products exhibited identical SDS-PAGE migration and antigenic properties with those of the corresponding proteins extracted from wheat grains. Wild-type and chimeric HMW-GS migrate significantly slower in SDS-PAGE than expected on the basis of their molecular weights. We found that this is in part due to the influence of conformational properties on their N- and C-terminal regions. *In-vitro* renaturation and reoxidation of the HMW-GS showed that the N- and C-terminal regions also influence their folding and assembly processes.

The participation of different cysteine residues within the different HMW-GS in intramolecular or intermolecular disulfide bonds were found to be allele specific. This indicates that the assembly process of HMW-GS may utilize specific disulfide bond formation between different alleles. Our findings suggest that the structure and the specific assembly of HMW-GS may play an important role in the baking quality of wheat flour.

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774 STRUCTURAL ANALYSIS AND PROTEIN ENGINEERING OF PHASEOLIN, BEAN SEED STORAGE PROTEIN. John M. Dyer[1], Jeffrey W. Nelson[1] and Norimoto Murai[2]. Louisiana State University, [1]Department of Biochemistry and [2]Plant Path./Crop Physiol, Baton Rouge, LA 70803

For the eventual goal of genetically engineering the phaseolin protein to make French beans a more complete source of nutrition, we have developed biophysical and computer assisted methods that can be used effectively to probe the stability of wild type and mutated phaseolin proteins. Biophysical analysis was performed by using absorbance, circular dichroism, fluorescence emission, and fluorescence polarization anisotropy to monitor denaturation induced by urea, guanidinium chloride (Gdm.Cl), pH changes, high temperature, or a combination thereof. The results demonstrated that this protein is exceptionally stable. The protein remained folded in 6.0M urea or Gdm.Cl. Thermal denaturation curves of the protein dissolved in 6.0M Gdm.Cl exhibited a melting point at approximately 65°C when probed with absorbance, circular dichroism, and fluorescence polarization anisotropy. To assist in the design of engineered proteins, the complete three dimensional structure of phaseolin was constructed from the alpha carbon coordinates established by Lawrence *et al.* (*EMBO J.* 9, 9-15). Construction was performed on a VAX system using the CHARMM, SYBYL, and Macromodel molecular analysis packages. The native structure was used as a template for simulating modifications in the protein structure. In addition, molecular cloning techniques have recently been employed to begin the construction of phaseolin cDNAs that are enhanced in their methionine coding capacity. Regions of the protein targeted for mutagenesis include surface exposed regions, an N terminal hypervariable region, and several beta strands in each of the beta barrel structures.

777 STRUCTURE,EXPRESSION AND EVOLUTION OF THE GENES CODING FOR THE CRUCIFEREAE STORAGE PROTEINS (NAPINS AND CRUCIFERINS) Michel Delseny, Monique Ravnal, Dominique Depigny, Lorette Aspart and Françoise Grellet. Laboratory of Plant Physiology and Molecular Biology ,University of Perpignan, 66860 Perpignan , France.

cDNA clones coding for the 2S napins and the 12S cruciferins storage proteins have been isolated from a library made with immature radish seed mRNA. Sequencing of 3 napins and 6 cruciferin cDNA clones demonstrated that these proteins are encoded by small multigenic families. The napin family can be divided into 2 closely related subgroups with more than 90% homology. The cruciferin family is made of 2 divergent groups of sequences, with approximately 65% homology. One of the cruciferin family can be divided into 2 subgroups with 85% homology. Copy numbers have been found to be 6-8 for napins and 8-10 for cruciferins per haploid genome. Preliminary evidence in radish and other Brassica suggest that there are several distinct loci, each corresponding to a subfamily. The initial napin gene occured following two dupplications and a deletion event. The dupplicated motif corresponds to the extra N-terminal peptide and the small subunit. A radish cruciferin genomic clone has been sequenced. In contrast to the published cruciferin gene from rapessed, it contains 3 introns in positions similar to those observed in Arabidopsis genes. Comparisons of the sequences between radish , rapeseed and Arabidopsis indicate that the dupplication giving rise to the 2 major subfamilies for napin and cruciferin arose before the separation between these species . Expression studies indicate that the various cruciferin genes are coordinately expressed with one of the napin group, whereas the other napin genes are expressed slightly earlier

775 STORAGE PROTEIN GENE EXPRESSION IN ZYGOTIC AND SOMATIC EMBRYOS OF INTERIOR SPRUCE. Barry S. Flinn[1,2], Craig H. Newton[1], Dane R. Roberts[1] and Iain E.P. Taylor[2]. [1]Forest Biotechnology Centre, B.C. Research, 3650 Wesbrook Mall, Vancouver, B.C., V6S 2L2 and [2]Botany Department, University of British Columbia, Vancouver, B.C., Canada.

Storage protein accumulation during zygotic and somatic embryogenesis of interior spruce was followed by SDS-PAGE and immunoblotting. Both embryo types display similar developmental specific accumulation of storage proteins, although accumulation is more prolonged during the later stages of maturation in somatic embryos. Several full length cDNA's encoding members of a multigene albumin storage protein family have been identified and characterized. They are closely related and encode polypeptides 449-466 amino acids in length with predicted molecular weights of 48-51 kDa. Northern analysis gives a transcript size of approximately 1.8 kb. The amino acid sequences show similarity with vicilin-type and to a lesser degree, legumin-type storage proteins of angiosperms. The temporal pattern of transcript accumulation was compared between zygotic embryos and somatic embryos differentiated on 10 μM and 40 μM ABA. Osmotic effects on storage protein expression were also explored.

778 TRANSCRIPTIONAL REGULATION OF A CLASS II PATATIN GENE. Jan-Peter Nap, Annelies Loonen, Petra Grondhuis and Willem J. Stiekema. Department of Molecular Biology, Centre for Plant Breeding and Reproduction Research CPRO-DLO, Wageningen, The Netherlands. Patatin accounts for up to 40% of the total soluble protein in potato tubers. Assumed to be a storage protein, patatin in addition is characterized by lipid acyl hydrolase activity. The protein is coded for by a large gene family, several members of which have been studied in considerable detail. The patatin genes have been divided into two classes, which differ in promoter structure and pattern of expression. Whereas class I gene expression contributes the bulk of tuber patatin, patatin class II genes are expressed to a much lower extent in both tubers and roots. We have isolated and characterized a member of the patatin class II gene family. Introduction into potato of a transcriptional fusion between the promoter sequence and GUS, by means of A. tumefaciens, yielded transgenic potato plants in which GUS activity was restricted to the tuber periderm, and the root and petiole vascular system. The transcriptional regulation of the patatin class II promoter is studied by introduction into potato of various 5'-deletions of the promoter fused to GUS. Promoter-directed GUS activity in transgenic potato will be reported. In addition, to gain insight in the physiological role of the highly localized patatin class II gene expression, experiments to identify biological and environmental parameters that influence patatin class II gene expression will be presented.

776 STUDY OF A MUTATED DIHYDRODIPICOLINATE SYNTHASE GENE LEADING TO A LYSINE OVERPRODUCTION IN *NICOTIANA SYLVESTRIS* RAEC-1. Marc Ghislain, Valerie Frankard, Marc Vauterin and Michel Jacobs. Laboratorium voor Plantengenetica, University of Brussels, B-1640 Sint-Genesius-Rode.

The strong inhibition of dihydrodipicolinate synthase (DHDPS) by lysine, the end-product of the diaminopimelate (DAP) pathway, is released in a previously isolated mutant RAEC-1, with as a consequence a higher lysine content (1). Such a modified gene would be a valuable tool to improve the nutritional value of crop plants, in particular cereals.

We have developed different studies :

1. the purification and characterization of the enzyme;
2. the expression of free lysine production in different organs and during the plant cycle;
3. ways to clone and to characterize the mutated allele dhdps-r1;
4. the cloning of dhdps as well as other genes of the DAP pathway through a functional genetic complementation of E. coli mutants.

These results will be discussed in the context of the genetic regulation of aspartate-derived amino acid biosynthesis in plants.

(1) Negrutiu I. *et al.*(1984) TAG 68 : 11-20.

779 TEMPORAL AND SPATIAL REGULATORY ELEMENTS IN THE -783 5' UPSTREAM REGION OF THE β-PHASEOLIN GENE OF *Phaseolus vulgaris*. Partha Sen, Mark D. Burow, Caryl C. Chlan and N. Murai Dep. Plant Path./Crop Physiol., Louisiana State University, Baton Rouge, LA 70803.

Six deletion mutants of the 783 bp 5'-upstream sequence were generated to identify the *cis*-regulatory elements responsible for developmental control of the β-phaseolin gene of *Phaseolus vulgaris*. These were ligated to the phaseolin coding and 3'-downstream sequences were used to transform tobacco. Analysis of phaseolin mRNA and protein in transgenic tobacco seeds indicated the existence of two positive regulatory elements in the -227 to -294 and -14 to -64 regions relative to the transcription initiation site. A weak positive and a negative element are present in the -425 to -783 and -296 to -424 region respectively. Temporal regulation of mRNA expression was maintained until -14 to -64 segment was deleted. However, deletion of the -106 to -294 segment changed the pattern of expression, so that maximum phaseolin mRNA occurs the second week after pollination instead of the third week as has been evidenced in the preceding deletions. A finer study with similar phaseolin promoter deletions fused to GUS reporter gene indicated that construct -783 showed maximum GUS activity on the 19th day after pollination (DAP) whereas in constructs -226 through -64 GUS activity was maximum on 15th DAP. Analyses of vegetative tissues samples of different constructs revealed that there is a silencer element upstream of -105 which suppresses phaseolin expression in shoot and root and that the -105 to -65 segment permits expression of the gene in shoot and root in equal amounts as in the seeds of the same construct. The results indicate that two temporal and at least three spatial regulatory elements are required for developmental control of the β-phaseolin gene.

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780 **TRANSGENIC RICE ANALYSES INDICATED THE EXISTENCE OF TWO QUANTITATIVE REGULATORY ELEMENTS IN THE 5'-UPSTREAM REGION OF THE STORAGE PROTEIN GLUTELIN Gt1 GENE.** N. Murai[1], Y. Kawagoe[1], S. Xiao[1], Z. Li[1] and T.W. Okita[2], [1]Dep. Plant Path./Crop Physiol., Louisiana State University, Baton Rouge, LA 70803 and [2]Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

To avoid the difficulties encountered in heterologous dicot transformation system, we developed a homologous system to generate transgenic rice plants and used this system to identify *cis*-regulatory elements responsible for developmental control of rice seed storage protein glutelin. We constructed a series of eleven 5'-deletion mutations of the Gt1 upstream regions, containing -8, -48, -101, -154, -213, -287, -399, -508, -1,800 and -5,100bp relative to the transcription initiation site. The deletion mutants were tanscriptionally fused to the GUS coding and *nos* terminator. Gt1/GUS fusion genes were used to transform homologous rice protoplast. Up to 30 transgenic plants were regenerated per construct and grown to maturity in greenhouse. Spatial and temporal regulation of the Gt1 expression were determined by use of the GUS assay and GUS mRNA levels in developing seeds and vegetative organs of mature transgenic plants. The results indicated the existence of two quantitative regulatory elements, one in the -508/ -1800 region and another in the -1800/ -5100 region. These two elements together increased by 100 fold the GUS expression in developing seeds. Both spatial and temporal control were maintained in -154 constructs, indicating spatial and temporal regulatory elements resides 3'-downstream of -154. However, one of -154 plants lost spatial regulation and the GUS activity was found in phloem of leaf, stem and roots. Thus, there appear to be a silencer element 5'-upstream of -154 that repress the GUS expression in vegetative organs.

781 **THE THREE-DIMENSIONAL STRUCTURES OF RESERVE PROTEINS FROM THE JACK BEAN.** T.-P. Ko, J. Ng, M. Greene, and A. McPherson Dept. of Biochemistry, University of California, Riverside, CA 92521

Canavalin, concanavalin A, and concanavalin B are the proteins of highest abundance in the seeds of the Jack Bean (*Canavalia ensiformis*). We have determined the three-dimensional structure of canavalin, a 150,000 Mr protein, in four different crystal forms, $P6_3$, R3, $C222_1$, and P23, using X-ray diffraction methods. Again using X-ray crystallography, we have determined the fold of the polypeptide chain of concanavalin B, a protein of Mr = 31,500. Canavalin is a trimer assembled from identical protein subunits about a perfect threefold axis of symmetry. Each subunit is in turn composed of two structurally homologous domains, consistent with an internally repeating gene sequence. Each domain consists of an eight-stranded β-barrel which exhibit the "Swiss Roll" topology. The disposition of these domains about the triad axis yields in appearance an aggregate quite similar to that of the coat protein in intact satellite tobacco necrosis virus (STNV). Four trimers can further assemble at low pH in a tetrahedral arrangement to form a duodecamer. This duodecamer comprises the contents of the cubic crystal unit cell. Concanavalin B on the other hand is an αβ barrel like that first seen in triose phosphate isomerase and subsequently in a number of other proteins. The known atomic structure of canavalin, concanavalin A and concanavalin B in conjunction with the cloned genes provide us with a unique and powerful system for the genetic enhancement of the nutritional properties of this important class of dietary proteins.

782 **UPSTREAM REGULATORY MOTIFS, CYTOSINE METHYLATION AND THE CONTROL OF RIBOSOMAL RNA GENE EXPRESSION IN CEREALS.** Richard B. Flavell, Stephen Jackson, Michael O'Dell and Ravinda Sardana, John Innes Institute, AFRC Institute of Plant Science Research, John Innes Centre, Norwich, UK.

The ribosomal RNA gene family is very large in cereals and not all genes are used in most cells. The fraction of genes used lies within the nucleolus. There is variation in regulatory DNA within the gene family but relative homogeneity in gene structure within a locus of reiterated genes. The variation affects the number of reiterated motifs upstream from the start of transcription. Loci with genes which have more such motifs are preferentially active over loci with genes which have fewer such motifs. These motifs bind a protein in nuclear extracts that also binds to a sequence close to where transcription is initiated. Some of the upstream motifs have unmethylated cytosine residues in active loci but not in inactive loci. A model has been proposed to explain the selection of subsets of active genes by the binding of a regulatory protein in limiting concentration to the upstream regulatory motif of the rRNA genes. The model predicts that changes in the number or the sequence of the upstream motifs influences the relative activity of an rRNA gene in competition with other rRNA genes. It also proposes that the inheritance of the subset of active genes from one cell generation to the next is determined by the cytosine methylation pattern.

783 **THE 94 KD VEGETATIVE STORAGE PROTEIN IN SOYBEAN LEAVES IS A LIPOXYGENASE.** Timothy J. Tranbarger, Vincent R. Franceschi, David F. Hildebrand, and Howard D. Grimes. Department of Botany, Washington State University, Pullman, WA 99164-4238. Department of Agronomy, Univ. of Kentucky, Lexington, KY 40546-0091.

To better understand its role in nitrogen storage and metabolism, we are examining the cell and molecular biology of the 94 kD vegetative storage protein (vsp94) in soybean leaves. The vegetative storage proteins (VSPs), including vsp94, are strongly induced by removing the sink tissue by depodding. Vsp94 was isolated and microsequenced using cyanogen bromide-derived peptide fragments. Useful sequence data was obtained from four peptides ranging in size from 8 to 20 amino acids. All four of these peptide fragments exhibited a high degree of homology to the soybean seed lipoxygenases (LOXs). To further confirm that vsp94 is a LOX, immunoblot assays were performed with polyclonal antibodies against soybean leaf LOXs. Results indicated that these LOX antibodies cross react with vsp94 specifically. Immunolocalization experiments demonstrate that a specific accumulation of vsp94 occurs in the paraveinal mesophyll layer. The other VSPs are also localized in this tissue. Furthermore, it is evident that this LOX is a vacuole-associated LOX. Protein extracts from depodded soybean (sink tissue removed) leaves have been assayed for LOX activity and there is a substantial increase in total activity upon depodding. Isoelectric focusing and "in gel" LOX activity staining indicates that there is at least one unique spot (with an acidic pI) appearing in the leaves after depoding. For these reasons, we now refer to vsp94 as the paraveinal mesophyll LOX. Current experiments are examining the molecular regulation of this unique LOX with respect to nitrogen supply and partitioning.

784 **AUXIN SENSITIVITY OF CYTOKININ HABITUATED TOBACCO CELL LINES.** Andrew N Binns[1], Rita A. Teutonico[1], and Robert C. Black[2] 1)Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018 and 2) Department of Biology, Pennsylvania State University, Media, PA 19063

Cytokinin habituated cell lines of tobacco are characterized by their ability to grow continuously on medium containing only an auxin, such as NAA. This phenomenon is likely to be the result of epigenetic change in the cell since it can be induced and reversed at high rates, and leaves the cells totipotent. The physiological basis of cytokinin habituation is unknown. In some cases the cytokinin habituated phenotype is not associated with an increase in cytokinin accumulation. Analysis of several habituated clones showed, however, that they accumulate newly identified cell division promoting substances, the dehydrodiconiferyl alcohol glucosides (DCGs) to levels seen in cytokinin requiring lines only when they are grown in the presence of cytokinin. This suggests that the habituated phenotype is the result of a 'coesstutive' activation of biosynthetic pathways that normally require cytokinin. Further, we examined the hormonal requirements for shoot initiation in these cell lines and found that they required identical cytokinin doses as cytokinin requiring cell lines. Interestingly, the cytokinin habituated cell lines were significantly more sensitive to the inhibitory influence of NAA on shoot initiation then were cytokinin requiring sibling clones. These results will be discussed in terms of a model of cytokinin habituation in which the primary change involves the conversion of the cell to a state of increased auxin sensitivity.

785 **SOMATIC EMBRYOGENESIS AS A DEVELOPMENTAL SYSTEM TO STUDY MODULATION OF AUXIN-BINDING CAPACITY.** Fiorella LoSchiavo, F. Filippini, D. Vallone and M. Terzi. University of Naples, Dept. Genetics, Via Mezzocannone 8, 80134 Napoli, Italy.

Embryogenic carrot cell lines follow variations in exogenous auxin concentration by modulating their auxin binding capacity. This capacity to modulate is a "sine qua non" for the formation of the earliest detectable embryos (Pro-Embryogenic Masses, PEM) but these PEM, once formed, become virtually insensitive to auxin. Sensitivity to auxin is regained at late embryonal stages. Cell variants were isolated that were altered in embryogenesis and altered in modulation. Those lines, that can be considered auxin-resistant as they formed colonies at 2,4-D concentrations as high as 0.1 mM, needed an auxin concentration 20-fold higher than wild type in order to reach an auxin-binding protein level such as the one needed for embryogenesis (26 pmol IAA/50 ug membrane protein).

In some of these lines the level of auxin-binding protein has been studied in relation with two auxin-induced effects: auxin-induced transcription and auxin-induced DNA methylation. The positive correlation found indicates that the auxin binding proteins have the physiological role of true receptors.

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786 THE INFLUENCE OF CALCIUM ANTAGONISTS ON IAA-INDUCED ADVENTITIOUS ROOT FORMATION IN HYPOCOTYL CUTTINGS OF PHASEOLUS VULGARIS L. Raj K.Kakkar and Vinay K.Rai Himachal Pradesh University, Department of Bio-sciences, Summer Hill,Shimla-171005, INDIA

Exogenously applied $CaCl_2$, $Ca(NO_3)_2$ and $CaCO_3$(10^{-6} to 10^{-3}M each) alone and with indole-3-acetic acid (IAA, 10^{-5}M) variably affected the adventitious root formation in hypocotyl cuttings of Phaseolus vulgaris L. cv. Kentucky Wonder, whereas Ca^{2+} chelator, EGTA and channel blockers, $LaCl_3$ and nifedipine (10^{-6} to 10^{-3}M each) significantly inhibited root formation and total root length over control. Also, when Ca^{2+} chelators and channel blockers (10^{-6} to 10^{-3}M) were applied along with IAA(10^{-5}M), significantly reduced IAA-induced adventitious root formation and total root length. EGTA or $LaCl_3$ (10^{-3}M) alone and with IAA(10^{-5}M) completely abolished root formation. The results will be discussed in relation to the role of calcium as a second messenger in regulating IAA-induced effects.

787 THE EFFECTS OF POLYAMINES ON CHLOROPHYLL LOSS Raj K.Kakkar and Vinay K.Rai, Himachal Pradesh University, Department of Bio-sciences, Summer Hill, Shimla-171005, INDIA

The present investigation was undertaken to study the effect of tetramine, spermine; triamine, spermidine and diamine putrescine, cadaverine and agmatine and phytochormone kinetin (10^{-6} to 10^{-4}M each) on chlorophyll retention in leaf discs of Phaseolus vulgaris L. cv. Kentucky Wonder after six days of excision. Polyamines including diamines and kinetin declined chlorophyll loss as compared to water (control) treated discs being maximum in spermine and putrescine (10^{-4} & 10^{-5}M each). However, decline was retarded by polyamine biosynthetic inhibitor viz. α -difluoro methyl arginine (αG-DFMA), α -difluoro methyl ornithine (αC-DFMO), irreversible inhibitors of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) and methyl glyoxal bis-guanyl hydrazone (MGBG) inhibited reversibly the S-adenosyl methionine decarboxylase (SAMDC) (10^{-6} to 10^{-4}M each) except αC-DFMO, which did not show any effect on chlorophyll retention. Polyamines and diamines (10^{-6} to 10^{-4}M) when applied together with αG-DFMA or MGBG (10^{-5}M) promoted the decline of chlorophyll loss. The findings will be discussed in relation to antisenescence properties of polyamines by studying their effect on ribonuclease and protease which further support them as new class of plant growth regulator.

788 GIBBERELLIN-REGULATED INCREASES IN SPECIFIC mRNAs IN THE DEVELOPING SHOOTS OF PISUM SATIVUM L. Norman S. Cohn, Chen-Zhao Jian, John P. Mitchell and Liu-Lai Wu. Ohio University, Department of Botany, Athens, Ohio 45701.

Changes in gene expression result following the application of exogenous gibberellin (GA) to dwarf peas, as demonstrated by the appearance of a small population of mRNAs not found in untreated plants. We have selected three clones by differential screening of a cDNA library (prepared with lambda gt10) derived from mRNA extracted from dwarf peas four hours after application of GA. The three clones hybridize with mRNAs having nucleotide lengths of about 900, 1100 and 1800 bases. The clones have been sequenced and further characterized. Genomic southern blot hybridization analyses suggest that, in each case, the clone is a member of a very small gene family. Northern blot hybridizations have demonstrated that the genes represented by these clones are expressed in both dwarf and wild-type peas. However, different members of the putative gene families are specifically expressed at different levels. A study of RNA kinetics over a period of from 15 min. to 48 hours after GA application was followed utilizing riboprobe analysis in Northern blotting for all three clones. The results point to a small array of genes that are differentially expressed over time following stimulation by GA. Moreover, comparisons of gene expression among the GA-treated and untreated dwarf and wild-type peas present interesting patterns of differences that reinforce the initial observation of differences in levels of expression between dwarf and wild-type peas in their responses to GA.

789 DIFFERENTIAL EXPRESSION OF TWO ACC-SYNTHASE GENES IN TOMATO FRUITS. David C. Olson[1], Joseph A. White[1], Leonard Edelman[1], Richard N. Harkins[2], and Hans Kende[1]. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824[1] and Berlex Biosciences, Alameda, CA 94501[2]

1-Aminocyclopropane-1-carboxylate(ACC) synthase is the regulated enzyme in the biosynthetic pathway of ethylene. A full-length cDNA encoding one form of this enzyme (ACC-synthase 1) has been cloned from tomato fruits by Van Der Straeten *et al.* [Proc. Natl. Acad. Sci. USA (1990) 87, 4859-4863]. We have cloned a cDNA encoding a second form of ACC synthase from tomato fruits (ACC-synthase 2) and have established its complete nucleotide and derived amino acid sequence. The cDNAs coding for both isoforms contain highly conserved regions that are surrounded by regions of low homology, especially at the 5' and 3' ends. Gene-specific probes were constructed to examine the expression of transcripts encoding the two ACC-synthase isoforms under two conditions of enhanced ethylene formation, namely during fruit ripening and in response to mechanical stress (wounding). The level of mRNA encoding both isoforms, ACC-synthase 1 and 2, increased during ripening. In contrast, wound stress caused only an increase in the level of mRNA coding for ACC-synthase 1. The effect of wounding on the accumulation of ACC-synthase 1 mRNA was evident within 40 min. Blot analysis of genomic DNA digested with restriction enzymes confirmed that ACC-synthase 1 and 2 are encoded by different genes.

790 METABOLIC AND HORMONAL REGULATION OF SOYBEAN VEGETATIVE STORAGE PROTEIN GENE EXPRESSION. Hugh S. Mason, Daryll B. DeWald and John E. Mullet. Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843.

Soybean vegetative storage protein (VSP) is a vacuolar glycoprotein which occurs as dimers of nonidentical subunits α and β, encoded by the genes *vspA* and *vspB*. The expression of *vsp* genes is high in growing shoot tissues, is modulated developmentally in response to sink demand, and is inducible in mature tissues by wounding and water deficit. We have investigated the roles of sugars and the putative plant hormone methyl jasmonate (JA-Me) in the regulation of *vsp* gene expression in soybean and transgenic tobacco. Accumulation of *vspA* and *vspB* mRNAs was induced in excised mature soybean leaves when treated in the dark with 0.2 M sucrose and 10^{-5} M JA-Me, but not by either compound alone. Glucose and fructose could substitute efficiently for sucrose in this induction. The time courses of *vsp* mRNA induction in excised leaves treated with sucrose/JA-Me and in wounded leaves were similar. However, the induction of *vsp* mRNAs in wounded leaves was strongly light dependent, suggesting a need for photoassimilate. A genomic clone of the *vspB* gene was obtained and a fragment of the putative promoter region, comprising nucleotides -787 to +46 relative to the transcription start site, was fused to the coding region of β-glucuronidase (GUS). Transgenic tobacco plants harboring this construct showed greatly increased GUS activity when excised leaves were treated with 0.2 M sucrose or glucose (but not mannitol) and 10^{-5} M JA-Me. These data indicate that the *vsp* genes are responsive to both metabolic and hormonal signals, which may be important in the regulation of sink activity as well. In order to delineate promoter sequences which are important in these responses, we have generated a series of 5' and 3' deletions of the *vspB* promoter and transformed tobacco with the GUS fusion constructs. Data from these experiments will be presented and the roles of sugars and JA-Me in the regulation of sink activity will be discussed.

791 EXPRESSION OF A *PSEUDOMONAS* ACC DEAMINASE GENE IN PETUNIA FOR REGULATION OF ETHYLENE BIOSYNTHESIS. Raymond E. Sheehy, Belinda Martineau, William R. Hiatt. Calgene, Inc., 1920 Fifth St., Davis, CA 95616.

Pseudomonas sp. ACP contains an inducible 1-aminocyclopropane-1-carboxylate (ACC) deaminase which allows the organism to grow on ACC as a nitrogen source by producing ammonia and α-ketobutyrate[1]. ACC is the immediate precursor of ethylene in the biosynthetic pathway of this hormone in higher plants. Ethylene regulates a variety of growth and developmental processes, in particular senescence and fruit ripening. We have isolated the gene encoding ACC deaminase and chimeric gene constructs utilizing an enhanced 35S promoter have been introduced into petunia. Expression of the ACC deaminase gene in petunia will be described, as will the effects of introducing an alternate pathway which shunts ACC away from ethylene biosynthesis.

1. M. Honma and T. Shimomura. 1978. Agric. Biol. Chem., 42, 1825-1831.

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792 A MUTANT OF *ARABIDOPSIS* RESISTANT TO ALPHA-METHYL TRYPTOPHAN. Joel A. Kreps and Christopher D. Town. Biology Department, Case Western Reserve University, Cleveland, OH 44106, U.S.A.

In both microorganisms and plant tissue culture, mutants resistant to 5-methyl tryptophan (5-MT) frequently have elevated levels of tryptophan and are sometimes auxin autotrophic in culture. Mutants of *Arabidopsis thaliana* have been selected for resistance to growth inhibition at the seedling stage by alpha-methyl tryptophan (aMT). One mutant, *amt1* has been characterized in detail. The appearance and growth rate of the mutant in the absence of the inhibitor are indistinguishable from wild-type, both as plants and callus. However growth of mutant plants is unaffected by 25 uM aMT and mutant callus by 50 uM aMT, concentrations which completely inhibit the growth of wild-type tissues. The mutant shows cross-resistance to 5-MT but is sensitive to 5-methyl anthranilic acid. Tryptophan levels in mutant and wild-type plants are 24.3 $\pm$ 2.7 and 4.7 $\pm$ 1.2 ug/g fresh weight respectively and in the corresponding callus 64.0 $\pm$ 2.6 and 31.8 $\pm$ 8.4 ug/g fresh weight respectively. Anthranilate synthase (AS) activity is approximately two-fold higher in crude extracts of mutant plants or callus than in wild-type. Preliminary experiments indicate that AS from *amt1* is also less sensitive than wild-type AS to feedback inhibition by tryptophan. Genetic analysis indicates that *amt1* segregates as a single nuclear mutation in *amt1* and shows incomplete dominance. Experiments are in progress to map the mutant and to further characterize the altered AS activity in *amt1*.

793 DIFFERENTIAL EXPRESSION OF AUXIN- AND WOUND-INDUCED 1-AMINO-CYCLOPROPANE-1-CARBOXYLATE SYNTHASE GENE. Naoki Nakagawa, Hitoshi Mori, Ken'ichi Yamazaki and Hidemasa Imaseki. School of Agricultural Sciences, Nagoya University, Nagoya, 464-01 Japan (NN, KY, HI) and National Institute for Basic Biology, Okazaki, 444 Japan (HM, HI).

1-Aminocyclopropane-1-carboxylate (ACC) synthase regulates the rate of ethylene biosynthesis in higher plants, and its endogenous activity greatly increases when auxin is supplied to vegetative tissues, when tissues are wounded and when climacteric fruits ripen. Wound-induced (W-type) ACC synthase is immunochemically different from the auxin-induced (A-type) enzyme, indicating that A-type and W-type enzymes may be coded in two different genes. A cDNA for W-type enzyme has been cloned from wounded fruits of Cucurbita maxima, but did not hybridize to mRNA of A-type enzyme. A-type enzyme was purified from auxin-treated slices of immature cucumber fruits, and its partial amino acid sequence was determined. Oligonucleotides synthesized based on the sequence were used as probe to screen a cDNA library constructed in gt10 for mRNA from auxin-treated hypocotyls of C. maxima. Three clones were isolated out of $5X10^4$ plaques, but they were identical. The cDNA subcloned into pUC119 expressed active enzyme in E. coli. The nucleotide sequence of A-type cDNA was significantly different from that of W-type cDNA, and both cDNAs did not cross-hybridize. A-type gene was expressed by auxin but not by wounding, whereas W-type gene was expressed by wounding but only slightly by auxin. A-type and W-type enzymes also were chromatographically separated. The results unequivocally indicate that A-type and W-type ACC synthase are coded in different genes and the two genes are expressed differentially by different stimuli.

794 DIFFERENTIAL GENE EXPRESSION IN RADIATION-INDUCED TUMORS OF *ARABIDOPSIS*. Bruce R. Campell, Young-ji Sun and Christopher D. Town, Biology Department, Case Western Reserve University, Cleveland, OH. 44106.

We are interested in the control of plant growth and development, and the role of auxin and cytokinin in these processes. We have used ionizing radiation to induce tumors on *Arabidopsis thaliana*, and from the primary tumors have established a number of tissue lines in culture. These cultured tumor lines are hormone autonomous and present a variety of shooty, rooty and undifferentiated phenotypes. They also differ from one another in their hormone content and in their response to exogenous hormones. Some tumor tissue lines have higher levels of free or conjugated auxin or cytokinin, while others do not. Some lines show the same growth response as normal callus when hormones are supplied in the growth medium, while others have increased or decreased sensitivity to hormones. In order to investigate the nature of the hormone autonomy at the molecular level, we have used subtraction hybridization to construct cDNA libraries enriched for sequences expressed in hormone autonomous tumor lines but not in hormone dependent callus. Screening of this library has yielded a number of "tumor-specific" cDNA clones. Results on the sequence analysis of these clones and their pattern of expression in other tumors and various plant organs will be presented.

795 **OSMOTIN-LIKE PROTEIN: STRUCTURE AND REGULATION BY ETHYLENE.** Fumihiko Sato, Satomi Takeda*, Soichi Okabe, Hisashi Koiwa, Takashi Hosoe, Nozomu Koizumi, Kazuko Ida* and Yasuyuki Yamada. Kyoto University, Dep. of Agricultural Chemistry, Kyoto 606-01 Japan. * Osaka Women's University, Sakai 590, Japan

Osmotin-like protein (OLP), having amino terminal amino acid sequence homologous to osmotin, accumulate with osmotin to substantial levels in cultured tobacco cells. To learn more about the regulatory mechanism(s) of gene expression of osmotin and osmotin-like protein and to clarify their functions in the stress response, we determined the primary structure of OLP and characterized the expression. The primary structure of an OLP was deduced from the nucleotide sequence of a cDNA clone made from mRNA isolated from cultured tobacco cells. The amino acid sequence indicated that OLP is synthesized as 29kD polypeptide with a hydrophobic leader peptide of 21 amino acids, and then processed to a 26kD polypeptide. The sequence analysis also suggested that OLP posessed a putative glycosylation site as well as Proline-Glutamate-Serine-Threonine rich sequence, neither of which were found in osmotin or tobacco pathogenesis-related protein (PR-S), although the amino acid sequence of OLP is 76% and 60% identical to the sequences of these other proteins, respectively. OLP mRNA was found to accumulate to high levels when cells approach stationary stage. The expression of OLP was suppressed when cells were treated with the ethylene biosynthesis-inhibitor Co(NO3)2, but this suppression was overcome by the injection of ethylene. Another plant hormone, abscisic acid had no effect on the expression of OLP. These results and previous findings indicated that ethylene is a potent regulator of gene expression in cultured cells.

796 ROLE OF CELLULASE IN THE ABSCISSION OF PEACH LEAVES. C. Bonghi*, N. Rascio*, A. Ramina* & G. Casadoro*. *) Inst. of Pomology, *) Dept of Biology, University of Padua, Padua, Italy.

Abscission is the controlled process of loss of whole parts of a plant. Leaf fall is the most common example of abscission, even though fruits as well as unfertilised flowers can be shed. The loss of these structures takes usually place within particular zones named abscission zones. The process of abscission appears to be under the control of plant hormones, being ethylene the major promoting agent. In our experiments abscission was activated in peach (Prunus persica L. Batsch) leaf explants by flushing them with exogenous ethylene. The enzyme cellulase (1,4-B-D glucan 4-glucanohydrolase E.C.3.2.1.4.) appears to be particularly important for the production of the cell wall damages which precede the leaf abscission. Two isoforms with pI of about 6.5 and 9.5 respectively are present in peach. While the acidic form is the only isoform detectable in control abscission zones, the basic isoform appears after the treatment with ethylene. Northern blot analyses of poly A^+ RNA from both control and ethylene activated abscission zones show hybridization bands with mRNAs of 2.2 and 1.9 kb respectively. A cDNA library, constructed using poly A^+ RNA from ethylene activated abscission zones, was screened with a cDNA probe coding for an avocado cellulase. We are now characterizing the positive clones.

797 MOLECULAR ANALYSIS OF BRASSINOLIDE ACTION IN PLANT GROWTH AND DEVELOPMENT. S.D. Clouse, D. Zurek, A. Hall, Department of Biology, San Diego State University, San Diego, CA 92182

Brassinolide, a plant steroid lactone, can substitute for auxin in a soybean epicotyl elongation assay and an *Arabidopsis thaliana* peduncle elongation assay. We have shown by two dimensional gel analysis of in vitro translated mRNA, that sub-micromolar concentrations of brassinolide alter the pattern of gene expression in elongating soybean and Arabidopsis stem sections, both in the presence or absence of added auxin. Northern blot analysis, using cDNAs corresponding to auxin-regulated genes as probes, has shown that the molecular mechanism of brassinolide-induced elongation is likely to differ from that of auxin-induced elongation. We have also found that nanomolar concentrations of brassinolide can enhance xylem differentiation in cultured explants of Jerusalem artichoke tubers. These molecular data provide further evidence that brassinolide is a true plant growth regulator. The use of subtractive hybridization and differential cDNA library screening to isolate genes regulated by brassinolide in soybean and Arabidopsis will be described.

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798 ISOLATION AND SEQUENCE OF A cDNA CLONE RELATED TO ETHYLENE BIOSYNTHESIS IN BRASSICA JUNCEA. Eng-Chong Pua, Guek-Eng Sim and Mee-Len Chye. Institute of Molecular and Cell Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Republic of Singapore.

Cell recalcitrance with respect to de novo shoot organogenesis from cultured explants of Brassica juncea has been investigated. Study on the effect of ethylene inhibitors, $AgNO_3$ and aminoethoxyvinylglycine, indicated that in vitro shoot regeneration from hypocotyls and leaf discs of B. juncea may be regulated by ethylene. Our current efforts have focussed on isolation of genes responsible for regulation of ethylene biosynthesis in recalcitrant Brassica. We have isolated a 1.3 kb cDNA clone, MU5, from a leaf cDNA library of B. juncea using a tomato cDNA clone, TOM13 (Holdsworth et al., 1987, Nucleic Acids Res. 15: 731-739), as a heterologous hybridization probe. Northern blot analysis showed that both MU5 and TOM13 hybridized to the same 1.8 kb transcript of B. juncea. In DNA sequence analysis, MU5 was 69% homologous to the TOM13 in nucleotide sequences and 56% in deduced amino acid sequences. Presently, work on characterization and construction of antisense gene of MU5 is in progress.

799 CLONING OF cDNAs ENCODING RIPENING & WOUNDING TYPE ACC SYNTHASE. Hitoshi Mori and Hidemasa Imaseki. National Institute for Basic Biology, Okazaki, 444 Japan.

ACC synthase is the key enzyme in ethylene biosynthesis and induced by auxin, tissue wounding and fruit ripening. We have shown that auxin- and wound-induced enzymes (A- and W-type, respectively) are products of different genes in winter squash. Now, a question arises, "Is the ripening-associated enzyme (R-type) different from others?" To answer this question, we cloned W- and R-type cDNA from tomato fruits. For W-type cDNAs, a cDNA library prepared from wounded tomato fruits was screened by the nucleotide sequence of the pyridoxal phosphate (PLP) binding site of winter squash W-type cDNA. Whereas R-type cDNA clones were isolated from another library prepared from ripening (non-wounded) tomato fruits by tomato W-type cDNA as probe. cDNA clones of both type expressed active enzyme in E. coli. The similarity between W- and R-type cDNA is 75% in the nucleotide and 77% in the amino acid sequence. The PLP binding site of tomato W-type enzyme is SLSKDMGLPGFRVG, same as that of winter squash W-type enzyme, however, that of R-type is SLSKDLGFPGFRVG that differs at two amino acids. Northern blot analysis indicated that W-type mRNA appeared to some extent during ripening, but increased 20 to 50-folds after wounding. By contrast, R-type mRNA appeared only during ripening, but rather decreased after wounding. We thus conclude that in tomato, genes for R- and W-type ACC synthase are different and are differentially expressed. As a similar relation also is found between auxin- and wound- induced ACC synthase, it is highly likely that tomato has three different genes for this enzyme; auxin-, ripening- and wound-inducible genes.

800 ROSETTE SENESCENCE IN *ARABIDOPSIS THALIANA*. Linda Hensel. David Baumgarten. Vojislava Grbic. and Tony Bleecker. University of Wisconsin. Department of Botany. Madison. Wisconsin 53706.

We are studying the developmental regulation of rosette leaf senescence in *Arabidopsis thaliana*. We compared the senescence pattern of "wild-type" lines to both known and newly obtained mutant lines. Bolting, anthesis, and individual leaf senescence were measured for individual plants. The timing of leaf senescence appears to be dependent on an interaction between the age of the leaf and the developmental state of the plant. Influences of specific hormones and flowering time are being studied using known single gene mutants and newly isolated EMS and T-tagged lines which show alterations in the timing of senescence. We isolated two types of lines: those with early rosette senescence and those with late rosette senescence. Individual leaf mortality curves show that the rate of senescence, initial signs of yellowing to fully brown, is altered in some cases; in other cases, the physiological timing of senescence is altered.

801 INDUCTION OF ACC SYNTHASE TRANSCRIPT BY AUXIN IN MUNGBEAN HYPOCOTYLS AND CULTURED APPLE SHOOTS. Woo Taek Kim, Wing-Kin Yip, Jian-Guo Dong, Aron Silverstone & S.F. Yang. Univ. of Calif., Veg. Crops Dept., Davis, CA 95616.

The plant hormone ethylene is synthesized in response to various stimuli, including auxin application, resulting in increased synthesis of ACC synthase. Employing the polymerase chain reaction (PCR) we obtained and sequenced a 290 bp and a 328 bp cDNA fragments encoding the active site domain of ACC synthase from auxin-treated cultured apple shoots and mungbean hypocotyls, respectively. In this PCR-based amplification we used RNAs as templates and mixed oligonucleotides as primers which corresponded to the two regions of ACC synthase proteins where their amino acid sequences (SNPLGTT and MSSFGLV) are conserved in apple, tomato and winter squash fruits. The deduced amino acid sequences of this region from both tissues show 51% to 70% homology to apple, tomato and winter squash enzymes. To study the temporal induction pattern of ACC synthase gene, poly(A)+RNAs were isolated from mungbean hypocotyls treated with IAA for various periods and analyzed by Northern blotting using the PCR fragment as a probe. ACC synthase mRNA was not detectable before treatment, and reached a maximum level at 2 hours and then declined during subsequent treatments. These results indicate that auxin induces ethylene production by increasing the ACC synthase mRNA level. Using similar PCR techniques, we have also obtained and sequenced cDNA fragments derived from tomato cell cultures and senescent petunia flowers. These results indicate that ACC synthases from various species and various organs exist in multiforms but share highly homologous regions.

802 CHARACTERIZATION AND PARTIAL PURIFICATION OF ETHYLENE FORMING ENZYME FROM AVOCADO FRUIT. Douglas J. McGarvey & Rolf E. Christoffersen, Biological Sciences Dept., University of California, Santa Barbara, CA 93106

Biosynthesis of ethylene in higher plants proceeds via the following pathway: SAM --> ACC --> ethylene. Ethylene forming enzyme (EFE), the enzyme responsible for conversion of ACC to ethylene, has never been fully characterized *in vitro*. We have obtained authentic EFE activity *in vitro* from extracts of avocado fruit (*Persea americana* Mill. cv Hass). This activity requires Fe^{2+} and ascorbic acid for activity. The enzyme has a low Km (50 μM) for ACC and discriminates between the two diastereomers of 1-amino-2-ethylcyclopropane-1-carboxylic acid, thus confirming its identity as authentic EFE. Activity is retained in a 100,000*g* supernatant and has a pH optimum of 7.5-8.0, thus suggesting a cytosolic localization. The enzyme responsible for this activity has been partially purified. Also presented is the relationship between AVOe3, a potential EFE gene, and this enzyme activity.

803 (+)S-ABA AND (-)R-ABA BOTH BLOCK GERMINATION, BUT HAVE DIFFERENT EFFECTS ON ABA-RESPONSIVE GENES IN WHEAT. M.K. Walker-Simmons[1], R.J. Anderberg[1] and S.R. Abrams[2].

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(+)S-ABA, found naturally in plant tissue, and the (-)R-ABA isomer were found to have equivalent biological activity as germination inhibitors of embryos isolated from mature dormant seed. Dose response tests showed that 1 to 2 μM concentrations of each isomer inhibited germination at the 50% level. To examine the role of specific ABA-responsive genes in ABA-inhibited germination, we determined the effects of both ABA isomers on gene expression. Application of 1 to 5 μM (+)S-ABA resulted in maximum induction by 24 hours of all three ABA-responsive genes examined: Em, dehydrin or rab, and a wheat group 3 LEA. In contrast, (-)R-ABA (10 μM) did not induce Em expression, but did fully induce dehydrin (rab) and the wheat group 3 LEA genes. The application of (-)R-ABA to the wheat embryos did not affect endogenous (+)S-ABA levels in the embryos as measured by immunoassay. These results indicate that Em expression is not necessary for ABA blockage of embryonic germination. The results also suggest that different ABA perception or signal mechanisms may exist for the Em gene compared to the dehydrin (rab) and group 3 LEA genes.

POSTER ABSTRACTS

804 AUXIN INDUCIBLE PROMOTER (SAUR PROMOTER)-IPT FUSIONS PRODUCE PITH-OFF TRANSGENIC TOBACCO PLANTS.

YIJI, Gretchen Hagen and Tom Guilfoyle. Department of Biochemistry, University of Missouri, Columbia, MO 65211

The isopentenyltransferase (ipt) and β-glucuronidase(GUS) genes were placed under the control of an auxin inducible, bidirectional SAUR promoter. This SAUR promoter is expressed under auxin control in a tissue-specific and organ-specific manner. The gene fusions were introduced into *Nicotiana tobacum* cv Xanthi by *Agrobacterium tumefaciens*-mediated transformation. The transgenic plants have a stunted, bushy appearance and a poorly-developed root system. The volume of stem cortex tissue in transgenics increases dramatically due to swelling of cortical cells. On the other hand, the number of the cortex cells in stems is reduced to 1/3 to 1/7 of that of non-transgenic plants. The number of pith cells is reduced significantly although the size of these cells remains unchanged. Xylem cells become much more centralized in the stems and thickness of vascular tissue also increases. These changes result in soft tissue on the outside of stems and woody tissue on the inside of stems. In magenta boxes, the transgenics show necrosis of apical meristems and chlorosis of leaves. Stomates of the leaves fail to close so that plants wilt if magenta boxes are opened and the plants are exposed to atmospheric humidity. Occasionally, multiple shoots are initiated from a single petiole without wounding, and cortex cells in the basal regions of stems dehydrate and die. After adaptation to open air, the transgenics that survive produce dark stems and leaves. Data from histochemical analysis of the GUS activity and quantification of cytokinin concentrations as well as other biochemical analyses at organ and tissue levels are used to explain these morphological changes.

805 HORMONAL REGULATION OF WOUND-INDUCIBLE PROTEINASE INHIBITOR II GENES by Robert Thornburg, Sanggyu Park, and Xiaoyue Li Department of Biochemistry and Biophysics, Iowa State Unviersity, Ames, Iowa 50011

We have previously prepared transgenic plants that express marker genes under control of the Proteinase Inhibitor II promoter (pin2-CAT and pin2-GUS). We are using these plants to assess the early biochemical events that occur following wounding of the foliage. We have found that indole-3-acetic acid (IAA) represses the expression of the pin2 gene system in our transgenic tobacco. We therefore examined the levels of IAA in whole plant tissues by gas chromatography-mass spectrometry using an isotope dilution technique. ^{13}C-labeled IAA was used as an internal standard. The endogenous levels of IAA declined 2 to 3 fold within 6 hours after a wound. The kinetics of auxin decline are consistent with the kinetics of activation of the pin2-CAT construction in the foliage of transgenic tobacco. To further establish the role of auxin in the wound-inducible activation of the pin2-CAT construction, we have utilized compounds that promote the intracellular accumulation of auxin by inhibiting cellular efflux of auxin such as triiodobenzoic acid (TIBA) When transgenic plants containing the pin2-CAT construction were sprayed with water alone the CAT gene was fully wound-inducible However, when these same plants were sprayed with 1 mM TIBA in water the CAT activity was not induced. Thus compounds which cause endogenous accumulations of IAA within cells result in decreased levels of wound-induced accumulation of CAT activity.

806 AUXIN INDUCTION OF A SAUR HOMOLOG IN TOMATO AND ITS ABSENCE IN AN AUXIN MUTANT. Steve Clouse and David Rayle. Department of Biology, San Diego State University, San Diego, CA 92182

Plant hormones can cause selective and rapid changes in the levels of specific mRNAs. A striking example are the Small Auxin Up Regulated (SAUR) genes isolated by McClure and Guilfoyle from elongating soybean hypocotyl sections. The availability of an auxin-insensitive mutant would facilitate studies directed toward establishing a causal relationship between molecular induction of a specific mRNA and a physiological process such as cell elongation. The diageatropica mutant (dgt) of tomato is insensitve to exogenously applied auxin and exhibits other symptoms of auxin insufficiency including lack of high specific activity auxin binding peptides in stems (Hicks, Rayle, Lomax.1989. *Science* 245:52-54). We have performed northern blot analysis with RNA from wild type (VFN) and mutant (dgt) tomato hypocotyls treated with or without auxin, using as the probe a 59-mer oligonucleotide complementary to a portion of the published sequence of SAUR 10A (McClure and Guilfoyle.1987. *Plant Molecular Biol.* 9:611-623). We have shown that in wild-type tomato (which elongates in response to exogenous auxin) a transcript of identical size to SAUR 10A is strongly induced within one hour by 50 μM 2,4-D while in the auxin-insensitve dgt mutant (which does not elongate in response to auxin) no transcript is expressed. Furthermore, the plant growth steroid, brassinolide, which causes stem elongation independently of auxin in many systems, leads to elongation of hypocotyls in both VFN and dgt tomatoes but does not induce the SAUR 10A homolog in either.

807 CYTOKININ PRODUCTION BY *RHIZOBIUM* STRAIN IC3342 AND POSSIBLE ROLE OF *lcr1* GENES. Narayana M. Upadhyaya[1], D. S. Latham[2], K. F. Scott[3] and P. J. Dart[4]. [1]CSIRO Division of Plant Industry, GPO Box 1600, ACT 2601; [2]Plant Cell Biology Group,Research School of Biological Sciences, The Australian National University, ACT 2601, Australia. [3]Pacific Biotechnology Ltd., 74, McLachlan Ave, Rushcutters Bay, NSW 2011; [4]Department of Agriculture, University of Queensland, Queensland 4072.

Growth hormones have been implicated in nodule morphogenesis and nodules contain higher levels of cytokinins than root tissue, but to date, no *Rhizobium* genes or genetic loci directly involved in cytokinin biosynthesis have been identified.

Rhizobium strain IC3342 induces a leaf curl syndrome in certain tropical legumes. We have demonstrated by radioimmunoassays the over-production of cytokinin ZR and DZR in plants nodulated by the strain IC3342. We have also confirmed by GC-MS the over-production of cytokinins Z and iP by IC3342 in the culture medium. Plants nodulated by a Tn5-induced mutant of IC3342 with Curl$^-$ phenotype had reduced levels of cytokinins in the sap. Wild-type DNA sequences of this mutant locus, designated *lcr1*, were shown to confer a Curl$^+$ phenotype on a closely-related, non-curl-inducing strain ANU240.

By DNA sequence analysis, we predict five putative genes in the *lcr1* region. Two of these genes show structural homologies with the *Escherichia coli* regulatory genes *ompR* and *fnr*. No DNA sequence homology was detected between *lcr1* genes and other known cytokinin biosynthetic genes. We suggest that *lcr1* genes are involved in the biosynthesis of cytokinins by pathways quite different from those of *Agrobacterium* or *Pseudomonas* and that the expression of these genes are symbiotically induced.

808 GENES INDUCED BY AUXIN IN TOMATO SEEDLING ROOTS Brian H. Taylor, Chantel Scheuring and Rasha Abou El-Kheir. Dept. of Biology, Texas A&M University, College Station, TX. 77843

Exposure of excised tomato seedling roots to exogenous auxin induces a proliferation of new lateral root initials. To examine the role of auxin-regulated gene expression in this phenomenon, we first identified root culture conditions that maximized the formation of new initials, then performed differential screening on a cDNA library prepared from RNA isolated from roots exposed to 1.6 uM napthaleneacetic acid for 36 hr to identify clones corresponding to genes that are induced by auxin. Two families of clones (represented by clones TR8 and TR132) have been identified and characterized with respect to their regulation by auxin and cytokinin and tissue specificity of expression. Sequence analysis indicated that one of the clones (TR8) corresponds to a gene that is related to, but not identical to, the proteinase inhibitor II gene of tomato and potato. A functional diageotropica gene is required both for auxin-induced lateral root formation and induction of the TR8 and TR132 genes.

809 DEVELOPMENTAL CONTROL OF THE FUSICOCCIN BINDING PROTEIN ACTIVITY. Leslie Basel and Robert Cleland, University of Washington, Department of Botany KB-15, Seattle, WA 98195

The phytotoxin fusicoccin (FC) binds to a binding protein (FCBP) and causes ATPase-mediated proton excretion from most higher plant cells. In order to study the developmental control of the FCBP and its relationship to the ATPase, FCBP and ATPase activities have been measured along the developmental gradient of a mung bean (Vigna radiata L.) hypocotyl. The ATPase activity varies about 2-fold along the hypocotyl, with the maximum activity in the region at the end of the elongation zone. FCBP activity is maximal in the most mature tissues. There is no apparent correlation between FCBP and ATPase activity, which suggests that the FCBP is not a subunit of the ATPase. FCBP activity is almost absent in the hook (meristematic) region, which suggests that it is not an essential protein for all cells. However, incubation of the tissues with FC results in a large (up to 10-fold) increase in FCBP activity. Thus, the phytotoxin has the ability to enhance the activity of its own receptor.

POSTER ABSTRACTS

810 ISOLATION AND CHARACTERIZATION OF THREE FAMILIES OF AUXIN DOWN REGULATED cDNAs. Neeraj Datta, Robert E. Wyatt, Ronald T. Nagao, Joe L. Key. Department of Botany, University of Georgia, Athens, GA 30602

We have isolated full-length cDNA clones corresponding to three classes (A, B, and C, previously referred to as 6, 12, and 11; Baulcombe and Key, 1980, J. Biol. Chem. 255, 8907-8913) of auxin down-regulated genes from a lambda zap library made from the mature hypocotyl of 4 day-old etiolated soybean seedlings. The RNA levels corresponding to these cDNA's, A, B, and C, are decreased 100, 10 and a 15 fold, respectively, in response to auxin. We will be reporting the sequences of these cDNA clones and the spatial-temporal expression patterns, as analyzed by Northern analyses, of the genes to which these cDNAs correspond.

811 EXPRESSION OF AUXIN AND CYTOKININ BIOSYNTHETIC GENES IN BRASSICA NAPUS AND NICOTIANA TABACUM UNDER THE DIRECTION OF AN ARABIDOPSIS THALIANA 2S PROMOTER. Tammy L. Oancia, Patricia Roeckel and Maurice M. Moloney. Department of Biological Sciences, University of Calgary, T2N1N4, Calgary, Canada.

A 1.2 Kb 2S promoter has been isolated by screening an *Arabidopsis thaliana* genomic library in vector EMBL3. This late embryogenic promoter has been sequenced and consensus regions such as RY repeats, TATA and CAT boxes have been identified Characterisation of the expression from this promoter is being tested using chimaeric constructs with GUS. Three hormone biosynthetic genes from *Agrobacterium* are being independently introduced into canola (*B. napus*) and tobacco (*N. tabacum*). Two of these genes, ipt and tzs, encoding isopentenyl transferase, and trans-zeatin synthetase respectively, are part of the cytokinin biosynthetic pathway, while the third, iaaH, encoding indole-3-acetamide hydrolase, is a member of the auxin biosynthetic pathway. We are studying the seed-specific expression of these genes under the control of the isolated 2S promoter. Our study of hormone action at the endogeneous level will serve as a first approximation towards understanding the relationship between hormone levels and physiological responses in plants. In addition, such seed-specific expression of these hormone biosynthetic genes may improve various parameters of yield such as seed size and number as suggested by previous studies in other laboratories involving external applications of auxin and cytokinin hormones.

812 EXPRESSION OF A MURINE ORNITHINE DECARBOXYLASE (ODC) GENE IN TRANSGENIC *NICOTIANA TABACUM. VAR. XANTHI*. Subhash C. Minocha and Richard A. DeScenzo, Department of Plant Biology, University of New Hampshire, Durham, NH 03824.

A full-length and a truncated murine ODC (ornithine decarboxylase) cDNA were cloned with the Cauliflower Mosaic Virus (CaMV) 35S promoter into *Agrobacterium tumefaciens* binary vectors. Using leaf-disc transformation methods, transgenic tobacco plants were obtained for each construct. Presence of the murine cDNA and mRNA was confirmed by Southern and Northern blotting. Western blots showed a polypeptide unique to the transformed plants which immunoreacted with anti-mouse ODC antibodies.

Enzyme assays were run at the pH optima for native plant ODC (pH 8.2) and murine ODC (pH 6.8). At pH 6.8, there was little activity in the control plants, but a significantly higher activity in the transformed plants. Difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC, completely inhibited enzyme activity in the transformed plants at pH 6.8. Almost 100% inhibition of ODC was observed in the transformed plants, at pH 6.8 by immunoprecipitation with the antibodies. The results show clearly that the activity of murine ODC can be detected and quantified even in the presence of the plant ODC. The transgenic plants containing truncated ODC cDNA had several-fold higher enzyme activity than those containing the full-length cDNA Transgenic plants containing the truncated ODC cDNA contained 10-12 times the levels of putrescine than the control plants.

813 THE *ROLB* GENE OF *AGROBACTERIUM RHIZOGENES* ALLOWS AUXIN TO POTENTIATE AUXIN ACTION IN TOBACCO. C. Maurel, H. Barbier-Brygoo, M. Bouvier-Durand, J. Tempé and J. Guern. Institut des Sciences Végétales, CNRS, F-91198 Gif-sur-Yvette Cedex, France.

The root-inducing *rolB* gene of *Agrobacterium rhizogenes* T-DNA is able to induce a 10,000-fold increase in the sensitivity to auxin of tobacco mesophyll protoplasts, as estimated by the hormonal effects on the transmembrane potential difference (Maurel *et al.*, 1991, Plant Physiol, in press). Immuno-inactivation of the electrical response of protoplasts to auxin by antibodies directed against ABP1, the major auxin-binding protein from maize, suggests that the increased sensitivity to auxin of *rolB*-transformed protoplasts could be due to an increased number of auxin receptors at the cell surface. The physiological and morphogenetic action of *rolB* was further investigated in the prospect of the accurate regulation of *rolB* expression in tobacco. For this we used plants containing both *rolB* and a *rolB*.GUS chimeric gene. At the protoplast level, auxin stimulated *rolB* expression (Maurel *et al.*, 1990, Mol Gen Genet, 223: 58-64) and consequently positively controlled the *rolB*-dependent increase in auxin sensitivity. In leaf explants, the *rolB*-dependent indirect rhizogenesis was controlled by auxin. In contrast, the direct rhizogenesis triggered by auxin on hypocotyls was not altered by *rolB*. We propose thus that the potentiation of auxin action by auxin itself, as mediated by *rolB*, might participate in prerhizogenic cell determination of transformed cells.

814 CHARACTERIZATION OF THE GA-REGULATION OF THE GAST1 GENE OF TOMATO. Shi Lifang, Robert Gast, Gopalraj Manjula and Neil Olszewski. Plant Biology Dept., University of Minnesota, St. Paul, MN 55108

GAST1 is a gene whose expression is regulated by GA in the shoots of both wild-type and GA-deficient *(gib-1)* tomatoes. In the mutant, GA application causes an increase in the level of GAST1 mRNA. This increase is first detectable 2 hrs after treatment and maximal accumulation (30-fold) occurs 12 hrs after treatment. GAST1 mRNA abundance is similar in untreated mutant and untreated wild-type plants. However, in wild-type plants GA treatment only causes a 4-fold increase in GAST1 mRNA levels. Auxins, cytokinins and ethylene have no effect on GAST1 mRNA abundance. Treatment with ABA alone reduces the abundance of GAST1 mRNA. In addition, ABA partially inhibits the GA-promoted induction of GAST1 mRNA. Nuclear run-on analysis indicates that transcription of the GAST1 gene is 3-fold higher in nuclei isolated from GA-treated shoots than in nuclei from control (untreated) shoots, suggesting that in this system GA is acting, at least in part, at the level of transcription. Present studies are directed toward the identification of promoter elements necessary for the GA responsiveness of the GAST1 gene. To study the function of this gene, we are studying the effects of altered GAST1 expression on plant growth and development. To do this, we are introducing constructs into tomato that express either sense or antisense GAST1 mRNA under the control of the 35S promoter.

815 FLOWER DEVELOPMENT IN A GIBBERELLIN-DEFICIENT (*gib-1*) MUTANT OF TOMATO. Steven E. Jacobsen and Neil E. Olszewski. Department of Plant Biology, University of Minnesota, St. Paul, MN 55108

The *gib-1* mutant of tomato initiates flower primordia but flowers do not complete development unless supplied with exogenous GA. To determine when *gib-1* flower development stops and if development can be restored following cessation, wild type and *gib-1* flowers of different lengths were treated with GA and then the number of days to petal reflex was recorded. In wild type, a nearly linear negative correlation exists between bud length and the number of days to petal reflex for all flowers tested (0.2 to 13.2 mm long). For *gib-1* flowers, this same negative correlation exists only for buds 0.2-2.5 mm long at treatment. Buds between 2.5 and 3.7 mm have a similar number of days to petal reflex and most buds longer than 3.7 mm do not develop normally. This suggests that mutant flowers reach a developmental block at a length of 2.5 mm and then remain developmentally arrested but rescuable by GA until they reach a length of 3.7 mm. Further analysis indicates that anthers of arrested flowers (2.5-3.7 mm) contain pollen mother cells that are in G1 of premeiotic interphase. When arrested buds are treated with GA, premeiotic DNA synthesis and callose accumulation are evident by 48 h post treatment, and within 66 h the first visible stage of prophase I of meiosis occurs. Developmentally arrested anthers are currently being used in differential screening experiments to identify clones to mRNAs that are more abundant in anthers 8 h after GA treatment.

POSTER ABSTRACTS

816 INVERTASE cDNA PREPARED FROM GIBBERELLIN-TREATED DWARF PEA PLANTS. John P. Mitchell, Liu-Lai Wu and Norman S. Cohn. Ohio University, Department of Botany, Athens, Ohio 45701.

It has long been known that exogenously applied gibberellin (GA) will induce the accumulation of invertase activity in internode tissue of dwarf pea plants as they begin the elongation process. In this study 'insoluble' invertase was extracted in 1 M NaCl from exhaustively water-washed dwarf pea stem tissue harvested 48h after GA treatment. This enzyme was purified by elution of a single band from a non-denaturing polyacrylamide gel after carboxy methyl- and diethylaminoethyl- Sephadex chromatography. After deglycosylation the purified protein was used to generate polyclonal anti-invertase antibodies in rabbits. This antiserum was utilized in screening a lambda gt11 cDNA library prepared from polyA mRNA isolated from stem tissue harvested 24h after GA treatment. Several cDNA clones were identified. As part of an investigation of precisely where, when and how the accumulation of invertase activity occurs, labelled invertase cDNA was used as a probe of RNA gel blots to document the accumulation of invertase mRNA during the period between fifteen minutes and 48h after GA treatment.

817 Separate pathways regulate the developmental and environmental pin 2 gene expression. Hugo Peña-Cortés, José J.Sanchez-Serrano and Lothar Willmitzer. Institut für Genbiologische Forschung, Ihnestr. 63, D-1000 Berlin 33, F.R.G.

Proteinase Inhibitor II genes (pin 2) belong to a small gene family of some 3-5 members per haploid genome which is constitutively expressed in potato tubers as well as in young floral buds of potato and tomato plants. Wounding of the leaves lead to an induction of expression of these genes in non-wounded upper and lower leaves as well as in the upper stem segment, whereas no expression is observed in non-wounded roots or in the lower stem segments. This systemic induction is likely to be related to the synthesis or release of a "wound signal" at the site of injury which migrates throughout the plant, activating pin 2 genes in the distal tissues. We have recently shown strong evidence for the involvement of the plant hormone abscisic acid (ABA) in the induction of the pin 2 gene expression upon wounding. Thus, spraying of potato plants with ABA leads to the accumulation of pin 2 mRNA in aerial tissue in the absence of any wounding, showing the same expression pattern as wounded plants. ABA-deficient tomato (*sitiens*) and potato plants (droopy) do not accumulate pin 2 mRNA upon wounding and it could be shown that this lack of induction was a consequence of the ABA deficiency. Upon ABA treatment, droopy and *sitiens* plants indeed accumulated pin 2 mRNA at levels similar to the wild-type parental plants. Results will be presented which indicate that ABA, at physiological concentrations, is able to induce the expression of the pin 2 gene family both locally and systemically. Additionally, the significance of the ABA involvement in pin 2 induction upon wounding has been further strengthened by analyzing the expression of a pin 2 promotor-β glucuronidase (GUS) gene fusion in transgenic ABA-deficient mutant potato plants. We have analyzed the developmental regulation of pin 2 gene expression in wild-type and ABA-deficient potato and tomato plants and observed that the pin 2 mRNA level is identical in mutant and wild-type plants. These results and others which suggest the action of separate pathways for the developmental and environmental regulation of pin 2 gene expression will be discussed.

818 ^{18}O LABELING PATTERNS OF ABSCISIC ACID AND XANTHOPHYLLS. Francoise Fantauzzo and Jan A.D. Zeevaart. Michigan State University, Plant Research Laboratory, East Lansing, Michigan 48824

Knowledge of biosynthesis and degradation of abscisic acid (ABA) is essential if one is to understand how its concentration is regulated at the tissue and cellular level. Our laboratory has made use of the stable isotope ^{18}O and mass spectrometry to study ABA biosynthesis. The ^{18}O-labeling patterns of ABA synthesized in various species and tissues in an atmosphere containing $^{18}O_2$ differ considerably (Zeevaart et al. 1989, Plant physiol. 91, 1594-1610.). The present experiments were undertaken to determine whether the ^{18}O-labeling pattern of ABA is related to that of the xanthophylls violaxanthin and neoxanthin. In apple tissue, 50% of violaxanthin and 25% of neoxanthin incorporated four oxygens which correlates with a high percentage of ring labeling in ABA synthesized during the labeling period (newly synthesized ABA). Like in stressed leaves, in unripe avocado fruit most of the newly synthesized ABA was labeled with a single ^{18}O in the carboxyl group, while in the same tissue the xanthophylls incorporated little ^{18}O. In ripe avocado fruit, most of the newly synthesized ABA was singly or doubly labeled in the ring oxygens. This corresponds to a high incorporation of ^{18}O into the xanthophylls of ripe fruits. Furthermore, ABA accumulated during ripening of avocado fruit, while the epoxy-carotenoids pool decreased by 80 to 90%. Taken together, these results establish a close correlation between ^{18}O labeling of the oxygens on the ring of ABA on one hand and ^{18}O incorporation by violaxanthin and neoxanthin on the other. They provide, therefore, further evidence that these epoxy-carotenoids are precursors to ABA.

819 DIFFERENTIAL EXPRESSION OF ALPHA-AMYLASE GENES IN GERMINATING RICE SEEDS. Raymond L. Rodriguez, Erik E. Karrer, Sunita Ranjhan and Ning Huang. Department of Genetics, University of California-Davis, Davis, CA 95616

Rice α-amylase gene family consists of 10 genes which have been classified into three subfamilies (RAmy1, RAmy2 and RAmy3) based on their sequence homology. There are three genes in RAmy1 (*RAmy1A-1C*), only one gene in RAmy2 (*RAmy2A*) and six genes in RAmy3 (*RAmy3A-3F*). These genes are mapped to 5 different chromosomes. The temporal and spatial expression of these genes are examined by Northern blot and *in situ* hybridizanon analysis. During seed germination, rice α-amylase transcripts are first appeared in the scutellum and later in the aleurone tissues with much high abundance. Not all α-amylase genes, however, are expressed at the same time and at the same level. *RAmy1* genes and *RAmy3B, C* and *E* genes are preferentially expressed in the aleurone layer. Expressions of *RAmy3A* and *RAmy2A* are not detectable at any time during germination. *RAmy3D*, on the other hand, is expressed only briefly (between 6 and 24 hours) and at low levels in the scutellum during rice seed germination. This apparent tight regluation of *RAmy3D* expression can be broken by detaching the embryo (with scutellum) from the intact seed. When isolated embryos are allowed to germinate for 48 hrs, the level of *RAmy3D* mRNA is 30 fold higher than that found in the embryos of intact germinating seeds. This implies that the rice endosperm produces a factor(s) which is transferred to the scutellum to selectively inhibit the the expression of *RAmy3D*. The nature of this factor is being investigated.

820 A XYLOGLUCAN-DERIVED NONASACCHARIDE AFFECTS THE REGENERATION OF CARROT PROTOPLASTS. Michael Emmerling*, Hildegard M. Warneck and Hanns-Ulrich Seitz, University of Tuebingen, Botanical Institute, W-7400 Tuebingen, Germany. *present adress: University of Melbourne, PCBRC, Parkville, Victoria 3052, Australia

Cell wall-derived oligosaccharides influence a variety of morphological and developmental processes in plants. Recently, a xyloglucan-derived nonasaccharide, XG9 ($Glc_4\ Xyl_3\ Gal\ Fuc$), was shown to act as an inhibitor of 2,4-dichlorophenoxyacetic acid (2,4-D)-induced growth of pea stem segments at nanomolar concentrations. We isolated a xyloglucan oligosaccharide from cell walls of *Daucus carota* L. suspension-cultured cells by controlled action of cellulase *in vitro*. The analytical data (gel permeation chromatography, thin layer chromatography, monosaccharide analysis, and methylation analysis) confirmed the identity of the oligosaccharide as XG9. The XG9-preparation was very effective in the pea stem-bioassay with up to 80% inhibition of 2,4-D-induced growth of etiolated pea stem segments at concentrations in the nanomolar range. The influence of XG9 on the regeneration of carrot protoplasts regenerated in the presence of 2,4-D as the only growth regulator was investigated. The results of the protoplast assays containing 4.52 µM 2,4-D and various concentrations (10^{-7}-10^{-11}M) of XG9 were compared to control protoplasts regenerated with or without 2,4-D, respectively. The oligosaccharide was added at the beginning of cultivation, the regenerating protoplasts were harvested after 96 hours. In the control experiments, the viability of the protoplasts was clearly reduced in the absence of 2,4-D. Application of nanomolar concentrations of XG9 in the presence of 2,4-D resulted in a suppression of the viability of 75% as compared to the controls. Lower and higher concentrations of the oligosaccharide were less effective indicating that a simple cytotoxic effect could be excluded. The regeneration of protoplasts not only depends on the viability of the cells but also on their ability to synthesize a new, functional cell wall. In none of the assays was the monosaccharide composition of cell wall preparations of the protoplasts significantly altered. The influence of XG9 on the activities of several glycan synthases was also investigated. The four glycan synthases tested were activated by nanomolar concentrations of XG9. The effect was similar to that achieved by the omission of 2,4-D from the regeneration medium. Again, lower and higher concentrations were less effective. Currently, the influence of XG9 in several other systems is investigated.

821 CYTOKININ-SPECIFIC cDNAs FROM *N. plumbaginifolia* TRANSFORMED BY THE *ipt* GENE FROM *Agrobacterium*. S. Harding and A. Smigocki, USDA/ARS, Plant Mol. Biol. Lab., Beltsville, MD, 20705

Transgenic tobacco plants in which overproduction of cytokinins can be regulated are being used for isolating cytokinin responsive cDNAs. The plants, which contain either the isopentenyl transferase (*ipt*) gene regulated by the *Drosophila* Hsp70 promotor (HS-*ipt*) or a non-functional *ipt* gene (t-*ipt*), received a 1 hour 45 °C heat shock to induce cytokinin synthesis. During heat shock, the *in vitro* translation products of several mRNAs accumulated in both genotypes but no differential changes in gene expression could be readily ascertained. Following a 3 hour recovery period at 25 °C, the probable heat shock messages declined in both while at least 7 other mRNAs increased only in HS-*ipt* plants. A cDNA library was constructed from HS-*ipt* tissue collected immediately after the 1 hour heat shock (1h AHS) and duplicate filters were probed with t-*ipt* cDNA (1h AHS) or with HS-*ipt* cDNA (4h AHS). A small group of up-regulated, potentially cytokinin-responsive cDNAs have been isolated. A subtracted cDNA library from HS-*ipt* tissue (4H AHS) has been constructed and is being screened for cytokinin specific cDNAs.

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822 TISSUE-SPECIFIC AND HORMONAL REGULATION OF BEAN CELLULASE EXPRESSION. Mark Tucker, Gail Matters, Susan Baird and Roy Sexton, USDA/ARS Plant Molecular Biology Lab, Beltsville, Maryland 20705

Tissue-specific expression and hormonal regulation of the basic (pI 9.5) abscission cellulase gene from bean (*Phaseolus vulgaris*) has been investigated. To identify cells that produce cellulase mRNA, ^{35}S-labeled antisense cellulase RNA probes were hybridized *in situ* to longitudinal sections of the petiole-abscission zone-stem region. Hybridization was limited to the abscission zone cells and adjacent vascular tissue. This indicates that all cells in the abscission zone, regardless of cell type, produce cellulase mRNA. An abscission cellulase cDNA was used to isolate clones from a bean genomic library. We identified overlapping clones spanning the entire coding sequence (3 Kbp), and an additional 12 Kbp of 5' and 1 Kbp of 3' flanking DNA. 1.2 Kbp of 5' flanking DNA was sequenced and comparisons were made between upstream regions of the cellulase and chitinase genes, both of which are transcriptionally induced by ethylene. Three areas of sequence similarity (>85% identity, 10-13 bp) were found. Deletions through these areas were made and the segments were fused to a β-glucuronidase (β-GUS) reporter gene. The constructs are being tested in a transient expression assay using particle gun transformation of bean petiole-abscission zone-stem sections. A 2 Kbp segment of the cellulase upstream region is sufficient to direct tissue-specific β-GUS expression.

823 CLONING THE *Arabidopsis thaliana ga-1* LOCUS BY GENOMIC SUBTRACTION. Tai-ping Sun & Frederick M. Ausubel Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

The *ga-1* mutants of *Arabidopsis* are gibberellin (GA)-sensitive male sterile dwarfs. The *ga-1* locus encodes *ent*-kaurene synthetase A or B which converts geranylgeranyl pyrophosphate (GGPP) to *ent*-kaurene in the GA biosynthetic pathway (Zeevaart, Plant Research'86, Annual Report of the MSU-DOE Plant Research Laboratory, 130-131, 1986). By genetic fine structure analysis, Koornneef et al. (Genet. Res., Camb. 41, 57-68, 1983) showed that one of the *ga-1* mutations (31.89) is an intragenic deletion. Using the genomic subtraction technique (Straus & Ausubel, Proc. natn. Acad. Sci. U.S.A. 87, 1889-1893, 1990) devised to clone DNA sequences corresponding to deletion mutations, we have isolated DNA fragments corresponding to a 5 kb deletion in the *ga-1* mutant 31.89. Three lines of evidence indicate that the sequence isolated by genomic subtraction corresponds to the *ga-1* region: (1) RFLP mapping showed that this sequence resides near the *ga-1* locus on chromosome four. (2) DNA sequence analysis showed that four other *ga-1* mutations (6.59, A428, d352 and Bo27) mapped within the deleted region in 31.89. (3) Cosmid clones containing the putative wild-type *ga-1* locus complemented the *ga-1* mutation in transgenic plants. We are presently characterizing the structure of the *ga-1* locus in order to study the regulation of the *ga-1* gene expression.

824 DEVELOPMENTAL AND ORGAN-SPECIFIC EXPRESSION OF AN ABA AND STRESS-INDUCED PROTEIN IN BARLEY. Bimei Hong[1], Rivka Barg[2], David THD Ho[1,1] Dept. of Biology, Washington University, St. Louis, MO 63130. [2] Dept. of Plant Genetics, ARO, Bet-Dagan 50250, Israel.

An mRNA species, HVA1, has been shown to be rapidly induced by abscisic acid (ABA) in barley aleurone layers. In the current work we have investigated the expression of HVA1 in other organs of barley. In developing seeds, HVA1 mRNA is never detected in starchy endosperm cells, yet this mRNA accumulates in both aleurone layers and the embryo starting at 25 day-post-anthesis, and its level remains high in these tissues of dry seeds. The levels of HVA1 mRNA and protein start to decline after the onset of germination, and HVA1 mRNA is barely detected in any organ of 3-day-old seedlings. The expression of HVA1 at both the mRNA and protein level can be induced by ABA treatment in 3-day-old seedlings. ABA-induction of HVA1 mRNA accumulation in barley roots starts within one hour and reaches a maximum at three hour of hormone treatment and the HVA1 protein becomes apparent after 4 hour of ABA treatment. Besides the HVA1 protein, a smaller protein of 20 kD (p20) which cross-reacts with antibodies raised against the HVA1 protein is also induced by ABA in barley. Experimental data indicate that these two proteins are encoded by different mRNAs. The p20 protein exhibits a unique pattern of expression. It is not expressed in seed tissues, hence, by definition, it is not a LEA protein. The expression of HVA1 is induced by various stress conditions including drought, NaCl treatment, cold and heat shock. Immunogold is used to localize the protein.

825 AUXIN-ALTERED EXPRESSION AND ACTIVITY OF LIPOXYGENASE IN SOYBEAN TISSUES. Wennuan Liu, David F. Hildebrand, W. Scott Grayburn and Glenn B. Collins, Dept. of Agronomy, University of Kentucky, Lexington, KY 40546

Lipoxygenases (EC 1.13.11.12) (LOXs) are commercially important enzymes that catalyze the peroxidation of molecules containing *cis,cis*-1,4-pentadiene moieties and appear to be ubiquitous among eukaryotic organisms. In soybean [*Glycine max* (L.) Merr.], the expression of LOXs are known to be developmentally regulated. Plant hormones have been firmly established as being involved in plant growth and development. Correlation between the expression of LOXs and the development and germination of soybean embryos suggests that plant hormones may affect the expression of LOXs. The aim of this study was to gain insight into the effect of exogenous auxins on the expression and enzymatic activities of LOX isozymes in cultured soybean tissues during somatic embryogenesis. The results showed that at least one of the more acidic LOX isozymes was induced by either NAA or IAA but not by 2,4-D after 4d exposure. This isozyme showed enzymatic activity and had the same MW and pI values as the germination-associated LOX found in germinating seedling tissues. A comparison of the peptide mapping patterns for the auxin-induced and germination-associated isozymes following digestion with the protease from *S. aureus* strain V8 revealed that the sequence of these isozymes were very similar but not identical. Levels of LOX-1, -2 and -3 proteins and activities were greatly decreased by 2,4-D 10d after explanting. S1 nuclease protection analysis showed that embryo LOX mRNAs levels were not apparently altered in the tisssues treated with each of the auxins. The reduced levels of the embryo LOX proteins may, therefore, be regulated at the levels of translation, post-translational modification or degradation.

826 CLONING AND CHARACTERIZATION OF A NOVEL ABA AND CYCLOHEXIMIDE REGULATED GENE IN BARLEY Qingxi Shen, Scott J. Uknes and T.- H. David Ho Department of Biology, Washington University, St. Louis, MO 63130

We have isolated a novel ABA inducible cDNA clone, designated HVA22. This cDNA represents a mRNA which is induced by ABA within 30 minute of treatment in barley aleurone layers. Cycloheximide, a protein synthesis inhibitor, also induces the expression of the gene to a significantly high level. In developing seeds, HVA22 mRNA is readily detectable in the aleurone layer as early as 18 days after anthesis and its level remains high in the embryo through all stages of seed development studied. However, it is barely detectable in endosperm during seed development. No significant homology was found between HVA22 and any reported *Lea* genes at both DNA sequence and deduced amino acid sequence level. In vegetative tissues/organs, HVA22 mRNA was detected in roots and leaves of the ABA-treated or drought-stressed three-day-old plants. In contrast, the mRNA is detectable only in the roots, but not the shoots of the seven-day-old or older plants treated the same way. Furthermore, cold stress induces the expression of HVA22 in the seedlings with a pattern similar to ABA and drought treatment. With wheat-barley chromosome addition lines, we have mapped the gene to chromosome 1 of the barley genome. HVA 22 homologues were detected in other species of *Gramineae*, including wheat, rye, oat, rice and maize.

POSTER ABSTRACTS

827 ISOLATION AND CHARACTERIZATION OF CYTOKININ RESPONSE MUTANTS IN *ARABIDOPSIS THALIANA*.
Wenpei Su and Stephen H. Howell. Boyce Thompson Institute, Ithaca, NY 14853
We have isolated mutants in *Arabidopsis thaliana* with altered responses to cytokinin in an effort to dissect the cytokinin signal transduction pathway. Two types of cytokinin response mutants were isolated: mutants resistant to cytokinin (benzyladenine) and mutants resistant to an anticytokinin, called tubercidin (7-deazaadenosine). A root screening assay was developed for selecting both types of the mutants. Primary root growth and root hair elongation are highly sensitive to exogenous cytokinin and *Arabidopsis* root exhibit a characteristic "cytokinin syndrome" when grown in medium containing BA. Therefore, we used a root growth assay to select for *Arabidopsis* seedlings which did not exhibit this cytokinin syndrome in 2.5 uM BA. Five cytokinin resistant lines were independently isolated, and genetic analysis indicated that the resistant traits were recessive in all five lines. Furthermore, the mutants failed to complement one another, suggesting that the mutations are allelic. A similar cytokinin resistant line was isolated from the Dupont collection of *Agrobacterium* T-DNA mutagenized *Arabidopsis* seeds. Tubercidin resistant mutants were isolated on the basis that tubercidin counteracts the effect of exogenously added cytokinin, and tubercidin produces root growth abnormalities on its own. Four tubercidin resistant mutant lines which grew normally in turbercidin were isolated. The tubercidin resistant character behaves as single Mendelian semidominant trait in all the mutant lines. These mutants are of interest because of the possibility that they might involve an alternation in a "cytokinin receptor". A cytokinin-protein binding assay has been developed to characterize these mutants.

828 EXPRESSION OF THE SAUR-AC1 GENE IN GRAVITY RESPONSIVE MUTANTS OF *ARABIDOPSIS*. Pedro Gil[1], Yang Liu[1], Vladimir Orbovic[1], Elizabeth Verkamp[2], Thomas C. Newman[1], Ken Poff[2], and Pamela J. Green[1]. MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824-1312; [2]Present address: Dept. of Biochemistry and Biophysics, Yale University, New Haven CT. The small auxin up RNA (SAUR) genes were originally characterized from soybean where they comprise a small gene family (McClure et al. 1989, Plant Cell, 1:229-239). During gravitropism, there is a rapid redistribution of the SAUR transcripts in soybean hypocotyls, which is presumably mediated at least in part by auxin. The kinetics of redistribution indicate that the SAUR transcripts are very unstable (McClure and Guilefoyle, 1989, Nature. 243:91-93). We have cloned an *Arabidopsis* SAUR gene, designated SAUR-AC1, using PCR primers corresponding to the most conserved region of the soybean genes. The region amplified by PCR was used as a hybridization probe to isolate the genomic clone. Like its soybean homologs, SAUR-AC1 is inducible by auxin in elongating seedlings. From amino acid 27 to 76 this clone is 80% homologous at the amino acid level with the prototype SAUR 15A gene from soybean and 74% at the nucleotide level. Sequences upstream and downstream of the coding region have also been determined. Interestingly, a sequence with similarity to the conserved DST element of the soybean SAUR transcripts exists in the 3' flanking region of SAUR-AC1. This sequence may be involved in the expression of SAUR-AC1 because other studies indicate that the DST element can act as an mRNA instability determinant (see abstract by T.C. Newman et al.). We are investigating the expression of the SAUR-AC1 gene in gravity-responsive mutants of *Arabidopsis*, to learn more about how this gene is controlled. Initial results indicate that some mutants with altered gravitropism exhibit markedly altered SAUR expression.

829 GENETIC AND MOLECULAR STUDIES OF ETHYLENE RESPONSES IN *ARABIDOPSIS THALIANA* Qianhong G. Chen and Tony Bleecker University of Wisconsin, Department of Botany Madison, Wisconsin 53706
We are investigating the molecular basis for ethylene response in *Arabidopsis thaliana*. In addition to the previously described etr mutant (Bleecker et al., 1988), we have isolated a number of additional ethylene-insensitive mutants representing at least 3 different complementation groups. In order to study the influence of these mutations on ethylene-regulated gene expression, we have transformed *Arabidopsis* with ethylene-responsive reported genes. GUS fusions of the E4 promoter from tomato (Fischer et al. 1989) and the chitinase promoter from bean (Broglie et al. 1989) have been transferred into wild type *Arabidopsis* (Gene fusion constructs were kindly supplied by R. Fischer and R. Broglie respectively). The chitinase promoter construct is expressed in vegetative tissues and up to 20 fold increase in GUS activity has been observed after ethylene treatment in some transgenic lines. Transgenic plants carrying the ethylene responsive reported gene are being crossed to the ethylene insensitive mutant lines in order to assess the effects of various mutations on ethylene-induced gene expression.

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830 THE EFFECT OF ABSCISIC ACID ANALOGS ON THE EXPRESSION OF THE Em GENE IN RICE SUSPENSION CELLS. Jennifer A. Schnall, Ralph Quatrano. University of North Carolina Department of Biology, Chapel Hill, North Carolina 27599-3280
Abscisic acid (ABA) analogs are powerful tools for the elucidation of the ABA response pathway. The ABA regulated Em gene from wheat was used as a marker to determine the effect of acetylenic, 2',3'-dihydroabscisic and new terpenoid ABA analogs on gene expression in rice cell suspension cultures. Slight changes in the conformation of the ABA molecule were found to profoundly influence the level of Em gene expression. This analysis provides insight into the functional components of the ABA molecule. Recent results from this laboratory demonstrate that NaCl and ABA have a synergistic effect on the expression of Em in rice cells, suggesting that salt appears to increase tissue sensitivity to ABA. Studies were therefore undertaken to determine whether the ABA analogs function along a similar pathway. Interestingly, the saturation kinetics of the analogs were found to be quite different from those of ABA. In addition to a molecular analysis of the ABA analogs, we present physiological data concerning an Arabidopsis mutant that is insensitive to several ABA analogs but responds normally to ABA.

831 QUANTITATIVE SYSTEM FOR EVALUATION OF AUXIN-INDUCED GENE EXPRESSION. Tim Ulmasov, Gretchen Hagen, and Tom Guilfoyle. University of Missouri-Columbia, Biochemistry Department, Columbia, MO 65211.

We have developed a convenient system for quantitative estimation of the auxin effects on the expression of an auxin-inducible gene. Protoplasts from transgenic tobacco plants carrying GH3-GUS-NOS gene fusion (GH3 promotor was derived from an auxin-inducible soybean gene) retain their capacity to respond to application of exogenous auxins by chimeric gene expression. As little as 10^4 protoplasts per assay may be used for measurement of GUS activity induced by auxin after 4-6 h of hormone application. Low background, rapidity of the response, simplicity, and accuracy of reporter activity measurements make this system attractive for studying the signal transduction mechanism which results in the hormone-specific gene expression. Using this system we compared dose-response relationships for three different auxins - NAA, IAA and 2,4-D. We found IAA to be the strongest inducer (inducing capacity retained up to 10^{-8} M) while 2,4-D was the least active one (response dropped to the non-induced level below 10^{-6} M).

832 ORGAN AND TISSUE SPECIFIC EXPRESSION OF THE AUXIN REGULATED GENE, GH1. Melissa A. Gee*, Carolynn Kerrleber, Gretchen Hagen, and Thomas J. Guilfoyle. University of Missouri, Departments of Biology* and Biochemistry, Columbia, Missouri 65211
The auxin responsive gene, GH1, from soybean encodes a protein of about 31kD and contains blocks at the amino acid level homologous to other auxin responsive genes, Aux1 and Aux2. GH1 transcripts have been shown to be auxin induced several fold above controls in soybean hypocotyls by RNA blot analysis and to be transcriptionally induced by auxin in plumules by nuclear run on experiments. We have further characterized GH1 by localizing expression using RNA *in situ* hybridization and by analyzing protein levels using western blot analysis. Localization at the organ and tissue levels by RNA *in situ* hybridization shows that GH1 is expressed under untreated conditions in roots, hypocotyls, plumules, apical buds, and flowers. In roots and hypocotyls, expression is strongest in the vascular cylinder, though expression also occurs in the pith, cortex, and epidermis. GH1 RNA is strongly localized to protoxylem ridges in basal hypocotyls and roots. In general, levels of GH1 expression are stronger in the elongating region of the hypocotyl compared to the basal hypocotyl or root. Expression in flowers occurs in vascular bundles and developing xylem elements. Treatment of hypocotyls and roots with 50uM 2,4-D for 4 hours increases GH1 RNA levels in all tissues similar to auxin induced expression patterns previously reported for another auxin responsive gene, GH3. Antisera raised against a T7 expressed GH1 fusion protein recognizes a protein of about 30kD from both root and hypocotyl crude extracts.

POSTER ABSTRACTS

833 FUNCTIONAL ASSAY AND PROTEIN BINDING REGIONS OF TWO RICE α–AMYLASE GENE PROMOTERS. Yogesh Mawal, Stanley Goldman, Ju–kon Kim, Jun Cao and Ray Wu. Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, N.Y. 14853

It is known that the α–amylase gene is induced to produce high levels of mRNA by the plant hormone gibberellin (GA). The tissue–specific expression of two rice α–amylase genes (OS*amy–b* and OS*amy–c*) was investigated using RNA blots (Northern) and transient assays of deletion mutants of the promoter sequences. The Northern blots of different rice tissues show that these two α–amylase genes are expressed in germinating mature seeds but not in root or leaf tissue. Transient expression study was carried out with a series of constructs in which 5'–deletions of the OS*amy–c* promoter were fused to the β–glucuronidase (GUS) gene. These constructs were introduced into rice aleurone cells by the biolistic method. We identified a putative negative regulatory sequence located between –231 and –50; deletion of this sequence resulted in a four–fold increase in GUS activity. To better understand the regulation of these genes, gel retardation and footprinting have been used to identify protein binding sequences in the 5' flanking region. For OS*amy–b*, regions between –284 bp and –54 bp showed specific binding. In DNase I footprinting gels three binding regions were seen which are located between –269 to –257, –227 to –220, and –165 to –153 upstream of the putative transcription start site. For OS*amy–c*, regions between –231 and –162 bp showed specific binding. Three footprints were found which are located between –218 to –203, –195 to –186 and –184 to –175

834 GENETIC EVIDENCE OF A ROLE FOR ABSCISIC ACID IN POLLEN DEVELOPMENT. Ruth R. Finkelstein, Monica Lier-Bolsinger and Noelle Roszkowski. UC Santa Barbara, Department of Biological Sciences, Santa Barbara, CA 93106

Mutations in the three ABA-insensitive (*ABI*) loci of *Arabidopsis* all have pleiotropic effects, but none of these mutations affects all ABA-inducible responses. Comparison of ABA sensitivity of monogenic mutants with those of all pairwise combinations of these mutations indicates that the *ABI* loci function in overlapping pathways of ABA response (Finkelstein and Somerville (1990) Plant Physiol. 94:1172-1179). Interestingly, the *abi2 abi3* double mutant was never recovered, even from the self-fertilized progeny of *abi2/+ abi3/abi3* plants. Genetic analysis and pollen viability staining assays suggest that the *abi2 abi3* genotype is lethal in pollen. A possible role for ABA in pollen development is induction of desiccation tolerance during maturation, similar to its effect in maturing seeds. To test this hypothesis, we are comparing wild type and mutant plants to identify the timing of pollen death and changes in gene expression during pollen maturation. We are especially interested in genes whose products are correlated with desiccation tolerance.

835 MOLECULAR ANALYSIS OF ARABIDOPSIS GENES CORRELATED WITH ABA SENSITIVITY. Nina Bang and Ruth R.Finkelstein. UCSB, Department of Biological Sciences, Santa Barbara, California 93106.

A major function of abscisic acid (ABA) in vegetative tissue is to mediate stress responses, yet the molecules in the signal response pathway of ABA are not known. Wild type and the ABA-insensitive mutants of Arabidopsis thaliana (ABI1 and ABI2) respond differently to both stress and ABA treatments, but appear similar under unstressed conditions. By comparing the mRNA populations in unstressed leaves of wild type and the abi1 and abi2 mutants we have identified gene products whose synthesis correlates with the ability to respond to ABA. Separation of the in vitro translated products by two-dimensional nonequilibrium pH gradient (NEPHGE) and isoelectric focusing (IEF) gel electrophoresis shows that at least 11 transcripts are less abundant in the abi1 mutant than in wild type, while only a subset of these are less abundant in the abi2 mutant.
We are using differential hybridization to identify cDNA clones whose expression differs between wild type and abi1 plants.

836 CHARACTERIZATION OF A GENE INVOLVED IN AUXIN ACTION Lawrence Hobbie, F. Bryan Pickett, and Mark Estelle Dept. of Biology, Indiana University, Bloomington, IN 47405

Mutations in the *AUX1* gene of *Arabidopsis* confer resistance to root growth inhibition by auxin and disrupt the gravitropic response of the root. Because the mutation does not appear to affect the aerial part of the plant, we believe that the *AUX1*gene is probably required in a root-specific pathway of auxin action. To begin to characterize the *AUX1* gene at the molecular level, we have identified RFLPs that are closely linked to the *AUX1* gene on chromosome 2, and plan to use these to start a chromosome walk to the gene. In addition, we are attempting to isolate second-site suppressors of *aux1* mutations to identify genes whose products may interact with *AUX1*.

837 ANALYSIS OF THE REGULATION AND FUNCTION OF THE ROOT INDUCING GENE *rolB*. I.Capone, M.Cardarelli, P. Filetici, M.Trovato and P.Costantino. Universita "La Sapienza", Dip. Genetica e Biologia Molecolare, Roma, 00185 Italy.

The gene *rolB* from the T-DNA of *Agrobacterium rhizogenes* is capable of inducing root morphogenesis in transformed plant cells. We have studied the regulation of expression of *rolB* in transgenic plants, during development and in transient expression assays on protoplasts. We have identified the promoter regions which control the level, tissue specificity, hormonal and developmental control of the gene and some of the proteins which specifically interact with these regulatory regions.
As an early event following *rolB* expression is an enormous increase in sensitivity to auxin of the plant cells, we have investigated and present data on the possible function of the *rolB* gene product, obtained by means of specific antibodies.

838 POLYAMINES MODIFY CLEAVAGE OF PLANT, BACTERIAL AND VIRAL DNA. Arie Altman, Nurit Rosenberg and Nitsa Levin. The Hebrew University of Jerusalem, The Otto Warburg Center for Biotechnology in Agriculture, P.O.Box 12, Rehovot, Israel 76-100.

Previous studies have shown that polyamines interact with bacterial and mammalian DNA to affect its cleavage by restriction endonucleases. A comparative study of the effect of polyamines on cleavage of plant, viral and bacterial DNA by several types of nucleases was performed. Polyamines were added to the in vitro reaction, either prior or after the enzymatic reaction. Spermidine, and especially spermine, at high concentrations (>1mM), inhibited cleavage of pBR322, while promoting its cleavage, in few cases, at lower concentrations. Spermine and spermidine inhibited cleavage of plant pCMA and pNT32 cloned DNA, and of corn and tobacco total chloroplast DNA. Spermine modified the degradation pattern of pBR322 by crude extracts from fresh and senescing leaves of mung bean and tomato, by interacting with specific cleavage sites. Similar effects of spermine were found in the cleavage of the pTYH cloned DNA of the tomato gemini virus by tomato leaf extracts.

The data suggest that polyamines modify DNA degradation and activity, by binding to specific cleavage sites. This may explain both the universality, and the divergence, of the biological effects of polyamines.

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839 DNA SEQUENCES REQUIRED FOR ETHYLENE-REGULATED GENE EXPRESSION IN RIPENING TOMATO FRUIT. Jill Deikman, Julie Montgomery, Linda J. Margossian, Rodrigo Lois and Robert L. Fischer. Department of Plant Biology, University of California, Berkeley, CA 94720. The plant hormone ethylene plays an important role in controlling fruit ripening. The regulation of gene expression in ripening tomato fruit has been studied by cloning mRNAs that accumulated both during fruit ripening and in unripe fruit exposed to exogenous ethylene. Genes that encode two of these mRNAs (E4 and E8) have been studied in detail and were shown to be regulated at the transcriptional level. We have constructed a nested set of deletions of the 5' flanking regions of both the E4 and the E8 genes, and by analysis of expression of these mutated genes in both stably transformed plants and in transient assay, we have identified cis-active DNA sequences required for the response of these genes to ethylene and for normal gene expression during tomato fruit ripening.

840 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL AUXIN RECEPTOR Paruchuri Prasad and Alan M. Jones, Dept. of Biology, University of North Carolina, Chapel Hill, NC 27599. While the molecular mechanism of action of auxins is not known precisely, its influence on cellular processes such as altered gene expression and membrane hyperpolarization is mediated through specific receptors. However, a receptor mediating these processes has not been unambiguously identified. We have used an anti-idiotypic antibody approach to identify putative auxin receptors. We have raised polyclonal anti-idiotypic antibodies against purified anti-IAA antibodies in rabbits. Using these antibodies, we have detected a novel 65-kDa auxin-binding protein (ABP) that specifically recognizes natural and synthetic auxins but not the inactive structural analogs. We have further shown that this ABP is present all along the etiolated shoot which is consistent with the known auxin effect on gene expression in this organ. We have localized the protein to the cytosol and nucleus suggesting that it may mediate important auxin-regulated processes such as gene transcription. We have detected a unique protein of 67-kDa in the nuclei using these antibodies which raises the possibility that this is a covalently modified form of the 65-kDa ABP or a different and specific nuclear protein sharing epitopes. The ubiquitous distribution of the 65-kDa ABP, its specific interaction with auxins, and its unique localization to the cytosol and nuclei suggest that it may play an important role in auxin-mediated processes.

841 RELATIONSHIP BETWEEN ABA AND OSMOTIC STRESS IN THE REGULATION OF STORAGE PRODUCT GENE EXPRESSION IN MICROSPORE EMBRYOS OF BRASSICA NAPUS. Ronald W Wilen, Gijs JH van Rooijen, Larry A Holbrook*, Suzanne R Abrams* and Maurice M Moloney. Department of Biological Sciences, University of Calgary, Calgary Alta, Canada, T2N 1N4; * Plant Biotechnology Institute, NRC, Saskatoon Sask, Canada, S7N. 0W9 *Brassica napus* seeds accumulate three major proteins, napin, cruciferin, and oleosin, during embryogenesis. We have utilized the microspore-derived embryo system of *B. napus* to investigate hormonal and environmental regulation of these storage protein genes. Microspore embryos express all three genes, and the accumulation of mRNA encoding these three proteins was dramatically increased with exogenous abscisic acid (ABA) if applied at heart stage or later. An ABA-like response in transcript accumulation was also noted when the embryos were cultured in the presence of an osmoticum (osmotic pressure = 25.5 bars). Measurement of endogenous ABA concentrations during osmotic stress, and time course experiments of mRNA induction by both hormone and osmoticum, suggested that the regulation of napin and cruciferin gene expression by osmoticum was mediated by ABA. When the time course RNA blots were reprobed with an oleosin clone, it was found that the induction of oleosin mRNA transcripts was faster in the osmotic treatment than in the ABA treatment. In an attempt to clarify these conflicting results regarding the involvement of ABA in osmotically regulated gene expression, we have employed the use of ABA analogues. One of these analogues, 4-hydroxy-4-(5-hydroxy-3-methylpent-3-en-1-ynyl)-3,3,5-trimethylcyclohexanone (PBI-11), has no effect on the induction of storage product mRNA when applied at a concentration of 10 µM to 50 µM. When PBI-11 was added to the embryos prior to the addition of ABA, the effect of ABA on storage product gene induction was drastically reduced. These results suggested that PBI-11 may be a competitive inhibitor of ABA. We are currently using PBI-11 to investigate the relationship between osmotic stress and ABA.

842 OCCURRENCE OF AN IMMUNORELATED AUXIN-INDUCED PEPTIDE IN HIGHER PLANTS SPECIES. Chris A. Wozniak, USDA-ARS, Northern Crop Science Laboratory, Fargo, North Dakota 58105. A peptide of approximately 27kDa in relative molecular mass (by 2-D PAGE) was found to accumulate concommittantly with auxin-induced callus formation in *Sorghum bicolor*. This callus-associated peptide (CAP1) accumulated to become the most abundant peptide in callus tissues as observed on silver stained 2-D gels. A 2-D PAGE screen of whole plant organs detected this peptide in crown tissues, but not in anthers, ovules, seeds, leaves, leaf sheaths, roots or stems of sorghum; this peptide was greatly enhanced in crowns following whole plant treatment with natural or synthetic auxins. A second callus-associated peptide (CAP2) was found to accumulate in callus which had lost the ability to regenerate. A polyclonal antiserum raised against purified CAP1 also reacted with this 44kDa peptide on 2-D immunoblots. Both peptides also bind Con A and are considered glycoproteins. This antiserum reacted with two bands of approximately 23kDa and 27kDa in etiolated coleoptiles of sorghum and maize as well. Examination of callus of other plant species revealed the presence of an immunorelated peptide of identical size (by SDS-PAGE/westerns) in 14 of 15 grass species evaluated; six cultivars representing the three subspecies of *Oryza sativa* failed to show any immunoreaction. The tribe in which rice is placed, the Oryzeae, is often considered an arbitrary grouping of uncertain affinities. None of the 17 species of dicots or 4 species of non-gramineous monocots tested indicated the presence of any immunoreactive peptide. CAP1 is being evaluated for a possible role in auxin metabolism or binding and any taxonomic relevance it may have. EM localization and cDNA construction are being pursued.

843 MOLECULAR CLONING OF AN ETHYLENE-RESPONSIVE FLOWER SENESCENCE-RELATED 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE mRNA FROM CARNATION. Ky Young Park, Amir Drory and William R. Woodson. Purdue University, Department of Horticulture, West Lafayette, IN 47907 A PCR amplified tomato ACC synthase cDNA clone was used to screen a cDNA library prepared from mRNA isolated from senescing carnation flower petals. Two cDNA clones were isolated which represented the same transcript based on restriction mapping and nucleotide sequence analysis. The longest of the two clones, CARACC3, contained a 1950 bp insert with a single open reading frame of 516 amino acids encoding a protein of 58 kDA. The predicted protein was 63% and 60% identical to the deduced proteins from tomato and winter squash ACC synthase cDNAs, respectively. RNA gel blot analysis indicated the carnation ACC synthase cDNA represents a transcript which accumulates in senescing petals concomitant with increased ACC synthase activity and ethylene production. An increase in mRNA for carnation ACC synthase was detected in young presenescent petals following exposure to exogenous ethylene. Further analysis indicated CARACC3 did not detect an increase in transcript levels in wound leaves which exhibit increased ACC synthase activity. Genomic DNA gel blot analysis indicated the presence of at least a second gene in carnation which hybridized to CARACC3 under conditions of reduced stringency. We conclude CARACC3 represents a flower senescence-related ACC synthase mRNA.

844 A FLOWER SENESCENCE-RELATED mRNA FROM CARNATION AND PETUNIA ENCODES THE PUTATIVE ETHYLENE FORMING ENZYME. Hong Wang and William R. Woodson. Purdue University, Department of Horticulture, West Lafayette, IN 47907 The programmed senescence of carnation flower petals is a highly controlled development process which is under the regulation of the phytohormone ethylene. We have isolated several senescence-related mRNAs which accumulate specifically in senescing carnation flowers. We have identified one cDNA clone, pSR120, as sharing significant nucleotide sequence homology (68%) with the putative ethylene forming enzyme (EFE) from tomato. This cDNA clone represents an mRNA which accumulates in senescing carnation petals concomitant with the increase in EFE activity and ethylene production. Furthermore, treatment of presenescent flowers with ethylene results in a rapid induction of pSR120 transcript accumulation and EFE activity. The mRNA represented by pSR120 is constitutively expressed as a moderate level in leaves which are capable of converting ACC to ethylene. Pollination of carnations results in a rapid accumulation of pSR120 transcripts in styles followed by a subsequent accumulation in petals, which correlates with ethylene production by these organs. We have recently isolated a pSR120 homolog from petunia (PET120) which represents a mRNA exhibiting similar senescence-related patterns of expression. Antisense constructs have been transformed into petunia for further evaluation of PET120 function.

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845 SPATIAL PATTERNS OF EXPRESSION OF FLOWER SENESCENCE-RELATED GENE EXPRESSION IN CARNATION PETALS. Shimon Mayak, Amir Drory and William R. Woodson. Purdue University, Department of Horticulture, West Lafayette, IN 47907

The senescence of carnation flower petals is associated with increased production of the phytohormone ethylene which plays a critical role in regulating programmed cell death. The increase in ethylene is the result of de novo synthesis of both ACC synthase and the ethylene forming enzyme (EFE). The basal portion of petals accounts for most of the ACC synthase activity and exports ACC to the upper portion which converts ACC to ethylene and senesces. In addition, only the basal portion of petals is capable of responding to ethylene exposure by the production of autocatalytic ethylene. Examination of senescence-related (SR) gene expression revealed both the upper and basal portions of petals respond to ethylene with an increase in steady-state SR transcripts. Accumulation of ACC synthase mRNA was largely confined to the basal portion of petals following ethylene exposure. Therefore, spatial patterns of autocatalytic ethylene in carnation petals does not appear to be the result of overall difference in ethylene responsiveness, but rather spatial regulation of ACC synthase gene expression.

846 MOLECULAR INVESTIGATION OF PLANT GENES OF SYNTHETIC AUXIN 2,4-D DEGRADATION. Markusheva T.V., Kusova L.V, Sultanbekova M.N., Zhurenko E.Yu., Katkova E.G., Sovenko O.S. Institute of Biology, USSR Academy of Sciences, Bashkir Branch, 69 pr. Octyabrya, 450054 Ufa, USSR

An original plasmid pMK16 comprising expressible genetic determinants of the acetic acid fragment degradation of synthetic auxin 2,4-D has been constructed on the basis of the plasmid pBR322. A study of 2,4-D catabolism genes in genomes of Triticum aestivum, Pisum sativum and Zea mays has been done using the method of Southern DNA molecular hybridization with the plasmid pMK16 as a probe. The results show a considerable similarity among 2,4-D catabolism genes of plants of of different taxonomic groups.

847 A GLUTATHIONE S-TRANSFERASE GENE IS REGULATED BY ETHYLENE IN *ARABIDOPSIS*. Jianmin Zhou and Peter B. Goldsbrough. Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

Many developmental and physiological processes in plants involve ethylene and are accompanied by specific changes in gene expression. We have initiated experiments to elucidate the mechanisms that regulate gene expression in response to ethylene in *Arabidopsis*. *In vitro* translation of poly(A)-RNA from ethylene-treated and control plants indicate that significant changes in mRNA populations result from exposure to ethylene. A cDNA library was screened by differential hybridization to isolate cDNAs representing mRNAs that were altered in abundance by ethylene. An ethylene up-regulated cDNA has significant homology with a gene encoding glutathione S-transferase (GST) from maize. The abundance of GST mRNA in *Arabidopsis* increases with maturity of the plant, being barely detectable in seedlings and maximal in flowering plants. Ethylene increases the level of GST mRNA in seedlings and in leaves of both pre-flowering and flowering plants, and these responses are ameliorated by treatment with 2,5-norbornadiene. We are currently examining expression of this gene in the ethylene-insensitive mutant, *etr-1*. Hybridization analysis of genomic DNA at reduced stringency indicates that the cloned GST cDNA is a member of a multigene family in *Arabidopsis*. A second cDNA clone was isolated that represented a mRNA that was down-regulated by ethylene. This cDNA encodes a chlorophyll *a/b* (Cab) binding protein. Cab mRNA levels decline in leaves exposed to ethylene. We are currently examining the expression of this gene during leaf senescence in normal and *etr-1* plants.

848 TRANSIENT OVERPRODUCTION OF CYTOKININ IN TRANSGENIC PLANTS BY RECONSTRUCTED IPT GENES. A. Smigocki. Plant Molecular Biology Lab. USDA/ARS, Beltsville, MD 20705-2350

Transgenic tobacco and tomato plants carrying the isopentenyl transferase (ipt) gene from A. tumefaciens are being analyzed for the effects of transient overproduction of cytokinins. Temporal and tissue specific expression of the cytokinin gene appears to directly influence the resulting plant phenotype. When the ipt gene was placed under the control of a wound (IIk-ipt) or a light (LR-ipt) inducible promoter, transgenic N. plumbaginifolia plants were characterized by extreme apical dominance with little release of axillary buds. The IIk-ipt plants bolted sooner and had larger leaves of lower specific weight than controls. The chlorophyll content of the leaves was similar to the controls even though the leaves were lighter in color. Accumulation of ipt messages was detected within 15 minutes after wounding. In contrast, plants transformed by the heat regulatable ipt (HS-ipt) gene exhibited phenotypes associated with excess cytokinin production even when thermally not induced (Plant Mol. Biol. 16:105-115, 1991). They had less developed root systems, increased growth of axillary buds, and in general, were shorter and greener than control plants. It appears that the effect of cytokinin on preprogrammed patterns of differentiation in plant cells is dependent on localization in specific cells, tissues, or organs as well as the stage of cell development.

849 HORMONAL REGULATION OF CELLULASE DURING ADVENTITIOUS ROOTING IN SOYBEAN. Elizabeth C. Kemmerer and Mark L. Tucker. USDA/ARS, Plant Mol. Biol. Lab., BARC-West, Beltsville, MD 20705.

Exogenously applied auxin induces adventitious rooting in seedling cuttings. Other factors, including auxin-induced ethylene production, affect this developmental process. Cellulase breaks down cell wall components in the stem, allowing the new roots to emerge. We are studying the regulation of cellulase enzyme(s) and gene(s) active during adventitious rooting in soybean. Cellulase enzyme activity increases 10-fold in hypocotyls from the day the soybean seedlings are cut and treated with auxin to the day of maximum hypocotyl swelling and root emergence, after which the cellulase enzyme activity decreases as the roots elongate. Epicotyl and leaf tissues, which do not swell or form roots, show no distinct pattern of cellulase activity. Plants treated with water, reacting only to endogenous auxin, show a similar pattern of cellulase enzyme activity accumulation but at half the level of activity. We have isolated and sequenced 688 bp of a soybean cellulase gene encompassing 241 bp of coding sequence and 447 bp of 3' flanking sequence. Compared with other cellulases, the partial coding sequence of the soybean cellulase clone displays 55.6% and 57.8% nucleotide sequence identity with avocado fruit and bean abscission cellulases, respectively. At the deduced amino acid level, the partial soybean clone shares 55.0% and 48.1% identity with avocado fruit and bean abscission cellulases, respectively. Preliminary RNA gel blot analyses using the soybean clone as a probe indicate that cellulase mRNA levels also correlate with stem swelling and root emergence. Studies at the enzyme activity and transcript levels are being performed with anti-auxin and anti-ethylene agents.

850 FRUIT SPECIFIC EXPRESSION OF A REPORTER GENE THAT IS FUSED TO 5' REGION OF AN AUXIN-REPRESSED GENE. A.S.N Reddy, M. Costa . G. An', and B.W. Poovaiah. Lab. Plant Mol. Biol. & Physiol., Dept. of Horticulture, 'Insutute of Biological Chemistry, Washington State University, Pullman, WA 99164

To study the mode of auxin action at the molecular level, we have isolated and characterized three auxin-regulated cDNA clones (λSAR1,λSAR2 and λSAR5) from strawberry fruit (Reddy *et al. Plant Mol. Biol.*, 14:643-653, 1990; Reddy and Poovaiah *Plant Mol. Biol.*, 14:127-136, 1990). The mRNA corresponding to SAR5 is repressed during auxin-induced fruit growth. Our studies indicate that there is a strong positive correlation between growth of strawberry fruit and repression of SAR5 mRNA. We isolated a genomic clone corresponding to the auxin-repressed cDNA by screening the strawberry genomic library. The 5' region of the SAR5 gene was identified by restriction endonuclease mapping and hybridization with oligo probes corresponding to the 5' untranslated region of the SAR5 cDNA. A binary vector Ti plasmid containing part of the 5' flanking sequence of the SAR5 gene and chloramphenicol acetyltransferase (CAT) reporter gene was constructed and used for transforming tobacco. Five independent transgenic plants were generated and analyzed for the reporter gene expression in leaves, flower buds, flowers and fruits. CAT activity was observed predominantly in the fruit of transgenic tobacco indicating tissue specific activity of the promoter. The effect of auxin on the expression of the reporter gene is under investigation.

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851 ISOLATION OF AN AUXIN-REPRESSED cDNA USING ANTIBODIES PRODUCED AGAINST A 52 kDa PROTEIN FROM STRAWBERRY FRUIT. S. Chengappa, A.S.N. Reddy, Y. Croissant-Sych, S. Patil & B. W. Poovaiah. Lab. of Plant Mol. Biol. & Physiol., Dept. of Horticulture, Washington State University, Pullman, WA 99164

In our earlier studies we demonstrated the accumulation of a 52 kDa protein in auxin-deprived fruit. Auxin treatment to the fruit resulted in the fruit growth and disappearance of the 52 kDa protein. Studies on the accumulation of this protein with normal and variant strawberry fruits that were treated with or with out auxin indicated that there is positive correlation between the accumulation of the 52 kDa protein and cessation of fruit growth. To isolate cDNAs that code for the 52 kDa protein, we constructed an expression library from Poly(A) RNA isolated from auxin-deprived fruits and screened with the antibodies raised against the 52 kDa protein. This has resulted in isolation of fifteen positive clones. Cross hybridization studies indicated that the majority of the isolated clones fall in to one group. One of the clones (# 8) that contained the longest insert was used to monitor the mRNA level in auxin-treated and auxin-deprived fruits. High level of mRNA corresponding to the clone #8 was observed in auxin-deprived fruit and no detectable mRNA was found in auxin-treated fruit. The level of mRNA corresponding to this clone at different stages of fruit growth and development and the overall significance of auxin-repressed gene expression will be discussed.

852 IDENTIFICATION OF A WHEAT PROTEINASE PROMOTER REGULATED BY GIBBERELLIN IN OAT ALEURONE LAYERS

F.J. Cejudo, P. Stabel, T.K. Ghose and D.C. Baulcombe. The Sainsbury Laboratory, John Innes Centre for Plant Science Research, Norwich, NR4 7UH, United Kingdom.

During wheat germination the plant hormone, gibberellin, induces the synthesis of hydrolytic enzymes in the aleurone layer. Induction is associated with increased levels of the corresponding mRNAs. The cDNA of one induced transcript was shown to be homologous to the mammalian thiol-proteinase, cathepsin B. Primer extension experiments, carried out to map the transcriptional start site, resulted in the synthesis of two major extended species, which were cloned employing a PCR-based procedure. Results from sequence analysis of the two extension products showed them to be closely related, consistent with transcription from at least two genes. An insert containing one of these genes was isolated from a phage genomic library. A 2.5 kb fragment upstream from the putative translational start site was fused to the ß-glucuronidase reporter gene, in order to test for promoter activity. Ballistic bombardment of oat (*A. sativa*) aleurone layers with this construct showed gibberellin regulated expression of the reporter gene. Deletion analysis is presently underway to identify the functional elements within the promoter which confer regulation by gibberellin.

853 HORMONE RESPONSIVE ELEMENTS IN PROMOTERS OF BARLEY α-AMYLASE GENES. Bhuvana Gopalakrishnan, Buracnai Sonthayanon and S. Muthukrishnan, Department of Biochemistry, Kansas State University, Manhattan, Kansas 66506 and Mahidol University, Bangkok, Thailand.

Protoplasts were prepared from barley aleurone layers using "Onozuka Cellulase" digestion and purification through a Percoll gradient. Protoplasts prepared by this procedure had high viability. They were responsive to gibberellic acid (GA) as measured by the stimulation of α-amylase synthesis. The stimulation by GA was counteracted by abscisic acid (ABA). In the presence of polyethyleneglycol, the protoplasts took up exogenously added plasmid DNA containing the reporter gene coding for chloramphenicol acetyltransferase (CAT) linked to a GUS-promoter from cauliflower mosaic virus or to barley α-amylase gene promoters and expressed CAT activity. Using this transient expression system, 5'-upstream fragments from high and low pI α-amylases genes have been shown to confer GA and ABA-responsiveness on the CAT reporter gene. Similar studies with progressive deletions of the promoter fragments have led to the identification of approximate locations of GA- and ABA-responsive elements in both high and low pI barley α-amylase genes.

854 HORMONE - SUCROSE INTERACTION ON INDUCTION OF HAPLOIDY IN SAFFLOWER (Carthamus tinctorius L.) B. RAJENDRA PRASAD AND S.Y. ANWAR, Department of Genetics, Osmania University, HYderabad - 500 007, India.

Considerable variation was noticed in the degree of response to callus induction and regeneration in the various combinations of growth regulators used. In general, the response of cultured anthers to added auxins was highly variable. Among the four auxins tested 2,4-D showed a high callusing frequency followed by NAA, IAA and IBA. The cytokinin type and concentration influenced greately the callusing and shoot regeneration frequency. BAP showed highest rate of callusing and shoot regeneration. Significant interaction between sucrose and hormone was observed. Optimum callus induction was observed in presence of BAP (0.5mg/l), NAA (0.1 mg/l) and 30% sucrose. Shoot regeneration was observed on MS supplemented with 2 mg/l BAP, 0.5 mg/l NAA and 2% surcose and rhizogenesis on MS (half-strength) medium supplemented with 0.1 mg/l NAA and 1% sucrose. Cytological studies of anther derived plants showed two ploidy levels, where the haploids were predominant (64%).

855 EXPRESSION OF A NITRILASE FROM AN ARABIDOPSIS cDNA SYNTHESIZING THE PLANT HORMONE INDOLEACETIC ACID. Dieter Bartling, Matthias Seedorf and Elmar W. Weiler. Ruhr-Universitat, Pflanzenphysiologie, Bochum, Germany

The auxin indoleacetic acid (IAA) is recognized as one of the major plant growth promoting hormones. It is involved in cell elongation, fruit growth, apical dominance, root formation, fruit and leaf abscission and many other processes. Little is known about the molecular basis of auxin action and biosynthesis. The pathway for IAA biosynthesis is not fully understood. In the Brassicaceae, IAA is believed to be synthesized from tryptophan via indoleacetaldoxime (IAOx) and indoleacetonitrile (IAN) in close proximity to the plasma membrane[1].

In a screening program for plasma membrane protein genes from A. thaliana, we have isolated a cDNA clone, PM81, which resembled a nitrilase gene encoded by Klebsiella ozaenae[2]. The induction of PM81 cDNA in bacterial cells resulted in the production of a fusion protein which was found to be enzymatically active. The PM81 cDNA encoded protein efficiently converts IAN to IAA. The identity of the reaction product was unambiguously proven by HPLC, monoclonal-antibody ELISA, HPLC-ELISA and HPLC-mass spectrometry. It is thus likely, that the last step in IAA formation in Arabidopsis is catalyzed by the nitrilase encoded by PM81. The PM81 clone is the first molecular tool to investigate the biosynthesis, spatial and temporal expression of an IAA synthesizing enzyme in plants.

1.Ludwig-Müller, J. & Hilgenberg, W. Physiol.Plantar. 74, 240 (1988)
2.Stalker,D.M., Malyj,L.D. & McBride, K.E. J.Biol.Chem. 263, 6310 (1988)

856 THE *AXR2* MUTANT OF *ARABIDOPSIS* IS DEFECTIVE IN AUXIN INDUCIBLE GENE EXPRESSION. Candace Timpte, *Pedro Gil, *Yang Liu, *Pam Green and Mark Estelle. Indiana University, Biology Department, Bloomington, Indiana 47405 and *MSU DOE Plant Research Laboratory East Lansing, MI 48824. The dominant mutation of *Arabidopsis thaliana ,axr2*, confers resistance to auxin and other plant hormones including abscisic acid, ethylene and cytokinin. The *axr2* mutation dramatically alters the phenotype of the plant, causing a dwarfed appearance and defects in both the root and shoot gravitropic response. One possible explanation for the multiple hormone resistance phenotype is that *axr2* plants are defective in a component which is required for transduction or regulation of several hormone signals. Examination of hormone regulated gene expression provides a rapid molecular assay for the response of the mutant to hormone. Auxin responsive genes have been described in soybean (McClure and Guilfoyle *Plant Mol. Biol.* 9:611-625 1987). Expression of the small auxin up RNAs (SAUR) genes increases within minutes of auxin application and is not induced by other plant hormones or a variety of stress conditions. A SAUR gene of *Arabidopsis* has been cloned and designated SAUR-AC1 (Gil *et al.* abstract this meeting). We have characterized the auxin response of wild type and *axr2* plant tissue using the SAUR-AC1 gene as a probe for both S1 nuclease analysis and RNA blotting. SAUR-AC1 expression increases dramatically in the hypocotyl of etiolated wild type seedlings upon treatment with 50 um 2,4-D. Expression of SAUR-AC1 is not detectable in etiolated tissue of *axr2* upon auxin application. Wild type rosette leaves express a low level of SAUR-AC1 which increases with exogenous auxin treatment. In contrast to the etiolated tissue, preliminary results indicate constitutive expression of SAUR-AC1 in *axr2* rosette leaves with or without application of 2,4-D. These experiments indicate that *axr2* mediates the primary response to auxin though involvement in the regulation of auxin responsive genes. We are also characterizing SAUR-AC1 gene response in the auxin resistant mutants *axr1* and *aux1*. The kinetics of SAUR-AC1 induction and expression in response to various concentrations of auxin are being characterized in wild type and mutant plants.

POSTER ABSTRACTS

857 REGULATION OF ETHYLENE FORMING ENZYME IN POLLINATION INDUCED SENESCENCE OF ORCHID FLOWERS
Jeanette Nadeau and Sharman D. O'Neill. University of California. Department of Botany, Davis, California 95616

Senescence of plant organs is a fundamentally important biological process that is regulated by a precise developmental program. Senescence of plant organs involves a number of coordinated changes in cell metabolism that ultimately lead to cell death. In the orchid flower, these processes are initiated by pollination and are manifested by changes in color of the perianth, followed by hyponastic closure and drying. Pollination induces ethylene biosynthesis by the flower and possibly enhances the sensitivity of the perianth to ethylene which, collectively, trigger senescence of the perianth. To elucidate the mechanisms by which pollination induces the suite of developmental changes associated with flower senescence, the role of the ethylene forming enzyme (EFE) in this process has been examined. Several cDNA clones with homology to the tomato fruit pTOM13 cDNA (putatively identified as encoding EFE) have been isolated from *Phalaenopsis* gynoecium and perianth libraries. Characterization of the temporal and spatial patterns of expression of these cDNA clones, as well as the pattern of EFE activity during the process of *Phalaenopsis* flower senescence will be presented. Data will also be presented that assesses the role of auxin and ethylene in regulating EFE gene expression.

858 PURIFICATION AND MOLECULAR BIOLOGY OF 5'-METHYLTHIOADENOSINE NUCLEOSIDASE FROM SEEDS OF *PISUM SATIVUM*.
S.M.Dunn and J.A.Bryant, University of Exeter, Dept. of Biological Sciences, Exeter, EX4 4QG, England. M.Kerr, Shell Research Ltd., Sittingbourne, Kent, ME9 8AG, England.

5'-Methylthio-5'-deoxyadenosine (MTA) is a by-product of S-adenosylmethionine (SAM) metabolism from which it is produced by at least five known biochemical pathways. These include i) the aminopropyl-transferase reactions of polyamine formation, necessary during rapid cell division and for the suppression of senescence related processes, and ii) synthesis of the volatile plant hormone ethylene via its precursor 1-amino-cyclopropane-1-carboxylic acid. Indeed these two systems appear antagonistic in their modes of action and, in some tissues, compete directly for SAM. The importance of MTA is two-fold. Firstly, MTA is highly toxic/regulatory to many enzyme systems including SAM-dependent methyltransferases, DNA methylases, and the polyamine-synthesising spermine synthase. Secondly, MTA locks up the methylthio moiety of methionine which is usually limiting in proliferating cells and those tissues actively evolving ethylene such as climacteric fruit. Plant tissues avoid this detrimental MTA accumulation by rapid metabolism involving MTA nucleosidase which cleaves the thioether hydrolytically yielding adenine and 5'-methylthioribose (MTR). This resulting MTR pool is then recycled back to SAM via the methionine salvage pathway. In addition, MTA nucleosidase levels are regulated by endogenous MTA production and may increase several-fold in preclimacteric fruit as a consequence of rapid cell-division. Our interest in the regulation of this enzyme has prompted us to purify it from seeds of *Pisum sativum* with a view to studying its molecular biology. The final chromatographic step involves affinity chromatography on Formycin A-sepharose and yields an activity which cannot be stained with Coomassie Blue or silver and yet can be excised from the gel following non-denaturing PAGE. A simple and rapid coupled assay was developed to replace the existing lengthy and expensive radio-TLC method. We have subjected the purified preparation to N-terminal sequence analysis and are currently engaged in the construction of oligonucleotides for probing cDNA libraries and to serve as primers for PCR

859 CYTOCHEMICAL, ULTRASTRUCTURAL, AND BIOCHEMICAL CHANGES IN SOYBEAN ABSCISSION ZONES.
Anxiu Kuang, Rownak J. Rahmatullah, Roland R. Dute, Narendra Singh, and Curt M. Peterson. Auburn University, Department of Botany and Microbiology, Auburn, Alabama 36849.

Leaf blade excision induces abscission in petioles of soybean, Glycine max (L.) IX93-100, that is accelerated by ethephon and inhibited by an auxin or cytokinin. The petiole distal abscission zone (AZ) is being used to determine the cytochemical, ultrastructural, and biochemical changes following induction of abscission. Cytochemical studies reveal enlarged cells within the AZ showing large nuclei and protein staining. Prior to abscission, prominent amyloplasts appear in the AZ, increasing with time. Simultaneously, starch grains disappear within amyloplasts of the starch sheath of the pulvinus distal to the AZ. The middle lamella between cells in the separation layer of the AZ breaks down followed by degradation of microfibrils in the cell wall as cell separation proceeds. In vivo labelling of proteins in the AZ with ^{35}S-methionine shows that 23, 26, and 70 kDa polypeptides appear during abscission with concomitant disappearance of 24 and 60 kDa polypeptides. The changes in polypeptide patterns are under hormonal regulation. Preliminary in situ hybridization and immunocytochemistry coupled with Northern and Western blot analysis using DNA probes and antibodies for cell wall degrading enzymes reveal a spatial pattern of gene expression at the AZ.

860 T-DNA INSERTIONAL MUTAGENESIS IN ARABIDOPSIS: THE DWARF MUTANTS.
Yewen Wu and Kenneth A. Feldmann, University of Arizona, Department of Plant Sciences, Tucson, AZ 85721.

More than 8,000 transformed lines have been generated, with the seed infection/transformation procedure, and screened for dwarf phenotypes. Dwarfs are characterized by a reduced stature (~5-9 cm in height), robust stem, and short dark green leaves. Nine segregating dwarf lines, that fit this description, were identified. These are not the gibberellic acid-type mutants described in the literature in that all are fertile, without the addition of GA. Of these lines one (dwarf #2) segregated for a deficiency of dwarf plants and has subsequently been shown not be be due to a functional T-DNA insert. The other eight lines all segregated 3:1 (wildtype:mutant) for the dwarf phenotype suggesting that all the dwarf mutants are due to recessive mutations. Four of the lines (#1, #13, #60, and #79) segregate for a single insert (1:2:1: KanR, dwarf: KanR, wt: KanS, wt), and two lines (#32 and #35) for two inserts (4:11:1). Additional genetic analyses indicate that these six mutants were induced by a T-DNA. Two lines (#28 and #87) segregate somewhat anomalously and we have shown that one of these mutants was not induced by a functional T-DNA insert. Phenotypic characterization of the dwarf mutants indicates that two of these eight (dwarfs #13 and #60) have organs, including the stem, petioles, pedicels, and siliques that are twisted. All dwarfs have short roots, internodes, hypocotyls, cotyledons, petals, stamens, and all other organs examined, relative to wildtype. The first mutant examined (#1) has small cells relative to the wildtype. The phenotypes of the other eight dwarfs suggest that they also have small cells. We are currently making all pairwise crosses of all dwarf the mutants to test for allelism. We are also testing these mutants in the presence of a number of hormones and hormone inhibitors.

861 The Modulation of T-DNA-encoded IAA Biosynthesis in Transformed Tobacco Cells.
Gary A. Kuleck[1], Robert C.Black[2] and Andrew N. Binns[3]
[1]USDA, Plant Hormone Lab, Beltsville, Md. 20705; [2]Pennsylvania State University-Media, Biology Dept., Media, Pa. 19063; [3]University of Pennsylvania, Department of Biology, Philadelphia, Pa. 19104

Although the control of IAA biosynthesis in plant cells is poorly understood, some insight can be gained by examining the indoleacetamide hydrolase (AH) activity which compnses the last step in the indoleacetamide pathway that has been defined in *Agrobactenum*-transformed plant cells. We have chosen to focus on the second enzyme in the pathway, from the *tms 2* locus of the T-DNA since this enzyme is easily assayable on a micro scale. Previously, we have shown that the AH activity levels undergo a consistent pattern of modulation in the growth cycle of transformed cells which is strongly correlated with the levels of free IAA in these cells, suggesting that IAA biosynthesis is regulated in these cells (Kuleck *et.al*, Plant Physiol. 95, 1004-1011). Furthermore, the timing and extent of the enhanced AH activity has been shown to be strongly influenced by the wounding of the cells, the availability of fresh nutrients in the media, and the initial presence of a low concentrations of IAA to stimulate AH activity.

AH activity has also been examined from the standpoint of determining the requirements for activating 'silent' or suppressed *tms 2* loci in plants regenerated from transformed cells which have intact, potentially functional T-DNA (Black *et. al*, Plant Physiol. 80, 145-151). The requirements for re-activation of the *tms* loci in the pith of one set these plants during culturing are the same as those of callus cultures: wounding of the tissues, a supply of fresh nutrients, and a brief IAA treatment. In comtrast, in another cloned line of plants with the same pattern of T-DNA integration, these conditions were necessary but not sufficient and re-activation occurred only in the presence of the DNA demethylating agent, 5-azacytidine. Thus, T-DNA-encoded IAA biosynthesis in these plant cells is regulated in two fundamentally different manners which reflect, not T-DNA integration site differences, but differences in host plant effects. In summary, the results reported here demonstrate that not only can the host affect T-DNA-encoded IAA biosynthesis, but that such interactions may be essential for the survival of the transformed plant cell.

862 ISOLATION AND PROPERTIES OF THE CYTOPLASMIC GIBBERELLIN RECEPTOR OF COTTON OVULES.
M.H.Abidova, G.T.Mavlanov, A.Abdukarimov. Sadykov's Institute of bioorganic chemistry Academy of Sciences Uzbek SSR, Tashkent, USSR.

Gibberellin dependent DNA-binding protein (GA_3-DNAbp) was isolated by consequent gel filtration, ion exchange and tandem affinity chromatography on DNA-cellulose (in the absence and than in the presence of GA_3) from cotton ovules. This protein was M_r 160 kDa, consist of two chains of 72 and 78 kDa, pI 4,6 and glicozilated (27±2 % of sugar, D-mannose and 2-amino-2-deoxy D-galactose). K_d of protein-GA_3 complexes are $0,85 \times 10^{-7}$M. GA_3-protein complexes are binding with /^{3}H/-DNA ($K_d = 0,4 \times 10^{-8}$M) and the protein without of GA_3 don't binding with DNA. Protein is localized in cytoplasm and partial in nucleus. GA_3-DNAbp in complex with hormon stimulates RNA synthesis by isolated nuclei, induces synthesis of new proteins and enhances incorporation of /^{14}C/glucose into the cell surfase cellulose.

POSTER ABSTRACTS

863 MOLECULAR CHARACTERISATION OF THE AXR1 LOCUS OF *ARABIDOPSIS THALIANA*. Ottoline Leyser, Cynthia Lincoln, Jocelyn Turner, Doug Lammer, and Mark Estelle. Indiana University, Dept. of Biology, Bloomington, IN 47405.

The AXR1 locus of *Arabidopsis* is defined by a series of mutant alleles conferring resistance to normally inhibitory levels of exogenously added auxins. The mutations are all recessive and pleiotropic, giving rise to plants which are dwarfed and show reduced apical dominance and defective root gravitropism. The mutant plants are also resistant to infection by the plant pathogen *Agrobacterium rhizogenes* which causes hairy root tumors on wild-type plants. The degree of auxin resistance conferred by the different axr1 alleles correlates with the severity of the other aspects of the phenotype. This strongly suggests that the AXR1 gene product is involved in auxin perception or response. In the hope of learning more about auxin action in plants we are cloning the AXR1 gene. Closely linked restriction fragment length polymorphisms (RFLPs) between the Columbia and Niederzenz genetic backgrounds have been identified. One such RFLP maps approximately 0.5 centiMorgans proximal of ARX1 and a second RFLP maps <1 centiMorgan distal to AXR1. The DNA probes which detect these RFLPs were used to initiate a chromosome walk in a genomic library constructed a Yeast Artificial Chromosome (YAC) vector. A YAC was detected which hybridizes to both the distal and proximal probes and hence encompasses the AXR1 gene. We are currently using DNA from this YAC to transform mutant axr1 plants in an attempt to rescue their phenotype and hence confirm that we have cloned AXR1. We are taking two approaches to this mutant rescue. Firstly, we are using conventional transformation of axr1 root explants with disarmed strains of *Agrobacterium tumefaciens* carrying sections of the YAC DNA cloned in binary vectors. Secondly we are transforming axr1 stem sections with virulent strains of *Agrobacterium rhizogenes* carrying the same binary vectors. We predict that cotransformation of the *rhizogenes* T-DNA and the wild-type AXR1 gene will lead to the development of hairy root tumors not seen on axr1 mutant plants. We are also using the YAC DNA to probe a cDNA library to identify transcripts in this region. Progress on these projects will be reported.

866 FUNCTIONAL ANALYSIS OF THE *AGROBACTERIUM TUMEFACIENS* T-DNA GENE 6^b. John M.E. Clement, R.A. Schilperoort and J.H.C. Hoge. Dept. of Plant Molecular Biology, Clusius Laboratory, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The T-DNA gene 6^b interferes with tumor formation and normal plant development. Stem infection experiments on *Nicotiana glauca* result in significantly larger tumors when the T-*aux* 1 and 2 genes or the T-*cyt* gene are transferred together with the $T\text{-}6^b$ gene; the $T\text{-}6^b$ gene also inhibits the shooty character of tumors induced by the T-*cyt* gene. *N. tabacum* SR1 plants transformed with the $T\text{-}6^b$ gene display several abnormal developmental features: *in vitro* propagated shoots show aberrant root formation along with abundant callus formation at the base of the stem while *in vitro* cultured seedlings show enhanced development with increased branching of the primary root. Translational fusion of the promoter of the $T\text{-}6^b$-gene to a GUS-reportergene and introduction of this construct in *N. tabacum* SR1 plants demonstrated that the $T\text{-}6^b$-promoter is highly active in the embryo and roots of seedlings and the root system of mature plants. These and other observations point to an auxin-like effect of the $T\text{-}6^b$ gene-product. Therefore we are analysing $T\text{-}6^b$ transformed plants and normal plants for differences in auxin content and auxin metabolism.

864 DOES PCH313 ENCODE THE ETHYLENE FORMING ENZYME? Ann M. Callahan, Ken E. Nichols Jr., Reuben Cohen, Deanna Fishel, Linda Dunn and Peter H. Morgens.

The ability to control the level of ethylene evolution in fruit could be a means to regulate the ripening/softening process since ethylene is involved in the regulation of some ripening genes. Hamilton et al. have suggested that pTom13 may encode the ethylene forming enzyme (EFE) in tomato (Nature, 346:284-287 1990). If it encodes EFE then there should be a strong correlation between the rates of ethylene evolution and the amount of related RNA accumulated. Two experiments were set up to examine the correlation of RNA accumulation with ethylene evolution using pch313 (a pTom13 heterologous gene in peach). The first involved measuring ethylene evolution and the amount of pch313 related RNA on individual peach fruit during the climacteric period (time of ethylene evolution and softening). The rate of ethylene evolution was higher in one cultivar of fruit but the amount of pch313 RNA was less. The relationship of softening to pch313 RNA accumulation and ethylene evolution will be presented. The second experiment utilized fruit from climacteric species (plum, apricot, pear) and non-climacteric species (strawberry, blackberry, cherry) which do not have an increase in ethylene during ripening. When pch313 RNA was measured during ripening it was found that cherry, blackberry and plum had no increase in pch313 RNA while apricot, pear and strawberry had an increase in pch313 RNA. Pch313 related RNA was present in all species tested but its correlation with the levels of ethylene evolved is not straightforward. There may be other forms of regulation acting or pch313 does not encode the EFE.

867 CYTOKININ-GENES INVOLVED WITH PLANT MORPHOGENESIS. Leech, Mark J., Cathie Martin and Trevor L. Wang. John Innes Institute, Colney Lane, Norwich, NR4 7UH, U.K.

We are interested in elucidating the molecular mechanisms involved in plant morphogenesis and have chosen leafy shoot (gametophore) formation in the moss *Physcomitrella patens* as a model system. The formation of these structures involves a switch in development from filamentous growth to one exhibiting radial symmetry, and requires cytokinin. Thus, we are attempting to isolate cytokinin-regulated genes that may be involved with morphogenesis in *P. patens*. Differential screening techniques have been used to screen a cDNA library constructed from poly(A)$^+$RNA isolated from a pooled tissue sample treated with cytokinin for 2, 4, 6 or 8 hours. The library was screened with a cDNA probe enriched for cytokinin-induced sequences, using a subtractive hybridization protocol, together with a probe from untreated tissue. Fifteen clones were purified which showed differential hybridization in the library screen; however, dot blot analysis of 10 of these clones showed no regulation after 6 hours treatment with cytokinin. Material from a mutant that cannot form buds unless cytokinin is added ot its culture medium is also being used to prepare probes for differential screening. As a second approach, we are seeking genes that encode transcription factors that may control differential gene expression in response to cytokinin. Using an oligonucleotide corresponding to the most highly conserved region of the *myb* DNA-binding domain, we have isolated 2 classes of *myb*-related genes from a *P. patens* cDNA library. Preliminary analysis of transcription of these genes suggests that transcripts are not regulated by cytokinin within 6 hours of treatment but the transcripts may be developmentally regulated since an up-regulation corresponds with the production of leafy shoots. Further experiments are in progress to analyse the function of the *myb* genes in *P. patens* including the manipulatoin of normal patterns of expression by transformation experiments.

865 PROMOTER ANALYSIS AND EXPRESSION OF AUXIN-REGULATED GENES FROM TOBACCO. Frans N.J. Droog, Cees J.M. Boot, Albert M. Mennes, Paul J.J. Hooykaas, Kees R. Libbenga and Bert J. van der Zaal. Leiden University, Dept. of Plant Mol. Biol., Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

After treatment of auxin-starved tobacco cells with auxins several mRNAs are rapidly and transiently induced prior to cell division (Van der Zaal et al. (1987) Planta 172, 514-519; Plant Mol. Biol. 10, 145-157). The level of 103-like mRNAs peaks around 6 hr after auxin treatment in a close correlation with the eventual cell division response, but a function for the encoded proteins could not be established. Since we are interested in the molecular mechanisms underlying auxin action we are investigating factors involved in 103-like gene expression. Analysis of two promoters of 103-like genes, fused with the gene for GUS, indicated that a 100 bp region around -300 prior to ATG is responsible for auxin-regulated and root tip-specific expression. Several nuclear proteins bind within this region. Their importance for the expression is under investigation. Compounds known to interact with signal transduction pathways were tested for their ability to induce or block 103-gene expression. Calcium and internal pH are possibly involved. We are also investigating the role of auxin-binding proteins in auxin signal perception at the plasmamembrane.

868 CLONING A FAMILY OF EARLY AUXIN-INDUCIBLE GENES IN *ARABIDOPSIS THALIANA*.* Steffen Abel and Athanasios Theologis. Plant Gene Expression Center, ARS-USDA, 800 Buchanan St., Albany, CA 94710 and Dept. of Plant Biol., UC Berkeley, CA 94720

Auxin controls various aspects of plant growth and development. The hormone is known to regulate the transcription of specific genes in a variety of plant tissues (1, 2, 3). The polypeptides encoded by these genes share four highly conserved regions. In order to gain insight into their structural diversity and functional significance, we have cloned the members of a gene family encoding such polypeptides in *Arabidopsis thaliana*. This was accomplished by PCR using degenerate primers homologous to the conserved protein domains and with genomic DNA as template. The PCR products were sequenced and used for RNA hybridization analysis with RNA from auxin treated and untreated hypocotyls. Subsequently, full length cDNAs and genomic sequences were isolated for those genes that respond to the hormone in a primary fashion (1).

(1) Theologis ,A., et al. (1985) J Mol Biol 183:53-68 ; (2) Ainley,W., et al. (1988) J Biol Chem 263:10658-10666; (3) Conner , T.W., et al. (1990) Plant Mol Biol 15:623-632

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POSTER ABSTRACTS

870 GENETIC TRANSFORMATION OF CITRUS PROTOPLASTS BY PEG-MEDIATED DIRECT DNA UPTAKE AND THE REGENERATION OF PUTATIVE TRANSGENIC PLANTS. J.L. Schell, G.A. Barthe and J.W. Grosser. Univ. Fla., Citrus Research and Education Center, Lake Alfred, FL 33850

Transformation of protoplasts by direct DNA uptake is a promising procedure for many major crop species. A reliable protoplast-to-tree system exists for many Citrus species and Citrus relatives, and this system can be easily modified for use in transformation of Citrus. Protoplasts isolated from Hamlin sweet orange (Citrus sinensis [L.] Osbeck) were transformed by PEG-mediated direct DNA uptake using plasmid pCB10-106, containing a bacterial resistance gene for spectinomycin at 100 µg/ml, as well as the GusA and NPTII genes as scoreable markers. Eleven putatively transformed plants (out of 850 tested) were identified by histochemical assay and polymerase chain reaction. Southern analysis is currently being performed to confirm transformation. These results mark the second reported transformation and recovery of putative transgenic plants of sweet orange. The groundwork has now been laid for the insertion of genes of interest into Citrus for the improvement of important Citrus cultivars.

871 IDENTIFICATION AND CHARACTERIZATION OF THE YEAST GENES THAT ARE HOMOLOGOUS TO THE S-PHASE SPECIFIC GENE *(cyc07)* IN SYNCHRONOUS CULTURES OF *Catharanthus roseus* CELLS. Masaki Ito, Akira Yasui and Atsushi Komamine, Biological Institute, Faculty of Science, Tohoku University, Sendai 980, Japan.

The S-phase specific cDNAs were isolated from the synchronous cultures of *Catharanthus roseus* cells. One of the obtained genes, which is named as *cyc07*, was analyzed. This gene, *cyc07*, is expressed specifically in the S phase in synchronous cultures. The nucleotide sequence of this cDNA contains one open reading frame, which encode a highly basic protein as are histones. In two-dimensional gels, *in vitro* translated products of *cyc07* mRNA showed similar mobility as histones. A data base search revealed that a sequence homologous to a nucleotide sequence of *cyc07* cDNA is present in the down stream region of the SIR3 gene in the genome of yeast (*Saccharomyces cerevisiae*). We could conclude that the nucleotide sequence homologous to *cyc07* is an unknown yeast gene that is different from SIR3 gene, because the homologous region was present down-steam from the reported 3' end of SIR3 mRNA. In this homologous region, we found one open reading frame (ORF) that is partly contained on a reported yeast genomic clone that contains entire SIR3 gene with its ORF. The entire coding sequence was cloned from a genomic library of yeast. The nucleotide sequence and protein sequence predicted from ORF had a significant similarity to those of *cyc07* through the entire coding region. In the synchronized culture of yeast cells, this homologous gene was expressed preferentially in the S phase during the cell cycle. this pattern of expression in yeast was very similar to that of *cyc07* in *C. roseus* cells. The function of this homologous gene will be discussed from the results of gene disruption and tetrad analysis in yeast.

872 CLONING AND CHARACTERIZATION OF THE GENES WHICH SPECIFICALLY EXPRESS IN CALLUS OF RICE. Shoshi Kikuchi and Kiyoharu Oono. National Inst. Agrobiological Resources, Tsukuba Ibaraki 305 JAPAN

In Japonica rice system, calli can be induced from mature seeds with addition of 2,4-D(2,4-dichlorophenoxyacetic acid) to the M.S medium at 10^{-6}M. Those calli can be regenerated into plants with some cytokinins. Our research interest is to elucidate the mechanism of callus formation and plant regeneration in molecular level. As a first step of this approach, we have tried to fish out some genes which would be good markers for these processes. In order to fish out some genes which are specifically expressing only in callus stage or after onset of regeneration, the differential screening analysis was made. cDNA libraries from callus (21days after callus induction) and regenerating callus (10days after transfer onto the regeneration medium) were constructed using lambda zap vector system. With the plus-minus replica method, one callus specific clone(pRC1) and several regeneration specific clones were isolated. The callus specific clone has 834bp insert. Intensive RNA blot hybridization analysis revealed the corresponding transcript(ca 800 bases) exists in actively proliferating calli such as 21 day after induction and in suspension culture. When the calli are transfered onto regeneration medium, the amount of transcript decreases quickly. In the leaves and roots and/or 2,4-D treated leaves and roots, the amount of the transcript is faint. Expression of this transcript was highly elevated when callus was treated at 37C for 3 days and slightly raised when callus was treated at 4C for 3 days. No change was observed under desiccation stress. Sequence analysis was made. There is one open reading frame corresponding to 205 amino acid residues. In the putative ORF region 17bp GC rich sequence repeats eight times. Data base homology seach was made but no significant homologous sequence was found.

873 TRANSFORMATION AND INHERITANCE OF A HYGROMYCIN PHOSPHOTRANSFERASE GENE IN MAIZE PLANTS. David A Walters, Clayton S. Vetsch, Diane E. Potts and Ronald C. Lundquist. Plant Science Research, Inc., 10320 Bren Road East, Minnetonka, Minnesota 55343

Embryogenic maize (Zea mays L.) callus cultures grown on solid medium were transformed by microprojectile bombardment with a chimeric E. coli hygromycin phosphotransferase (HPT) gene. Transformed callus lines were obtained using a step-up selection protocol, wherein the bombarded callus was cultured first on solid medium containing hygromycin at 15 mg/L for 2 weeks and subsequently on hygromycin at 60 mg/L for 6-8 weeks. Three independent transformed callus lines were shown by Southern blot to have the HPT coding sequence. The three lines were found to be competent for regeneration into fertile plants; all Ro plants tested showed the presence of the HPT gene. Genetic analysis of R_1 and R_2 outcross progeny showed the expected 1:1 ratio for $HPT^+ : HPT^-$ for one of the lines, whereas two lines gave progeny which showed a ratio significantly less than 1:1. Cotransformation of two genes introduced on separate plasmids was demonstrated and the pattern of inheritance in the progeny showed the two genes to be linked. These results demonstrate the utility of the HPT gene as a selectable marker for recovering transformed plants from regenerable maize callus. The data also suggest that not all transgenic lines will display Mendelian inheritance patterns.

874 SELECTION AND CHARACTERIZATION OF NaCl-TOLERANT CALLUS LINES OF SAFFLOWER (CARTHAMUS TINCTORIUS) CV.A-1. T.D. Nikam* and M.G. Shitole, Department of Botany, University of Poona, Pune-7 * Department of Botany, Modern College, Pune 5, India.

A NaCl-tolerant callus line of safflower cv.A-1 isolated on MS medium supplemented with 0.75% NaCl, when grown at 00, 0.1, 0.25, 0.5 and 0.75% NaCl concentrations, growth of calli decreased at 0% NaCl in comparison with non-selected calli. These results revealed the need of NaCl for growth of calli. NaCl-tolerant calli survived upto 0.75% NaCl while non-selected calli survived upto 0.5% NaCl. At all levels of salinity, Na/K ratio was increased more in NaCl-sensitive calli than NaCl-tolerant calli thereby indicating efficient K uptake mechanism in NaCl-tolerant calli under saline conditions. In selected calli reducing sugars, non-reducing sugars, starch, protein, proline contents and TAN values were better under saline conditions than salt sensitive calli. Selected calli exhibited NaCl-tolerance when they were cultured away from the selection pressure for four passages. Attempts were made for regeneration of NaCl tolerant calli on MS medium with NaCl and without NaCl, alongwith BAP, Kin, IAA, NAA alone and in combinations. However, only rooting observed alongwith IAA alone from salt-tolerant calli which suggests that with further manipulation safflower crop could be improved for growing under saline conditions through tissue culture.

875 THE CONSTRUCTION OF UESFUL GENE EXPRESSION VECTOR AND AGROBACTERIUM MEDIATED TRANSFORMATION OF RICE Shen Daleng,Li Y.,Ren D.,Bashir M.,Qing R.,Tan C.C. Fudan University,Institute of Genetics,Shanghai 200433

The cecropin B gene encodes a peptide,which can kill many kinds of bacteria.This man-made gene encodes with plant favoring codons and consists of 153bp.Therefore,it has possibility to express in plants.The artificial gene was ligated to PBI121. The newly constructed vector PBW1 was introduced into A.tumefaciens(PGV2260)by triparent matings in YEB medium and transformant(PBW1/PGV2260)was selected with Rif.,Ap.and Km. A.tumefaciens treated with phenolic compounds and was co-cultured with rice mini adventitious buds in the presence of dicot extraction for 24 hours. After 10 subcultures, the test of Kanamycine resistance,the activity of β-glucuronidase and Dot blotting have indicated that the mini buds and plantlets have been transformed.

Acknowledgment
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POSTER ABSTRACTS

876 POTATO SUSPENSION CULTURE PROMOTES TRANSFORMATION EFFICIENCY IN SOYBEAN (Glycine max L. Merril) MEDIATED BY Agrobacterium Tumefaciens. Hsin-Hsiung Chang* and Ming-Tsair Chan. Department of Agronomy, National Taiwan University, Taipei, Taiwan, R.O.C.

We have established an effective system for improving the transformation efficiency of soybean mediated by Agrobacterium. Soybean cotyledons were excised and infected with Agrobacterium tumefaciens strains C58Cl(pGV2260::NG1) and C58Cl(pGV2260::NG3) which contain both of the GUS and NPT II genes. Established potato suspension cells(PSC) was found to be able to increase significantly the transformation efficiency when added in the infection medium. Transformed callus was increased as high as 70% after treated by PSC in this study, the frequency was higher than that treated by acetosyringone(AS). NPT II activity and GUS protein of transformed callus and plants were analysed. These results confirmed the fact that the chimeric genes were successfully integrated and expressed in the transgenic tissues. High levels of phenolic compounds sinapinic acid and acetosyringone were found in the potato suspension cells. Their roles on promoting the transformation efficiency were discussed.

877 EVALUATION OF PROTOCLONAL VARIATION WITH CHEMICAL MUTAGENESIS IN BRASSICA NAPUS (L.) VAR. WESTAR. S. Mohan Jain, Department of Crop Husbandry, University of Helsinki, Helsinki, Finland.

The potential application of protoclonal variation has been lacking in improving crop plants. In this investigation, our objective was to study protoclonal variation and compare with chemical mutagenesis in B. napus (L.) var. westar. Leaf mesophyll protoplasts were isolated from 3-week-old-seedlings and mutagenized with 0.0005% ethylmethane sulfonate for 48 hr. In greenhouse, mutagenized protoclones and plants were self-pollinated and seeds of each individual plant were collected and evaluated for seed shape and colour in the R1 generation and for flowering time (days to first flowering) in the subsequent R2 and R3 generations. Yellow and shrivelled seeds were obtained in the R1 generation of mutagenized protoclones and plants and their germination rate was very poor. The frequency of seed quality of protoclones was better and their germination rate was over 95%. The flowering times of R1 families (derivatives of R0 single regenerant) did not change significantly with the control in R2 and R3 generations. However protoclone 19 (R1 family) flowered a week earlier in the R2 generation and did not differ significantly with control in the R3 generation. Our results indicate that mutagenesis of protoplasts produced higher rate of variability and useless variations as compared with protoclones and it may not be an ideal approach for the induction of useful variations for the crop improvement programme.

878 *RICE PROTEIN EXPORT MUTANT (4C) EXPRESSED IN LIQUID CULTURES. Gideon W. Schaeffer. USDA, ARS, Plant Molecular Biology Lab, Beltsville, Md. 20705-2350*

Liquid cultures of a rice mutant (4C) have unique protein transport properties. Mutants were recovered from anther calli grown for 3 cycles at inhibitory levels of lysine + threonine (L+T) and one cycle of S-aminoethylcysteine (S-AEC). Plants were regenerated and progeny (seed) had increased lysine (10-15%) and protein (10-20%) over the wild type seed of the starting cultivar. Cell suspension cultures were started from scutellar tissue of mature embryos subcultured at 14-21 day intervals for over 3 years. The mutant and control lines are similar in appearance, growth rates, and activities of some enzymes. The lines differ dramatically in the amount of protein synthesized relative to the total cell weight, and substantial portions of the proteins synthesized are exported from the cells into the suspension medium. The mutant cells exported double the protein compared to the wild type parental cells. At this time it is clear that the mutant is higher in protein and lysine, as are the seeds from which the lines were derived. The chitinase activities are slightly lower in the mutant and some hydrolases (glucanases and glucosidases) are higher in the mutant than the controls. The goals are to isolate and characterize the gene(s) which prescribe the high protein export phenotype.

879 UTILIZATION OF THE RICE ACTIN 1 GENE FOR FOREIGN GENE EXPRESSION IN TRANSGENIC RICE. David McElroy[1], Wanggen Zhang[1], Alan D. Blowers[2], Barnabas Jenes[3], Kin C. Kong[3] and Ray Wu[1,3]. [1]Field of Botany, [2]USDA-NSF-DOE Plant Science Center, and [3]Section of Biochemistry, Cornell University, Ithaca, New York 14853, USA.

One of the major limitations in the application of gene transfer technology to rice (and other cereal crops) has been the lack of efficient promoters for the constitutive expression of foreign genes in transgenic monocot plants. With a view towards overcoming this limitation we set out to identified, characterized, and evaluated a promoter from a rice actin gene for use in rice transformation. RNA gel blot analysis of four rice actin genes revealed that they can display either a constitutive or developmentally differentiated pattern of transcript accumulation. By such analysis the rice actin 1 gene (*Act1*) was found to code for a transcript that is relatively abundant in all rice tissues and stages examined. By characterizing *Act1* genomic and cDNA clones we were able to determine the complete genomic structure of the rice *Act1* gene, thus facilitating the creation of a number of *Act1-Gus* reporter gene constructs. Results of *Act1-Gus* gene expression assays showed that *Act1* 5'-region activity is relatively high in transformed rice and maize cells and that *Act1-Gus* gene expression is ubiquitous in transgenic rice plant tissues. It was found that sequences from both the 5'-flanking and 5'-transcribed regions of the *Act1* gene play a role in the level of *Act1* 5'-region activity in transformed rice and maize cells and that such sequence can stimulate foreign gene expression from heterologous plant promoters.These results suggest that the rice *Act1* 5'-region may have general utility for the expression of agronomically important gene in transgenic cereal plants. As a first step toward exploiting the utility of the rice *Act1* 5'-region as series of *Act1*-based expression vectors have been constructed for regulating foreign gene expression in transgenic rice plants.

880 APPLICATION OF EMBRYO RESCUE TECHNIQUE IN RICE WIDE HYBRIDIZATION. M.A.A. Miah,M.S. Pathan, A.T. Barai, A.K. Saha and N.M. Miah. Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh.

Wide crosses were done involving diploid and tetraploid wild relatives O. nivara, O. latifolia and O. minuta (male parents) with BR breeding lines (female parents) to transfer blast, RTV, WBPH and BPH resistant genes to the cultivated rice. Abnormal seed development was obsered due to failure of endosperm development in some crosses. Therefore, embryo rescue technique was employed to obtain F1 plants. The plantlets thus obtained were grown in vitro until four leaf stage prior to transfer in earthen pot at the net house. Plants developed from the cross of oryza nivara were fertile and showed normal seed setting. O. latifolia and O. minuta are tetraploid male parents. The F1s obtained through embryo rescue process involving O.latifolia were completely sterile. Spikelets from some of the rescued plants were not only sterile but also abortive. Microscopic examinations revealed that about 97% pollens are non functional and this is one of the possible reasons for such sterility. Preliminary attempt for backcrossing with cultivated rice did not improve the situation. Cytological observations confirmed that PMC from sterile F1s possessed 36 univalents at metaphase I. Attempts are being taken to make these sterile F1s fertile.

881 TRANSFORMATION OF RICE PROTOPLASTS WITH CATIONIC LIPOSOMES. Zhen Zhu (1), Karen Hughes (2) and Xianghui Li (1), Institute of Genetics, Academia Sinica, Beijing China (1) and University of Tennessee, Department of Botany, Knoxville, Tennessee 37996-1100.

Recently, a cationic liposome that binds DNA with high efficiency was synthesized by Felgner et al. (1). The cationic lipid (N-[1-2,3-dioleyloxy)propyl]N,N,N-trimethylammonium chloride) or DOTMA, together with phospholipids, forms forms positively charged liposomes that interact spontaneously with DNA or RNA to form complexes. The cationic liposome preparation is available commercially under the name Lipofectin (BRL). Rice protoplasts were incubated with a liposome-DNA complex in the presence of PEG 6000. Protoplasts were recovered, mixed with low melting temperature agarose and cultured in selective media. Calli derived from protoplasts non-selective medium were examined for the presence of transforming DNA sequences and activity of the transforming gene. Based on the results of this study, the transformation frequency was estimated at 14.3%. Plants were regenerated from transformed calli.

1. Felgner et al., (1987) PNAS 84:7413.

POSTER ABSTRACTS

882 IMPROVEMENT OF IN VITRO PLANT REGENERATION EFFICIENCY FROM RICE EXPLANTS FOR TRANSFORMATION Heng Zhong, C. Srinivasan and Mariam B. Sticklen. Departments of Crop and Soil Sciences and Entomology, Michigan State University, East Lansing, MI 48824-1311. High frequency regeneration and germination of somatic embryos are a prerequisite for obtaining transformed rice (*Oryza sativa* L.) plants. A tissue culture protocol has been developed to efficiently regenerate large numbers of somatic embryos and fertile rice plants from mature seeds. Twelve rice cultivars representing three *Oryza sativa* subspecies responded to this protocol. Somatic embryos arose either directly from the root/shoot junction of excised seedlings, or from scutella through somatic embryogenic callus induction. The efficiency of regeneration was over 30 somatic embryos per junction tissue within six weeks, or over 50 somatic embryos per scutellum within eight weeks in culture. Secondary embryogenesis was observed in all cultures. Hundreds of rice plants have been regenerated from somatic embryos. A system was also developed to efficiently induce *in vitro* shoot primordial multiplication from cultured rice shoot tips. Each shoot tip produced 8-10 shoot primordia within 4 weeks of culture. Also, adventitious buds were induced from calli derived from rice scutellum. Over 30 adventitious buds were formed from each scutellum culture. These regenerative pathways may improve rice transformation as well as regeneration frequency using themicroprojectile bombardment and electroporation methods as compared to transformation of protoplasts and cell suspension cultures. Microprojectile bombardment and electroporation were used to introduce several genes into rice, including two reporter genes [Gus gene (pAct1-F) or Luciferase gene (pWB196)], one *Bacillus thuringiensis* (Bt) toxin gene for lepidopteran pest resistance (pWB139), one proteinase inhibitor gene for pests and diseases resistance and two genes for herbicide resistance, [5-enolpyruvylshikimate-3-phosphate synthase gene for glyphosate resistance (pDM104) or acetolactate synthase (ALS) gene for chlorsulfuron resistance (pGH1)]. Explants for transformation included root/shoot junction tissue, somatic embryos, shoot meristems, and cell suspension cultures. Over one hundred rice plants have been regenerated from bombarded or electroporated explants. Work is in progress to confirm the integration and expression of these foreign genes in regenerated rice plants.

883 CELL CYCLE ANALYSIS IN SUSPENSION CULTURES OF *ARABIDOPSIS* AND SOYBEAN. James J. Campanella, Alan P. Fields* and Christopher D. Town, Departments of Biology and Pharmacology*, Case Western Reserve University, Cleveland, OH 44106.

We have initiated experiments to analyze cell cycle control in suspension cultures of *Arabidopsis* and soybean. Using polyclonal antisera raised against synthetic peptides representing highly conserved epitopes of $p34^{cdc2}$ (EGVPSTAIREISLLKE) and cyclin (KYEEMYPPEIG), we have identified cross-reactive proteins of the appropriate molecular mass (ca. 34 kd and 51 kd respectively) in western blots of extracts from a suspension culture of an *Arabidopsis* tumor cell line (2.10A) and from a soybean suspension culture SB1. In immunoprecipitation experiments, the same antisera demonstrate co-precipitation of the cyclin-like protein with $p34^{cdc2}$. The soybean culture is amenable to synchronization by a double phosphate starvation procedure described by Amino et al. (Physiol. Plant. 59, 393-396, 1983; L.R. Griffing, personal communication). Application of the same protocol to the *Arabidopsis* line is in progress. Using synchronized cultures, cell cycle variations of $p34^{cdc2}$, cyclin, and their complexes will be investigated.

884 CULTURE OF PROTOPLASTS FROM EMBRYOGENIC CALLI OF RICE AND FOXTAIL MILLET. Z.A.XIA, G.Y.WANG and J.J.DONG. Shanghai Institute of Plant Physiology, Academia Sinica

Protoplasts isolated directly from the selected embryogenic calli(EC) of rice(Oryza sativa L. subsp.japonica cvv. Long Hu no.6, Hao Shan no.1 and Shang Hon Shan Nou), foxtail millet (Setaria italica L. cv.Jin Gu no.10). The protoplast yields are around 10^5-10^6/gFW. The protoplasts were cultured in N_6 or MS media with liquid,liquid/solid double layers or agarose block methods. The mature,fertile,regenerated plants via somatic embryogenesis have been obtained. By using the image analysis system we grouped and counted the EC cells of foxtail millet and found that the glutamate dehydrogenase content of single cells can be used as a parameter for cell type classification. The relation between the EC cell type trend and the protoplast clones were studied. The results showed that the state of EC clones which are suitable for protoplast culture could be determined more accurately by virtue of the EC cell type trend.

885 TRANSFORMATION OF *ANTIRRHINUM MAJUS*: REGENERATION OF TRANSGENIC PLANTS AND DEVELOPMENT OF A TRANSIENT EXPRESSION SYSTEM.

Manfred Klemm, Zs. Schwarz-Sommer, H. Saedler, MPI, Plant Breeding, 5- Cologne 30, FRG

Deficiens (defA$^+$) is one of the homeotic genes which are involved in the genetic control of *Antirrhinum majus* flower development [1][2]. Mutation of this gene (defA-1) causes homeotic transformation of petals into sepals and of stamina into carpels in flowers displaying the 'globifera' phenotype. According to Northern blot analyses the defA$^+$ gene is expressed at elevated levels in petals and stamens of flowers but its basal expression is not restricted to these organs. The overall expression of DefA is nearly constant during all stages of flower development. Analyses of several morphoalleles of the deficiens gene indicate that expression of this gene is under genetic control. The contribution of transcriptional control can be elucidated by eliminating the specific signals for posttranscriptional controls and replacing them with sequences from a readily assayed reporter gene such as ß-glucuronidase (GUS). This type of experiments can be performed either in stably transformed transgenic plants or by utilizing assays for transient gene expression. We made major efforts to establish these two experimental systems for future work with *Antirrhinum majus*. Phosphinothricin-resistant *Antirrhinum* plants were obtained by *Agrobacterium*-mediated transformation using a chimeric bar gene plasmid. Transient gene expression assays provide an alternative system to analyze genetically modified plant genes. We are in the progress to establish microprojectile bombardment of DNA-coated tungsten particles to deliver DNA into the organs of young flower buds of wildtype and mutant *Antirrhinum majus* plants. With this tool we intend to investigate the properties of defA$^+$ promoter-GUS gene fusion vectors such as the influence of putative factors involved in the control of def gene expression.

1) Sommer, H.; Beltran, J.; Huijser, P.; Pape, H.; Lönning, W.; Saedler, H. and Schwarz-Sommer, Zs. (1990) EMBO J 9:605-6132)
2) Schwarz-Sommer, Zs.; Huijser, P.; Nacken, W.; Saedler, H.; Sommer, H. (1990) Science. 250:931-936

886 AN IN VITRO SELECTION METHOD AS A TOOL FOR GENETIC TRANSFORMATION IN MELON. Victor Gaba, Ester Feldmesser and Yehesekal Antignus, Dept of Virology, The Volcani Center, ARO, Bet Dagan 50250, Israel

To develop a genetic transformation system for melon (Cucumis melo L. cv. Galia) we tested the effect of a common selection agent, the antibiotic kanamycin, on regeneration in vitro. Primary explants of the seed-derived cotyledons of cv. Galia displayed several responses to the antibiotic. The massive callus growth typical of this species in culture is blocked by 100 mg/l kanamycin. Adventitious bud production on the same medium is not greatly inhibited by up to 250 mg/l kanamycin. However, cutting the primary explant into pieces greatly increases the effectiveness of the kanamycin at that concentration. Cutting the primary explants into fragments that are too small reduces regeneration. Approximately 20% of halved explants develop shoots at concentrations of kanamycin from 300 mg/l to 800 mg/l, which bleach and die by the end of the culture period. Shoot survival is therefore more sensitive to the antibiotic than is shoot production. Because this cultivar regenerates in vitro by adventitious buds rather than via callus, selection requires both a high kanamycin concentration and a small explant.

887 REGENERATION OF *MEDICAGO TRUNCATULA* AND TRANSFORMATION USING *AGROBACTERIUM* WITH GENETICALLY MODIFIED Ri AND DISARMED Ti PLASMIDS

Mark R. Thomas, Ray J. Rose, Kim E. Nolan and Carol J. Wang. University of Newcastle, Dept. of Biological Sciences, NSW, 2308 Australia.

M. truncatula is a diploid self fertilizing annual legume used for improving pasture and has also been proposed as a suitable model plant for investigating the molecular genetics of the *Rhizobium*-legume symbiosis. Since our initial report of regeneration of *M. truncatula* we have developed a highly regenerable seed line from a plant (cv. Jemalong) possessing a high embryo-producing capability from tissue culture and have improved the regeneration protocol. We have also regenerated *M. truncatula* from isolated protoplasts embedded in agarose discs. These systems are being used for the production of transgenic plants and somatic hybrids.

In the case of *Agrobacterium rhizogenes* - mediated transformation only a Ri plasmid with an inactivated *rol* A gene regenerated transgenic plants. The Ri transformed roots however did not regenerate fertile plants.

Fertile transgenic plants were obtained from *Agrobacterium tumefaciens* - mediated transformation of leaf tissue using a disarmed Ti plasmid and a binary vector containing the kanamycin resistance gene NPT II under the control of the cauliflower mosaic virus 35S promoter. Leaf explants from the regenerated plants and their progeny callussed and produced embryos at kanamycin levels that blocked proliferation in control tissue. The NPT II gene was identified by Southern hybridisation in calli, regenerated plants and their progeny.

The GUS gene has been introduced using an *A. tumefaciens* system and a disarmed Ti plasmid. Regenerated plantlets show GUS activity in root and shoot tissue.

POSTER ABSTRACTS

888 TISSUE CULTURE SYSTEMS FOR CANOLA TRANSFORMATION. M.Arnoldo, N.MacLean, L.Burnett, M.Westecott, R.Keats, S.Roxakis, B.Huang, L.Fallis, E.Barbour, V.Armavil, C.Baszczynski, D.Macrae. & R.Kemble. Allelix Crop Technologies, 6850 Goreway Dr. Mississauga, Ont. L4V 1P1, CANADA.

Various tissue culture systems have been established, optimized and compared for their efficiency in Agrobacterium-mediated canola transformation. Of all the variables tested, explant source and concentration & combinations of plant growth regulators have the greatest effect on plant regeneration as well as transformation efficiency. For Brassica napus, stem segment transformation appeared to be the most genotype-independent system whereas cotyledonary petioles and hypocotyls were very efficient in some genotypes but not efficient in others. Microspore-derived, haploid embryos were another explant source for transformation. All tissues require cytokinin for shoot induction (e.g. BAP, 4.5ppm for cotyledonary petioles, 1ppm for stem segments and hypocotyls, 0.05ppm for microspore-derived embryos), but not necessarily auxin. Cotyledonary explants were used primarily for transformation of B.campestris. Inclusion of plant growth regulators in seed-germination media stimulated shoot regeneration from seedling organs derived from these seeds. Each of the tissue culture system provides certain advantages for different applications.

889 AGROBACTERIUM-MEDIATED TRANSFORMATION OF CUCUMBER (*CUCUMIS SATIVUS*, L. (CV. CLINTON) WITH THE COAT PROTEIN OF ZYMV. John Forsman, Robert Balint, Christopher Steele and Juanita A.R. Ladyman. Plant Cell Research Institute, Inc., Dublin, CA 94568

Transformation and regeneration of cucumber tissue was accomplished using *Agrobacterium tumefaciens*, strain LBA4404. The preparation of the bacteria containing the genes for kanamycin resistance, β-glucuronidase (GUS) and the ZYMV coat protein (plasmid = pZBIN) was as follows: Media containing acetosyringone and kanamycin was inoculated with LBA 4404 (pZBIN). The bacteria were left to multiply overnight and the bacterial suspension centrifuged. The pellet was resuspended in conditioned media, i.e., in media in which LBA 4404 carrying the pBI121 plasmid had been multiplied overnight. Cotyledonary discs from germinated seeds were incubated on tobacco nurse cell cultures for 24 hours. The discs were then inoculated with the suspension of LBA 4404 (pZBIN), returned to the nurse cultures and cocultivated with the bacterial preparation. After 48 hours the discs were incubated on a medium that induced somatic embryogenesis in the presence of kanamycin (40-75 mg/l). After 10 days the somatic embryos were sliced away from the cotyledon tissue and placed on a medium that induced tissue multiplication, also in the presence of 40-75 mg/l kanamycin. After 4 weeks on multiplication medium, a selection pressure of 40 mg/l yielded tissue that was 10% GUS positive; on 75 mg/l the tissue was 95% GUS positive. After 8-10 weeks the embryos were matured for 5 days in suspension culture and then the tissue was transferred to a germination medium. Plants were obtained that were GUS positive (histochemical assay), contained the NPTII protein as measured by ELISA and contained the coat protein gene as identified by Southern blot analysis.

890 THE INFLUENCE OF ANTIBIOTICS ON THE TISSUES OF M.LITTORALIS. M.N.Nasretdinova, V.I.Negruk. A.S.Sadikov's Institute of Bioorganic Chemistry,Academy of Sciences Uzbek SSR, Tashkent, USSR.

We studied the influence of kanamycin (Km) and cefotaxime on the induction of M.littoralis primary leaf callus and its growth and seedlings development. It was found that the induction of primary callus was most depressed in the presence of 25 μg/ml of Km and 500 μg/ml of cefotaxime in medium. The differs were observed when the influence of antibiotics on the seedlings development was investigated. In this case the root system formation was most depressed. For example, roots didn't develope when 25 μg/ml of Km was added to medium. These data were utilized in obtaining of transgenic alfalfa plants.

891 ISOLATION AND CHARACTERISATION OF A PLANT CYCLIN GENE. Heribert Hirt, Mathyas Mink, Janos Györgyey[#], Martin Pfosser[$], Denes Dudits[#] and Erwin Heberle-Bors. Inst. of Microbiology and Genetics, Univ. of Vienna, Althanstr.14, 1090 Vienna, Austria; [$]Inst. of Botany, Univ. of Agriculture and Forestry, Gregor-Mendelstr.33, 1190 Vienna, Austria and [#]Inst. of Genetics, Biological Res. Inst., Hung. Acad. of Sciences, 6701 Szeged, POB 521, Hungary

Cell division in eukaryotes is mediated by the action of MPF (mitosis promoting factor) which is composed of the cdc2 protein kinase and one of various mitotic cyclins. We have recently isolated a plant cdc2 kinase gene and showed it to be differentially expressed during development (Hirt et al., PNAS (1991), 88:1636-1640). Here, we report on the isolation of a plant cyclin gene. The predicted amino acid sequence showed highest homologies to the sea urchin cyclin B protein. In synchronized alfalfa suspension cultures, the mRNA of this cyclin was only expressed in late G2 and M phase, an expression pattern typical for cyclin B genes. Hybridization at lower stringency revealed two additional transcripts that were both expressed in S and G2 phase, but disappeared well before onset of M phase. This pattern is characteristic for cyclins of the A type. Currently, we are producing peptide antibodies directed against a specific domain of the cyclin B protein and will investigate the expression and regulation of this gene at the protein level.

892 CELL CYCLE-SPECIFIC EXPRESSION OF THE PLANT CDC2 GENE. Vilmos Czismadia, Claudia Jonak, Martin Pfosser*, Anikó Páy, Heribert Hirt and Erwin Heberle-Bors. Institute of Microbiology and Genetics, University of Vienna, Althanstr. 14, 1090 Vienna, Austria. *Institute of Botany, University of Agriculture and Forestry, Gregor-Mendelstr. 33, 1190 Vienna, Austria

The cdc2 protein kinase plays a central regulatory role in the control of the eukaryotic cell cycle of animals and yeasts. We have recently isolated a cDNA clone for the plant homolog of the yeast cdc2/CDC28 genes from alfalfa (Medicago sativa), cdc2Ms (Hirt et al. 1991, PNAS 88:1636-1640). In an alfalfa suspension culture, synchronized by aphidicolin, the cdc2Ms gene is transcribed continuously but with a clear maximum in G2 phase. After mitosis, expression decreases strongly and starts to recover by the end of G1 phase. A similar expression pattern is observed for the cyclin B gene (abstract by Hirt et al.). Experiments on the cell cycle-specific expression of the cdc2Ms gene on the protein level are underway.

893 A CHIMERIC STREPTOMYCIN RESISTANCE GENE AS A DOMINANT NUCLEAR MARKER FOR PLANT CELL TRANSFORMATION. D.K. Srivastava*, T. Ulmasov and E.S. Piruzian Institute of Molecular Genetics, USSR Academy of Sciences, Moscow 123182,USSR, *Department of Biotechnology, Dr.Y.S.Parmar University of Horticulture & Forestry, SOLAN-173230, INDIA.

Transformation of plant cells has been achieved using chimeric genes that confer resistance to toxic drugs such as kanamycin, hygromycin, methotrexate and bleomycin. A new genetic nuclear marker for plant cell transformation has been developed that is based on the expression of the enzyme streptomycin phosphotransferase-I(SPT-I). The coding sequences of SPT-I from the bacterial transposon Tn5 were placed under the control of 35S promoter of the cauliflower mosaic virus in a plasmid pAP2034. The plasmid pAP2034 carries the chimeric SPT-I gene (1.63kb) between EcoRI and Sal-I site downstream of the octopine synthase polyadenylation signal. The chimeric SPT-I gene is linked with NPT-II which is located downstream of SPT-I gene that confers kanamycin resistance in plants. This plasmid was mobilized into Agrobacterium tumefaciens pGV3850 strain using the mobilizing plasmid pRK2013(HB101)(Figurski and Helinski 1979) and selecting transconjugants for kanamycin resistance (12.5 μg/ml) on carbenicillin (100 μg/ml)LB plates. The plant cells transformed with this modified strain(Agrobacterium-mediated transformation) can be selected on kanamycin and tested for streptomycin phosphotransferase-I.

POSTER ABSTRACTS

894 IMPROVEMENT OF *Arabidopsis thaliana* PROTOPLAST CULTURE AND PLANT REGENERATION. Jean Masson and Jerzy Paszkowski. Institute of Plant Sciences, ETH-Zürich, CH-8092 Zürich, Switzerland.

Regeneration procedures for mesophyll protoplasts of different *Arabidopsis thaliana* genotypes have been established (Damm et al., 1988, MGG 213, Karesch et al, Pl. Cell Rep., 1990). Regeneration efficiencies were up to 5 plants per 1000 plated protoplasts.
Although this frequency is sufficient for direct gene transfer (Damm et al., 1989, MGG 217, Karesch et al., Pl. Cell Rep. 1990), it is still too low for the detection of rare events e. g. homologous recombination or shot gun mutant complementations.
Therefore, we examined additional parameters of cell culture. Significant differences in protoplast division rates were found by varying growth conditions of the donor plants and changing several components of culture media. The crucial variables were day length for donor plant and nitrogen sources in protoplast media. These and others *Arabidopsis* protoplast culture conditions will be discussed.

895 CYTODIFFERENTIATION OF MAIZE SUSPENSION CULTURES. Albert Kausch, Thomas R. Adams, Mary Lou Mangano, Susan Zachwieja, Nancy G. Willetts, Sheryl Chambers, Whitney Adams, Jr., Richard Daines, Catherine Mackey. DEKALB Plant Genetics, Eastern Point Road, Groton CT 06340

Patterns of development in embryogenic suspension cell cultures capable of producing fertile transgenic maize plants by particle bombardment (Gordon-Kamm et al.,1991,Plant cell 2: 603-618) have been observed histologically and by scanning electron microscopy. Various cytochemical and viable stains were used to establish a developmental sequence which was subsequently substantiated in microcloning experiments. Cluster growth measurements for the various developmental stages were made by packed cell volume measurements and cell number estimates. A diagram depicting developmental patterns within maintainable embryogenic suspensions will be presented. Autoradiographic analysis was conducted on serially sectioned 3H-thymidine labelled suspension cell clusters to examine spatial, temporal, and populational relationships of of cell cycle events in these cultures. The accessibility of mitotically active cells and their response to penetrations by microprojectiles was evaluated. This information addresses the developmental patterns in maize embryogenic suspension cultures which allow formation of transformed clonal sectors that retain their morphogenetic competance allowing for selection and recovery of fertile transgenic maize plants.

896 ANALYSIS OF POSSIBLE INVOLVEMENT OF RolC AND IaaL PROTEINS IN IAA METABOLISM. Mattias Eriksson, Göran Sandberg, Ove Nilsson and Olof Olsson, Dep. of Forest gen. and Plant phys., Swedish Univ. of Agr. Sci., S-901 83 UMEÅ, SWEDEN

We have opumized the conditions for transient gene expression in protoplasts of *Nicotiana tabaccum*. Transformation of protoplasts was carried out by electroporation in 0.4 M mannitol and 8% PEG. Parameters like resistance of the medium, pulse properties and post-pulse incubation conditions were investigated. The luciferase enzyme from *Vibrio harveyi*, both wildtype dimeric form as well as an engineered monomeric fusionprotein (*luxF*), was used as a reporter. Tobacco protoplasts were transformed with different DNA constructs expressing genes with known or unknown function in IAA metabolism. To ensure a high transcription of these genes, they were put behind a strong, engineered 35S promoter preceded by 4 35S enhancers. *iaaM* and *iaaH* from the T-DNA of *A. tumefaciens*, which catalyze the sequential formation of IAA from tryptophan, were separatly and simultanously introduced into protoplasts and the cells were analyzed by GC-MS for IAA and IAA conjugates. In other experiments protoplasts were transformed with *iaaL* from *P. syringae* subsp. *savastanoi* or *rolC* from the T-DNA of *A. rhizogenes* separatly and together with *iaaM* and *iaaH*. Levels of IAA and IAA conjugates were investigated.

897 ENHANCEMENT OF PLANT REGENERATION, CELL SUSPENSION GROWTH AND PROTOPLAST ISOLATION IN SOME INDICA RICE VARIETIES. S.S. Gosal, M.S. Khinda, J.S. Sandhu, M.S. Gill and H.S. Dhaliwal, Biotechnology Centre, P.A.U., Ludhiana-141004, India.

Callus cultures were established in 9 indica rice varieties, PR 103, PR 106, PR 109, IR 8, IR 54, Vaigai 7, Jaya, Basmati 370 and Pusa Basmati 1 from mature seeds on MS (1962) medium supplemented with various combinations and concentrations of 2,4-D and kinetin. Seed placement was a critical factor affecting callusing frequency. Callus induction up to 90% was achieved in some varieties by placing seeds with endosperm inserted into MS medium supplemented with 2,4-D (2 mg/l) + kinetin (0.5 mg/l). During subculturing, selection for a particular type of callus, frequent transfer of calli to fresh medium, gradual decrease in the level of 2,4-D and addition of $AgNO_3$, prevented necrosis and enhanced root as well as shoot regeneration, upto 50% (Jaya) and 90% (IR 54) with 15 to 20 shoots per callus/test tube. Addition of tyrosine (50 mg/l), tryptophan (50 mg/l) and alanine (50 mg/l) into MS liquid medium containing 2,4-D (2 mg/l) + kinetin (0.5 mg/l) improved the growth of cell suspension cultures. In some varieties, cells harvested from suspension cultures after 3-4 days of subculturing and incubated in Cellulase R-10 (1.5%) and Macerozyme R-10 (0.5%) for 8-10 hours, gave better yields of protoplasts as compared to the actively growing calli.

898 THE TWO CULTIVATED JUTE SPECIES ARE QUITE POSSIBLY NOT CLOSELY RELATED. S. Majumdar, N.S. Banerjee, T. Saha, S.K. Sen PMCG,Bose Institute,P1/12,CIT Scheme VII-M,Calcutta 700054,India.

The potentiality of jute as a vegetable fibre yielding crop is next to cotton. Corchorus olitorius and C. caosularis represent for two cultivated jute species. Plant scientists interested in further improvement of this crop recognised the neccessity for genetic introgression of desirable attributes of both the species into single plant type. All past attempts in this direction failed to produce sexual hybrids. Sexual incompatibility and/or apomictic seed production hindered in the process of recovering true sexual hybrids through plant breeding. Protoplast fusion and cell culture techniques were applied to bypass the sexual barrier. Though, in due course a large number of somatic embryos were recovered none of them germinated into a full plant. Additionally, molecular insight of the nuclear genomic organisation of the two jute species revealed interesting results. The pattern of distribution of repetative DNA of the two jute species turned out to be significantly different. Presence of species specific unique segments of DNA appear to be prevalent in each of the two species. The extent of difference in the genomic DNA organisation between the jute species seems to be paradoxical to the relationship that was comprehended on the basis of phylogenetic status. Afterall, past attempts for genetic introgression between the two jute species were based on their presumptive phylogenetic closeness.

899 AGROBACTERIUM MEDIATED TRANSFORMATION OF CASSAVA LEAF DISCS AND EXPRESSION OF VIRAL COAT PROTEIN GENES IN CALLUS TISSUE. C. Franche[1], D. Bogusz[1], C. Fauquet[1], A. Sangare[2], C. Schöpke[2] and R. N. Beachy[2]. ORSTOM[1]/Washington University[2]. Department Biology. BOX 1137. Saint-Louis. Missouri 63130. USA.

Cassava (*Manihot esculenta* Crantz) which is cultivated for its starchy tuberized roots, is an important African food crop and the third largest source of calories in the world. The goal of our research is to genetically engineer cassava for resistance to ACMV (African Cassava Mosaic Virus) and CCMV (Cassava Common Mosaic Virus) using the coat protein mediated protection strategy.

Several chimeric genes including the ß-glucuronidase (GUS) gene, the ACMV coat protein gene, and the CCMV coat protein gene were introduced in cassava leaf discs using the binary vector pMON 505. Cassava leaf discs from two month old plants were cocultivated for three days with the avirulent *Agrobacterium* strain GV3111 carrying the pMON 505 derivatives. The explants were then tranferred to fresh medium supplemented with 1 mg/l BA, 1 mg/l NAA, 100 mg/l kanamycin and 500 mg/l carbenicillin. Calli emerging from the cut edge of the leaf discs were excised 30 days after cocultivation and transferred every three weeks for a further 90 days on the selection medium. 70 to 80 % of the leaf discs developed an average of three transformed calli. Southern blot analysis was used to prove that the T-DNA was inserted into the cassava genome of the kanamycin resistant calli. Some calli were found to express a high level of CCMV coat protein as indicated by Western analysis. The level of expression of the different GUS constructs and of the ACMV coat protein constructs will be discussed. The efficient transfer and expression of foreign genes in cassava offers promising possibilities for improvement of cassava by means of genetic manipulation. Successful regeneration of transgenic cassava will depend on the availability of a regeneration procedure in which *Agrobacterium* transformation can be targeted to cells capable of regeneration.

POSTER ABSTRACTS

900 INTRA- AND INTERSPECIFIC SOMATIC HYBRIDISATION IN POTATO.
S. Cooper-Bland, E. Baird, M. De,Maine, A. Kumar and R. Powell. Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland.

The technique of somatic protoplast fusion has special significance for potato. The tetraploid and heterozygous nature of potato (Solanum tuberosum L. ssp. tuberosum, 2n = 4x = 48) coupled with its tetrasomic inheritance, means that conventional genetical analysis is difficult. However, the production of dihaploid potato lines (2n = 2x = 24) provides an opportunity to simplify the identification of useful genotypes. Somatic hybridisation via protoplast fusion is a potentially useful technique for the potato breeder since it offers a novel way of resynthesising tetraploid cultivars/genotypes by combining complimentary agronomic traits of the dihaploids at the intraspecific level. Additionally, these dihaploid lines could be used to produce interspecific somatic hybrids with wild Solanum species in order to introduce novel sources of genetic variability (i.e. disease resistance) into the cultivated potato gene pool. We have produced several intraspecific (PDH 40 + PDH 727)and interspecific (PDH 40 + S. brevidens) somatic hybrid plants using the electrofusion method. Nuclear hybridity of these somatic hybrid plants have been confirmed using randomly amplified polymorphic DNA markers (RAPD). The technique is more convenient to use than RFLP analysis because it uses smaller amounts of DNA and hence, less plant material is required for DNA isolation. The technique has also the advantage that it does not necessitate the use of radioactivity.

901 ORGANOPHOSPEROUS PESTICIDE INDUCED RESISTANT CELL LINES IN CLUSTER BEAN BY TISSUE CULTURE.
Ch.Ayodhya Ramulu and Digamber Rao, Department of Botany, Kakatiya University, Warangal-506009, A.P., INDIA.

Tissue culture has made tremendous progress in area of plant research at basic and applied level. Recent advances in genetics and biotechnology have made tremendous change in crop improvement. Gene transfer technique is a tool through which plants of desirable characters can be established. Cluster bean is an agronomically important crop for its rotation, high protein content, guar gum is used for food additive and industrial uses. Callus was successfully induced from various seedling explants of Cyamopsis tetragonoloba (L.) Taub. PNB cultivar on B5 medium supplemented with 2.50 mg/L 2,4-D and 2.0 mg/L 6-furfuryl aminopurine. The greenish and friable callus was subjected to induced stress of Anthio (60,80 and 100 ppm) pesticide by subculturing it to medium. After 6-weeks of growth period surviving cell lines at 80 ppm have been isolated for further investigation of growth index, cell viability and mitotic index were found to be enhanced. At 40 ppm concentration effect is not significant, but 100 ppm concentration showed poor cell growth and mortality of cell are abundent. The enzyme activity of α-amylase, peroxidase is increased. The successfully isolated, induced resistant cell clones and callus was kept for Morphogenetic studies.

902 BIOTECHNOLOGY FOR INDUCTION OF PESTICIDE STRESS TOLERENCE IN CERTAIN GRAIN LEGUMES BY TISSUE CULTURE.
Digamber Rao and Ch.Ayodhya Ramulu, Department of Botany, K.U.C. Warangal 506 009, INDIA.

Recent advances in genetics and biotechnology have made tremendous changes in crop improvemet. Molecular genetics and genetic engineering have potential application to crop improvement. In modern agricultural system use of pesticides has become indispensable. Modifying plant to become resistant to pesticides would allow their selective use for crop protection. The effect of Monocrotophos induced stress were studied in callus, suspension cultures. Callus cultures were induced from seedling explants of Greengram, Chickpea and Cluster bean on B5 medium with combinations of 2.50 mg/L 2,4-D, 1.50 mg/L kinetin, 1.0 mg/L IAA and 1.75 mg/L BAP. The greenish and friable callus was subjected to Monocrotophos (40,60,80 ppm) induced stress by transferring into medium. The surviving cell lines were isolated and growth was determined with packed cell volume, growth index, cell viability. The α-anylase enzyme activity in stressed cells have been increased. The pesticide resistant cell lines have been isolated for its morphogenetic studies for regeneration. The growth index and cell viability was increased at 60 ppm of monocrotophos stressed cells. In 80 ppm of moocrotophos prooved to be inhibitory and mortality of cells were separated.

903 USE OF *AGROBACTERIUM RHIZOGENES* - MEDIATED TRANSFORMATION TO IMPROVE ROOTING OF EUCALYPTS
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The success of *Eucalyptus* clonal nurseries in South Africa is dependent on the rooting ability of the clonal material. Poor rooting results in inadequate field establishment and economic loss. Optimisation of physiological and environmental conditions in the nursery has resulted in a remarkable increase in rooting capacity of some *Eucalyptus* clones (up to 80%). However, rooting of certain clones exhibiting desirable wood properties cannot be increased significantly in this way. Our approach has been to transform the bases of *in vitro* propagated shoots of three *Eucalyptus* species via the Ri plasmids of three *A. rhizogenes* strains. This resulted in root initiation and the production of numerous secondary functional roots. The results show that there is considerable variation in transformation frequency with the various helper strains on different *Eucalyptus* species. Transformed root cultures were produced and plantlets with transformed roots and normal shoots were hardened off and further root development was monitored. The use of a similar procedure in clonal nurseries has been investigated.

904 COLONY FORMATION FROM MESOPHYLL PROTOPLASTS OF INDICA RICE.
Hari S. Gupta and Arunava Pattanayak. ICAR Research Complex for NEH Region, Barapani, India.

Culture of mesophyll protoplasts of cereals has been the object of much attention because until recently they were considered extremely recalcitrant to, if not incapable of, reentering the cell cycle. We report induction of high frequency sustained division in mesophyll protoplasts isolated from leaf base-derived meristematic tissue of indica rice line, RCPL 1-3C-an advance progeny of Pusa-33 x Khonorullo. The tissue incubated in an enzyme mixture containing 1% Cellulase R10 and 0.1% Pectolvase Y-23 in CPW 7M released protoplasts after seven hours. They were purified and cultured in modified N6 liquid medium without nitrate ions. The protoplasts regenerated cell wall, enlarged in size and divided. First division was seen after 11-12 days. The medium was replenished after 21 days, after which division frequency increased dramatically. The divided cells tend to separate from each other resulting into extremely friable cell colonies. Microcolonies composed of 20-30 cells were visible after 30-35 days. Results on the effects of feeder cells on division and induction of embryogenic calli for plant regeneration would be presented.

905 IMPROVED MEDIA FOR ESTABLISHING FRIABLE, EMBRYOGENIC CULTURES OF ELITE, INBRED MAIZE GENOTYPES.
Janet Suttie, Erik Dunder, Martha Hill, Gary Pace. CIBA-GEIGY Agricultural Biotechnology, POB 12257, Research Triangle Park, NC 27709.

Cell cultures of maize have become a prime target for genetic manipulation *in vitro*. Friable, embryogenic cultures from elite, inbred genotypes have the genetic background, morphology and regeneration characteristics highly desirable for these procedures. Although limited success has been achieved using published media to establish elite cultures, modifications of these media resulted in higher initiation frequencies across several different lines. The modifications included lowering the nitrate to ammonium ion ratio, increasing the total inorganic nitrogen concentration and varying the sulfate ion concentration. The synthetic auxin type and concentration was also modified to augment the higher frequencies achieved with this new formulation. The initiated cultures developed an ideal morphology for plant transformation work, could be maintained longterm and showed high regeneration potential and fertility.

POSTER ABSTRACTS

906 STABLE, HERITABLE TRANSFORMATION OF WINTER BARLEY (HORDEUM VULGARE)

Judy P. Freeman, Andrew P. McQueen and Tina L.Barsby. Nickerson International Seed Company, Cambridge Science Park, Milton Road, Cambridge, England.

Genetic transformation of barley was achieved using an electrostatic discharge gun. Freshly isolated microspores of Hordeum vulgare variety Igri were bombarded with gold microprojectiles carrying plasmid DNA containing the marker gene *bar* which confers resistance to the herbicides Basta and Bialaphos. Embryos were recovered on medium containing the active ingredient of these herbicides, glufosinate ammonium. Regenerants were tested for the presence of the introduced gene using PCR and Southern blotting. Biological assays for expression of the introduced gene were also carried out. A second seed generation has been recovered from the transformed plants. Field trials are in progress in the UK.

907 FIELD TRIALS WITH TRANSGENIC RICE PLANTS: MOLCULAR AND GENETIC EVALUATION. Zhijian Li and Norimoto Murai, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803-1720.

An efficient PEG-mediated protoplast transformation system has been established to generate a large number of fertile transgenic rice plants. To evaluate the growth and morphological development of transgenic plants, 117 F1 progeny plants from 8 selfed primary transgenic plants containing plant expressible hygromycin-resistance genes (*hph*) were grown in field conditions. In an attempt to initiate novel gene tagging system in rice, maize *Ac*-containing transgenic rice plants (547 F1 progeny plants from 13 primary transgenic plants) were also tested in the same conditions to investigate the phenotypic diversity associated to *Ac* transposition in rice genome. Field trial with transgenic rice plants was conducted under the supervision of the U.S. Department of Agriculture (Permit No. 90-029-01). Major agronomic traits including plant height, tiller number, panicle length, spikelet number and seed fertility were compared among seed- and protoplast-derived control plants, and transgenic plants. No significant differences in major agronomic traits were found in the majority of primary and derived progeny transgenic plants with *hph* genes. One out of 8 transgenic plants produced progeny plants showing a 3:1 segregating dwarf phenotype. DNA analysis suggests that the observed dwarfism may be derived from a recessive somaclonal mutation. In contrast, considerable phenotypic changes were observed in *Ac*-containing transgenic plants. Predominant variations occurred in plant height and seed fertility. Southern hybridization analysis indicated that one dwarf and one sterile phenotype were associated with the appearance of unique *Ac*-containing fragments in two transgenic progeny plants. Further genetic and molecular analysis will be conducted to determine the relationship of apparent *Ac* mobility and new phenotype.

908 GENE EXPRESSION DURING CELL CYCLE REACTIVATION IN TOBACCO MESOPHYLL PROTOPLASTS. Sigrid Carle and George Bates, Florida State University, Department of Biological Science, Tallahassee, Florida 32306.

We are interested in the pattern of gene expression that accompanies the developmental changes that occur in tobacco mesophyll protoplasts during the first few days of culture. Changes that characterize this transition include induction of DNA synthesis and cell division, and alterations in transcription. Reactivation of the cell cycle is of particular interest and is dependent on auxin and cytokinin. We are using histone expression as a marker for cell cycle reactivation; in animal cells histone expression is coupled to DNA synthesis. Protoplasts were placed in culture. RNA was isolated from them and nuclear division was assessed every 24 hr. Nuclear division begins between 24 and 48 hr of culture. By the third or fourth day, 70 percent of the protoplasts divided at least once. Northern analysis shows no histone H3 expression in the leaves from which the protoplasts were made, but H3 expression begins after 24 hr of protoplast culture and precedes cell division. Once H3 expression begins it remains more or less constant. We also examined expression of the PR1 gene (pathogenesis-related protein gene) as a control for stress-induced gene expression. PR1 expression was not detected until 48 hr of culture and continued to rise at later times. When either auxin or cytokinin were omitted from the culture medium, neither histone expression nor nuclear division occurred.

909 TRANSFORMATION OF *GLYCINE ARGYREA* BY *AGROBACTERIUM RHIZOGENES* AND DIRECT DNA UPTAKE. V.Kumar, B.Jones and M.R.Davey. Plant Genetic Manipulation Group, Department of Botany, University of Nottingham, Nottingham NG7 2RD, UK.

Transformation of *G.argyrea* G1420 following inoculation with *A.rhizogenes* LBA9402, A4T (wild type Ri plasmids) and R1601 and A4TIII (*nptII* gene cointegrated into the TL-DNA) was influenced by seedling age and bacterial concentration. Hypocotyls excised from 9-14 d-old seedlings and bacterial concentrations of 1.0 x 10^8/ml resulted in the highest transformation frequencies. Transformed roots produced callus which regenerated shoots on B5 medium with 1.1 mg/l BAP and 0.005mg/l IBA (SC2). Opine synthesis and TL-DNA dot blot hybridization confirmed the transgenic nature of roots and callus regenerating shoots. Protoplasts isolated from cotyledons of 14 d-old seedlings of accession G1626 were suspended at 1.0 x 10^6/ml in a solution of 9% mannitol, 0.5mM $CaCl_2$ and 0.5mM MES with 20 µg of pCaMVNEO, and exposed to 2 square wave DC pulses, each of 75 V/cm for 2 µs. Electroporated protoplasts were suspended in K8P medium at 1.0 x 10^4/ml (16 h, dark, 27°C), followed by plating at 4.0 x 10^3/ml in K8P medium containing 6.0 g/l Sigma Type VII agarose. Protoplasts were dispensed in 55 µl droplets (total of 3 ml/9 cm dish) overlaid by 10 ml of liquid K8P medium. Subsequently, the latter was diluted with SC2 medium. Transformed tissues were selected by exposure to 25-100 µg/ml of kanamycin sulphate. Selected tissues regenerated shoots on B5 based medium containing 0.2 mg/ml BAP, 0.005 mg/ml IBA and 2.5 g/l Phytagel. Shoots derived from roots transformed by *Agrobacterium* strains R1601 and A4TIII and those regenerated from protoplasts, expressed NPTII enzyme activity, confirming the stable integration and expression of the *nptII* gene in transgenic tissues.

910 TOWARDS THE STABLE TRANSFORMATION OF BARLEY USING PARTICLE BOMBARDMENT

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The research of our group is focussed on stable transformation of barley. In order to achieve this we have used microprojectile bombardment on suspension cultured cells, microspore derived embryo's and immature zygotic embryo's. Several parameters have been optimized, including the coating method of the microparticles with DNA and the type of the cellcultures used for bombardment.
The plasmids that were used in transfection experiments encoded ß-glucuronidase (GUS) as a reporter and hygromycin phospho transferase or phosphinotricin acetyl transferase as selectable markers. The GUS expression under control of different promoters was compared using both PEG-induced gene transfer and particle bombardment. The sensitivity of a number of cellcultures for the selecting agents hygromycin and phosphinotricin has been determined. Results of our studies aiming at stable transformation will be presented.

911 GIBBERELLIN BIOSYNTHESIS IN NORMAL AND DWARF-1 MAIZE CALLUS CULTURES. Anthony Talo, Paul Gaskin*, JakeMacMillan*, and Bernard O. Phinney. Dept. of Biology, UCLA, Los Angeles, CA 90024; *Long Ashton Res. Sta., Bristol BS18 9AF, UK.

We are interested in the use of single gene recessive mutants (d1, d2, d3, d5, and an1) to analyze the factors controlling shoot elongation in maize. The dwarf-1 gene results in a plant 1/4 to 1/5 the height of the wild-type; the mutant gene is expressed from the seedling stage to maturity (Emerson et al., Cornell U. Mem. 1935) While the cells from d1 seedlings are appreciably smaller and fewer in number than those from the wild-type, there are only minor differences when the two genotypes are grown in callus culture. The goal of the study reported here was to determine if the d1 gene is expressed biochemically in callus. Endogenous gibberellins (GAs) and the GA metabolites from [^{3}H, ^{13}C]-GA_{20} feeds were identified from d1 and wild-type callus by full-scan gas chromatography (GC-MS). While the kinds of GAs identified from the callus cultures are similar to those found in seedlings, preliminary experiments suggest quantitative differences between wild-type and d1 cultures that differ from those found in intact plants. Like wise, the preliminary data from [^{3}H, ^{13}C]-GA_{20} feeds do not conform to the results obtained from intact seedlings where [^{3}H, ^{13}C]-GA_{20} feeds have shown the d1 gene blocks the step, GA_{20} to GA_1.

POSTER ABSTRACTS

912 STUDIES ON GROWTH, PROTEIN SYNTHESIS AND GENE EXPRESSION IN ASPEN CULTURES: Barry L. Nadel, Arie Altman, Lisa Shoseyov, and Oded Shoseyov. Dept. of Horticulture and the Otto Warburg Center for Biotechnology in Agriculture. Faculty of Agriculture, Hebrew University of Jerusalem, P.O.B. 12, Rehovot, Israel

The effects of growth regulators (IAA, GA_3, BA, ABA)on growth and development in vitro of plantlets of Aspen (Populus tremula) was studied. IAA considerably increased shoot growth, and leaf development and enhanced root formation, whereas BA reduced internode length and inhibited root formation. Solubilized proteins of hormone-treated shoot cultures were analyzed by 1 and 2 D SDS polyacrylamide gel electrophoresis. Significant changes were observed in polypeptide profiles of BA-treated shoots. This included modifications in the intensity of many bands, and the appearance of novel polypeptides. In vivo protein synthesis was studied by ^{14}C-leucine incorporation, showing significant effects of the growth regulators. IAA enhanced considerably protein synthesis, whereas BA and ABA inhibited it.
RNA, isolated from hormone-treated shoot cultures, was used for both in vitro translation (^{35}S-methionine) and for northern blot analysis using 1,4-ß-endoglucanase gene as a probe. Data of these experiments will be discussed.

913 RECOVERY OF TRANSFORMED PLANTS FROM EMBRYOGENIC CULTURES OF CORN. Fionnuala M. Morrish[1], Rosalind Williams[2], Charles Armstrong[1] and Micheal E. Fromm[1]. Monsanto Company 700 Chesterfield Village Parkway, St Louis MO 63198[1], PGEC-USDA, 800 Buchanan St. Albany CA 94710[2].

The use of microprojectile bombardment has allowed the recovery of transformed plants from corn. Actively dividing suspension cultures have been the primary targets for bombardment due to their rapid cell division rate. The establishment of these cultures takes time and results in loss or reduction in fertility of the transformed plants due to the long tissue culture phase. To reduce the period of tissue culture we have used callus as a target tissue and have successfully recovered a number of independent events using Basta selection Callus was shown to contain the BAR gene and express PAT activity. Plants were regenerated from these calli under Basta selection and were also positive for PAT activity and the presence of the BAR gene. An analysis of these plants will be presented.

914 ESTABLISHMENT OF A TRANSFORMATION SYSTEM TOWARDS OBTAINING TRANSGENIC RICE PLANTS EXPRESSING RTBV COAT PROTEIN GENE Liangcai Li*, Rongda Qu, Alexandre de Kochko and Roger N. Beachy. Department of biology, Washington University, St. Louis, MO 63130, USA and *Institute of Genetics, Academia Sinica, Beijing 100101, China

We are designing a strategy to use coat-protein-mediated approach to protect rice from infection by agents that are responsible for producing rice tungro disease in Southeast Asia.
An efficient regeneration system from suspension cell clusters and small calli of two rice strains (one *japonica* and one *indica*) were established. Up to 50% of the calli (or clusters) could regenerate green plants. In most cases, multiple plantlets(5-20) were produced on each of the calli (clusters) indicating that many cells were involved in the regeneration. Transformation experiments showed that GUS gene delivered by particle bombardment was transiently expressed in each type of tissue at a high efficiency (hundreds to thousands of blue spots per shot).
Sequences that included open reading frames encoding the coat protein(s) of rice tungro bacilliform virus (RTBV) were constructed in a pMON999 derivative. Co-transformation with the gene constructs and a selectable marker (*hph*, hygromycin resistance gene) into the cell lines is currently underway.

915 HETEROLOGOUS GENE EXPRESSION AND INHERITENCE OF FOREIGN GENE IN RICE. Swapan K. Datta, Alex Peterhans, Karabi Datta, Nouchine Soltanifar and Ingo Potrykus. Institute of Plant Sciences, ETH-Zurich, CH-8092, Zurich, Switzerland.

We have established plant regeneration from protoplasts of a number of varieties of rice (both Japonica and Indica subspecies including commercial Indica-cultivars of IRRI-lines). Transgenic fertile Indica-rice has been obtained expressing a bacterial gene (hygromycin phosphotransferase) driven by 35S prompter of Cauliflower mosaic virus (CaMV). The transgene has also been inherited in the subsequent progeny. Heterologous gene expression was carried out with a dicot intron (soybean phaseolin gene) which was processed in rice correctly and efficiently and an accuracy comparable to other dicot plants. The average hybrid gene copies integrated in rice genome was 5 to 10 fold higher than in tobacco. The amount of mature mRNA was not influenced by the presence of intron. Transposition of maize Ac (stable and transient) is now in progress in haploid rice protoplast cultures derived from microspore-embryogenic cell suspension which should provide heterologous transposon for mutagenesis and gene tagging in rice.

916 THE RolC PROTEIN OF A.RHIZOGENES ALTERS PHYTOHORMONE LEVELS IN TRANSGENIC TOBACCO. Ove Nilsson(1,2), Anders Ostin(2), Thomas Moritz(2), Nadine Imbault(2), Göran Sandberg(2) and Olof Olsson(2). (1) Department of Plant Physiology, University of Umeå, S-901 87, Umeå, Sweden; (2) Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83, Umeå, Sweden. Tobacco plants (Nicotiana tabacum cv. W38) were transformed with the rolC gene from Agrobacterium rhizogenes fused behind the strong Cauliflower mosaic virus 35S promoter. The regenerated plants showed severe developmental alterations with shortened internodal length, lanceolate leaves and reduced apical dominance. The plants were analyzed for free and conjugated IAA, cytokinins, gibberellins and abscissic acid. The metabolism of IAA was studied by feeding radioactively labeled IAA to the apex, and in other feeding experiments the response to naphtaleneacetic acid (NAA), 6-benzylaminopurine (BAP) and gibberellic acid (GA_3) was studied. The results from these experiments are discussed in relationship to IAA synthesis and degradation experiments, where radioactively labeled tryptophan and IAA was fed to an Escherichia coli culture overproducing the RolC protein.

917 *ipt* TRANSFORMED POPLAR AND TOBACCO PLANTS AS A MODEL TO STUDY IAA METABOLISM. Staffan Eklöf, Nadine Imbault, Göran Sandberg and Olof Olsson. Department of Forest Genetics and Plant Physiology, The Swedish University of Agricultural Sciences, S-901 83 UMEÅ, Sweden.

A promoterless gen 4 cassette was fused close to the right border of a binary T-DNA vector, which also carried a HPT gene from *E. coli*. Tobacco leaf discs and Poplar stem segments were cocultivated with *A. tumefaciens* carrying this vector, and hygromycin resistant transformants were regenerated. Many of these plants displayed abnormalities associated with an elevated endogenous cytokinin biosynthesis. One tobacco plant produced flowers with reduced stamen size, but gave fertile offspring upon backcrossing to wildtype. This plant is now used as a model plant to study cytokinin effects, interaction between cytokinin and IAA metabolism, effect of endogenous cytokinin levels on IAA induced promoters etc.

POSTER ABSTRACTS

918 MULTIPLE SHOOT FORMATION AND PLANTLET PRODUCTION OF Phaseolus vulgaris. Siramut Jamseejan[1], A. Wongpiyasatid[1], T. Tongutnaisri[2] and S. Unsrisong[1]. Kasetsart University[1], Maejo Inst. of Agric. Tech.[2], Thailand

A large number of plantlets of red kidney bean (Phaseolus vulgaris) were routinely obtained by germinating mature seeds in MS medium containing 11 mg/l BA for 12 days, culturing the excised cotyledonary nodes of the seedlings on the MS containing 5 mg/l BA and 0.05 mg/l NAA for 7 days, keeping these cultures for another 14 days in dim light for shoot elongation, then subculturing shoot clusters to MS containing 0.2 mg/l BA, 0.02 mg/l NAA and 0.03 mg/l GA_3 at every 10 days for two subcultures. Elongated shoots were then rooted in MS containing 0.01 mg/l 2,4-D for 7 days and finally the rooted shoots were transferred to the medium containing MS (salts) and B_5 (vitamins) until the complete rootings were obtained. The whole procedure took 2.5 months from the culture initiation to the soil-ready plantlet. In addition to naturally occurring somaclonal variation, the seeds were treated with gamma rays before germinating in the induction medium. Gamma rays at 50 Gy was suitable for seed treatment of red kidney bean for in vitro culture. It was dose that caused 5.0% reduction in shoot number production as compared to the control (no radiation treatment).

919 AS NONTYPICAL ACTIVATION OF GENE EXPRESSION IN PLANT CELLS BY "IVIN YAN" & "METYUR". Larysa A.Galkina, Vladimir N.Zaetcs and Dmytry P.Prudiev. Department of Bioengineering, Institute of Bioorganical Chemistry, Murmanskaya 5, Kiev-94,253660

In our experiments it was shown that synthetical plant growth regulators used in agriculture in the S.U. & synthesied in our Institute reduced the period of emergence of seedlings twice as much from (24 to 12 hours). We have found that this integrative biological effect is connected with abrupt non-typical activation of gene expression in germ axis of haricot (Phaseolus vulgaris) emergence. "Ivin Yan" causes increase of synthesis by rybosomal & matrix RNP-particles & connected with this sharp synthesis increase of all cell proteins without change in its correlation. On the non-cell systems of wheat seedings emergence it was found that "Ivin-Yan" provided stimulation effect on the translation process. In contrast, "Metyur" induces positive charge protein synthesis with molecular mass of 30 KD, evidently of regulatory nature, which is supposed which is supposed to be drawn in the process of gene expression activation by stage mechanism.

920 A TISSUE CULTURE SYSTEM FOR MOLECULAR ANALYSIS OF ROOT-KNOT NEMATODE DISEASE. D. Radin, X. Zhang, D. Weissenborn, J. Eisenback and C. Cramer. Departments of Crop and Soil Environmental Sciences and Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061.

A root culture system has been developed for achieving high frequency infections of tomato by Meloidogyne incognita. Epidermal thin-cell-layer explants from floral stems of Lycopersicon esculentum produce up to 40 adventitious roots per culture within 7 to 10 days on Murashige and Skoog medium. When rooted cultures are transferred to Gamborg's B-5 medium and exposed to infective juvenile nematodes, gall formation is apparent within five days in genetically susceptible (mi-/mi-) tomato cultivars, Rutgers, Red Alert and Pearson. Root-knot resistant genotypes (Mi+/Mi+), LA655, LA656, LA1022, exhibit a characteristic hypersensitive resistance response in thin-cell-layer roots. A gene transfer method mediated by Agrobacterium tumefaciens is being developed for tomato thin-cell-layers to analyze the effects of specific introduced genes on the response of roots to nematode attack. Preliminary experiments utilizing a disarmed Agrobacterium binary GUS plasmid system has demonstrated expression of the beta-glucuronidase (GUS) reporter gene driven by the CaMV 35S promoter in tomato thin-cell-layers. The expression of specific genes occuring as part of the host defense response to nematode attack is also being investigated using in situ hybridization of nematode infected thin-cell-layers with RNA probes.

921 ISOLATION OF AUTONOMOUSLY-REPLICATING PLASMIDS FROM TRANSFORMED SORGHUM CELLS. Alan D. Blowers, Takashi Hagio,[1] and Elizabeth D. Earle[2]. *Plant Science Center and [1]Department of Plant Breeding, Cornell University, Ithaca, NY 14853*

Cells from a suspension culture of *Sorghum vulgare* (sorghum) have been transformed to either hygromycin or kanamycin resistance following uptake of plasmids introduced on DNA-coated high-velocity microprojectiles. Genomic DNA isolated from hygromycin- and kanamycin-resistant calli contained *hph*- and *npt*II-hybridizing restriction fragments of the expected size, respectively. A second introduced, but unselected for, reporter *uidA* gene was also detected by DNA gel blot analysis in these transformants. However, detectable levels of β-glucuronidase expression (mRNA and protein) were observed in only two transformants, both of which had a high copy number of plasmid molecules integrated into their genome. In addition to chromosomally-integrated copies of the introduced DNA, putative autonomously-replicating extrachromosomal plasmids were detected in sorghum genomic DNA gel blots. Ten of these plasmids were characterized after recovery of plasmid DNA from ampicillin-resistant *E. coli* cells transformed with sorghum genomic DNA isolated from transformed plant cells. Preliminary characterization of these recovered plasmids has revealed that they are structurally derived from the introduced transforming DNA. We have found that i) more than one type of plasmid could be recovered from the same sorghum transformant; ii) the same plasmid, judged to be identical as determined by restriction enzyme analysis, was isolated from independent sorghum transformants; and iii) the plasmid population within the sorghum transformants had not reached an equilibrium as changes in both plasmid copy number and structure were detected by DNA gel blot analysis. A further characterization of these plasmids will be presented.

922 TRANSFER OF A HERBICIDE TOLERANCE GENE BY DIRECT GENE TRANSFER OF *ARABIDOPSIS* GENOMIC DNA INTO TOBACCO PROTOPLASTS. P.Gallois, R. Malone, K.Lindsey, M. Kreis, MGK Jones. Universite de Perpignan, Laboratoire de Physiologie et Biologie Moleculaire Vegetale. U.A. 565 du CNRS. 66860 Perpignan, France. To study the possibility of gene rescue in plants by direct gene transfer we chose the *Arabidopsis* mutant GH50 as a source of donor DNA. GH50 is tolerant to chlorosulfuron a herbicide of the sulfonylurea class. Tobacco protoplasts were cotransfected with genomic DNA and the plasmid pHP23 which confers kanamycin resistance. A high frequency of cointegration of the plasmid and the genomic DNA was expected, which would allow the tagging of the plant selectable trait with the plasmid DNA. After transfection by electroporation the protoplasts were cultivated on regeneration medium supplemented with either chlorosulfuron or kanamycin as a selective agent. Selection on kanamycin yielded resistant calli at an absolute transformation frequency (ATF) of 0.8×10^{-3}. Selection on chlorosulfuron yielded resistant calli at an ATF of 5×10^{-6}. When a selection on chlorosulfuron was subsequently applied to the kanamycin resistant calli, 8% of them showed the tolerance to this herbicide. Southern analysis carried out on the herbicide tolerant transformants detected the presence of the herbicide tolerance gene of *Arabidopsis* into the tobacco genome. Segregation analysis showed the presence of the tolerance gene and the marker gene in the progeny of some transformants. A few transformants showed evidence of genetic linkage between the two genes.

923 INFLUENCE OF GENOTYPEAND MEDIA ON ANTHER CULTURE RESPONSE IN RICE(Oryza sativa L.). Nibedita Lenka and G.M.Reddy,Department of Genetics, Osmania University, Hyderabad-500 007,A.P. INDIA.

Efficiency of callus induction from anthers of seven indica rice genotypes was tested with different media. Frequency of callusing varied with the type of medium used and the time of cold pre-treatment. The most responsive genotype was the var.PTB-33, with 40% of the anthers showing callusing on N6 medium fortified with 2mg/l NAA and 0.5mg/l KN. The callus was highly embryogenic on N6 medium supplemented with 2mg/l 2,4-D and 0.5mg/l KN, although growth was more in the presence of NAA. Anthers of var.PTB-33 exhibited maximum green plant regeneration on MS medium containing 2mg/l BAP+1mg/lNAA/IAA and 0.6mg/lKN. Higher concentration of sucrose (6%) in the medium was found to be better for both callus initiation and shoot(green) regeneration. Rooting of the shoots was poor in this medium. Effect of different amino acids viz., Alanine, Glutamine, tyrosine and Proline on callusing revealed that, except proline all the other amino acids promoted both callus proliferation and shoot regeneration. About 2-3 fold increase in the shoot regeneration was noticed when alanine was included in the medium.

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924 PEROXIDASE AND RESPIRATORY ENZYMES OF SALT SENSITIVE AND SALT TOLERANT CALLI OF SAFFLOWER (CARTHAMUS TINCTORIUS L.) CV. A 1. M.G. Shitole and * T.D.Nikam Department of Botony, University of Poona, Pune 7 *Modern College Pune, India

Salt tolerant callus of safflower cv. A 1 was raised on M S medium supplemented with 1.5 mg. NNA/1, 0.5 BAP/1 and 0.75 % NaCl and activities of enzymes hexokinase (HK), succinic dehydrogenase (SDH), glucose 6-phosphate dehydrogenase (G 6-PDH), acid phosphatase and peroxidase were investigated in the salt tolerant and salt sensitive calli growing at 00, 0.1, 0.25, 0.5, 0.75 % NaCl. It was found that activities of HK, SDH, G 6-PDH and acid phosphatase decreased at all higher (more than 0.1 %) concentrations of NaCl whereas their activities were less than the control under non saline conditions and were more than the control at all levels of salinity in the salt tolerant calli. The results revealed need of NaCl for optimum activities of these anzymes in the salt tolerant calli thereby indicating shift towards halophytic mode of life. Peroxidase activity was more than the control at all levels of NaCl in the salt tolerant and salt sensitive calli. However, its activity was much more in the salt tolerant calli than the salt sensitive calli which suggested that peroxidase plays important role in the salt tolernace of both the calli and there is a correlation between peroxidase activity and salt tolerance capacity of the calli.

925 SALT STRESS EFFECT ON COTTON CALLUS. F.Muhamedhanova, M.Muminova, A.Abdukarimov. A.S.Sadykov's Institute of Bioorganic Chemistry, Academy of Sciences Uzbek SSR, Tashkent, USSR.

The physiological activity of salt stress and the possibility of phenotype identification on cells tolerant to salting on cotton (G.hirsutum L., G.klotzschianum Andress.) callus were studied. For obtaining NaCl tolerant cell clones we used two traditional methods: 1 - the straight selection, when cultivated tissue was transfered to high salt concentrations at once (1,4-1,6%), and 2 - step selection, i.e. callus was been transfering step by step from low concentration (0,2; 0,4; 0,6; 0,8; 1,0; 1,2; 1,4; 1,6 %) demonstrated the undoubted physiological NaCl effect. The gradually adaptation to NaCl concentrations reduced the callus salt sensitivity and increased its salt tolerance and the straight selection selected the most vital and adapted cells by the help of stress. But very high NaCl concentrations (more 2,0 %) are toxic even for potential salt tolerant lines.

926 A GENOTYPE INDEPENDENT APPROACH TO SELECT SHOOT COMPETENT CELLS FROM WHEAT SUSPENSION CULTURES. Wen Chung Wang. Department of Agronomy, Horticulture and Entomology, Texas Tech University, Lubbock, Texas 79409

Application of current genetic manipulation approaches in vitro for crops depend on the ability of plant cells to regenerate into fertile plants. A novel approach were reported previously for efficient plant regeneration from long-term suspension culture of wheat Mustang. Protoplast derived from this cell line also maintained regeneration competence. However, plants regenerated were sterile. This approach is very reproducible which have been applied to other 4 wheat genotypes to isolate young shoot competent cells from the cell populations where both rooting cells and non-morphologic cells coexisted. Among the genotypes, variations were observed for the date of root initiation and the amount of the shoot competent cells presence in the cell populations. Some genotypes has more shoot competent cells than others, some with lots of root competent cells and only very small amount of the shoot competent cells. The quantity of the shoot competent cells correlated well with the genotypic response when immature embryos were plated for regeneration. The selected cells can be proliferated with 0.1-0.25 mg/l 2,4-D with very limited differentiation. The cells can be triggered to differentiate into shoot with lower 2,4-D level after 5-7 subcultures. These shoot competent cells can be proliferated at 1-4 mg/l 2,4-D M. S. medium at a much faster growth rate with much friable callus for protoplast culture. Somatic embryogenesis events were observed from the cell population of the protoplast derived culture. Plant regenerated from these young cultures were under the evaluation for genomic stability and fertility, and was used for transformation studies.

927 USING PCR TO CONFIRM *AGROBACTERIUM* TRANSFORMATION OF AMERICAN CHESTNUT (*CASTANEA DENTATA*).

Charles A. Maynard. SUNY, College of Environmental Science and Forestry, Faculty of Forestry, Syracuse, New York 13210.

Oligonucleotide primers for the neomycin phosphotransferase (*neo*, Beck et al. 1982. Gene, 19:327-336) were used to detect *Agrobacterium* transformation. The technique was effective on shoot tissue and callus of petunia (*petunia hybridia*) and on callus from willow (*Salix* spp.), black cherry (*Prunus serotina*), and American chestnut (*Castanea dentata*).

To simulate a rare transformation event in chimeric tissue, transformed petunia callus was added to non-transformed callus before DNA extraction. Even at high dilution, PCR amplification was capable of detecting the presence of *neo* DNA. PCR amplification of introduced genes will be especially useful with long-breeding-cycle tree species.

928 SURFACTANT EFFECTS ON THE GROWTH OF *SOLANUM DULCAMARA* TRANSFORMED ROOTS AND PROTOPLASTS IN CULTURE.

V. Kumar, L. Laouar*, M.R. Davey, B.J. Mulligan and K.C. Lowe* University of Nottingham, Departments of Botany and *Zoology, Nottingham NG7 2RD, U.K.

The non-ionic surfactant, Pluronic F-68 (Poloxamer 188; ICI/Atochem), has been used as a cell-protecting/growth stimulating agent in animal cell cultures; its effects on plant cells have been poorly studied. *Solanum dulcamara* roots transformed by *Agrobacterium rhizogenes* A4TIII were grown on hormone-free MS liquid medium containing 0.001-1.0% (w/v) Pluronic F-68. Commercial grade (COMM) Pluronic was dissolved in $d.H_2O$; a Pluronic fraction (FRAC) was prepared by passage through silica-Amberlite resin (SAR). Maximum growth stimulation (2 x control) occurred with 0.01% (w/v) COMM Pluronic after 25d. Growth stimulation also occurred when COMM Pluronic was used in a membrane-raft system. Pluronic (0.01% w/v) stored for 5d markedly inhibited growth, which was more pronounced with solutions stored at 4°C than at 22°C. Freshly-prepared, FRAC Pluronic also inhibited growth at all concentrations tested. An increase in mean protoplast plating efficiency of up to 26% occurred when cell suspension protoplasts were cultured in agarose-solidified K8P medium with 0.1% (w/v) freshly-prepared COMM Pluronic at a density of 1.0×10^5/ml. The results support our findings with animal cells that a SAR-bound fraction is the growth-promoting component in COMM Pluronic preparations.

929 INDUCTION OF SOMATIC EMBRYOGENESIS FROM MESOPHYLL PROTOPLASTS OF ATYLOSIA SP. AND CAJANUS.

S. Narasimha Chary, V.V. Ramana Rao and J.K. Bhalla. Cytogenetics and Tissue Culture Laboratory, Department of Botany, Osmania University, Hyderabad 500007, A.P., INDIA.

Wild relatives are of potential use in crop improvement, because of their desirable traits. An attempt has been made to isolate mesophyll protoplasts from a wild species Atylosia volubilis and a cultivar UPAS-120 pigeonpea and embryogenesis was induced. The mesophyll protoplasts were isolated using enzymatic method. The enzyme mixture consisting of 0.5% pectinase + 1% cellulase was used. The isolated protoplasts were cultured on three different modified liquid media viz., B_5, MS and Blaydes in which B_5 was more effective in induction of embryogenesis. The fusion of protoplasts of cultivar and wild species was carried out by chemical method using polyethylene glycol. The fused products were cultured as suspension media which have formed protoclones, and subsequently callus was formed.

POSTER ABSTRACTS

930 ANTHER CULTURE OF COLD TOLERENT RICE OF NEPAL. V.P. Agrawal and N. Joshee, Research Laboratory for Agricultural Biotechnology and Biochemistry (RLABB), P.O. Box 2128, Kathmandu, Nepal. Five allegedly cold tolerant rice cultivars cultivated at higher altitudes (1500-3000m) in Nepal and their F_1 hybrids were grown both in open fields and hydroponically in green house. Rice boots of physiological growth condition corresponding to uninucleate pollen stage were excised and pretreated at 8°C for 0-21 days. For callus induction, anthers from cold pretreated boots were incubated in dark in a modified N_6 media (FJ) cotaining 2.5 ppm NAA, 0.5 ppm kinetin, 0.5 ppm 2,4-D, 500 ppm casein hydrolysate and 4% sucrose. For plant regeneration, resulting calli were incubated in light (1500 lux) in a modified MS media (SKII) containing 1 ppm each of kinetin, NAA and BAP, 500 ppm of casein hydrolsate and 4% sucrose. FJ and SKII were developed in FJ. Zapata's laboratory in International Rice Research Institute, Philippines. Callus induction and subsequent plantlet regeneration were done at 25°C. Plantlets could be obtained for all five cultivars. Time period and frequency for callus induction and plantlet (green and albino) regeneration depended upon rice genotypes (cultivars), duration of cold pretreatment and growth conditions of rice plants. This work has been supported by the Rockefeller Foundation International Rice Biotechnology Program (RF No. 90032 No. 77).

931 DNA METHYLATION CHANGES, POLYPEPTIDE PATTERN AND LSU RUBISCO LEVELS RELATED WITH PHASE CHANGE AND REJUVENATION OF HAZELNUT (Corylus avellana L.). Díaz-Sala, C.; Rey, M., Rodríguez, R. Lab. Fisiología Vegetal. Fac. Biología. Univ. Oviedo. Oviedo. SPAIN.

Phase change in woody species has received attention because of the maturation-related decrease in the ability to clone selected individuals using explants from adult plants. The fact that "in vitro" subcultures and severe pruning of trees may favour rejuvenation was tested based on the characterization of "in vitro" morphogenic capacities and molecular differences among mature clones, juvenile clones, "in vitro" plantlets from mature clones after several subcultures and mature clones after severe pruning of hazelnut. The results obtained showed that "in vitro" sequential subcultures of explants taken from adult plants in the presence of high concentrations of cytokinins and severe pruning induce transient phenotypic and physiological changes which increase morphogenic competence of this explants, similar to those for juvenile materials. The increase of morphogenic potential after several subcultures and severe pruning is the result of a reinvigoration process. Total protein electrophoresis reveals the existence of specific polypeptide that can be correlated with the cronological and physiological age of the plant material. Rubisco large subunit detection, using polyclonal antibodies, shows a decrease of activity with aging; however the lowest activity is detected in "in vitro" plantlets. The latter result can be related with the presence of sucrose in the culture medium. There are not significative differences between juvenile and mature clones for DNA methylation degree. However, there is a great increase in "in vitro" plantlets for both CCGG and CC(A/T)GG restriction fragments.

932 **Genetic Studies on Callus induction in *Vicia faba*.**

1- The role of amino acid composition and seed protein.

SOLIMAN, M., ABOU-SALHA, A.E., MAHMOUD, S., and MADKOUR, M. A.
National Agricultural Genetic Engineering Laboratory (NAGEL), Giza, Egypt.

Explants from six cultivars of *Vicia faba* were cultured on different kinds of media. callus production was recorded for each cultivar and correlated with the amino acid composition. Significant variations were found among *Vicia faba* cultivars in their ability to induce callus on different media. The cv. "Giza 2" produced more calli on all kinds of media whereas "Giza 3" produced a very limited number of calli on most of the media. The protein and amino acid composition analysis showed an absence of a single subunit in the protein banding pattern of the cv "Giza 2" on SDS-PAGE and a low content of glycine and alanine. A significant negative correlation was found between callus induction and Glycine and cystine content of the cultivar.

933 TRANSFORMATION AND REGENERATION OF SWEET PEPPER WITH COAT PROTEIN GENES OF TMV AND CMV. Yu-Wen Wang, Mei-Zhu Yang, Nai-Sui Pan and Zhang-Liang Chen. National Laboratory of Protein Engineering and Plant Genetic Engineering, Biology Department, Peking University, Beijing 100871, China

Sweet pepper is an important vegetable in the world and has been regarded as a difficult plant for genetic transformation. Three different cultivars of sweet pepper were screened out for their high capacity of regeneration. The normal flowering and fertile regenerated plants have been obtained from cotyledons of 10-16-day old seedlings by a four-step culture procedure, shoot inducing, shoot elongation, rooting of excised shoots and transplanting into soil. MS was the basal medium in all steps, supplemented with different kind and different concentration of phytohormone. Optimal shoot inducing medium is MS+4-6mg/l BA with the shoot regeneration frequency of 100%. 35% of the induced shoots elongated on the medium of MS+2mg/l Zeatin+ 1-2mg/l GA_3 and subsequently rooted on MS medium or with 0.1-0.5mg/l NAA. The regenerants were transplanted into soil and developed into normal plants. These cultivars were transformed with Agrobacterium containing genes encoding coat protein of TMV and CMV we cloned driven with 35S promotor of CaMV and transgenic plants were regenerated. The expression of these genes in transgenic sweet pepper were detected and further analysis of cross-protection to virus infection are in progress.

934 A COMPARATIVE STUDY OF REGENERATION FREQUENCIES AND EFFICIENCY OF AGROBACTERIUM-MEDIATED TRANSFORMATION BETWEEN COTYLEDON AND STEM EXPLANTS OF FOUR BRASSICA VARIETIES.
Jason M. Pickering, C. L. Trapnell, Susan A. Marlow & Linda A. King. School of Biological & Molecular Sciences, Oxford Polytechnic, Oxford, U.K.

Growing concerns about the environmental effects of chemical insecticides have increased interest in the development of biological methods of pest control. The current study investigates the use of insect-specific viruses as a novel method for the biological control of pests using transgenic plants as the delivery system. We will initially use Cricket paralysis virus (CrPV), a small insect picornavirus, as a model to assess the potential of such a system.

A preliminary study was undertaken to maximise shoot regeneration from cotyledon and stem segment explants of four brassica varieties, using methods modified from Molony et al., (1) and Fry et al., (2). Cotyledons and stem segments of B. napus cv. 'cobra' and 'samouri', and stem segments of B. oleracea were cultured on solidified Murashige and Skoog medium suplemented with varying concentrations of 6-Benzyl-aminopurine (BAP) and alpha-Napthaleneacetic acid (NAA), and shoot regeneration frequencies were compared.

Transformations of cotyledon and stem segments were carried out by co-cultivation with the Agrobacterium binary vector PJIT 59, containing the CMV 35S promoter and the GUS reporter gene (kindly provided by the John Innes Institute, U.K.). Transgenic shoots were selected by three successive cultures on kanamycin supplemented medium. Sample tissues from regenerated plantlets were then analysed using the beta-glucoronidase fluorometric assay.

935 EXPRESSION OF THE PPi DEPENDENT PHOSPHOFRUCTOKINASE IN BEANS. Sandra du Plessis and Frederik C. Botha
Dept. of Botany & Genetics, U.O.F.S, Bloemfontein, South Africa.

The PPi:D-fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.90 PFP) activity sharply increases during the exponential growth phase in *Phaseolus vulgaris* cell suspension cultures. Maximum activity was present at the midlog phase of growth. The changes in PFP activity during the growth cycle were not due to the presence of an activator or inhibitor of PFP. The PFP of beans contains the typical α- and β subunits of higher plant PFP. Monospecific antibodies against the α- and β subunits of PFP were used to quantify the amount of PFP protein on protein blots. The change in PFP activity was the direct consequence of changes in specific PFP protein content. The pattern of change in the PFP subunit content indicated that the subunits are not coordinately expressed. Northern blot analysis of total RNA, using a cDNA probe against PFPβ from castor bean, indicated that prevailing PFP activity is primarily controlled by the level of transcription of PFP. Immunoprecipitation of *in vitro* translation products with anti-PFPα and anti-PFPβ also showed that PFP activity is controlled by the prevailing PFP-mRNA levels.

POSTER ABSTRACTS

936 ON THE LOCALIZATION OF PRECURSOR PROTEINS TO THE CHLOROPLAST ENVELOPE MEMBRANE. Karsten Fischer, Susanne Brink and Ulf-Ingo Flügge. Institut Botanik I der Universität Würzburg, Mittlerer Dallenbergweg 64, 8700 Würzburg, Germany

Most of the chloroplast proteins that are destined to the different compartments of the chloroplast (e. g. outer and inner envelope, intermembrane space, stroma, thylakoid membrane and lumen) are coded for in the nucleus and are synthesized in the cytosol as higher-molecular-mass precursor proteins containing a N-terminal transit peptide which is cleaved off during the import process. In contrast to transit peptides of higher plant proteins destined for the stroma and the thylakoids those of the inner envelope membrane proteins contain an amphiphilic α-helix (1). In order to determine which structural domains of the transit peptides are involved in the localization of the attached protein to the different chloroplast compartments we have fused the transit peptides of inner envelope membrane proteins to the mature parts of stromal and thylakoid proteins and *vice versa*. The cloning strategy will be outlined and experiments concerning the localization of the fusion proteins will be shown.

1. Dreses-Werringloer, U., Fischer, K., Wachter, E., Link, T. and U. I. Flügge (1991) Eur. J. Biochem. 195, 361-368

937 MOLECULAR MECHANISM OF NUCLEAR TARGETING OF THE R-PROTEIN, THE MAIZE TRANSCRIPTIONAL ACTIVATOR Mark W. Shieh,[1], Susan R Wessler,[2] and Natasha V Raikhel[1]. [1] MSU-DOE Plant Research Laboratory, East Lansing, Michigan 48823-1312, [2] Department of Botany University of Georgia, Athens, GA 30602.

The proteins that function in the nucleus are synthesized in the cytoplasm and are transported into the nucleus. The nuclear import of proteins larger then 50 kD is mediated by specific nuclear localization sequences (NLS) within the proteins. There is no conserved sequence for the NLS but they are known to be both necessary and sufficient for nuclear uptake. We are interested in understanding the molecular mechanism of nuclear targeting in plants. To study nuclear transport we are using the recently cloned R-gene of maize. Genetic and structural evidence indicates that the maize R gene encodes a nuclear transcriptional activating factor. The R-gene encodes a protein of 610 amino acids which contains a helix-loop-helix motif which is homologous to regions of the transcriptional activators *myo D1* and *myc*[1]. The reporter gene β-glucuronidase (GUS) has been fused to the R gene to facilitate the localization of the protein within the plant cells. The GUS-R gene fusion and the GUS genes were transiently expressed in Nicotiana tobacum suspension cells. Histochemical analysis of the tobacco cells revealed that the GUS-R fusion protein is localized in the nucleus, however, the GUS protein is retained in the cytoplasm. Further studies will reveal specific NLS that are capable of redirecting the GUS protein to the nucleus.

1 S.R. Ludwig, L.F. Habera, S.L. Dellaporta, and S.R. Wessler, 1989. PNAS. 86:7092-7096

938 THE PROCESSING OF CONCANAVALIN A IN JACKBEAN (*CANAVALIA EINSIFORMIS*) SEEDS
Philip S. Sheldon and Dianna J. Bowles
Centre for Plant Biochemistry and Biotechnology, Department of Biochemistry and Molecular Biology, The University of Leeds, LS2 9JT, U.K.

The post-translational processing of the lectin ConA is complex and involves a number of unusual events including deglycosylation, endo-proteolytic cleavage C-terminal to asparagine residues and a religation or transpeptidation. This results in the N-terminal and C-terminal halves of the mature polypeptide chain being reversed from their order in the initial translation product. In order to study the enzymology of these processes, the glycoprotein precursor of ConA has been purified to electrophoretic homogeneity, radio-labelled with ^{125}I, and used as a substrate for processing *in vitro*. The precursor does not possess carbohydrate-binding activity. Removal of the oligosaccharide by treatment with N-glycanase from *Flavobacterium meningococcum* in the absence of the subsequent proteolytic and ligation steps will convert the precursor into an active lectin. The labelled precursor is a substrate for the jackbean N-glycanase and asparagine protease involved in processing. It has been used to assay these enzymes and enable their purification.

939 IMMUNOCYTOCHEMICAL IDENTIFICATION OF SPECIFIC FUNCTIONAL ACTIVITIES OF CIS, MEDIAL AND TRANS GOLGI CISTERNAE. L. Andrew Staehelin, Margaret A. Lynch, Patricia J Moore, Kathleen M. M. Swords and Guo-Feng Zhang.
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347.

The plant Golgi apparatus is the site of synthesis of complex polysaccharides and glycoproteins, and both types of molecules are transported to the cell surface in secretory vesicles. However, little is known about how these different synthetic pathways are organized within Golgi stacks, and where sorting of the different types of molecules occurs. To address these problems, we have used highly-characterized antibodies in conjunction with immunogold staining of high pressure frozen and freeze-substituted sycamore suspension culture cells to localize the appearance of specific chemical groups to structurally defined Golgi cisternae. The antibodies employed recognized both backbone and sidechain domains of the pectic polysaccharides, polygalacturonic acid/rhamnogalacturonan-I (PGA/RG-I), and of the hemicellulose, xyloglucan (XG), as well as O-linked arabinose residues of extensin, and the xylose residue of complex N-linked glycans of glycoproteins.

Our micrographs demonstrate that 1) individual Golgi stacks can process simultaneously glycoproteins and polysaccharides, 2) O-linked arabinosylation of glycoproteins occurs in cis cisternae, 3) addition of xylose to N-linked glycans and methyl-esterification of pectic polysaccharides occurs in medial cisternae, 4) synthesis of the backbone of XG, addition of sidechains to PGA/RG-I, and some fucosylation of XG occurs in trans cisternae, and 5) completion of XG fucosylation takes place in the trans Golgi network. In other studies, we have found that the functional organization of Golgi stacks may differ between cell types in clover root tips. Supported by NIH grant GM 18639 to LAS.

940 EXPRESSION OF MAIZE b-70, AN ENDOPLASMIC RECTICULUM MOLECULAR CHAPERONE HOMOLOG. Russell Wrobel, Gregory R. OBrian, and Rebecca S. Boston. North Carolina State University, Raleigh, NC 27695

Plants carrying *floury-2 (fl2), Defective endosperm-B30 (De*-B30),* or *Mucronate (Mc)* mutations overproduce b-70. Amino acid sequence homology and similar biochemical characteristics identify b-70 as a homolog of the mammalian molecular chaperone of the ER lumen, binding protein (BiP). During endosperm development in these mutants, levels of b-70 protein and mRNA increase dramatically between 14 and 20 days after pollination paralleling the accumulation of the major endosperm storage proteins, zeins. This increase in b-70 mRNA accumulation is endosperm-specific and dependent on gene dosage in the *fl2* mutant. In all three mutants, the abundance of b-70 mRNA is inversely proportional to the abundance of zeins. Treatment of maize suspension cultures with tunicamycin, a potent inhibitor of glycosylation, also results in increases in b-70 RNA and protein. We suggest that induction of b-70 in the mutants is a cellular response to abnormally folded or improperly assembled storage proteins. This is reminiscent of abnormally folded or assembled proteins within the ER lumen inducing BiP in mammalian cell cultures. Investigations are underway to determine the molecular basis for the induction of b-70 in maize endosperm.

941 IMMUNO-MICROSCOPY ANALYSIS OF PROTEIN BODY FORMATION IN MAIZE MUTANTS OVEREXPRESSING THE MOLECULAR CHAPERONE HOMOLOG b-70. Fan Zhang and Rebecca S. Boston. North Carolina State University, Department of Botany, Raleigh, North Carolina 27695

A maize protein, b-70, bears striking similarity to a group of polypeptide chain binding (PCB) proteins that have been implicated in folding and/or assembly of nascent proteins in the endoplasmic reticulum (ER). To characterize b-70 and determine its possible role in protein body formation in maize endosperm, immuno-microscopy studies were conducted in three maize mutants W64A *floury-2*, B37 *Mucronate*, and B37 *Defective endosperm-B30*, in which the level of b-70 protein is highly elevated. Our results showed that protein body morphology in all three mutants had been altered. Immuno-localization of the different types of zeins (α, β and γ) in protein bodies indicated that the pattern of deposition observed in normal protein bodies is changed in these mutants. The degree of abnormality in protein body morphology is positively correlated with increased amounts of b-70 protein. Additional investigation of the possible involvement of b-70 in protein body formation in maize endosperm is in progress.

POSTER ABSTRACTS

942 CHROMATOGRAPHIC CHARACTERIZATION OF THE CHLOROPLAST SOLUBLE PROCESSING ENZYME USING AS A SUBSTRATE THE LHCP PRECURSOR SYNTHESIZED IN *E COLI*

John E Oblong, Mark S Abad, and Gayle K. Lamppa. The University of Chicago Department of Molecular Genetics and Cell Biology, Chicago, Illinois 60637

The light-harvesting chlorophyll *a/b*-binding protein (LHCP) is synthesized as a 31 kD precursor protein (preLHCP) containing an N-terminal extension termed a transit peptide. Upon translocation into the chloroplast, preLHCP is proteolytically processed to two mature forms of ~26 and 25 kD. The processing enzyme that produces the 25 kD form has been characterized as a ~240 kD soluble metalloprotease. A wheat gene coding for preLHCP was used in an *E coli* expression system to generate milligram amounts of this substrate. preLHCP expressed in *E coli* is sequestered into inclusion-like bodies that can be solubilized in 6 M urea. Sequential dialysis of preLHCP out of urea yielded a soluble substrate that was cleavable in an *in vitro* organelle-free processing reaction. In order to better understand the requirements for recognition of the preLHCP substrate by the processing enzyme, dialyzed preLHCP was purified by Superose 12 gel filtration chromatography, and, was found to be a high molecular weight aggregate. The purified aggregate has both α-helix and β-sheet secondary structure, as determined by tryptophan fluoresence and circular dichroism. Purification of the soluble processing activity from chromatographic columns was monitored using the *E coli* expressed preLHCP as a substrate. Evidence indicates that the 240 kD enzyme is a multi-subunit protease. Six protein bands remain as potential candidates for the 240 kD processing enzyme as determined by silver-stained SDS-PAGE gel analysis.

943 A SINGLE CARBOXYL-TERMINAL PROPEPTIDE OF PROBARLEY LECTIN IS SUFFICIENT TO REDIRECT A SECRETED PROTEIN TO THE VACUOLES IN TRANSGENIC PLANTS Sebastian Y. Bednarek and Natasha V. Raikhel, MSU-DOE Plant Research Laboratory, East Lansing, Michigan 48824-1312

Secretory proteins are sorted to the plant cell vacuoles or are retained within the secretory pathway by mechanisms which require specific targeting information contained within the structure of the protein. It has been demonstrated, that secretory proteins lacking such information follow a default pathway and are secreted. We have previously shown using a transgenic system that the 15 amino acid carboxyl-terminal propeptide (CTPP) of probarley lectin (proBL) is necessary for the proper sorting of this protein to the plant vacuole. Mutant forms of the protein lacking the CTPP are secreted. The active N-acetylglucosamine binding form of proBL is a dimer consisting of two identical subunits. ProBL is initially synthesized and assembled into a dimeric protein containing two CTPPs within the endoplasmic lumen. To investigate whether one or both of the CTPPs of proBL dimer are necessary for proper sorting we have engineered plant cells to express both a wild type and mutant forms of proBL lacking the CTPP coding region. Dimers of proBL were assembled in the cells which contain either zero, one or two CTPPs. Preliminary results indicate that proper sorting of proBL is dependent on the presence of only one CTPP. To test the hypothesis that the CTPP is sufficient as a vacuolar sorting signal, we have prepared a chimeric gene containing the region encoding the barley lectin CTPP fused with the gene encoding cucumber chitinase, a secreted protein. Organelle fractionation and EM immunocytochemistry have demonstrated that the cucumber chitinase-CTPP fusion protein is redirected to the plant vacuole confirming that the CTPP is a vacuolar sorting determinant.

944 TARGETTING OF PROTEINS TO DIATOM PLASTIDS: A NEW ROUTE

D. Bhaya[1] and A. Grossman[2], [1]Centre for Biotechnology, JNU, New Delhi-67, India. [2]Carnegie Inst. of Washington, 290 Panama St., Stanford, CA 94305, USA.

Diatoms, unlike higher plants, have a xanthophyll-dominated light harvesting complex (LHC) and an endoplasmic reticulum (ER) network surrounding the plastid. We have demonstrated that the polypeptides of the LHC from the diatom Phaeodactylum tricornutum (these polypeptides are designated Fcp) are nuclear-encoded, synthesized as precursors in the cytoplasm and constitute a small multi-gene family. Three different cDNAs coding for these proteins were isolated, sequenced and shown to have homology to the genes encoding the chlorophyll a/b binding polypeptides of higher plants (Grossman A.R. et al, Mol. Gen. Genet (1990) 224:91-100]. Recently we have also isolated and characterized Fcp encoding genomic clones. The genomic sequences characterized have no introns and some of the members of the gene family are clustered. The amino termini of the precursor Fcp, as deduced from gene sequences, have features of a classic signal peptide. We have demonstrated that these precursor polypeptides can be cotranslationally imported and processed by an in vitro microsomal membrane system, suggesting that cytoplasmically-synthesized proteins destined for the plastid require a signal peptide to traverse the ER that surrounds the plastid. This mode of transport contrasts with that of protein transport into the chloroplasts of higher plants which is a post-translational process mediated by a transit sequence, a sequence that bears little resemblance to a signal peptide.

945 INSERTION OF CHLOROPHYLL BINDING PROTEIN INTO A MEMBRANE LACKING CHLOROPHYLL. Bruce D. Kohorn, Andrea H. Auchincloss, Fenbio Gao. Botany Dept. Duke University, Durham NC 27706.

Some integral membrane proteins do not accumulate to normal levels when certain pigments are deficient, and this has been interpreted to mean that such proteins may be rapidly degraded when not in a correct complex. Alternatively, pigments could facilitate the movement of some proteins from an aqueous to a lipid environment. To determine whether chlorophyll is absolutely required for the membrane integration of the Light Harvesting Chlorophyll Binding Protein (LHCP) of chloroplast thylakoid membranes, we have expressed LHCP in E. coli that lacks photosynthetic pigments. LHCP is targeted in vitro to the bacterial inner membrane by the addition of a bacterial signal peptide and cannot be extracted from these membranes by NaOH, NaBr or Na_2HCO_3, but is extracted by 0.2% Triton X-100. Treatment of isolated right side-out and inside-out bacterial inner membrane vesicles with trypsin reveals that only the amino-terminus of LHCP is exposed on the cytoplasmic face and the remaining portion of the protein is inaccessible. Treatment of the inside-out vesicles with trypsin followed by alkaline extraction shows that LHCP is intrinsic to the membrane and is not anchored solely by the bacterial signal peptide. In addition, LHCP that lacks a bacterial signal peptide will insert into isolated E. coli membranes, and we are investigating the influence of added pigments on the process. While chlorophyll is therefore not essential for integration, the specificity of LHCP for a particular membrane may well lie in LHCP's ability to accumulate only in locations of chlorophyll abundance.

946 CHLOROPLAST PROTEIN IMPORT OF DHFR FUSION PROTEINS.

Jinggiu Li and Alan L. Friedman. Dept. of Biology, Marquette University, Milwaukee, WI 53233.

Most Chloroplast proteins are synthesized in the cytosol as higher molecular weight precursors and posttranslationally imported into the organelle. However, the mechanism of protein translocation across the chloroplast envelope membranes remains unclear. To study the conformation of proteins during membrane translocation, we have constructed a series artificial fusion proteins which can be stabilized as a tightly folded complex upon specific ligand binding. These fusion proteins contain the precursor of ribulose bisphosphate carboxylase small subunit (prSS) fused to mouse dihydrofolate reductase (DHFR). The fusion proteins were expressed in an *in vitro* wheat germ translation system, and presented to isolated pea chloroplasts. All but one of the fusion proteins import into chloroplasts and were processed by the chloroplast stromal protease. Methotrexate, a competitive inhibitor of DHFR, has little effect on the import of fusion proteins but does lead to aberrant processing. Methotrexate affinity chromatography of the fusion protein indicates that the fusion proteins translated in the *in vitro* translation system fail to bind to methotrexate. We recently cloned the prSS-DHFR fusion proteins into *E.coli* expression vectors. Upon induction, transformants can be selected on trimethoprim, a folate antagonist, and the isolated protein binds specifically to methotrexate affinity column. These data demonstrates that prSS-DHFR fusion proteins, expressed in *E.coli*, are enzymatically active and capable of methotrexate binding. The effects of methotrexate on the import of the over-expressed fusion proteins will be presented.

947 **Targeting of fusion proteins towards the thylakoid lumen of chloroplasts.** **Douwe de Boer[1], Hans Bakker, Peter Weisbeek.**
Department of Molecular Cell Biology and Institute of Molecular Biology, University of Utrecht, P O.Box 80 056, 3508 TB Utrecht, The Netherlands. [1]present address: Agrotechnological Research Institute (ATO) P O Box 17, 6700 AA Wageningen, The Netherlands

Routing of fusion proteins to the thylakoid lumen of the chloroplast was compared *in vitro* and *in vivo*. The *Escherichia coli* protein ß-lactamase was used as a passenger to study this intraorganellar sorting process. The first step, translocation of ß-lactamase into the chloroplast stroma, occurs properly both *in vitro* and *in vivo* and is dependent on the presence of a transit peptide in the protein construct. The second step, targeting towards the thylakoid lumen, is more complicated as was also observed previously when other passenger proteins were used. *In vitro* the presence of a thylakoid transfer domain is not enough for routing and proper processing. Only when the complete thylakoid lumen precursor plastocyanin was fused to ß-lactamase the fusion protein was processed adequately, but routing was still incomplete. However, *in vivo* the information present in the thylakoid transfer domain was the only requirement for proper transport towards the thylakoid lumen. These data show that *in vivo* the only requirement for targeting of passenger proteins towards the thylakoid lumen is the presence of a transit peptide and a thylakoid transfer domain. Furthermore, we demonstrate that the *in vitro* import system does not necessarily reflect the *in vivo* situation with respect to intraorganellar sorting.

POSTER ABSTRACTS

948 FUNCTIONAL ANALYSIS AND REGULATION OF THE GENES ENCODING THE LUMINAL BINDING PROTEIN (BIP) OF TOBACCO. Jurgen Denecke, Maria Helena Goldman, Jan Demolder*, Jef Seurinck and Johan Botterman, Plant Genetic Systems NV, Plateaustraat 22 and *Rijksuniversiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium

We have cloned cDNAs of the tobacco homolog of the luminal binding protein (BiP) that has been described in other higher eukaryotes. In contrast to the mammalian and yeast protein, tobacco BiP is encoded by a multigene family. The gene products of all the cloned members of this family contain a carboxy terminal HDEL peptide that may form the signal for retention in the endoplasmic reticulum (ER). A chimeric gene containing the coding region of one of the tobacco BiP genes is able to complement a mutation in the Saccharomyces cerevisiae BiP gene. BiP transcripts are shown to be predominantly present in tissues with high rates of cell divisions, in secretory tissues and in cells treated with tunicamycin. Data illustrating the effect of heterologous proteins on BiP expression will be presented.

949 SECRETION OF AN ENDOPLASMIC RETICULUM RESIDENT PROTEIN HYPOTHESIZED TO BE AN AUXIN RECEPTOR. Alan Jones and Eliot Herman. Dept. of Biology, Univ. of North Carolina, Chapel Hill, NC 27599 (AMJ) and Plant Molecular Biology Lab, USDA/ARS, Beltsville, MD 20705 (EH).

Maize contains a 22-kDa subunit auxin-binding protein (ABP) that resides largely in the endoplasmic reticulum (ER) and is hypothesized to be an auxin receptor mediating auxin-induced cell elongation. However, indirect evidence (e.g. Barbier-Brygoo, et al. 1989 PNAS 86, 891) suggests that the cellular site of action of this ABP is the plasma membrane (PM). We addressed the relationship of the ER and putative PM pools of this ABP using electron microscopic immunocytochemistry of a black mexican sweet maize cell line. We have directly shown that the ABP is localized on the ER, golgi apparatus, PM/cell wall space, and the vacuole. The ABP appears to be accumulated and sequestered in the cell wall space and can be lost to the medium. This apparent secretion of the ABP is blocked by brefeldin A indicating that the normal secretory pathway is utilized. Furthermore, as it has been shown for other KDEL-containing proteins in animal cell lines, the secretion of the ABP is enhanced by calcium. Finally, monensin increases the ABP in the medium. Since monensin disrupts endocytosis among many other processes, an intriguing and speculative interpretation of this last observation is that the ABP is recycled into the cell.

950 EXPRESSION IN LEAVES OF TRANSGENIC TOBACCO PLANTS OF SEED PROTEIN GENES CODING FOR THE BIFUNCTIONAL α-AMYLASE/PROTEINASE INHIBITOR FROM MAIZE AND ORYZACYSTATIN FROM RICE. Sameer Masoud Lowell Johnson, Frank White and Gerald Reeck[2]. Kansas State University Departments of Plant Pathology and Biochemistry[2], Manhattan, KS 66506-5502

Genes coding for the bifunctional α-amylase/proteinase inhibitor (12K-CI) and a thiol-proteinase inhibitor (oryzacystatin) from corn and rice cDNA libraries respectively, were used. Various constructs of both genes were cloned under control of the cauliflower mosaic virus 35S promotor and the *nos* 3 region in pMON410-A. The 12K-CI constructs included several combinations of the tobacco mosaic virus 5' untranslated sequence, the 12K-CI signal peptide, and 12K-CI untranslated sequences with various deletions. Plants were regenerated after *Agrobacterium*-mediated transformation. Plants were assayed for vector-encoded nopaline synthase as an indicator of transformation. Both 12K-CI and oryzacystatin genes were transcribed in some plants, as shown by Northern blot analysis, using the appropriate inhibitor cDNA clone hybridized to either total or poly A$^+$ mRNA blots. Protein extracts of some transformed plants gave a positive immunological reaction to antibodies raised against seed 12K-CI or recombinant oryzacystatin protein expressed in *Escherichia coli*. The mobilities of the expressed 12K-CI protein isolated from the transgenic leaves and 12K-CI isolated from corn seed were identical in both native and denaturing gel electrophoresis. Thus, correct processing of the signal peptide appeared to have occurred. Protein extracts of plants expressing mRNA of oryzacystatin showed a band corresponding to the molecular weight of oryzacystatin in denatured Western blots. Both expressed proteins showed biological activity when assayed for inhibitor activity with insect α-amylase and trypsin for 12K-CI and papain for oryzacystatin.

951 AN INVESTIGATION INTO THE INTRACELLULAR PROCESSING OF ACTINIDIN, A PLANT PROTEASE. J C Amiss[1], U M Praeker[1] and H Smith[2]. Leicester University, Leicester Biocentre[1] and Department of Botany[2], Leicester, England.

Actinidin is a cysteine protease which contributes about 50% of the total soluble protein in the kiwi fruit (Actinidia deliciosa). We are interested in the intracellular processing and localisation of this abundant plant protease. A full length cDNA has been isolated, which predicts a polypeptide that is 159 amino acids longer than the mature actinidin, with an amino-terminal extension of 126 amino acids and a carboxy-terminal extension of 33 amino acids. The hydrophobicity plot of the precursor suggests the presence of a signal sequence at the amino terminus. This suggests that actinidin is initially synthesised as a preproprotein which is co-translationally translocated into the endoplasmic reticulum. We demonstrate by in vitro transcription and translation of the actinidin cDNA that a precursor is synthesised which is larger than the mature protein, and show results of experiments to determine the co-translational cleavage of the putative signal sequence. We discuss the problems involved with the detection, by immunological methods, of actinidin in kiwi fruit cells. To further study actinidin processing, tobacco plants have been transformed with actinidin cDNA and we show progress made with these transgenic plants. We have also explored a baculovirus expression system as a means of producing preproactinidin suitable for in vitro processing studies, to identify factors which mediate the cleavage of the immature to the mature actinidin.

952 ISOLATION OF MYOSIN GENES FROM ARABIDOPSIS. John Schiefelbein, Mark Kinkema, Xiaopei Huang, and Sin-Hang Chan. University of Michigan, Department of Biology, Ann Arbor, Michigan 48109.

Myosins are a group of motor proteins that transduce energy stored in ATP into motion along actin filaments. In plant cells, myosins are believed to play a role in cell shape determination, vesicle transport, and cytoplasmic streaming. Two classes of myosins have been identified in animal and fungal cells: double-headed myosins (myosin-II) used in muscle contraction and cytokinesis, and single-headed myosins (myosin-I) implicated in organelle transport and cell motility (1). Oligonucleotide primers derived from conserved head sequences of myosin heavy chains from Acanthamoeba, Dictyostelium, Drosophila, and yeast were used to generate polymerase chain reaction products from Arabidopsis genomic DNA. Sequence analyses of PCR products and genomic clones indicate that at least four myosin-like genes have been identified. The predicted product of each gene possesses sequence characteristics of myosin-I molecules, including a putative phosphorylation site present in most known myosin-I's but not myosin II's (2). The recent results of gene expression studies using these myosin-I sequences will be presented.

1. Korn, Hammer (1988) Ann Rev Biophys Biophys Chem 17:23-45.
2. Brzeska, Lynch, Martin, Korn (1989) J Biol Chem 264:19340-19348

953 POST-TRANSLATIONAL ASSEMBLY OF COFACTOR-CONTAINING PROTEINS: A ROLE FOR THE CYT b6f COMPLEX IN THE ADDITION OF Fe-PROTOPORPHYRIN IX TO APOCYTOCHROME c6. Gregg Howe and Sabeeha Merchant. Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA 90024-1569.

The post-translational steps in which plastidic c-type cytochromes are assembled into functional, thylakoid membrane-localized electron transfer catalysts involves a number of steps including proteolytic processing, membrane insertion and/or translocation, and the covalent attachment of heme to the apoprotein. We have taken a combined genetic and biochemical approach to define both the identity of cytochrome (cyt) c6 processing intermediates in *Chlamydomonas reinhardtii* as well as the temporal order of the processing events. A screen of acetate-requiring strains for holocyt c6 deficiency identified one mutant, called F2D8, that accumulates 1 - 5% of the wild-type level of holocyt c6 but nevertheless accumulates normal levels of plastocyanin (the alternate, functionally equivalent catalyst). "Pulse"-labelling experiments indicate that F2D8 synthesizes a polypeptide that is immunoreactive with antibodies raised against cyt c6. However, the immunoprecipitated product migrates with an electrophoretic mobility that is similar to that of chemically-prepared apocyt c6 but slightly higher than that of holocyt c6. We suggest that the F2D8 is defective in the attachment of heme to apocyt c6. Since apocytochromes are known to be unstable *in vivo* and susceptible to proteolysis *in vitro*, it is not surprising that the anti cyt c6-reactive polypeptide in F2D8 has a short half-life. F2D8 also fails to accumulate polypeptides of the cyt b6f complex. In analyzing several previously characterized PSII, PSI and cyt b6f mutants (of both nuclear and chloroplast origin), we find that all the cyt b6f mutants are phenotypically identical to F2D8 with respect to the deficiency in cyt c6 whereas the PSII, PSI, or double (PSII − PSI) mutants exhibit normal accumulation of holocyt c6. We suggest that the cyt b6f complex is required for the maturation of cyt c6.

POSTER ABSTRACTS

954 CLONING AND CHARACTERIZATION OF cDNA CODING FOR THE MITOCHONDRIAL RIESKE IRON-SULFUR PROTEIN OF HIGHER PLANTS Jintai Huang, Friedhelm Struck, Dale M. Matzinger and Charles S. Levings, III North Carolina State University, Department of Genetics, Raleigh, NC 27695-7614

cDNAs coding for the mitochondrial Rieske Fe-S protein have been cloned from maize and tobacco by using a gene-specific probe amplified by polymerase chain reaction with degenerated primers. Southern blot analysis revealed that there are several copies of the mitochondrial Rieske protein gene in the maize and tobacco genome. Expression of this gene in higher plants appears to be tissue specific. The precursor to the plant mitochondrial Rieske Fe-S protein has a significantly longer transit peptide compared to its lower eukaryote counterpart Complementation analysis in yeast of hybrid proteins consisting of different domains of plant and yeast Rieske Fe-S proteins showed that the carboxyl two thirds of the plant protein is functionally equivalent to that of the yeast protein However, the amino terminus of the Rieske Fe-S protein seems to determine its species specificity because this region is not interchangeable between plant and yeast proteins. A structurally conserved domain essential for the function of possibly all the "*bc*1 complex type" Rieske Fe-S proteins has been identified. We have shown that yeast can be used as a model system to study the structure-function relationship of plant gene products that are enzymatic components of the mitochondrial respiratory chain

955 The Barley Lipid Transfer Protein is targeted to the Lumen of the Endoplasmic Reticulum. Susan M. Madrid. Department of Physiology, Carlsberg Laboratory Gamle Carlsberg Vej 10. DK-2500 Copenhagen-Valby, Denmark

Lipid transfer protein (LTP) is a prominent aleurone protein present in developing, mature, and germinating grains of barley (*Hordeum vulgare* L.) Its coding sequence contains an N-terminal extension with three in frame methionine codons in a putative signal sequence of maximally 26 residues LTP was synthesized by in vitro transcription and translation and analyzed by radiosequencing Initiation was shown to take place at both the first and second methionine codons The in vitro synthesized precursor LTP was shown to be cotranslationally translocated across the ER membrane and processed correctly to the mature LTP. Posttranslational translocation was less efficient The results show that LTP is not involved in intracellular lipid transfer as previously assumed but more likely involved in intercellular processes. In agreement with this conclusion, preliminary immunocytochemical studies indicate that barley LTP is released from the aleurone cells during germination. Apparently, the LTPs isolated from plants operate in a different compartment than those studied in yeast and mammalian cells. Secondary structure prediction and amphiphilicity analysis identified three amphiphilic regions consisting of two α-helices and one ß-strand in LTPs from barley and other plants The amphiphilic structural elements have sizes similar to the acyl groups of lipids and may provide a hydrophobic binding site for lipid in the otherwise hydrophilic barley LTP

956 VACUOLAR TARGETING OF SEED STORAGE PROTEINS IN YEAST. Gerhard Saalbach, Gotthard Kunze, Veselin Christov, Klaus Adler and Klaus Muntz. Institute of Genetics, Gatersleben, O-4325, Germany. Seed storage proteins are transported via the secretory pathway (ER, Golgi) into storage vacuoles (protein bodies). Three different proteins, legumin and vicilin of field bean, and hordein of barley were expressed in yeast. Using immunocytochemistry the vacuolar localization of legumin was demonstrated. To identify targeting signals, gene fusions were constructed from segments of these proteins, and from the 2S-albumin of brazil nut with the SUC2 gene encoding secretory yeast invertase. Targeting of the fusions was studied in yeast. With legumin, increasing the length of the N-terminal segment increased the portion of invertase directed to the vacuole. Only the complete α-chain (281 aa) directed over 90% to the vacuole. A short C-terminal legumin segment (76 aa) fused to the C-terminus of invertase caused efficient targeting to yeast vacuoles. The N-terminal half (222 aa) of vicilin was not sufficient for vacuolar targeting. Eighty percent of the fusion was secreted. A similar result was obtained with the prolamin hordein. An N-terminal segment of 86 aa allowed 70% secretion. A segment with the signalpeptide, the 3kD propeptide, and the 3kD subunit of the 2S-albumin allows 100% secretion of invertase. The addition of 22 aa of the 9kD subunit results in 30% retention inside the cells. The results indicate that legumin contains multiple targeting information and that the information in the studied proteins is formed by higher structures of long sequences.

957 STRUCTURAL AND FUNCTIONAL ANALYSIS OF YPTm4, A NEW MEMBER OF THE RAS SUPERGENE FAMILY FROM *Zea mays*: Ernst-Günter Hoppe, Ian Moore, Thomas Diefenthal[1], Klaus Palme and Jeff Schell
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YPT genes form a subfamily of small GTP binding proteins apparently involved in vesicular transport. The structure and function of a cDNA clone from *Zea mays* encoding a novel YPT homologue, YPTm4, was analysed. Three previously described YPT clones of *Zea mays* , YPTm1, YPTm2 and YPTm3, were compared with the differential expression patterns of YPTm4. Complementation studies with a YPT1 deficient mutant of *S. cerevisiae* show a functionally different behavior of YPTm4 concerning its complementation potential. Mutational analysis of the fatty acylation site and of the GTP-binding domain of YPTm4 were carried out in maize and yeast cells. Antibodies were raised against a synthetic C-terminal peptide of YPTm4 to obtain a clone-specific probe for subcellular immunolocalisation.

958 NONESSENTIALITY OF LYSINE AND ARGININE RESIDUES OF RuBPCase SMALL SUBUNIT TRANSIT PEPTIDE IN CHLOROPLAST PROTEIN IMPORT Kathryn K. Kolacz, Mary L. Taylor Gerard F. Barry, Karen L. Fincher, Stephen R. Padgette and Ganesh M. Kishore Monsanto Company 700 Chesterfield Village Parkway, St. Louis, MO 63198

The transit peptide sequences of cytoplasmically-synthesized, chloroplast-targeted proteins contain a preponderance of hydrophilic and basic residues and few acidic amino acid residues. In the case of mitochondrial transit peptides, a critical role for the basic residues has been proposed for recognition and import of the preprotein by mitochondria The importance of the analogous basic residues (lysine and arginine) in the chloroplast transit peptide was investigated by replacing these residues in the transit peptide of RuBPCase small subunit with either glutamic acid (acidic) or glutamine (neutral). These mutant transit peptide sequences were fused to a bacterial coding sequence and tested *in vitro* for import into lettuce chloroplasts. Fusion proteins composed of the mutated transit peptides are imported into chloroplasts, apparently with the same efficiency as the wild type fusions, suggesting at least in the case of this transit peptide, that the basic residues may not have a distinct role in recognition and import. This is the first report that a transit peptide with acidic residues is capable of functioning in protein import by the chloroplast. These data will be reviewed in the context of current models for protein import by this organelle.

959 LEAF CELL PROTEIN TARGETING - In vitro IMPORT AND PROCESSING OF PRECURSOR PROTEINS WITH SPINACH LEAF MITOCHONDRIA Elzbieta Glaser, Carina Knorpp, AnnaCarin Eriksson and Marie Hugosson. Department of Biochemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, 106 91 Stockholm, Sweden

Both mitochondria and chloroplasts contain their own specific DNAs and protein synthesizing systems. However, most of the organellar proteins are nuclear encoded, synthesized in the cytoplasm and require a specific intracellular trafficking and import machinery.Recently, we have demonstrated high organellar specificity for the *in vitro* import of the *in vitro* transcribed/translated mitochondrial and chloroplast precursor proteins using homologous organelle system i.e. isolated spinach leaf mitochondria and chloroplasts. Moreover, we have also shown that processing system of mitochondria is highly specific for the mitochondrial precursors and that the chloroplast processing system has lower specificity. In the present study we have investigated efficiency of the *in vitro* import at different temperatures. Studies of the kinetics of the separate events of the *in vitro* import as binding of the precursor to the outer membrane, translocation and processing revealed that the extent of the imported of precursor proteins can be limited by different events at different temperatures.These studies have also shown that there exists substantial degradation of both the precursor and its mature form inside the mitochondria. This degradation was not related to the processing activity. We have also further investigated processing system of spinach leaf mitochondria. In vitro transcribed/translated cDNA clones of *Nicotiana* and *Neurospora* F1ß and *Neurospora* Riesky protein were investigated.Submitochondrial particles of spinach leaf mitochondria were disrupted by sonication and the membrane fraction was separated from the matrix by centrifugation. Surprisingly, the processing activity was found to be associated with the membrane fraction. The activity could not be dissociated from the membrane by washing with 100mM KCl, 5Murea or 0.4% Triton X-100. Fractionation of the membranes on the MonoQ FPLC column revealed that two proteins of molecular masses of 39kD and 71kD were responsible for the processing activity. Studies concerning localization of the processing activity in the membrane as well as the mechanism of processing are in progress.

POSTER ABSTRACTS

960 SEQUENCE OF A PRECURSOR POLYPEPTIDE DESTINED TO CROSS THE PEPTIDOGLYCAN WALL OF CYANELLES

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The flagellated protist, *Cyanophora paradoxa*, harbors 2-6 cyanelles, that combine morphological and structural features of cyanobacteria with a genome of plastid size. Notably the cyanelles are surrounded by an inner membrane, a murein sacculus and an outer membrane. Their limited genome size of 127 kb necessitates the import of approximately 90% of their constituent polypeptides. In earlier experiments *C.paradoxa* polyA$^+$mRNA directed translation products could be imported into isolated pea chloroplasts *in vitro* but not into isolated cyanelles under comparable conditions. To in vestigate the import process more thoroughly we characterized a full-length cDNA clone of Ferredoxin-NADP$^+$reductase. It codes for a precursor of 363 amino acids cor responding to a protein of Mr 40.445. The aminoterminal part of the mature FNR protein was determined by peptide sequencing. We inter a transit peptide, 66 amino acids in length, that shows some similarities to chloroplast transit peptides. The bulk of the sequence containing 8 basic and 5 acidic residues is interdispersed with 13 hydroxylated amino acids and preceded by a relatively hydrophobic aminoterminal region. However it is rather different from the three known FNR transit peptides from pea, spinach and ice plant, which show a substantial degree of sequence conservation. Interestingly a putative processing signal appears to be conserved in higher plants as well as in *C.paradoxa*. The mature FNR protein containing 297 amino acids shows about 60% identity to both its cyanobacterial and higher plant counterparts.

961 SECRETION OF PEPTIDES FROM TRANSFORMED PLANT CELL CULTURES. Kaye S. Bateman[1], Adrienne E. Clarke[1], Geoffrey W. Tregear[2] and Marilyn A. Anderson[1].

The University of Melbourne ([1]School of Botany; [2]The Howard Florey Institute of Experimental Physiology & Medicine) Parkville, 3052, Australia.

Plant cells, like other eucaryotic cells, are useful for the expression of peptides, which often require post-translational modification and disulphide bonding for biological activity. We are exploring the potential of plant cells to secrete biologically active peptides. We have transformed *Nicotiana plumbaginifolia* suspension-cell cultures with DNA encoding the animal peptide hormone, arginine vasopressin (AVP). The gene sequence transferred consisted of the 35S promoter from Cauliflower Mosaic Virus, the signal sequence from the S_2-glycoprotein from *N. alata* to direct secretion of the AVP peptide into the culture medium, a synthetic AVP coding sequence, and the termination sequence from the S_2-glycoprotein. AVP was detected in the culture medium of the isolate with the highest level of AVP mRNA, but the peptide was unstable in the presence of plant cells. We are attempting to enhance the stability of the secreted peptide by incorporating it into a hybrid protein.

962 IMPORT AND ASSEMBLY OF THE CHLOROPLAST ATP SYNTHASE COMPLEX.

Johnathan A. Napier, Hakan K. Larsson and John C. Gray
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The ATP synthase of the chloroplast thylakoid membrane is a typical H^+ translocating ATPase, sharing considerable homology with its prokaryotic and mitochondrial counterparts.In the case of the chloroplast ATP synthase, three of the nine component subunits are cytoplasmically synthesised and are transported across the chloroplast envelope, prior to assembly into the complex. The regulation of assembly of this complex is poorly understood, but it has been postulated that multimeric complexes like the ATPase are regulated by the abundance of the nuclear gene products. To test this idea, we have attempted to alter the levels of these polypeptides by either over-expression or antisense in transgenic plants. To that end, we have isolated and characterised full length cDNA clones from tobacco encoding the γ and δ subunits, the two nuclear components of CF_1. These have been cloned in both orientations into Bin19, under different promoters, and introduced into tobacco. The effects of this on the ATP synthase complex will be discussed. We have also examined the role of several envelope proteins in the binding and translocation of the ATPase subunits and other, unrelated proteins into the chloroplast. The role of the chloroplast chaperone, cpn60, and its post-translocational association with chloroplast-targetted proteins is also discussed.

963 REGULATION OF NUCLEAR TRANSPORT OF A PLANT VIRAL PROTEIN. James C. Carrington, Maria A. Restrepo, and Deon D. Freed Texas A&M University, Department of Biology, College Station, Texas 77843

The genome of the plant potyvirus, tobacco etch virus (TEV), encodes a large polyprotein precursor from which mature proteins arise by proteolytic processing. Several TEV proteins are involved directly or indirectly with viral RNA replication. Two replication-associated proteins (NIa and NIb) accumulate within the nucleus of infected cells, whereas others (e.g CI protein) are found in the cytoplasm. Using a ß-glucuronidase fusion strategy, we have mapped the amino acid sequences required for nuclear transport of NIa, which functions both as a polyprotein-specific proteinase and as a factor that attaches covalently to newly-synthesized RNA strands. Two sub-sequences, located at positions 1-11 and 40-68 from the N-terminus, were found to be necessary for efficient nuclear transport of NIa/GUS fusion proteins in protoplast transient assays and in transgenic plants. Both regions contained relatively high numbers of arginine and lysine residues, a feature characteristic of animal and yeast nuclear transport signals. Interestingly, differential proteolytic processing of the viral polyprotein appears to modulate nuclear transport of NIa. A 6-kDa protein, the function of which has not been characterized, is located adjacent to the N-terminus of NIa in the polyprotein. A 6-kDa/NIa/GUS fusion protein in which cleavage of the 6-kDa/NIa junction was inhibited accumulated in the cytoplasm, whereas a 6-kDa/NIa/GUS protein that was able to proteolytically process accumulated in the nucleus.

964 PARTIAL SEQUENCES OF ALANYL-TRNA SYNTHETASE CDNAS FROM *A THALIANA*.

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Aminoacyl synthetases are essential for protein synthesis in chloroplasts, mitochondria and in the cytosol. As no aminoacyl synthetase genes have been found in organellar genomes, it is likely that the organellar enzymes are nuclear-encoded and post-translationally imported from the cytosol. To investigate the intracellular targetting of these enzymes (and eventually their interaction with tRNAs) we have attempted to clone and sequence plant cDNAs coding for alanyl-tRNA synthetases PCR amplification using primers based on the published alanyl-tRNA synthetase sequences from *E. coli* and *Bombyx mori* (1) has allowed us to obtain several sequences from *Arabidopsis* cDNA which show very high homology to a conserved region of the protein. Clone-specific probes are being used to screen a cDNA library to obtain full-length clones of different members of what appears to be a small multigene family.

(1) Chang and Dignam (1990) *J. Biol. Chem.* 265, 20898-20906

965 pLHCP RETENTION BY CHLOROPLAST *hsp70*: UNFOLDED CONFORMATION FACILITATES INSERTION INTO THYLAKOID MEMBRANES. *R. Nechushtai, S. Yalovsky, H Paulsen*[1], *D Michaeli*.

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The major light-harvesting complex of photosystem II (LHCIIb) is a chlorophyll-protein complex, located in the thylakoid membranes of the chloroplast. The apoproteins constituting this complex, pLHCP, are encoded by nuclear genes and are in turn synthesized in the cytoplasm as, precursors which comprise a transit sequence required for their posttranslational import into the plastids. The insertion of pLHCP or LHCP into the thylakoid membranes requires the presence of MgATP and a stromal protein factor which does not contain RNA. We show that for the insertion to occur, pLHCP must be unfolded, i.e in a competent, denatured state. This competence is lost when the protein is renatured. Members of the *hsp 70* protein family have been suspected to confer transport competence to precursors. Following their identification in the chloroplast, *hsp 70* was purified from chloroplast stromal extract. Its effect on the folding state of pLHCP and on the insertion of the latter into the thylakoids was tested. The presence of *hsp 70* during incubation of denatured pLHCP, in renaturing conditions, was sufficient to enable correct insertion of pLHCP into isolated thylakoids. *hsp 70* acted directly on denatured pLHCP, by retarding its refolding and thereby maintaining its competence for insertion.

POSTER ABSTRACTS

966 THE C-TERMINUS END OF PHASEOLIN IS NECESSARY FOR TRIMERIZATION BUT NOT FOR RETENTION OF MONOMERS IN THE ENDOPLASMIC RETICULUM. Emanuela Pedrazzini, M. Serena Fabbrini, Roberto Bollini, Aldo Ceriotti and Alessandro Vitale: Istituto Biosintesi Vegetali, CNR, via Bassini 15, 20133 Milano

We are studying relationships between assembly and intracellular transport of phaseolin (PHSL), the maior bean storage protein, which is a soluble homotrimeric glycoprotein Assembly occurs in the endoplasmic reticulum (ER) and PHSL is then transported to protein storage vacuoles through the Golgi complex. Using Xenopus oocytes we have previously shown that assembly is necessary for PHSL intracellular transport (Ceriotti et al., manuscript in preparation). The published three-dimensional structure of PHSL allows the identification of regions of contact between monomers (Lawrence et al., EMBO J. 9, 9-15, 1990). We have deleted one of these regions (the last 58 aa at the C-terminus; the mutant has been termed Δ363) Expression in Xenopus oocytes indicates that Δ363 does not form trimers and does not exit from the ER The stability of Δ363 in the ER is very high. These results indicate that the C-terminus of PHSL is necessary for trimerization but not for retention of monomers in the endoplasmic reticulum. Coexpression of wild type PHSL and Δ363 indicates that the two molecules do not interact permanently in the ER

We thank J Slightom for the generous gift of PHSL clone cDNA-31. Supported in part by the PF "Biotecnologie e biostrumentazione" and the "PS Meccanismi responsabili per la destinazione intracellulare di proteine", of the CNR

967 ASSOCIATION OF BIP WITH SOLUBLE STORAGE (VACUOLAR) PROTEINS IN THE ENDOPLASMIC RETICULUM OF BEAN COTYLEDONS TREATED WITH TUNICAMYCIN Leone D'Amico, M Serena Fabbrini, Roberto Bollini, Aldo Ceriotti and Alessandro Vitale. Istituto Biosintesi Vegetali, CNR, via Bassini 15, 20133 Milano, Italy

Under a variety of stress conditions, including inhibition of glycosylation by tunicamycin (Tm), proteins inserted into the endoplasmic reticulum (ER) can be unable to fold or oligomerize properly. In animal cells this induces a dramatic increase in the synthesis ER-resident proteins which are believed to have the function of preventing or disrupting inappropriate interactions of newly synthesized proteins. The ER of bean cotyledonary cells synthesize large amounts of a few soluble glycoproteins destined to be accumulated in the protein storage vacuoles We investigated whether inhibition of glycosylation induces the synthesis of proteins which may play a role in the folding and assembly of plant storage glycoproteins Treatment of developing bean cotyledons with Tm enhanced the synthesis of at least two proteins, with Mr 78 kD and 97 kD. Pulse-chase experiments and subcellular fractionation indicated that these are ER residents Mr 78 kD protein was identified as the bean analog of the immunoglobulin binding protein (BiP or GRP78) by immunoprecipitation with anti-mammalian BiP antibodies When newly synthesized storage glycoproteins phaseolin, phytohemagglutinin or α-amylase inhibitor were immunoprecipitated from an ER preparation of Tm-treated tissue, BiP was always co-precipitated. This suggests that, at least in Tm-treated cotyledons, BiP plays a role in the early stages of the synthesis of storage proteins

Supported in part by the "PS Meccanismi responsabili per la destinazione intracellulare di proteine", of the CNR

968 A MUTATIONAL ANALYSIS OF THE CARBOXYL-TERMINAL PROPEPTIDE OF BARLEY LECTIN DETERMINATION OF THE FUNCTIONAL ELEMENTS NECESSARY FOR PROPER PROCESSING AND SORTING TO VACUOLES OF TOBACCO. Jim E. Dombrowski, Martin R. Schroeder, and Natasha V. Raikhel MSU-DOE Plant Research Laboratory, East Lansing, Michigan 48824-1312.

Barley lectin (BL) is synthesized as a preproprotein with a 15 amino acid glycosylated carboxy-terminal propeptide (CTPP) that is removed before or concomitant with deposition of the mature protein in the vacuoles. Expression of a cDNA clone encoding BL in transformed tobacco plants results in correct processing, maturation and accumulation of active BL in vacuoles [Wilkins T.A., Bednarek, S.Y.,and Raikhel, N.V.,(1990) Plant Cell 2, 301-313] We have previously shown that the glycan of the propeptide is not essential for vacuolar sorting, but may influence the rate of post-translational processing. The deletion of the CTPP from BL resulted in the missorting and eventual secretion from the cell Therefore specific mutations were constructed to address the following: a) deletion mutations to define specific regions of the CTPP necessary for proper sorting to the vacuole b) mutations directed at the cleavage site to disrupt processing, c) mutations which effect the secondary structure of the CTPP, d) the alteration of the acidic residues and e) the addition of a second glycosylation site Preliminary data obtained from transient expression of these mutant constructs suggest that the disruption of the secondary structure of the CTPP or the addition of another putative glycosylation site resulted in partial missorting and secretion of proBL into the incubation media We have screened transformed tobacco plants for the expression of the BL CTPP mutant constructs, of which several transgenic lines were selected. Further analysis of these mutants will reveal structural features important for correct localization and processing of these constructs in transgenic tobacco plants

969 EXPRESSION OF HEVEIN GENE IN TRANSGENIC TOMATO: PROCESSING LOCALIZATION AND POSSIBLE FUNCTION Hyung-Il Lee[1], Willem Broekaert[2] and Natasha Raikhel[1] [1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312 and [2]F.A. Janssens Laboratory of Genetics, Catholic University of Leuven, W.de Croylaan 42, B-3001 Heverlee, Belgium. Hevein is a 43 amino acid chitin-binding protein which is found in the lutoid bodies of rubber tree latex. In vitro analysis showed that hevein had an inhibitory effect on the growth of several chitin-containing fungi. Recently, we isolated and characterized a hevein cDNA clone (HEV1) This clone encodes a 204 amino acid polypeptide which consists of three distinct domains a signal peptide (17 aa), an N-terminal domain (hevein) whose 43 amino acid sequence is well-conserved in a variety of chitin-binding proteins, and a C-terminal domain (144 aa) whose peptide sequence is highly homologous to that of proteins encoded by potato win genes. To understand processing events involved in hevein formation, polyclonal antisera were raised against N-terminal C-terminal and both domains In vitro translation experiments demonstrated that the first 17 amino acids could function as a signal peptide Immunoblot analysis of lutoid body proteins suggests that the hevein preproprotein is post-translationally processed in vivo to N-terminal (hevein) and C-terminal polypeptides The chitin-binding experiments and microsequencing data further confirmed this post-translational processing event. To investigate precursor-product relationship and in vivo function of the HEV1-encoded protein, the HEV1 gene was introduced into tomatoes under the control of the CaMV 35S promoter Northern blot analysis shows that the introduced HEV1 gene is constitutively expressed in transgenic tomatoes Further analysis of the hevein preproprotein in transgenic tomatoes will be presented

970 NUCLEAR LOCALIZATION SIGNAL(S) REQUIRED FOR NUCLEAR TARGETING OF THE MAIZE REGULATORY PROTEIN, OPAQUE 2. Marguerite J Varagona[1], Robert J. Schmidt[2] and Natasha V. Raikhel[1] [1]MSU-DOE Plant Research Laboratory, East Lansing, MI 48824-1312, [2]Department of Biology, University of California, San Diego, La Jolla, CA 92093

Nuclear targeting has been studied extensively in animal and yeast systems It is known that large proteins, greater than 40 kD, require ATP and nuclear localization signal(s) (NLS) for transport to the cell nucleus. Smaller molecules are thought to diffuse freely through the nuclear pores. We are interested in studying the general mechanism of nuclear targeting in plant cells Previous studies show that the Opaque 2 (O2) protein from maize regulates the transcription of certain numbers of α-zein storage protein gene families We have recently shown that the O2 protein is localized in the nucleus of both maize and transgenic tobacco plants. The 47 kD O2 protein is relatively small, and it is not clear whether NLS are required for nuclear targeting of this protein. We tested for the NLS requirement by fusing the O2 cDNA clone to the reporter gene encoding β-glucuronidase (GUS) and expressing the fusion protein in transgenic plants. The 115 kD O2.GUS fusion protein is targeted to the nucleus in both monocot and dicot transgenic systems whereas the 68 kD GUS protein is cytoplasmically localized. Thus region(s) of the O2 protein must contain NLS used to target the fusion protein to the nucleus. In frame deletions of the O2 protein were then fused to the reporter gene and putative NLS sequences were identified. A detailed functional analysis of these NLS in stably and transiently transformed plants will be presented

971 INTRACELLULAR TRANSPORT AND TARGETING OF PEA VICILIN IN TRANSGENIC TOBACCO. Allison R. Kermode, Stuart Craig, Don Spencer and Thomas J.V. Higgins. Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada and C.S.I.R.O. Division of Plant Industry, Box 1600, Canberra AC 2601 Australia

Transport of proteins to the plant cell vacuole is mediated by the secretory system (ER and Golgi complex); correct targeting requires active sorting information. A major objective of the present study is to identify the protein domain that allows a pea storage protein (vicilin) to be targeted from the rough ER (its site of synthesis) to its site of deposition within the developing seed - the protein body/vacuole. The specific approach utilizes recombinant genetic techniques to engineer proteins and is a two-pronged one: to examine the fate (eg. targeting and stability) of deletion mutant and chimeric proteins in heterologous host (tobacco) cells. In-frame deletions within the region of the vicilin gene coding for the mature protein (both N-terminal and C-terminal) have been made as well as chimeric genes encoding the vicilin signal peptide plus various portions of mature vicilin protein linked to a bacterial cytosolic "reporter" protein (GUS, β-glucuronidase). This approach allows an identification of sequences (or domains) in vicilin which are both necessary and sufficient for vacuolar targeting. The mutant vicilin proteins have been detected in the leaves and seeds of transgenic tobacco by Western blotting. In general, their relative amounts in the leaf and seed are low (as compared to intact vicilin), perhaps due to their reduced stability. Enzyme activities of the vicilin-GUS fusion proteins are reduced as compared to "free" GUS. Biochemical and structural approaches are being applied to determine the subcellular localization of the engineered proteins.

POSTER ABSTRACTS

972 CHARACTERIZATION OF NUCLEAR TARGETING SIGNALS IN HIGHER PLANTS. Elizabeth A. Pierson, David W. Galbraith and Claudio L. Afonso. University of Arizona, Department of Plant Sciences, Tucson, Arizona, 85721

We have previously isolated and characterized a plant cDNA encoding a protein bearing structural resemblance to the mammalian transcriptional factor Sp1. Features of this protein include, in order from the N- to the C-terminus, a glutamine-rich domain, a serine-rich domain, three tandem zinc-finger motifs, and an acidic domain. This protein is found in the nuclei of proliferating plant cells, but is absent, as is the cognate mRNA, from non-proliferating cells. We have found that a 188 amino acid C-terminal fragment of this protein was sufficient, when translationally fused N-distal to beta-glucuronidase, to target this large cytoplasmic marker protein to the nuclei of transfected *Nicotiana tabacum* protoplasts.

We now are investigating whether a specific topogenic motif responsible for nuclear targeting exists within this fragment, through PCR-based construction of translational fusions of these individual subdomains with beta-glucuronidase. In our initial experiments, we have divided the fragment into three regions, each comprising approximately 60 amino acids. Nuclear targeting activity appears restricted to the C-terminal region. This region contains an oligopeptide sequence that is similar to the nuclear localization signal found in human c-myc. We are currently attempting to determine whether this oligopeptide performs an analogous targeting function in plants, and to identify those proteins that this targeting sequence might recognize.

973 PROTEIN TARGETING TO GLYOXYSOMES AND LEAF-TYPE PEROXISOMES. Laura J. Olsen and John J. Harada. University of California, Department of Botany, Davis, CA 95616

Higher plants contain several classes of peroxisomes that are similar morphologically, but differ metabolically and are found during different developmental stages of the life cycle. Glyoxysomes are involved in storage lipid mobilization during oilseed germination and early seedling growth, and contain enzymes of the glyoxylate cycle. During "greening" of cotyledons, glyoxysomes are converted into leaf-type peroxisomes, which function in photorespiration. We are using isocitrate lyase, a glyoxylate cycle enzyme that is localized exclusively in glyoxysomes, to study protein import into glyoxysomes/peroxisomes. The carboxyl-terminal three amino acids (ser-lys-leu) of some peroxisomal proteins have been shown to be necessary and sufficient to direct these proteins to yeast and mammalian peroxisomes. To determine whether this region functions as a glyoxysomal targeting sequence in plants, we have constructed genes (from Brassica napus) encoding modified isocitrate lyase proteins from which the carboxyl terminus has been deleted. Analysis of the cellular location of these modified proteins in transgenic plants indicates that the targeting information for the transport of these proteins to glyoxysomes is contained in the carboxyl terminus. In addition, since a constitutively-expressed promoter has been used in these constructs, we have examined the transport of glyoxysomal proteins into leaf-type peroxisomes. Glyoxysomal proteins are not endogenously expressed in green tissue. However, leaf-type peroxisomes from tobacco plants transformed with full-length isocitrate lyase clearly contain this enzyme. Thus the targeting determinant for glyoxysomal proteins is able also to direct transport of proteins to leaf-type peroxisomes.

974 THE VACUOLAR TARGETING INFORMATION OF PHYTOHEMAGGLUTININ IS LOCATED IN DIFFERENT POLYPEPTIDE DOMAINS FOR YEAST AND PLANT CELLS. Antje von Schaewen and Maarten J. Chrispeels, Department of Biology, University of California, San Diego, La Jolla, CA 92093-0116

Targeting proteins to vacuoles requires specific vacuolar sorting information in a polypeptide domain of the protein. To define the polypeptide domain that targets phytohemagglutinin (PHA) to the vacuole, we made 3' deletions of the PHA-L coding sequence and fused the truncated portions to a yeast invertase gene that encodes invertase without its signal peptide. The constructs with appropriate 5' and 3' regulatory regions were introduced into *Arabidopsis* and tobacco cells and the subcellular location of invertase determined. The results show that a short domain between amino acids 34 and 43 of PHA, which we identified earlier as being sufficient for targeting invertase to the yeast vacuole, is not sufficient for targeting to the plant vacuole. Targeting to the plant vacuole requires the presence of a different domain between amino acids 91 and 117. Whether both domains are required in plants is currently under investigation. The presence of targeting information in these internal domains of PHA, a protein which is not proteolytically processed, differs markedly from recent findings that in other vacuolar proteins targeting information is located in prodomains (e.g. sporamin and wheat germ agglutinin).

975 **Targeting of an intrinsic membrane protein to the tonoplast in plant cells.**

Herman Höfte and Maarten J.Chrispeels. Department of Biology, University of California, San Diego, Jolla, Ca 92093.

We previously isolated a cDNA encoding an abundant intrinsic tonoplast protein (Tonoplast Intrinsic Protein or TIP) in bean seeds. The deduced amino acid sequence shows a highly hydrophobic protein with six predicted membrane spanning domains connected by short hydrophilic loops and cytoplasmically oriented N- and C-termini. TIP shows significant amino acid sequence similarity with a solute transporter protein in bacteria, and a number of putative solute transporters in animals and plants. To identify sequence elements that function as tonoplast targeting signals, we first expressed the TIP cDNA in a heterologous system. Constructs containing the cDNA behind the constitutive CaMV-35S promoter were used to generate transgenic tobacco through *Agrobacterium*-mediated transformation. The protein is highly expressed in leaf cells of the transgenic plants and correctly targeted to the vacuole as shown with immuno-cytochemistry and immuno-blots of isolated vacuoles. Next, deletion mutants of TIP, as well as fusions between parts of TIP to a reporter protein (Phosphinotricin acetyl transferase, PAT) were expressed in transgenic tobacco cells. The results so far indicate that the C-terminal 49 amino acids of TIP, containing the 6th membrane-spanning domain and the cytoplasmic tail, suffice to target PAT to the tonoplast when fused to its C-terminus. We are currently analyzing additional TIP mutants in order to investigate whether this part of the protein is also *necessary* for tonoplast targeting and to further narrow down the targeting signal.

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POSTER ABSTRACTS

976 **ANTIBODIES AS A MEANS FOR THE STUDY OF PROTEIN CONFORMATION DURING IMPORT OF PROTEINS INTO CHLOROPLASTS.** Adelheid Gauly and Hans Kossel, Institut für Biologie III, Universität Freiburg,Schänzlestr. 1, D - 7800 Freiburg, Germany.

For studying the *in vitro* import of proteins into chloroplasts a fusion protein consisting of the transit peptide and 81 N-terminal amino acids of the mature chlorophyll a/b-binding protein from mustard fused to ß-glucuronidase (GUS) was constructed as a model system. Antibodies against GUS inhibit the import of this fusion protein. It could also be demonstrated that the translocation step rather than binding of the precursor to the envelope membrane is affected by the action of the antibodies. From this we conclude that a protein multimer such as the complex between the fusion protein and the antibodies cannot be translocated through the chloroplast envelope and that proteins that assemble to multimeric complexes within the chloroplast can only be translocated as monomeric units.

Translocation intermediates arising by antibody mediated translocation arrest should be expected if a protein is imported in an unfolded linear form. In this case the C terminus should still be exposed on the outside of the chloroplast and should, therefore, be susceptible to protease degradation. In contrast, the N terminus should be resistant towards external proteases but susceptible to the internal transit peptidase. We observe that part of the Cab-GUS fusion protein under translocation arrest conditions can be degraded simultanously by both, internal transit peptidase and external protease. This leads us to conclude that precursor proteins during their passage through the chloroplast envelope are in an extended and at least partially unfolded conformation.

977 ER TARGETING ENHANCES VICILIN ACCUMULATION IN LEAVES Christine I Wandelt M Rafiqul I Khan Stuart Craig, Hartmut Schroeder, Donald Spencer and T J V Higgins CSIRO Plant Industry GPO Box 1600, Canberra, Australia

Two chimeric genes, one encoding vicilin the other encoding a mutant form with the tetrapeptide lys-asp-glu-leu (KDEL) at its carboxyterminus were expressed under the control of the cauliflower mosaic virus 35S promoter in transgenic tobacco and alfalfa In tobacco leaves the level of vicilin-KDEL protein was 100 times higher than that of vicilin, and in alfalfa leaves, the difference was 20-fold An examination of the leaves of transgenic plants by electron microscopy showed the presence in the ER of protein aggregates that could be labeled with antibodies to vicilin The aggregates were found only in the plants transformed with the vicilin-KDEL construct and not in the plants transformed with the unmodified vicilin coding sequence The stability of the vicilin and vicilin-KDEL proteins in alfalfa leaves was measured in pulse-chase experiments with ^{14}C-amino acids. Normal vicilin had a half-life of 4-5 h, whereas vicilin-KDEL was much more stable with a half-life of more than 48 h No difference was found in the abundance of the mRNAs for vicilin and vicilin-KDEL in tobacco plants that differed 100-fold in their accumulation of the respective proteins. In alfalfa leaves, the level of vicilin-KDEL mRNA was, on average, 4-fold higher than that of the unmodified vicilin but the respective protein levels differed 20-fold *In vitro* translation of total mRNA from the leaves of vicilin and vicilin-KDEL alfalfa plants resulted n a 4 to 5-fold higher level of vicilin-KDEL protein in the cell-free system These results show that there was no difference in the efficiency of translation of the two mRNAs We conclude that the carboxyterminal KDEL peptide caused the retention of the modified vicilin in the ER, thereby greatly increasing the stability of the protein *in vivo* which in turn resulted in the accumulation of vicilin-KDEL protein to high levels in the leaves of transgenic plants

978 TARGETING OF CHLOROPLASTIC ENVELOPE PROTEINS Hsou-min Li and Kenneth Keegstra, University of Wisconsin Department of Botany, Madison, WI 53706

The chloroplastic envelope consists of two membranes, the inner and the outer membranes, each with a distinct set of polypeptides. Like other proteins in chloroplasts, most envelope proteins are encoded by the nuclear genome and synthesized in the cytosol. Despite the metabolic importance of the chloroplastic envelope, knowledge has been lacking concerning the targeting of envelope proteins from the cytosol to their correct membrane compartments in chloroplasts. In order to better understand the biogenesis of chloroplastic envelope proteins, we have isolated cDNA clones encoding proteins of the envelope. Detailed investigations on the import characteristics of an 14 kDa outer membrane protein, OM14, have been performed. It was concluded that OM14 used an ATP independent pathway for its insertion into the chloroplastic outer membrane. A thermolysin-sensitive component on the chloroplastic surface is also not required for this insertion. Efforts are now being focused on the characterization of a 30 kDa inner membrane protein, IM30. Current data indicate that IM30 uses an import pathway similar to that used by proteins destined for the stroma. Information on the location of the targeting sequence and the intraorganellar sorting pathway of IM30 will be presented in the poster.

POSTER ABSTRACTS

979 INHIBITION OF CHLOROPLASTIC PROTEIN TRANSPORT BY SYNTHETIC ANALOGS OF TRANSIT SEQUENCES. Sharyn Perry, Wilson Buvinger*, John Bennett+ and Kenneth Keegstra. University of Wisconsin-Madison, Department of Botany, Madison, WI 53706. *Olympus, Biomedical Research Center, East Setauket, NY 11733. +International Center for Genetic Engineering and Biotechnology, NII Campus, Shaheed Jeet Singh Marg, New Delhi 110 067, India. Transit peptides at the amino terminus of chloroplastic precursor proteins are necessary and sufficient for import into chloroplasts. However, transit peptides from various precursors lack sequence homology raising the question of the import machinery's specificity. We used twenty-residue synthetic peptides corresponding to regions of the transit peptide of the small subunit of ribulose-1,5-bisphosphate carboxylase (prSS) as competitive inhibitors for the transport of full-length precursor proteins into chloroplasts. We also investigated whether there may be functional domains within the primary sequence of the transit peptide. We showed that synthetic peptides of sequences from the ends of the transit peptide had little to no effect on binding of prSS, but reversibly inhibited translocation into chloroplasts. Synthetic peptides of sequences from the central region of the transit peptide inhibited binding of prSS to chloroplasts. Binding and translocation of several precursor proteins were inhibited to the same extent by the synthetic peptides, suggesting shared components in the import apparatus. Attempts are currently underway to identify components of the import apparatus by cross-linking the synthetic peptides or affinity purified prSS to chloroplasts

980 THE VACUOLAR TARGETING SIGNAL OF THE BASIC CHITINASES OF TOBACCO. Jean-Marc Neuhaus,Liliane Sticher§, Fred Meins, Jr§ and Thomas Boller,Botanical Institute, Hebelstrasse 1, 4056 Basel and §Friedrich Miescher Institute, P.O.Box 2543, 4002 Basel, Switzerland

Tobacco (*Nicotiana tabacum*) plants infected by pathogens produce several different chitinases. The basic isoforms are localized in the vacuole while the acidic isoforms are extracellular. The basic isoforms differ from the acidic isoforms not only by many single amino acid changes but also by additional sequences at the C-terminal and N-terminal ends. The N-terminal sequence of the basic chitinases is homologous to hevein (a vacuolar polypeptide from hevea latex), to the four domains of the vacuolar lectins of wheat, rice and barley and to the N-terminus of a wound-induced potato protein of unknown function and localization.

By oligonucleotide mutagenesis and subsequent in vitro rearrangements the N- and C-terminal sequences of the basic chitinase were individually removed and added to another unrelated protein, the extracellular chitinase of cucumber. The various constructions were placed under the control of the CaMV 35S promotor and introduced into *Nicotiana sylvestris* plants by *Agrobacterium* -mediated leaf disc transformation.

The transgenic plants were analysed by the extraction of intercellular washing fluid and by the isolation of protoplasts and vacuoles. The hevein domain was found to be irrelevant for both localization and enzymatic activity of the basic chitinase. Deletion of the 7 C-terminal amino acids caused secretion of the tobacco chitinase. Addition of this C-terminal sequence to the cucumber chitinase caused the vacuolar accumulation of the modified chitinase. Experiments are in progress to determine the minimal sequence required for vacuolar targeting.

981 DELETION OF C-TERMINAL DOMAINS CAN INFLUENCE IN VITRO IMPORT AND INTRAORGANELLAR TARGETING OF CHLOROPLAST PRECURSORS. Kenton Ko and Zoenka W. Ko. Queen's University, Department of Biology, Kingston, Ontario, Canada K7L 3N6

The transit peptide of the lumenal 33 kD oxygen-evolving polypeptide (OEE1) is capable of importing and targeting mouse dihydrofolate reductase (DHFR) into the thylakoid lumen. The first set of experiments in this study indicate that methotrexate cannot block import or targeting of OEE1-DHFR in contrast to that reported for mitochondria. These results suggest that the OEE1 transit sequence interacts with DHFR in a manner affecting the ability to bind methotrexate. We further examined and compared the transport characteristics of a number of carboxy-terminal truncated chloroplast precursors to determine whether carboxy domains affect the import and targeting mechanism of these proteins. The plastid precursors chosen for this study are targeted to one of the chloroplastic compartments-the stromal, the thylakoid membrane and the lumen. In most case depending on the extent of the deletions, removal of the carboxy domains can influence import, processing and targeting of the precursors. In one case, even extensive deletions had little effect on import into the chloroplast. These results suggest that carboxy domains in the mature part of the protein do interact with the transit peptide and as a result play an important role in determining the import and targeting competence of chloroplast precursors.

982 THE TARGETING ASPECTS OF A 70 KD HEAT SHOCK PROTEIN (SCE70) OF THE SPINACH CHLOROPLAST ENVELOPE. Chengbiao Wu and Kenton Ko, Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Recent evidence has indicated that members of the heat shock protein HSP70 family play important roles in protein translocation and assembly processes in the endoplasmic reticulum, mitochondria and chloroplast. A homologue of HSP70, SCE70 has recently been isolated from the envelope membrane of the spinach chloroplast through screening of a cDNA expression library. Since its location is very different from other widely studied chloroplast proteins we are interested in elucidating the targeting mechanism of SCE70. Our initial results show that the SCE70 transit peptide (TP) is not located at the C-terminal end. To determine the precise N-terminal location of the SCE70 TP, various deletion constructs were cloned into transcription plasmids to generate fusion proteins using in vitro expression systems. Isolated chloroplasts were then employed to assay the targeting competence of these fusion proteins. Comparison between the SCE70 TP and other chloroplastic TPs was also made.

983 BIOCHEMICAL CHARACTERIZATION OF THE 70 KDa OUTER ENVELOPE PROTEIN SCE70. Lauralynn Kourtz, William C. Plaxton and Kenton Ko. Queen's University, Department of Biology, Kingston, Ontario, Canada, K7L 3N6

A cDNA encoding a 70kD protein from the spinach chloroplast envelope (SCE70) has been recently characterized. SCE70 is highly homologous to HSP70 proteins from other organisms which may be involved in protein translocation. Western blotting techniques in combination with chelator washes and protease treatments were employed to investigate the location of SCE70 in the outer envelope membrane. The deduced amino acid sequence of SCE70 reveals a putative calcium/calmodulin binding site and an ATP binding domain. The ability of the SCE70 protein to bind calmodulin is currently being investigated using gel shift assays. The ATP binding and hydrolyzing activity of SCE70 is currently being examined.

984 EXPRESSION AND TARGETING OF STRICTOSIDINE SYNTHASE IN TRANSGENIC TOBACCO PLANTS. Daniel R. Bergey and Thomas D. McKnight. Department of Biology, Texas A&M University, College Station, TX 77843.

Strictosidine, a precursor to over 1000 indole alkaloids, is produced by the condensation of tryptamine and secologanin. Strictosidine synthase, the enzyme responsible for this condensation, is the first committed step in the indole alkaloid pathway. We have introduced a modified cDNA encoding strictosidine synthase from Catharanthus roseus (McKnight et al. [1990] Nucl. Acids Res. 18:4939) driven by the CaMV 35S promoter into tobacco. Transgenic tobacco plants expressing this construct had from 3 to 22 times greater strictosidine synthase activity than C. roseus plants. Ultrastructural immunolocalization demonstrated that strictosidine synthase is a vacuolar protein in C. roseus and is correctly targeted to the vacuole in transgenic tobacco. We are currently examining targeting of strictosidine synthase deletions and fusions to locate vacuolar targeting signals on this protein.

POSTER ABSTRACTS

985 STUDIES ON THE TRANSLOCATION OF PROTEINS INTO AND ACROSS THE THYLAKOID MEMBRANE. Kenneth Cline[1], William F Ettinger[2], and Steven M. Theg[2]. [1]Fruit Crops Department, University of Florida; Gainesville, FL 32611 and [2]Department of Botany, University of California, Davis, CA 95616. Approximately 50 % of thylakoid membrane polypeptides are encoded on nuclear genes and synthesized in the cytosol as precursors. Studies in several laboratories have demonstrated that these proteins are compartmentalized via sequential membrane translocation steps. The precursor proteins are initially transported across the two envelope membranes into the stroma and then subsequently transported into the thylakoids. We have analyzed the thylakoid translocation/integration requirements of the integral membrane protein, LHCP, and 2 lumenal proteins, OE23 and OE17, using an in vitro assay for transport into isolated thylakoids. Our results suggest that these two classes of proteins utilize different transport machinery. Whereas LHCP integration requires a stromal protein factor, transport of the OE23 and OE17 polypeptides occurs maximally with washed thylakoid membranes in the absence of stroma or any other soluble plant-derived protein. The energy requirements for membrane translocation/integration also differ. LHCP integration is absolutely dependent upon the hydrolysis of ATP and is enhanced by the presence of a trans-thylakoid ΔpH. Conversely, transport of OE23 and OE17 is entirely dependent upon the presence of a trans-thylakoid ΔpH. These differing requirements have important implications for the mechanisms by which proteins are transported into thylakoids and also for the evolution of organellar translocation processes.

986 A MUTANT PHOTOSYSTEM I CAB PROTEIN INTEGRATES INTO THYLAKOIDS BUT DOES NOT ASSEMBLE INTO PSI
Zach Adam and Neil E. Hoffman. Carnegie Institution of Washington, Department of Plant Biology, Stanford, CA 94305.

A chlorophyll a/b binding protein (CAB) from photosystem I (tomato CAB7) was modified such that residues 194 and 195 were switched from Val and Gly to Lys and Ile. This modification occurs in the stromal exposed loop between the second and third membrane spanning regions. The mutant polypeptide was imported into isolated chloroplasts and integrated into the thylakoid membrane in a form resistant to alkali extraction. Unlike the wild type protein which is fully protease resistant despite numerous potential cleavage sites in the stromal loop and N-terminus, the mutant polypeptide is only partially resistant to protease. Based on the size and intensity of labelling of protease protected fragments we concluded that the mutant protein was integrated into the membrane with the same topology proposed for PSII-CAB polypeptides. However it failed to assemble into the PSI-core complex. It is likely that the substitution interferes with the folding of the polypeptide in the stromal loop thereby rendering this region protease sensitive. This interpretation suggests that CAB protein integrates into the thylakoid prior to attaining its final conformation, or that incorrectly folded CAB protein can correctly integrate into the thylakoid. Schwartz *et al.*(FEBS Lett: in press) noted that the stromal exposed loop of PSI CAB proteins contains a sequence of 20 amino acids not found in PS II CAB proteins. This region is proposed to be involved in targeting the protein to PSI. The mutation that we created is located in this 20 amino acid sequence, and the failure of this mutant protein to associate with PSI is consistent with the proposed function of this region.

987 CHARACTERIZATION OF SATURABLE COMPONENTS INVOLVED IN THE SORTING OF THYLAKOID PROTEINS.
Zach Adam and Neil E. Hoffman, Carnegie Institution of Washington, Department of Plant Biology, Stanford,CA 94305

The integration of CAB protein (CABp) into thylakoid membranes requires a stromal factor and ATP. The translocation of OEEp (33 kD-oxygen evolving enhancer protein) to the luminal side of the thylakoid also requires ATP but not stroma. PSADp is a peripheral membrane protein associated with photosystem I and found on the stromal side of the thylakoids. Assembly of PSADp into PSI occurs in isolated thylakoids in the absence of both stroma and ATP. These membrane specific processes may require thylakoid proteins that either recognize CABp in association with stromal factor or in the cases where stromal factor is not required, distinct structural elements in OEEp and PSADp. We are testing these ideas by characterizing saturable components involved in the sorting pathways. We have over-expressed CABp and OEEp in E. coli and found that these radiolabeled proteins are integration and translocation competent, respectively, after they are denatured in 8M urea and diluted into stroma. If we decrease the specific activity of labelled CABp by adding unlabelled CABp, we inhibit the integration of the labelled protein into thylakoids. If we add comparable amounts of unlabelled CABp to radiolabelled OEEp, OEEp translocation is unaffected. Similarly, unlabelled OEEp inhibits the translocation of labelled OEEp but has no effect on the integration of CABp. These findings indicate that the pathways for sorting CABp and OEEp have distinct saturable components. We are determining whether the sorting of PSADp requires a third saturable component. To localize the saturable components, we are examining the effect of increasing levels of thylakoid and/or stroma on sorting processes. Our initial results, using CABp, indicate that saturable components are found in both the stroma and thylakoids.

988 DELETION MUTANTS OF CAB PROTEINS ARE EFFICIENTLY IMPORTED INTO CHLOROPLASTS BUT ARE INCORRECTLY ASSOCIATED WITH THYLAKOIDS.
Laiqiang Huang, Zach Adam, and Neil E. Hoffman. Carnegie Institution of Washington, Dept. of Plant Biology, Stanford, CA 94305

CAB polypeptides (p) are integral membrane proteins containing three membrane spanning helices. We have created a series of mutations in tomato CAB4p to test whether individual membrane spanning helices with hydrophilic flanking sequences, when fused to a transit peptide, can be imported into chloroplasts and correctly targeted to thylakoid membranes. All of the mutated precursors, including those with large C-terminal deletions, were imported efficiently and processed. All mutant polypeptides were found primarily in the stroma. Imported protein associated with the thylakoid was resistant to alkali extraction but was protease sensitive indicating that the hydrophobic regions did not span the membrane. We also found that both wild type and the mutant CABp can non-physiologically insert into membranes in the absence of stroma. Presumably the wild type CABp, in the absence of stroma, is misfolded resulting in hydrophobic regions that can non-specifically insert into membranes but that do not span the membrane. We suspect that CAB deletion mutants that have been imported into chloroplasts are unable to interact with all the components of the sorting machinery and hence cannot integrate into the thylakoid. The ability of deletion mutants to insert into thylakoids in a non-physiological way suggests that such mutants are not suitable for studies aimed at elucidating thylakoid targeting signals.

989 IMMUNOLOGICAL CROSS REACTIVITY BETWEEN PROTEINS FROM *SYNECHOCOCCUS* CYTOPLASMIC MEMBRANES AND SPINACH CHLOROPLAST ENVELOPES
Amie Franklin, Arthur Grossman, and Neil E. Hoffman. Carnegie Institution of Washington, Department of Plant Biology, Stanford CA 94305

Most chloroplast envelope proteins have not been assigned a function. We are exploring whether the proteins in the cytoplasmic membrane from *Synechococcus sp* PCC 7942 can be used in the examination of chloroplast envelope polypeptides. In contrast to higher plants, *Synechococcus* is amenable to many of the techniques of bacterial genetics and can be used to explore gene function *in vivo*. The rationale for such an approach is derived from the theory that the plant chloroplast arose from an endosymbiotic association between a free-living photosynthetic prokaryote and a non-photosynthetic host cell. Conceivably the cytoplasmic membrane of the prokaryote evolved into the chloroplast envelope and a number of proteins located in this membrane have counterparts in the chloroplast envelope. We are using an immunological approach to test this idea. Antibodies were raised against cytoplasmic membrane and cell wall proteins from *Synechococcus* and envelope proteins from spinach chloroplasts. *Synechococcus* antibodies recognize spinach-envelope-polypeptides of: 76kd, 68kd, 65kd, 62kd, 49kd, 47kd, 40kd, 39kd, 35kd, 31kd, and 19kd. The spinach envelope antibodies recognize cytoplasmic membrane polypeptides from *Synechococcus* of: 87kd, 70kd, 66kd, 58kd, 56kd, 50kd, 40kd, and 36kd. We are attempting to isolate the genes encoding these proteins from both plant and cyanobacterial expression libraries.

990 CHARACTERIZATION OF THE ER LOCALIZED MOLECULAR CHAPERON BiP IN TOMATO. David J. Meyer & Alan B. Bennett. Univ. of California, Mann Lab., Dept. of Veg. Crops, Davis, CA 95616.
Members of the HSP70 class of stress proteins form a ubiquitous family of proteins, members of which have been identified in all organisms examined to date. In eukaryotes it appears that multiple genes encode different members of this family, and further that different members are targeted to specific subcellular locations. Recent evidence from mammalian and yeast systems implicates an ER localized HSP70 in the processes of ER targeting, protein folding and multimer assembly, and nuclear division. We have isolated cDNA and genomic DNA clones from tomato corresponding to the ER localized HSP70, BiP. The open reading frame encodes a 75 kD polypeptide which has high similarity to BiP from other organisms: the product from this ORF encodes a protein with a functional ER-targeting signal sequence and also the C-terminal sequence -H-D-E-L-COOH. Several lines of evidence suggest that these clones correspond to the single BiP gene in tomato. A polyclonal antiserum raised against the C-terminal 72 amino acids encoded by the cDNA strongly reacts with a 75 kD protein on western blots; subcellular fractionation experiments confirm that this protein is found in vesicles of the ER. We are presently purifying this protein from suspension cultured tomato cells in order to analyze its biochemical properties and to further investigate the role of BiP in plant cell growth and development.

POSTER ABSTRACTS

991 VACUOLAR TARGETING OF TOMATO PROTEASE INHIBITOR I.
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Vacuolar targeting signals are currently among the least understood of the plant organellar targeting signals. Proteins targeted to the vacuole appear to be transported through the ER-Golgi-secretory pathway. Therefore, vacuolar proteins must contain signals for ER import, and selection from the secreted bulk flow. We are presently focusing on the targeting signals of tomato Proteinase Inhibitor I. The mature form of Inhibitor I, which is found in the vacuole, is 42 amino acid residues shorter than that encoded by the full length cDNA. The mature form lacks not only the ER signal sequence, but also an additional 19 amino acid residue "pro" sequence. Investigations are aimed at determining the possible role of this "pro" sequence in vacuolar targeting. Plasmid constructs have been made which contain a copy of the Inhibitor I cDNA both with and with out the "pro" sequence. Results from transient expression assays which use tobacco protoplasts to evaluate the sorting properties of the proteins encoded by these Inhibitor I constructs will be discussed. Future work will center on identification of other possible targeting regions and the effects of point mutations within these targeting domains

992 THE USE OF FLOW CYTOMETRY TO STUDY CHLOROPLAST PROTEIN IMPORT
Barry D. Bruce, and Kenneth Keegstra
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Flow cytometry has become a very powerful tool in many areas of cell biology. Even though the chlorophyll based autofluorescence associated with plant cells and chloroplasts make them ideal for the use in fluorescence activated cell sorting (FACS), its use has been very limited in the plant sciences. The power of this technique over other static techniques is that it can very rapidly analyze cells (>3000 particles/sec) for up to six different parameters simultaneously. These parameters can include measurements on size, shape, DNA content, chlorophyll content, [Ca], and with the appropriate immunofluorescent reagents up to four different cell surface markers. We have begun to use flow cytometry to investigate the early steps of protein transport into isolated chloroplasts. Using flow cytometry it may be possible to rapidly and accurately measure the number of bound precursors, look at the kinetics of precursor internalization, measure the level of competitive inhibition between various precursors or peptides, and to test for the requirement of cytosolic factors for protein import. To date we have shown that the flow cytometer can provide much information on plastid heterogeneity, plastid intactness, and using various immunofluorescent reagents we can detect various chloroplast outer envelope proteins such as OM-14. We are now in the process of trying to detect the presence of *in vitro* synthesized RUBISCO small subunit as a bound precursor to isolated chloroplasts where import has been blocked. If this can be done successfully we hope to be able to answer many questions on the import pathway for this protein.

993 INVOLVEMENT OF HSP70 HOMOLOGUES IN CHLOROPLAST PROTEIN TRANSPORT
Barry D. Bruce, Jerry S. Marshall, and Kenneth Keegstra
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How a folded protein crosses a biological membrane remains one of the major problems in protein translocation. The hsp70 class of molecular chaperones, composed of many closely related members, has been implicated in this ATP-dependent process. It has been proposed that hsp70's assist in protein translocation by interacting with precursor proteins and altering their folding both before and after translocation. By directing or facilitating protein folding the hsp70 protein maintains the precursor in an "open" conformation and only in this form is the precursor competent for translocation. We are investigating the role of both cytosolic and chloroplastic hsp70's in chloroplast protein transport. We have shown that isolated pea chloroplasts contain three distinct hsp70 homologues. To begin investigating the role of these chaperones in chloroplast protein transport we have isolated a full length cDNA clone for the major 78 kD stromal hsp70 homologue. The sequence for this protein shows highest homology (>80%) with the hsp70 homologue, DnaK, from the cyanobacterium *Synechocystis*. We are also investigating the role of the cytosolic hsp70 found in the wheat germ *in vitro* protein synthesis system. Mono-specific antibodies were raised against the wheat germ hsp70 and have been used to immunoprecipitate a precursor protein/hsp70 complex. The extent of cytosolic hsp70 involvement in chloroplast protein transport is being determined with various precursors.

994 ON THE IDENTITY OF THE MAJOR CHLOROPLAST ENVELOPE MEMBRANE PROTEIN.
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The chloroplast envelope membrane is the site of different metabolite translocators coordinating the metabolism in the cytosol and the stroma as well as that of the protein import machinery translocating nuclear-coded precursor proteins from the cytosol into the chloroplasts. Recently, from the isolation and sequence analysis of cDNA clones the primary sequence of the phosphate translocator from both spinach and pea chloroplasts (M_r ~36 000; p36) became available (1,2). On the other hand, Schnell et al. have argued against the assignment of p36 as the chloroplast phosphate translocator and have provided evidence that this cDNA clone codes for the chloroplast import receptor (3). To clarify this point experiments on the identity of p36 (e.g. function, localization, protease sensitivity, inhibition studies) were performed and the results will be shown.

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2. Willey, D.L., Fischer, K., Wachter, E., Link, T.A. and Flügge, U.I. (1991), Planta 183, 451-461
3. Schnell, D.J., Blobel, G. and Pain, D. (1990) J. Cell Biol. 111, 1825-1838

995 EFFECTS OF TRANSIT PEPTIDE AMINO ACID SUBSTITUTIONS ON THE CHLOROPLAST IMPORT AND PROCESSING OF RUBISCO.
Kathleen Archer[1] and Kenneth Keegstra[2]. [1]Biology Department, Trinity College, Hartford, CT 06106. [2]Botany Department, 430 Lincoln Drive, Birge Hall, University of Wisconsin, Madison, WI 53703.

Transit peptides from different chloroplast proteins within a species show little sequence conservation. Between species, however, a specific protein tends to show some conservation in certain regions. One such region for the small subunit of rubisco involves approximately 10 amino acids at the carboxy-terminus of the transit peptide. This region is predicted to form a reverse turn followed by a beta strand with hydrophobic moment. We have dissected this region using in vitro mutagenesis, creating a set of mutants with changes in primary or predicted secondary structure. Our results show that introduction of a negative charge has profound effects on processing, and that some of the aberrantly processed forms assemble into holoenzyme while others do not. Chloroplast fractionation studies indicate that correctly and aberrantly processed forms and internalized but un-processed precursors are all located in the soluble compartment. The mutation expected to affect secondary structure by substituting a beta strand for the reverse turn (and thus extend beta strand structure thoughout the region) strongly affects the kinetics of import.

996 **"Phytoantibodies": a general vector for the expression of immunoglobulin domains in transgenic plants.**
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Recently, the successful expression of complete murine immunoglobulin genes in plants provided the opportunity to exploit the potential of the antibody repertoire in plant physiology and pathology studies.
The basic building block of any antibody-based antigen-binding structure is represented by the heavy (VH) and light (VL) chain variable domains. Considering that 'single domain antibodies' (polypeptides constituted of VH domains only) are often able to bind antigen with good affinity and represent a versatile alternative to whole antibody molecules, we have engineered the sequences encoding only the immunoglobulin heavy chain (VH) variable domains in a new general purpose vector to transform plant via *Agrobacterium*.
We demonstrated that the transgenic plants produced high levels of correctly processed "single domain antibodies'. The cellular distribution was studied by immunoistochemistry, confirming that dAbs can indeed be expressed efficiently in plants.
The success of the approach will depend on several factors, such as the availability of specific antibodies, their antigen-binding capacity as single domain antibodies', their functionality in different cell compartments, the effectiveness of the immune-complex in terms of stability and interactions with the normal physiological processes of the engineered plant.

POSTER ABSTRACTS

997 INDUCTION AND CLONING OF A TRIAZOLOPYRIMIDINE-RESISTANT ALS GENE OF ARABIDOPSIS. George S. Mourad*, Banibrata Pandey*, William L. Crosby**, and John King*. *University of Saskatchewan, Department of Biology, Saskatoon, SK. Canada, S7N 0W0 and ** Plant Biotechnology Institute, National Research Council, Saskatoon, SK. Canada S7N 0W9.

By EMS mutagenesis, we have isolated a dominant mutation at the CSR1 locus of Arabidopsis thaliana that confers high levels of resistance to the herbicide triazolopyrimidine sulfonamide (tzp) at both the whole plant and acetolactate synthase (ALS) levels. Taking advantage of the published sequence of the Arabidopsis wild-type ALS gene (Mazur et al., Plant Physiol. 85: 1110-1117. 1987), we have isolated the tzp-resistant ALS gene by amplification using the polymerase chain reaction (PCR). Because ALS functions in the chloroplast we have amplified the region of the gene, a 1.7 kb fragment, encoding the presumptive mature chloroplastic form of the enzyme. The PCR products were cloned in a vector carrying a single 3'-overhanging dideoxythymidine triphosphate at both ends. The sequencing of this mutant gene is in progress to locate the site of the mutation. Our eventual objective is to use this, and other, similar herbicide-resistant mutants of Arabidopsis, to investigate the influence modifying ALS has on the functioning of the branched-chain amino acid pathway.

998 AN ORGAN-SPECIFIC GENE FROM A TREE TRUNK
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A tree trunk is a heavily lignified stiff organ, which contains high levels of soluble polysaccharides, phenolics and RNases. Because the stiff organ with these inclusions hinders RNA extraction and cDNA synthesis, none of the organ-specific genes have been reported. At present study, following points were examined for studing the organ-specific genes in a tree trunk. First, purification and characterization of a gene product in the oragan. Secondary, extraction of intact RNA from a tree trunk. Finally, detection of an organ-specific gene. Concerning to the first place, one of the most abundant protein in the oragan was purified and characterized as a lectin. The lectin is closely related to the storage protein in the organ because of its localization and annual variation of the level. Concerning to the second place, after several trials, the cDNA was synthesized from the isolated poly(A)+ RNA. It showed maximal chain length ca.2.2kb, which ranges up to 81.5Kdalton polypeptides. Finally, the lectin gene was detected by PCR and Sorthern blot. The findings obtained suggest that intact RNA was extracted from a tree turnk. Because a cDNA library from a tree trunk carries information for a life form, i.e. a tree but not herebaceous plant, it serve as a key tool for analyzing such life forms. Constructing such a library will open the gate to the control of life form.

1000 REGULATION OF THE TONOPLAST ATPASE FROM BARLEY ROOTS BY SALINITY STRESS. Tom Berkelman, Frances M. DuPont and Peter J. Morrissey. USDA, ARS, WRRC, 800 Buchanan St., Albany, CA 94710

Sodium uptake into the vacuole of barley root cells is driven by the pH gradient generated by the tonoplast H^+-ATPase. This enzyme therefore plays a key role in the ability of barley to tolerate saline soils. When barley roots are grown in the presence of 100 mM NaCl the rate of proton transport catalyzed by the tonoplast ATPase increases 2-fold. This change occurs over the course of several days and is not accompanied by an increase in the rate of ATP hydrolysis by the enzyme or any apparent change in the purified enzyme or its subunits when analyzed by SDS-PAGE. The time course for the change in activity is consistent with a change in gene expression. We are testing the hypothesis that salinity causes a change in the expression of ATPase genes resulting in the synthesis of an ATPase isozyme with different catalytic properties. We have focused on the 57 kD subunit because it is implicated to play a regulatory role, and because a cDNA clone is available for this subunit from Arabidopsis. We are examining the possibility of differentially regulated isoforms of this subunit and we intend to characterize the genes.

1001 PURIFICATION OF A 29kDa PROTEIN FROM WHEAT MITOCHONDRIAL MEMBRANES EXHIBITING PORE FORMATION IN VITRO.
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Voltage dependent anion channels (VDAC) named also porins have been identified in the mitochondrial outer membrane of many eukaryotic organisms (Colombini 1989). The VDAC protein has a molecular mass of ~30 kDa and exhibits selective permeability pores for hydrophilic small metabolites by forming water filled pores. In this study a 29 kDa protein was purified from the membranal fraction of wheat mitochondria. The isolation and purification procedure consisted of passage of solubilized mitochondrial membranes through a hydroxylapatite celite column followed by a DE52 column. The purified protein was integrated into liposome lipid bylayers. The liposomes were characterized as being permeable to an anionic fluorescent dye ANTS (Mr 445) but neither to a cationic antiquencher DPX (Mr 442) nor to dextran fluorescein (Mr 40000). These properties suggest that the wheat mitochondrial protein is the putative VDAC. Since this is the first mitochondrial channel forming protein purified from plant mitochondria, the 29kDa protein will be an instrumental tool for further studies on its relevance for mitochondrial function in plants.

Colombini M. 1989 J.Membrane Biol.111, 103-111.

1002 ROLE OF INVERTASES IN INTERNODAL ELONGATION AND SUCROSE ACCUMULATION IN SUGARCANE STALKS.
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Role of invertases in maturation and sucrose accumulation in sugarcane stalk parenchyma cells has been examined. Activity of vacuolar invertases, generally, remains higher than cytoplasmic invertases. The following observations suggest that the invertase activity is related to cell growth and expansion: (a) The peak invertase activity co-incides with the period of fastest growth (b) Late-maturing cultivars show higher invertase activity during September, compared to early maturing cultivars that have completed growth phase by then (c) The activity of elongated internodes is 4 to 6 times lower than the internodes that are elongating (d) Within an internode, cell expansion zone had higher invertase activity than cell maturation zone. The absence of invertases in mature, sucrose accumulating internodes suggests that the enzyme may not be involved in cleavage of sucrose prior to its accumulation in vacuoles and lends support to a recent hypothesis suggesting entry of intact molecules into vacuoles of stalk parenchyma.

1003 CLONING AND EXPRESSION OF *ARABIDOPSIS THALIANA* GENES ENCODING HOMOLOGS OF MAMMALIAN P-GLYCOPROTEINS Robert Dudler, Christoph Ringli and Cecilia Hertig. University of Zurich, Institute for Plant Biology, CH-8008 Zurich, Switzerland.

Resistance of mammalian tumor cells to many lipophilic cytotoxic drugs has been shown to result from an increased drug efflux which in turn is caused by the overexpression of the P-glycoproteins. These membrane proteins are encoded by the multi-drug resistance (*mdr*) genes and show homology to the high affinity transport systems and, in particular, to the hemolysin export system, of bacteria. This has led to the suggestion that P-glycoproteins are drug-efflux pumps. The function of P-glycoproteins in normal cells is not known.

We have become interested in possible plant homologs of P-glycoproteins and have succeeded in cloning two different classes of *Arabidopsis* genes which both are homologous to the mammalian *mdr* genes. Sequence analysis of one of these genes showed that it encodes a protein which exhibits 55% sequence conservation to the mouse *mdr1* gene product. Surprisingly, none of the intron positions are conserved between the *Arabidopsis* gene and the only other mammalian P-glycoprotein gene characterized so far, the human *mdr1* gene. Studies on the expression pattern and localization of the products of these genes will be presented, and the role these proteins possibly play in plants will be discussed.

POSTER ABSTRACTS

1004 PLASMA MEMBRANE H⁺ATPase EXPRESSION AND BIOGENESIS

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In some plant cells a plasma membrane proton pump will consume as much as 1/4 to 1/2 the cellular ATP to generate a pH and electrical gradient across the plasma membrane An energized membrane is necessary to drive proton coupled cotransport systems and mediate signal transduction pathways Our objective is to understand the biogenesis and regulation of the plasma membrane proton pump

In *Arabidopsis* we have identified at least 10 H⁺-ATPase genes (AHAs) by a PCR method that utilizes degenerate DNA primers corresponding to regions conserved in all P-type ATPases Our working hypothesis is that individual members of this family are differentially regulated and/or encode biochemically unique isoforms AHA2 and AHA3 have been analyzed by GUS reporter gene analysis AHA2 appears to be expressed predominantly in root epidermal tissue while AHA3 is expressed predominantly in phloem tissue

The mechanism by which AHA1 is targeted to the plasma membrane is being investigated by constructing GUS fusion proteins containing different domains of AHA1 and determining where the fusion proteins become localized Evidence indicates that the N-terminal region of AHA1 including the first transmembrane domain is sufficient to cause association of the GUS fusion protein with the microsomal membrane fraction

The proton pump is thought to be regulated by kinase-mediated phosphorylation A full length cDNA encoding a Calcium Dependent Protein Kinase (CDPK) has been isolated from *Arabidopsis* This gene (AK1) is analogous to the soybean CDPK (Harper et al Science 252 951 1991) with a serine/threonine type kinase domain and a calmodulin-like regulatory domain The AK1 enzyme has been expressed as a fusion protein in *E coli* , and the purified fusion protein displays calcium stimulated protein kinase activity

1005 MOLECULAR CLONING OF A 62 KD PLASMA MEMBRANE PROTEIN IN SOYBEANS AND CARROTS: POSSIBLE ROLE IN SUCROSE METABOLISM

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Using independent experimental approaches with different species, we have obtained polyclonal antibodies to a 62 kD plasma membrane protein. This protein appears to be widespread in the plant kingdom as cross-reactivity at the antibody level has been shown in soybean, carrot, spinach, tomato, and corn. Both the soybean and tomato antibodies recognize the same 62 kD protein in a purified plasma membrane fraction from carrot suspension cells. Further, western analysis demonstrates that the tomato antibody reacts with an identical protein in microsomal preparations from developing soybean seed. Both the method of identification of the soybean 62 kD protein (sucrose based affinity labeling) and immunolocalization data obtained with both antibodies indicate that the proteins may be associated with sucrose metabolism at the plasma membrane. Antibody staining is highly correlated with developing phloem tissues in carrot embryos and there is a high degree of staining at the surface of embryogenic carrot cell clumps. Both antibodies have been used to screen cDNA expression libraries from soybean and carrot and common plaques were found, again indicating conservation of this plasma membrane protein. This 62 kD protein is probably not an invertase since sequence analysis of the protein deduced from the soybean cDNA does not show homology to conserved regions of invertases and secondly, neither the tomato nor the soybean protein appears to be glycosylated by conA affinity blotting. The possible roles of this protein in sucrose metabolism are under investigation.

1006 **ISOLATION OF PLASMODESMATA: A PROTOCOL FOR ISOLATING CLEAN CELL WALLS CONTAINING EMBEDDED PLASMODESMATA.**

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In the past, our understanding of the biochemical structure and function of the plasmodesmata has been hampered by the lack of a reliable isolation procedure. In previous studies of plasmodesmata performed in our lab, a relatively simple cell wall isolation procedure was employed. Although this procedure was used for the primary characterization of two plasmodesmatal associated proteins (PAP 26 and PAP 27), the resulting preparation was not entirely free of non-cell wall components. Thus, following the digestion of the cell wall, a plasmodesmatal fraction was obtained which contained many contaminating membranous structures. The presence of such contaminants interfered in the identification and characterization of plasmodesmatal composition. We report here for the first time on a protocol for the isolation of clean cell walls which are free of intracellular debris but which contain embedded plasmodesmatal structures. PAP 26, a plasmodesmatal associated protein is highly enriched by the protocol. A number of other proteins are also enriched and are suspected to be plasmodesmatal components. PAP 27 a second plasmodesmatal protein which was shown to be associated with the neck region was however, lost as a result of shearing during extrusion from a nitrogen pressure bomb.

1007 USE OF THE PATCH CLAMP TECHNIQUE TO DETECT ION CHANNELS IN THE NUCLEAR ENVELOPE OF HIGHER PLANT CELLS.

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As several patch clamp studies have shown, nuclear envelopes (NEs) of animal cells appear to contain large, cation-selective channels, which may be the same as the nuclear pores (1, 2). To extend these investigations to plant cells, we have performed patch clamp experiments on nuclei isolated from the gelatinous endosperm of immature coconuts (Cocos nucifera). These nuclei were used because they can be prepared rapidly without homogenization and in the absence of detergents; therefore, they frequently retain an intact double membrane (characteristic of NEs) which is suitable for patch clamp experiments. The nuclear pore density in these NEs was determined by freeze-fracture to be approximately 7-8 pores/μ^2. Patch clamping of whole nuclei and excised patches revealed a large channel with a maximum conductance of approximately 1000 pS in symmetrical 200 mM KCl. A further characterization of this channel will be presented.

1. Mazzanti et al. (1990) Nature 343, 764.
2. Matzke et al. (1990) FEBS Lett. 271, 161.

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1008 ISOLATION AND CHARACTERIZATION OF cDNA CLONES ENCODING PLASMA MEMBRANE H⁺-ATPase FROM *NICOTIANA TABACUM* AND *ATRIPLEX NUMMULARIA*.

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A cDNA clone encoding a plasma membrane H⁺-ATPase has been isolated from a *N. tabacum* c.v. Wisconsin-38 λ-ZAP cDNA library. The nucleotide sequence of the cDNA insert shares greater than 90% sequence identity with the sequence of the plasma membrane H⁺-ATPase of *Nicotiana plumbaginifolia* Northern blot analysis indicates that this cDNA hybridizes to a single mRNA of approximately 3.7 kb. The expression of this transcript can be induced by NaCl shock in tobacco cells adapted to 428 mM NaCl growing in media without salt, but not in unadapted cells growing under the same conditions. Southern blot analysis indicates that the plasma membrane H⁺-ATPase in tobacco cells is encoded by several genes. Using the *N. tabacum* cDNA as a probe, an apparent full length cDNA clone was isolated from an *Atriplex nummularia* λ-ZAP library. The sequence of this clone indicates that it encodes a plasmamembrane H⁺-ATPase. Preliminary results indicate that the *Atriplex* gene encodes a 3 7 Kb transcript that is highly regulated by salt exposure.

1009 A MUTANT OF *ARABIDOPSIS* DEFICIENT IN XYLEM LOADING OF PHOSPHATE.

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A mutant of *Arabidopsis* deficient in the accumulation of inorganic phosphate (Pi) was isolated. The mutant plants accumulate 21 times less Pi in the shoots than wild type. Although the mutant plants are fertile, growth is severely affected. The phosphate-deficient phenotype is caused by a single nuclear recessive mutation at a locus designated *pho1*. The rate of phosphate uptake into the roots was similar between mutant and wild type plants over a wide range of external phosphate concentrations. In contrast, translocation of phosphate to the shoot was reduced by 10 to 39-fold in the mutant grown in media with low phosphate (<200μM). At higher phosphate concentrations in the growth media, the defect in shoot translocation was overcome. Translocation of sulfate to the shoots was normal in the mutant, indicating that the lesion was not a general defect in anion transport. The rate of efflux of phosphate from the roots was low for both wild type and mutant plants. The results suggest that the mutant is deficient in activity of a protein required to load phosphate into the xylem.

POSTER ABSTRACTS

1010 VACUOLAR ATPase SUBUNIT GENES ARE DIFFERENTIALLY EXPRESSED IN ELONGATING COTTON FIBERS Ann L T. Powell, Ching-Yi Wan and Thea A Wilkins
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Cotton fibers are formed by individual, rapidly elongating cells that differentiate from the outer integument of the ovule. During the first ten days of elongation, the enlarging central vacuole distinguishes the differentiating primordial fiber cells. Numerous molecular and enzymatic changes must occur to result in the several thousand-fold expansion of fiber cells. Previous research has shown high levels of ATPase activity in the tonoplasts of elongating fibers, suggesting that vacuolar ATPases (V-ATPase) function to transport osmoregulatory solutes into the vacuole, thereby increasing vacuolar turgor pressure, one of the postulated forces responsible for fiber cell elongation. We have investigated the expression of genes encoding the major subunits of the multimeric V-ATPase enzyme complex during the elongation phase of fiber development. cDNA clones have been isolated from libraries constructed from mRNA obtained from ovules collected at three days preanthesis and at anthesis. Clones encoding the 70 kD catalytic subunit, the 60 kD regulatory subunit and the 16 kD integral membrane subunit of V-ATPases were isolated from cotton ovules using heterologous probes from carrot, *Arabidopsis* and yeast, respectively. Four separate cDNA clones have been identified for the 70 kD catalytic subunit and the 16 kD integral membrane subunit indicating that the V-ATPase subunits are encoded by small gene families. Southern blot analysis of cotton DNA shows that the catalytic subunit is encoded by at least four genes in Upland cotton. RNA slot blot analysis has shown that the amount of mRNA homologous to V-ATPase cDNA clones is developmentally regulated in elongating cotton fibers in addition to the constitutive expression throughout the plant. The amount of V-ATPase-specific mRNA attains maximum levels coincident with the maximum rate of fiber elongation and the localization of ATPase activity in the vacuole of fibers. Therefore unlike V-ATPase gene expression in other plants, these data suggest that the cell-specific accumulation of V-ATPase is a consequence of novel regulation of gene expression of the V-ATPase subunits in developing cotton fibers.

1011 ISOLATION AND MOLECULAR CLONING OF VACUOLAR H^+-PUMPING INORGANIC PYROPHOSPHATASE. Vahe C. Sarafian[1,2], Christopher J. Britten[1], Yongcheol Kim[1], Ronald J. Poole[2] and Philip A. Rea[1].
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The vacuolar membrane of plant cells contains two primary H^+ pumps: an ATPase and an inorganic pyrophosphatase (H^+-PPase). The H^+-PPase belongs to a new category of H^+-translocase and is implicated in the electrogenic translocation of both H^+ and K^+ into the vacuole [Davies, J.M., Rea, P.A. and Sanders, D. (1990) *FEBS Letters, 278*: 66-68; Davies, J.M., Rea, P.A. and Sanders, D. in preparation]. We have recently identified, isolated, reconstituted, cloned and sequenced the major M_r 64,000-72,000 subunit of the H^+-PPase. The M_r 64,000 subunit of the enzyme has been purified to near-homogeneity by a combination of LC and FPLC of Triton X-100-solubilized membranes and its catalytic and/or MgPPi-binding function deduced from its kinetics of interaction with N-ethylmaleimide. The M_r 64,000 polypeptide isolated from *Beta* has been microsequenced by *in situ* tryptic digestion of the polypeptide after electrotransfer onto nitrocellulose. cDNA clones for the corresponding subunit from *Arabidopsis* were obtained in the first instance by antibody screens of the expression library λZAP and subsequently by filter hybridization screens of an *Arabidopsis* λgt10 library with λZAP cDNA clone. Since the microsequence data from *Beta* show nearly 100% identity to the deduced sequence of the translation product of the *Arabidopsis* clone, the cDNA clones are inferred to encode the catalytic subunit of the translocase. Models for its organization in the vacuolar membrane together with a hypothetical scheme for the phylogeny of this novel eukaryotic H^+/K^+ pump will be presented.

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1012 ISOLATION OF A VANADATE SENSITIVE ATPase FROM GROUNDNUT SEEDLINGS. Vinay Sharma and Jaya Bhusnan. Department of Biosciences and Biotechnology, University of Roorkee, Roorkee, (India)-247 667.

A membrane fraction enriched in vanadate sensitive ATPase has been identified from 6 day old etiolated groundnut seedlings by a combination of differential and density gradient centrifugation techniques. The fraction separates out as a pellet when 12,000/105,000 x g pellet was loaded on a 20/34% two step sucrose gradient. This fraction is distinct from chloroplast, mitochondrial and tonoplast membranes as it did not result in any significant inhibition (<13%) by the addition of the inhibitors for the ATPase known to be associated with these membranes, e.g. azide for mitochondria, phloridzin for chloroplast and nitrate for tonoplast. Addition of 75 mM KCl shows 40% stimulation in the ATPase activity associated with this fraction whereas, addition of vanadate (0.2 mM) and molybdate (0.2 mM) caused 38% and 19% inhibition of ATPase activity respectively. The ATPase activity associated with this fraction was further characterized and found to have a pH optimum of 5-5.5. Kinetic studies of the enzyme followed a simple Michaelis-Menten relationship. The enzyme exhibited a Km of 0.21 mM and Vmax of 82.4 µM $Pi.mg^{-1}$ protein. min. An organwise distribution showed that radicle is most enriched in ATPase activity followed by cotyledons, hypocotyl, leaves and epicotyl in the descending order. A comparative study of vanadate-sensitive ATPase activity levels versus seedlings of various ages showed a peak in 5th and 6th day old seedlings. The order of divalent cations requirement for the optimal activity was $Mg SO_4 > Mn SO_4 > Zn SO_4 > Fe SO_4$.

1013 TOWARDS PROTEIN ENGINEERING OF *CHLAMYDOMONAS* CHLOROPLAST ATP SYNTHASE. Jacob Schlesinger, Ronith Motzery, Noun Shavit, Allan Michaels and Stefan Leu, Department of Life Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

A system to study functional aspects of chloroplast ATP synthase by site directed mutagenesis is being developed in our laboratory. The genes atpA, atpB, and atpC, encoding subunits α, β, and γ of *Chlamydomonas* CF_1, have been subcloned into expression plasmids. The atpA gene from the chloroplast DNA of *Chlamydomonas reinhardtii*, coding for the α subunit of CF_1, was cloned and sequenced. The deduced amino acid sequence showed 75% identity to the sequence of the α subunit from tobacco CF_1. The largest open reading frame of the *Chlamydomonas reinhardtii* gene encodes for 508 amino acids, which is one amino acid more than the corresponding sequence in tobacco. There is an apparent contradiction in the peptide sizes since the observed Mr of α-CF_1 from *Chlamydomonas reinhardtii* is significantly lower than the Mr of higher plant CF_1 α subunits determined by SDS-PAGE. The atpA gene was subcloned into the expression vector pT7. The gene was transcribed *in vitro*, then translated in the reticulocyte lysate in the absence or presence of isolated photosynthetic membranes. The translation product co-migrated with *Chlamydomonas* α subunit on SDS-PAGE. The level of translation was not affected by the presence of photosynthetic membranes. About 50% of the α subunit translated in the presence of photosynthetic membranes was recovered with the membrane fraction.

The atpB gene was overexpressed in *E. coli* and accumulated to more than 10% of the cell protein. The overexpressed β subunit, recovered as insoluble aggregate, was enriched to about 75% purity by subsequent washing steps and dissolved in 4 M urea. The protein remained soluble after urea was removed by dialysis. This enriched preparation of subunit β specifically bound the photoreactive ATP analog 3-O-(4-benzoyl) benzoyl-ATP (BzATP). The binding of BzATP was prevented by competition with ATP.

The atpC gene was subcloned into an overexpression plasmid to yield a protein identical to the mature chloroplast γ subunit with the addition of three amino acids. The protein was overexpressed in *E. coli* and was found mainly in the insoluble cell fraction. The overexpressed γ subunit, which has an apparent molecular weight of about 38 kDa, was purified to near homogeneity by washing of the pellet with several buffers and finally dissolved in 4 M urea. We will use these overexpressed subunits to reconstitute active ATPase complexes.

1014 APPROACHES TO THE ISOLATION OF pH MUTANTS IN ARABIDOPSIS. Brian W. Tague and Howard M. Goodman. Department of Molecular Biology, Massachusetts General Hospital, Boston MA 02114.

The maintenance of pH is central to cellular metabolism and influences important physiological processes in plants. A molecular genetic approach to pH homeostasis is being undertaken using the crucifer, *Arabidopsis thaliana*. Three major compartments of the plant cell are involved in pH homeostasis and can be perturbed by various treatments: the cytoplasm (pH 7), the cell wall/extracellular space (pH ~6) and the large central vacuole (pH ~5). The Landsberg *erecta* strain has been used to establish the range of external (medium) pH that will support *Arabidopsis* growth. *Arabidopsis* will germinate at a pH as low as 4.0 in liquid culture and as high as 8.0 on agar; continued growth only occurs in the pH range 5.0 to ~7.0. A population of gamma-irradiated *Arabidopsis* seed was sown at pH 5.5 or pH 8.0 to screen for mutant lines resistant to elevated pH and for mutant lines more sensitive to elevated pH than wild-type. A number of potential mutant lines are now being analyzed genetically; results of these experiments will be reported. Treatments which perturb vacuolar pH (the application of weak bases) and cytoplasmic pH (weak acids) are also being studied to determine there usefulness in isolating *Arabidopsis thaliana* mutants altered in pH homeostasis.

1015 A MOLECULAR GENETIC ANALYSIS OF IRON UPTAKE IN *ARABIDOPSIS THALIANA*. Ying Yi and Mary Lou Guerinot.
Department of Biological Sciences, Dartmouth College, Hanover, N.H. 03755

Iron is an essential nutrient for all higher plants, with iron-containing compounds figuring prominently in the electron transport systems of photosynthesis and respiration. Although iron is abundant in the soil, the acquisition of iron is problematic due to its low solubility at biological pH under aerobic conditions. We have chosen to study iron uptake mechanisms in the model plant system, *Arabidopsis thaliana*, using biochemical, genetic and molecular genetic techniques. The focus is on the reduction of ferric iron which takes place at the root surface. Ferric iron reduction is enhanced under iron deficiency and is probably the primary factor in making iron available for absorption by dicots and non-graminaceous monocots. The reduction has been shown to take place at the plasma membrane of root epidermal cells via a transmembrane redox system which can be activated when iron is in short supply. Using a cloned fragment from the *FRE-1* gene of yeast to screen a lambda library of *Arabidopsis* DNA, we have identified two lambda phage which presumably carry an *Arabidopsis* homolog of the *FRE-1* gene. *FRE-1* is thought to encode either a (subunit of) ferric reductase, or a gene product necessary for expression of the ferric reductase. We are currently determining the nucleotide sequence of the *Arabidopsis FRE* gene. Analysis of *FRE* gene expression is also underway, using RNA prepared from roots and green tissues of *Arabidopsis* grown in the presence or absence of iron. In addition to our molecular analyses, we have also initiated screens for mutants of *Arabidopsis* which lack ferric reductase activity or which lack regulation of ferric reductase activity using an assay based on the dye FerroZine. A study of iron uptake in *Arabidopsis* should have application to general transmembrane permeation processes and their regulation and may ultimately lead to the construction of iron efficient plant varieties. These studies should also shed light on root specific gene expression and the regulation of gene expression by metals.

POSTER ABSTRACTS

1016 POLYPEPTIDES INVOLVED IN β-GLUCAN SYNTHESIS IN COTTON FIBERS
Andrawis Andrawis, Yehudit Amor, and Deborah Delmer. Dept.of Botany The Hebrew University, Jerusalem 91904, Israel

Higher plants contain two plasma membrane-localized UDP-glc: β-glucan synthases. Activity for one of these, the cellulose synthase, has not been detected in plants, but, in the bacterium Acetobacter xylinum, it has been characterized as a Mg^{2+}-dependant synthase activated by c-di-GMP. In recent work in collaboration with R. Mayer and M. Benziman of our institute, we have identified cotton fiber membrane polypeptides of 48 and 83 kD which bind c-di-GMP with high affinity and specificity, show cross-reaction with an antibody against the bacterial synthase, and show developmental regulation consistent with their being components of a c-di-GMP-dependant cellulose synthase. Preliminary PCR experiments indicate that cotton cDNA does contain sequences with homology to the cloned bacterial gene which codes for the c-di-GMP-binding polypeptide. In other work on the second glucan synthase, the Ca^{2+}-dependant callose synthase, we have discovered that washing membranes with EDTA releases a characteristic set of polypeptides and considerably enhances the callose synthase activity in the membranes. Analysis of the polypeptides released shows the presence of at least one very active Ca^{2+}-dependant protein kinase and a very abundant 34 kD polypeptide which undergos rapid phosphorylation/dephosphorylation, precipitates in uM Ca^{2+}, and shares sequence homology with a class of animal proteins called annexins and protein kinase C. The possible role of these polypeptides in regulating glucan synthesis is under investigation. (Supported by the U.S. Dept. of Energy and German-Israeli Foundation for Research and Development-GIF.)

1017 PURIFICATION AND CHARACTERIZATION OF A XYLOGLUCAN OLIGOSACCHARIDE DEGRADING PEA STEM CELL-WALL LOCALIZED α-FUCOSIDASE. Christopher Augur, Peter Albersheim, and Alan Darvill. Complex Carbohydrate Research Center and the Dept. of Biochemistry, University of Georgia, 220 Riverbend Rd. Athens GA 30602

Oligosaccharins (oligosaccharides with regulatory properties), released from the cell wall hemicellulosic polysaccharide xyloglucan e.g. the undecasaccharide, XG11, and nonasaccharide, XG9, at low concentrations (10^{-8} M) inhibit auxin-induced growth of pea epicotyls. The terminal fucosyl residues of XG11 and XG9 have been shown to be essential for their biological acuvity We have detected and purified from pea stems a cell-wall-localized α-L-fucosidase that is able to remove the terminal fucosyl residue from the XG oligosaccharides. The fucosidase destroys the growth inhibitory activity of the oligosaccharides We wish to determine whether the enzyme is under auxin control and what role it plays in the control of plant growth. Thus, the fucosidase has been purified to homogeneity by selecuve extraction of pea tissue followed by a combination of cation-exchange chromatography and isoelectric focusing. The purification steps were monitored by SDS-PAGE. The enzyme has an apparent molecular weight of 20 kd. N-terminal and internal amino-acid sequences have been obtained and used to design synthetic oligonucleotides. These oligonucleotides were used to generate via PCR, a 380 bp cDNA fragment that codes partially for the α-fucosidase which was then sequenced. Using this molecular probe, we are presently determining whether expression of the α-fucosidase gene is under auxin control. Acknowledgements: This work is supported in part by U.S. Department of Energy grant DE-FG09-85ER13425, and by U S. Department of Energy grant DE-FG09-87ER13810 as part of the USDA/DOE/NSF Plant Science Centers Program

1018 BIOLOGICAL ACTIVITY OF XYLOGLUCAN OLIGOSACCHARIDES ON CELL GROWTH OF DIFFERENT PLANTS.
M.Beatrice Bitonti, Concetta Aquino, Rossella Bosco, Paola Campolongo, Rosanna Canino, Silvia Mazzuca and Enzo Perri*. University of Calabria, Department of Ecology, 87030 Arcavacata di Rende(Cs), Italy; Istituto Sperimentale per l'Olivocoltura, 87030 Rende(Cs), Italy.

It is well known that cell wall-derived oligosaccharides show various biological activities when supplied to living plant tissues (Darvill and Albersheim 1984, McNeil et al. 1984, Albersheim and Darvill 1985, Ryan et al. 1985, Tra thanh Van et al. 1985, West et al. 1985, Branca et al. 1988, Fry 1989). It has also been reported that specific products of xyloglucan degradation exibit both an inhibitory effect on cell elongation, as shown by antiauxin activity of a xyloglucan nonasaccharide (XG9)(York et al.1984, McDougall and Fry 1988), and an auxin-like activity as reported for xyloglucan epta- to nonasaccharide (McDougall and Fry 1990). We now report the first evidences of biológical acitivity of xyloglucan oligosaccharides extracted and purified by Olea Europaea L. on cell growth of differents plants.

1019 EXPRESSION OF A PETUNIA GLYCINE-RICH PROTEIN GENE IN TRANSGENIC PLANTS EXPRESSING ANTISENSE RNA.
Shu-Hua Cheng and Carol M. Condit. Depts. of Plant Science and Biochemstry. Univ. of Nevada, Reno, NV 89557.

Glycine-rich proteins (GRPs) are thought to be cell wall proteins. Several studies have shown, at the mRNA and protein level, that the expression of GRPs is high in young tissue and is little or none in old tissue. GRPs have been immunolocalized in xylem and/or phloem in french bean, petunia and soybean. Furthermore, ongoing immunocytochemical studies show that petunia GRP1 is highly expressed in apical meristem and promeristematic cell of undifferentiated lateral buds. These results indicate a cell specific expression of GRPs, and suggest that GRPs play an important role (or roles) in plants. However, the precise function(s) of GRPs in vivo is unknown. In this report, we use antisense RNA as a tool to elucidate the function of GRPs in petunia. A 1200-base-pair Hind III fragment from the coding region of petunia GRP1 gene was fused in the inverted orientation behind the cauliflower mosaic virus 35S RNA promoter in a PBI vector. This construct was then introduced to petunia via *Agrobacterium* mediated gene transfer. The results of the effects of antisense RNA on the endogenous expression of petunia GRP1 gene will be presented.

1020 A NEW CONCEPT OF THE POLYMERIC NATURE OF CELLULOSE IN SECONDARY CELL WALLS.
C. R. Benedict, R. J. Kohel and G. M. Jividen. Texas A&M University, Department of Biochemistry & Biophysics, College Station, TX 77843-2128

The current concept of cellulose synthesis by cellulose synthase is based in part on polymer analysis of derivatized cellulose isolated from secondary walls of cotton fibers (Marx-Figini, 1982). The DP of the cellulose polymer remained constant throughout the period of cellulose synthesis indicating a structure controlled polymerization reaction. The polymerization reaction is a chain elongation reaction of pre-existing cellulose chains at the end of the microfibrils. We have re-examined the polymeric structure of cellulose by dissolving secondary wall cellulose of cotton fibers in a non-degrading solvent of N,N-dimethylacetamide/LiCl (DMAc/LiCl) (McCormick, 1985) followed by gel permeation chromatography of the dissolved cellulose on three linear columns of $1x10^5$ Å to 500 Å of Ultrastyragel in a Waters 150C GPC equipped with a computer program for calculating mol wt and mol wt distribution (Ekmanis, 1987, Timpa and Ramey, 1989). The ^{13}C-NMR spectrum of the dissolved cellulose showed 5 chemical shifts: C-1 of glucose in the cellulose chain is assigned the peak at 103.5 ppm and C-6 at 60.7 ppm. The four peaks between C-1 and C-6 are assigned to C-2, C-5, C-3 and C-4 carbons. The sharpness of the chemical shifts indicate the cellulose DMAc/LiCl is a true solution. GPC analysis show the $\bar{M}_w$ and MWD of the cellulose polymer are $2.7x10^6$ and 10^4 to $5x10^7$ The normal distribution of the different mol wt chains in the cellulose polymer indicates a statistical chain and step polymerization reaction. The data suggest that the polymerization reaction consists of chain initiation, chain elongation and termination reactions prior to the incorporation of the different size cellulose chains into the cellulose microfibrils.

1021 Elicitor induced ultra-rapid protein cross-linking in the cell wall of soybean cells. Bradley D., Brisson L. and Lamb C.J. Plant Biology Laboratory, Salk Institute for Biological Studies 10010 North Torrey Pines Road, La Jolla, Ca 92037

Early events of elicitation have been investigated in soybean cell cultures. Following elicitation with diutathione or fungal elicitor, biochemical analyses have shown the disappearance of two SDS-extractable proteins of 33 and 100 kDa in the cell wall and in the extracellular fluid. This response is initiated within the first minutes upon addition of elicitor and is complete in ten minutes under optimal conditions. Immunofluorescent observations have revealed a similar distribution of these proteins in control and elicited cells. Therefore, the rapid disappearance could be attribute to their cross-linking in order to toughen the cell wall. Interestingly, the cross-linking was induced by hydrogen peroxide This suggests the importance of hydrogen peroxide as a substrate and its subsequent use by peroxidase(S) in the regulation of cross-linking. Inhibition of cross-linking by anti-horseradish peroxidase antibodies support the involvement of peroxidases in these events. However, kinetic studies have shown that the concentration and the activity of peroxidases initially present seem to be sufficient to induce the rapid insolubilization, which seems, therefore to be controled by the concentration of hydrogen peroxide.

POSTER ABSTRACTS

1022 EXPRESSION OF THE PHENYLACRYLIC ACID DECARBOXYLASE (PAD) IN TRANSGENIC PLANTS AND ITS EFFECT ON DEVELOPMENT. Monika Clausen, Christopher J. Lamb, Roland Megnet and Peter W. Doerner. Salk Institute, Plant Biology Department, La Jolla, California 92037

Lignin is one of the most abundant biopolymers. Its role as structural polymer in xylem during plant development and its participation in stress responses make it a very interesting subject to study.
The biosynthetic precursors for lignin formation are provided by the phenylpropanoid pathway. It was shown previously that exogenously applied cinnamic acid affects transcription and activity of other members of the pathway like PAL and CHS.
To study the effect of reduction of endogenous phenylacrylic acid levels on plant development the yeast gene encoding for phenylacrylic acid decarboxylase (PAD) was cloned. The gene product catalyzes the decarboxylation of cinnamic acid and its homologs to styrene.The function of the cloned gene was confirmed by complementation analysis in Saccharomyces cerevisiae. The open reading frame was fused to different plant promoters employing the pBI vector system. Agrobacterium tumefaciences mediated transformation gave rise to transgenic tabacco and Arabidopsis thaliana plants. Copy numbers for different transgenic individuals were determined by Southern blot analysis and by segregation analysis of the kanamycine resistence marker. The transcribed gene was identified by Northern blot analysis. We will describe the development of transgenic plants observed in primary transformants and F1 progeny.

1023 GENETIC MANIPULATION OF CELL WALL STRUCTURE BY ENGINEERING THE EXTENSIN NETWORK. J.B. Cooper, D. Halverson and P. Sullivan. UCSB, Dept of Biological Sciences, Santa Barbara, CA 93106

The cell wall is an important regulator of plant growth and development, and a major component of dicot primary cell walls is the family of hydroxyproline-rich glycoproteins called extensins. The rod-shaped extensins are thought to form a covalently crosslinked network which may be important in wall function. We have utilized a well-characterized extensin gene (pDC5A, from carrot) to construct defined mutations in both the expression and the structure of the extensin network. Expression mutants utilize transcriptional fusions between the coding and 3' sequences of carrot extensin (in both sense and antisense orientations) and heterologous promoters (constitutive CaMV 35S and light-regulated soybean SSU). The mutant extensin alleles were linked to a Kan^r marker in the *Agrobacterium* binary vector,pARC10, and transgenic *Nicotiana tabacum* plants containing these constructs were regenerated. Preliminary results indicate that abnormal expression of extensin in stems and leaves leads to abnormalities in shoot development. We are constructing homozygous lines with abnormal developmental phenotypes for further analysis. This lines will be used to check for cosegregation of the phenotype with Kan^r. Structural mutations designed to alter the length of extensin glycoproteins from 80 nm (wild type length) to between 20 and 150 nm were made using in-frame deletions and insertions of proline-rich extensin repeats. Transgenic carrot roots containing constitutively expressed mutant extensins are being examined. The results of these experiments should lead to a better understanding of the function of extensins in plant growth and development.

1024 CHANGES IN GENE EXPRESSION DURING POD DEVELOPMENT IN OILSEED RAPE. S.A. Coupe[1], J.E. Taylor[1], Isaac, P.G.[2] and J.A. Roberts[1]. [1] Department of Physiology and Environmental Science, University of Nottingham, Sutton Bonington, Leics. LE12 5RD. U.K. [2] Nickerson International Seed Co. Ltd. Cambridge. CB4 4GZ. U.K.

The phenomenon of abscission occurs at precise locations in a plant and is the result of cell separation brought about by cell wall dissolution. It has been proposed that the loss of adhesion between adjacent cells at the site of abscission is regulated by the activity of several cell wall hydrolases.
The development of the pod in oilseed rape is accompanied by the degradation of cell walls along a discreet layer of cells that run the length of the pod. This layer of cells, termed the dehiscence zone, has similar characteristics to an abscission zone. As the pods mature, a rise in the activity of the hydrolytic enzyme cellulase accompanies cell separation and is confined to the deniscence zone.
Analysis of ethylene production by intact pods has revealed an increase in ethylene production during maturation which appears to be temporarily correlated with a tissue specific increase in cellulase activity.
Further work has been carried out to examine changes, occurring at the protein and gene level, which accompany pod maturation and subsequent dehiscence. The results of these findings will be discussed.

1025 POSITION AND REGULATION OF EXTENSIN IN THE STRUCTURE OF MAIZE PERICARP Elizabeth E. Hood, Kendall R. Hood, William McManus and Jenifer M. Murphy. Utah State University, Department of Biology, Logan, UT 84322-5305

The structural extensins of the plant cell wall are hydroxyproline-rich glycoproteins. Our interest is in the role of extensin in cell wall architecture of maize pericarp. The pericarp was chosen because it is a maize tissue quite rich in extensin (van Etten CH et al., 1961 J Agr Food Chem 9 433-435; Hood,EE et al., 1991 Plant Physiol. in press). We have studied protein accumulation in the pericarp of 3 maize varieties, a sweet corn and 2 popcorns. Extensin accumulates to high levels throughout seed development. The popcorns accumulate more extensin per pericarp than the sweet corn correlating with their greater water impermeability for proper popping. We are further investigating the potential function of different protein levels in these maize pericarps. Our first approach is to determine the regulation of the extensin gene. The tissues we are using are from the sweet corn, from one popcorn variety with a particularly high protein level, and from the periderm of a kernel derived smut gall which is the equivalent of a mutant pericarp with reduced levels of extensin. Preliminary evidence indicates that transcript amounts probably do not account for the differences in extensin in these 3 maize tissue systems. Indeed, the smut periderm has levels of transcript nearly equivalent to sweet corn at similar stages of development and 20-fold less protein. Our second approach is to view these peridermis by electron microscopy to investigate the differences in spatial patterns of extensin protein associations at molecular resolution. Preliminary results from these experiments indicate that the cells from normal maize pericarp are elongated and have thick walls at maturity while those from the smut gall periderm are hexagonal and thin walled. Experiments are currently under way to localize the protein to a specific wall layer and identify patterns.

1026 GLYCINE-RICH PROTEINS IN THE CELL WALL OF XYLEM: DEPOSITION OF THE PROTEIN IN THE ABSENCE OF LIGNIN AND ULTRASTRUCTURAL LOCALIZATION WITHIN THE WALL.

Beat Keller[1], Nikolaus Amrhein[2] and Ueli Ryser[3]. [1]Swiss Federal Research Station for Agronomy, 8046 Zürich. [2]ETH-Zentrum, 8092 Zürich. [3]Université de Fribourg, 1700 Fribourg, Switzerland.

Lignin biosynthesis was inhibited in young bean seedlings by 2-aminoindan-2-phosphonic acid (AIP). AIP is a specific and potent inhibitor of phenylalanine ammonia-lyase, an enzyme involved in lignin biosynthesis. At a concentration of 100 µM AIP in the growth medium, no lignin could be detected in roots and hypocotyls of 7- or 9-day-old seedlings when stained with phloroglucinol/HCl. Glycine-rich protein GRP 1.8, a cell wall protein of bean, was studied by immunocytochemistry in hypocotyls grown in the presence of 100 µM AIP. No difference of the GRP deposition pattern at sites of normally lignified secondary cell wall thickenings, as well as along the protoxylem vessels, was found in unlignified tissue when compared to controls. The cell-type specific synthesis of GRP 1.8 was not affected by AIP. Thus, deposition of the GRP 1.8 structural cell wall protein is not dependent on lignification, and the glycine-rich protein may be a scaffold for correct lignin deposition in the complex wall structure of xylem. We have recently detected GRP 1.8 also in cell walls of metaxylem (reticulate and pitted vessel members) where the antibody labelled border regions between lignified and unlignified parts of the cell wall. Experiments using immunoelectron microscopy confirm the localization of GRP 1.8 in the cell wall. The ultrastructural localization will be presented.

1027 THE COMPLETE PRIMARY STRUCTURE OF A SOYBEAN METALLOPROTEINASE: STRUCTURAL SIMILARITY TO HUMAN COLLAGENASE. John S. Graham, Jin Xiong, Jeffery W. Gillikin, William Burkhart[1], and Jerry M. McGeehan[1], Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403 and [1]Glaxo Research Laboratories, Glaxo, Inc., 5 Moore Dr., Research Triangle Park, N.C. 27709.

A metalloendoproteinase from leaves of *Glycine max* has been purified to homogeneity. The complete amino acid sequence of the 171 residue monomeric protein (19kD) has been determined by automated Edman degradation. Amino acid sequence analysis reveals regions of structural similarity to the collagenase family of extracellular metalloproteinases. Indeed, the soybean metalloendoproteinase (SMEP 1) shares significant biochemical properties with the human enzyme. Western analysis shows that SMEP 1 is most abundant in older leaves of the plant. Preliminary data based on analysis of intercellular wash fluids shows that SMEP 1 resides in the extracellular compartment of the leaf. This work represents the first clear demonstration of the presence of a metalloproteinase from a plant source and is the Azocollase A activity first described by Ragster and Chrispeels (Plant Physiology 64: 857-862; 1979).

POSTER ABSTRACTS

1028 **A MONOCLONAL ANTIBODY THAT BINDS TO SYCAMORE MAPLE XYLOGLUCAN RECOGNIZES A FUCOSE-CONTAINING EPITOPE.** J. Puhlmann, N Dunning, P Albersheim, A. Darvill and M. G. Hahn Univ of Georgia, Complex Carbohydrate Research Center, Depts of Botany and Biochemistry, 220 Riverbend Road, Athens GA 30602, USA

A library of murine monoclonal antibodies (McAbs) that recognizes plant cell wall polysaccharides has been generated. One of these McAbs (CCRC-M1) binds strongly to xyloglucan, a hemicellulosic polysaccharide isolated from Acer pseudoplatanus cell walls, but does not recognize xyloglucan prepared from tamarind seeds, which can be distinguished from sycamore xyloglucan by the absence of fucose. McAb CCRC-M1 also binds weakly to the acidic polysaccharide rhamnogalacturonan I, isolated from A. pseudoplatanus Competition binding studies, using purified oligosaccharides structurally related to xyloglucan, were done to further define the epitope recognized by CCRC-M1. The oligomers tested included xyloglucan oligosaccharides, ranging in size from trimer to nonamer, which were prepared either by enzymatic hydrolysis of xyloglucan or by chemical synthesis. Several commercially available fucose-containing oligosaccharides derived from mammalian glycoproteins were also tested Oligosaccharides lacking the terminal fucosyl residue or those containing terminal fucosyl residues linked $\alpha(1\rightarrow3)$ to glucose did not compete. Only those oligosaccharides containing a Fuc$\alpha(1\rightarrow2)$Gal disaccharide unit at the nonreducing end were able to compete with the intact xyloglucan polysaccharide for the antibody binding site Of the fucose-containing xyloglucan oligosaccharides, a nonasaccharide was the most effective These results suggest that the terminal fucosyl residue is essential for McAb CCRC-M1 to recognize sycamore maple xyloglucan, but may not be sufficient for maximum affinity. McAb CCRC-M1 is being used to locate fucosyl-galactosyl side chains in the walls of plant cells (Supported by DOE grant DE-FG09-85ER13426; and in part by the USDA/DOE/NSF Plant Science Program through funding by DOE grant DE-FG09-87ER13810)

1029 **ANALYSIS OF MAIZE PEROXIDASES: CLONING AND CHARACTERIZATION OF A ROOT CATIONIC PEROXIDASE cDNA** Michael A Menke and Otto Folkerts, Agricultural Biotechnology Laboratory, DowElanco, 1701 Building, Midland, Michigan, 48640

We have cloned and characterized a cDNA (*per5*) encoding a maize cationic peroxidase (calculated pI = 9.1) Degenerate oligonucleotide PCR primers based on two conserved amino acid domains from plant peroxidases were used to generate a 387 bp PCR peroxidase probe Two classes of cross hybridizing cDNA clones were isolated from a maize root cDNA library The majority of cDNA clones (class I) contained inserts of approximately 1 3 - 1 4 kb . One other cDNA clone (class II) contained a 1 85 kb insert and differed from class I clones as shown by restriction mapping (*per11*) The longest class I cDNA (*per5*) was completely sequenced. The 1354 nt cDNA insert encodes a putative 333 amino acid open reading frame. The deduced amino acid sequence of the *per5* clone revealed a putative 28-amino acid endoplasmic reticulum signal peptide and a 305-amino acid mature protein (M_r 32,847). The amino acid sequence was compared to the amino acid sequences of the anionic peroxidase of tobacco (47% sequence similarity), and the cationic peroxidases of horseradish (51%) and turnip (51%) and found to possess all three conserved amino acid domains of peroxidases. By RNA gel blot analysis the mRNA for *per5* was found to be present at 5 5-fold higher steady state levels in root tissue compared to stem tissue, while no expression was detected in either leaf, kernel or tassel tissue. Genomic DNA gel blot analysis showed that the *per5* and *per11* cDNAs are both encoded by single copy genes RFLP mapping using gene-specific probes showed that *per5* maps to chromosome 8S and *per11* maps to chromosome 3L

1030 **PECTIN-DEGRADING ENZYMES FROM THE ROOT CAP OF PISUM SATIVUM** M C Hawes and M Stephenson, University of Arizona, Departments of Plant Pathology and Molecular & Cellular Biology, Tucson AZ 85721

Root tips of pea can shed 3400 ± 500 individual living cells daily. In the soil, these root "border" (BRD) cells form a sheath around the root. Cell separation and release is a temperature sensitive, developmentally regulated process that can be "reset" and synchronized by washing BRD cells from the root. We are studying enzymes in the root cap that may be involved in degrading the middle lamella so that cells separate from each other and from the root. Pectolytic enzyme activity was detected using Nelson's reducing sugar activity, and was found to be correlated with BRD cell separation (Plant Physiology 94: 1855). The enzyme is inactivated by treatment for 5 min at 65 C, and has a pH optimum of 5.5. Properties of the enzyme are consistent with those of an endopolygalacturonase. A second enzyme activity has the properties of a pectinmethylesterase (PME): extracts of the cap exhibit temperature-dependent induction of a gel when incubated with pectin. Gel formation also requires the presence of a second temperature-labile, nonenzymatic factor from root cap extracts.

1031 **BIOSYNTHESIS OF CYANELLE PEPTIDOGLYCAN IN CYANOPHORA PARADOXA.**

Michael Kraus[1], Barbara Plaimauer[1] Beatrix Pfanzagl[1], Wolfgang Loffelhardt[1], Jose Berenguer[2], Miguel A.de Pedro[2]. [1] Institut fur Allgemeine Biochemie der Universitat Wien und Ludwig-Boltzmann-Forschungsstelle fur Biochemie, A-1090 Vienna, Austria. [2] Centro de Biologia Molecular CSIC-UAM & Facultad de Ciencias UAM, E-28049 Madrid, Spain.

Cyanelles, the photosynthetic organelles of *Cyanophora paradoxa*, are unique with respect to the occurrence of a murein wall between the two envelope membranes. The overall composition of the peptidoglycan is similar to that from *E.coli* and about 20 enzymes usualy not present in eukaryotic cells (including seven previously detected penicillin binding proteins) are necessary for its biosynthesis. Our interest was the subcellular location of the enzymes involved and the distribution of the respective genes between the nuclear and the cyanelle genome. (a) Enzymes performing the first steps i.e UDP-N-acetyl-muramic acid (UDP-mur) synthesis, and the last step (D-ala-D-ala ligase) of the biosynthesis of the "building block", UDP-mur-pentapeptide, were shown to be confined to the cyanelle stroma, with the implication that this monomer has to cross the inner membrane. The enzymes either are synthesized within the cyanelles or have to be imported as novel import precursors. (b) Enzymes involved in the last steps of the biosynthesis of high molecular peptidoglycan (DD- and LD-carboxypeptidases and endopeptidases) were localized in the periplasmic space. This implies translocation of these proteins through the inner membrane if they are encoded by the cyanelle genome. Alternatively they might be the products of nuclear genes, with the two options of being targeted directly through the outer membrane to the periplasm or being imported to the stroma and in a second step translocated through the inner membrane. We will also present data from antibody screening of expression libraries for proteins involved in cyanelle wall biogenesis.

1032 **COMPUTER SIMULATIONS OF THE STATIC AND DYNAMIC MOLECULAR CONFORMATIONS OF XYLOGLUCAN.** Samuel Levy[1], William S. York[2], Rainer Stuike-Prill[2], Bernd Meyer[2] and L. Andrew Staehelin[1,]. [1] Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado, CO 80309-0347, USA.[2]Complex Carbohydrate Research Center, University of Georgia, 220 Riverbend Road, Athens, GA 30602, USA.

The hemicellulosic polysaccharide xyloglucan binds with a strong affinity to cellulosic cell wall microfibrils, the resulting heterogeneous network constituting up to 50 % of the dry weight of the cell wall in dicotyledonous plants. To elucidate the molecular details of this interaction, we have performed theoretical potential energy calculations of the static and dynamic conformations of xyloglucan using the GEGOP software.

In particular, we have evaluated the preferred sidechain conformations of model fragments of xyloglucan for molecules with a cellulose-like, flat, glucan backbone, and a cellobiose-like, twisted, glucan backbone conformation. For the flat backbone conformation the determination of static equilibrium molecular conformations revealed a tendancy for sidechains to fold onto one surface of the backbone, defined here as the $H1^s$ face, in the fucosylated region of the polymer. This folding produces a molecule that is sterically accessible on the opposite face of the backbone, the $H4^s$ face. In contrast, the trisaccharide sidechain folds onto the $H4^s$ face of xyloglucan fragments with a twisted backbone conformation. Preliminary NMR data on nonasaccharide fragments isolated from sycamore suspension-cultured cell walls are consistent with the hypothesis that the twisted conformation of xyloglucan represents the solution form of this molecule.

Metropolis Monte Carlo (MMC) simulations performed on the flat, rigid, backbone xyloglucan indicate that the trisaccharide sidechain is less mobile than the terminal xylosyl sidechains. MMC calculations on a fully relaxed molecule revealed a positive correlation between a specific trisaccharide sidechain orientation and the "flatness" of the backbone glucosyl residues adjacent to this sidechain. Based on these conformational preferences we suggest the following model for the binding of xyloglucan to cellulose. Nucleation of a binding site is initiated by the fucosylated, trisaccharide sidechain that flattens out an adjacent region of the xyloglucan backbone. Upon contacting a cellulose microfibril this region spreads by stepwise flattening of successive segments of the backbone. Self-association of xyloglucan molecules in solution may be prevented by the low frequency of formation of these nucleation sites and the geometry of the molecules in solution. Supported by NSF grant DCB 8615763 and NIH grant GM 186639 to LAS, by DOE grant DE-FG09-85ER 13426 to Dr. Alan Darvill[2] and DOE grant DE-FG09-87ER13810 to CCRC[2].

1033 **STRUCTURE OF THE ACTIN GENE FAMILY IN *ARABIDOPSIS*.** John M. McDowell, Elizabeth C. McKinney, and Richard B. Meagher. Department of Genetics; University of Georgia; Athens, GA 30605.

It is well established that actin is present in a variety of plant cell types. Localization studies and studies with inhibitors indicate numerous potential roles, which include cytoplasmic streaming, determination of the plane of cell division, maintenance of cytoskeletal structure, pollen tube growth, organellar movement, and graviperception. Actin is encoded by a multigene family in all organisms examined with the exception of yeast. Six to eight diverse actin isoforms are present in Petunia, soybean, maize, and *Arabidopsis*. Molecular genetic analysis reveals a Petunia actin gene superfamily of at least 150 members. Preliminary data indicates that the actin gene families in rice and soybean are also extremely complex. An analysis of the rate of nonsynonymous substitution indicate that plant actin gene families are comprised of several ancient subfamilies. The date of divergence of plant actin genes coincides with the emergence of vascular plants (350-400 MY). Given the great diversity of potential functions of plant actins, we have proposed that the ancient classes of plant actin genes arose with the diverse cell types present in vascular plants and have been preserved due to a requirement for diverse functions and/or tissue-specific and developmental expression.

Our lab is undertaking a molecular genetic analysis of actin divergence, differential expression, and function *Arabidopsis thaliana*. Genomic southern blots indicate an actin gene family of 11-13 members. 11 unique *Arabidopsis* actin genes have been isolated and partially sequenced. Sequence alignments indicate that the majority of the family members are quite divergent. A gene tree of the *Arabidopsis* actin sequences will be presented and a method for PCR amplification and sequencing of actin genes will be outlined. This method can be used to characterize highly divergent actin genes and to determine the actin mRNA phenotype in individual cell types.

POSTER ABSTRACTS

1034 A CELL WALL PROTEIN OF LOBLOLLY PINE. Wuli Bao, David M. O'Malley and Ron R. Sederoff. North Carolina State University, Department of Forestry, Raleigh, North Carolina 27695

The calcium chloride protein extract from xylem cell walls of nonlignified differentiating wood of loblolly pine (P. taeda L.) yields a single major TCA soluble glycoprotein. The protein is rich in proline and threonine and is abundant in primary cell walls. Polyclonal rabbit antibodies raised against the deglycosylated protein were used for ELISA, Westerns and immunolocalizations for both the deglycosylated and native glycoprotein. Antigen is found in cambium, phloem and new xylem tissues. Ray cells and resin duct epithelium of wood are rich in this protein. Antigen is also found in cell walls of mature wood sections which were treated with cellulase. Both immuno-gold stain and alkaline phosphatase stain show antigen existing in secondary cell walls of early wood. The milled dry wood shows increased availability of antigen in wood by treating with hydrogen floride and cellulase. We propose that this extensin-like protein is present in xylem cell walls during lignification and that the protein remains in wood as a structural component of the primary and secondary cell walls in wood for a long period of time.

1035 WHAT IS THE CHARACTER OF THE ASSOCIATIONS WHICH INSOLUBILIZE EXTENSIN INTO CELL WALLS? LIBERATION OF EXTENSIN FROM COTTON SUSPENSION CULTURE CELL WALLS.

Xiaoyang Qi and Andrew J. Mort. Oklahoma State University, Department of Biochemistry, Stillwater, Okalhoma 74078 USA. It is well known that the hydroxyproline (hyp)-rich cell wall glycoprotein, extensin, becomes insolubilized into the wall. In order to learn about the nature of the insolubilization we have tested the ability of a variety of selective hydrolytic methods and (combinations of them) to liberate extensin or fragments of extensin from cell walls. These methods include: 1) endopolygalacturonase digestion to digest homogalacturonans, 2) cellulase digestion to degrade cellulose and xyloglucan, 3) HF at -73°C to remove specifically arabinose residues from the hyp residues, 4) HF at 0°C to remove all sugars from the walls and 5) trypsin to cleave the proteins at lysine and arginine residues. None of the treatments alone liberated significant quantities of extensin as judged by release of hyp-containing peptides. Considerations of the extent of release by combinations of treatments show that the arabinose side chains on the hyp residues are important for preventing trypsinization of the protein but also that the presence of pectin and cellulose inhibit the action of trypsin on extensin. As was noted many years ago, removal of all sugars by treatment in HF at 0°C did not solubilize the protein. However a subsequent trypsinization solubilized ~ 80% of it. After sequential treatment of walls with methods 1, 2, and 3 only protein and sugars indicative of rhamnogalacturonan I (RGI) remained insoluble. Treatment of this residue with trypsin liberated what appear to be large glycopeptides containing sugars indicative of RGI which react with antibody to deglycosylated extensin precursors on Western blots. From our observations we suggest that extensin becomes insolubilized into cell walls by direct protein-protein (or protein-phenolic-protein) crosslinks and by some as yet uncharacterized protein-pectin crosslinks. (Supported in part by NSF grant DMB-9006052)

1036 STABLE TRANSFORMATION OF NORWAY SPRUCE EMBRYOGENIC CALLUS THROUGH MICROPROJECTILE BOMBARDMENT. Dominique Robertson(1), Rhonda Ackley(2), Arthur K. Weissinger(1), Anne-Marie Stomp(3) and Ronald R. Sederoff(3) Depts of 1. Crop Science, 2. Genetics and 3. Forestry, North Carolina State University, Raleigh, North Carolina 27695

We have obtained stably transformed callus from embryogenic cultures of Norway spruce through microprojectile bombardment of mature somatic embryos with pRT99gus (Topfer et al. 1988 Nuc. Acids Res. 16:8725), containing genes for kanamycin resistance (*neo*) and beta glucuronidase (*gusA*) driven by the 35S-CaMV promoter. Twelve lines of callus from three experiments selected on 10 mg/l kanamycin have npt II protein concentrations of 25 to 1880 ng/mg tissue, as determined by ELISA. GUS activity, determined fluorimetrically, is present in each of these lines and ranges from 55 to 1525 pM 4-MU/min ug protein. DNA/DNA hybridizations of cesium-purified DNA from control and one of the transformed lines with an internal, random primed PCR product from *neo* showed multiple integrations with copy numbers between 1 and 10. Polymerase chain reaction (PCR) analyses, using primers designed to amplify the *neo*, *gusA* and *amp* genes, have shown novel bands in addition to the expected bands, suggesting that stable integration of plasmid sequences into the host genome has occurred. Most of this material is no longer embryogenic however some material (proembryos and suspensors) from these experiments growing on 10 mg/l kanamycin has given positive ELISA results for npt II. We are currently amplifying this material on medium promoting maturation of embryos

1037 PROPERTIES OF A NOVEL 17 kD PEPTIDE INHIBITOR OF TOBACCO CELL WALL INVERTASE. Marion Weil and Thomas Rausch; Botanisches Institut, J.W.Goethe Univ., D-6000-Frankfurt, F.R.G.

Cell wall invertase (CWI) from Agrobacterium tumefaciens-transformed Nicotiana tabacum cell clones has been characterized and its glycosylation pattern established using an antiserum directed against the deglycosylated CWI of Daucus carota (a gift from Dr.Arnd Sturm, Basel). Upon cation exchange (sulfopropyl-Sephadex/NaCl-gradient) and gel permeation (Ultrogel AcA 54) chromatography CWI activity and Western Blot immunosignal did not correlate. In purified CWI fractions with strong immunosignal but low or even absent CWI activity a 17 kD peptide was consistently observed. This putative inhibitor of cell wall invertase (ICWI) has 1) an pI of < 7.0, 2) coprecipitates with CWI upon immunoprecipitation using the anti-CWI-antiserum and protein A-Sepharose, 3) elutes from sulfopropyl Sephadex (pH-gradient) in the same fractions as CWI, 4) dissociates from CWI at pH 9.5 but reassociates at pH 5.5, 5) reduces the mobility of CWI in native PAGE , and 6) shows a strong unusual staining reaction with $ZnCl_2$ (after SDS-PAGE). The results suggest that the anionic ICWI binds (reversibly) to CWI (pI 9.5) by ionic interactions. The ICWI has been purified for N-terminal sequencing. The possible physiological role of the ICWI will be discussed.

1038 IDENTIFICATION OF GENES FOR CELLULOSE BIOSYNTHESIS IN ACETOBACTER XYLINUM. Inder M. Saxena and R. Malcolm Brown, Jr. University of Texas at Austin, Department of Botany, Austin, Texas 78713.

An operon consisting of four open reading frames has been identified in Acetobacter xylinum to code for polypeptides involved in cellulose biosynthesis. The first gene in the operon has been identified as the cellulose synthase catalytic subunit gene, based on amino acid sequencing of an 83 Kd polypeptide shown by photoaffinity labelling to be the cellulose synthase catalytic subunit. The second gene in the operon codes for an 86 Kd polypeptide, which co-purifies with the 83 Kd polypeptide during purification of cellulose synthase. The function of the 86 Kd polypeptide is not clearly understood at present; however, it has been suggested to be involved in the activation of cellulose synthase activity. The remaining two polypeptides in the operon have not yet been identified. The expression of the cellulose synthase catalytic subunit gene and other genes in the operon has been studied under the control of various E. coli promoters. Comparison of the cellulose synthesizing polypeptide sequences with protein sequence databases has revealed no significant homology with any known protein sequence. Screening of an Arabidopsis thaliana cDNA library with the cellulose synthase catalytic subunit gene did not lead to identification of any homologous sequences. Since considerable variation is observed for the cellulose synthesizing gene sequences even among different strains of A. xylinum, we believe that the sequence of a conserved region such as the substrate-binding site of cellulose synthase would be more suitable for identifying cellulose synthesizing genes in other organisms including higher plants.

1039 IMMUNOLOCALIZATION OF LECTIN IN PISUM SATIVUM ROOT CELLS. D. Janine Sherrier and Kathryn A. VandenBosch. Texas A&M University, Department of Biology, College Station, Texas 77843

Lectins are believed to function in the recognition between leguminous host plants and Rhizobium during the establishment of a symbiotic state. We will report the ultrastructural distribution of glucose/mannose isolection 2 in root cells of 5- and 7-days old garden peas (Pisum sativum L. cv. Sparkle). Lectin will be localized using indirect immunogold labeling. The distribution of lectin will be evaluated in elongating root hairs, epidermal cells apical to root hairs, cortical cells subjacent to developing root hairs, and cap cells. Distribution will be compared in tissue prepared by chemical or cryofixation methods. Further work will document changes in the distribution of lectin upon interaction with Rhizobium.

POSTER ABSTRACTS

1040 ANALYSIS OF THE 5'-REGULATORY SEQUENCES OF PRP1, A SOYBEAN PROLINE-RICH CELL WALL PROTEIN GENE. Hideki Suzuki, Thomas J. Fowler and Mary L. Tierney. Ohio State University, Biotechnology Center & Agronomy Department, Columbus, Ohio 43210

In soybean, proline-rich cell wall proteins (PRP's) are structural proteins encoded by a small gene family which is differentially expressed during plant development. PRP1, encodes a 256 amino acid protein which is highly expressed in root. We have begun studies on the transcriptional regulation of this gene by fusing 1.1 kb of the PRP1 promoter to a gene encoding β-glucuronidase (GUS) and analyzing GUS expression in transgenic plants. We have determined that this 1.1 kb of PRP1 5'-flanking sequence is sufficient for GUS expression in transformed tobacco roots, indicating that the regulation of this gene is conserved among plant species. In order to more carefully define the PRP1 promoter, we have initially analyzed a nested set of deletions using the *Agrobacterium rhizogenes*-mediated hairy root transformation as a rapid assay system. Nine 5'-deletion constructs, spanning 1.1 kb of the PRP1 promoter, were transformed into *Vigna aconitifolia*, and hairy roots were analyzed for GUS activity. These studies indicated that at least three cis-acting regions contribute to the PRP1 promoter activity in root tissue. Total nuclear extracts prepared from soybean roots were also shown to have DNA binding activities with several regions within -1062 to -391 of the PRP1 promoter. Nuclear extracts isolated from soybean leaves, a tissue which does not express PRP1, did not exhibit these binding activities. Our current efforts are focused on identifying specific DNA sequences which are necessary for promoter activity and transacting factor interactions.

1041 RECOMBINANT ENDO-1,4-BETA-GLUCANASE FROM A PROCARYOTIC SOURCE, PROMOTES POLLEN TUBE ELONGATION IN PEACH (Prunus persica). [1]Oded Shoseyov, [2]Mara Dekel-Reichenbach and [2]Rov H. Doi. [1]Dept. of Horticulture, Faculty of Agriculture, The Hebrew University of Jerusalem, P.O.Box 12, Rehovot 76-100, Israel, [2]Dept. of Biochemistry and Biophysics UC Davis, Davis CA. 95616.

Endo-1,4-beta-glucanase (EGase) was cloned from Clostridium cellulovorans expression library that was constructed in lambda gtII. The gene (engC) was subcloned into E. coli and was found to express an active Carboxy Methyl Cellulase (CMCase) enzyme. The nucleotide sequence of engC was determined. Out of more then thirty bacterial EGase genes none was homologous to the plant EGase genes. It was found that engC is highly homologous to the avocado EGase ("cellulase"). Recently it was proposed that EGase plays a major role in plant cell elongation. We developed a plant cell elongation model system in peach pollen in order to test in vitro the effect of recombinant EGase on peach pollen tube elongation. It was found that recombinant EGase promotes pollen tube elongation more then twice as the control.

1042 EXPRESSION OF THE *cel*1 GENE DURING AVOCADO FRUIT ABSCISSION Pietro Tonutti[1,2], Laura G. Cass and Rolf E Christoffersen, Department of Biological Sciences, University of California Santa Barbara, CA 93106 Characterization of cellulase genomic clones has identified two closely related genes in the avocado, *cel*1 and *cel*2 (Cass et al.,1990 M.G.G. 223, 76-86). Because these genes are highly similar within protein coding regions, RNA transcripts derived from either gene would hybridize to cDNA probes in RNA blot analysis. To date, cellulase cDNA clones isolated from ripe fruit libraries correspond to the *cel*1 sequence suggesting that *cel*1 is responsible for the majority of cellulase mRNA present in ripe fruit RNase protection assays using a probe that distinguishes between *cel*1 and *cel*2 RNA transcripts have shown no detectable *cel*2 RNA in ripening fruit. Another tissue that is known to contain cellulase enzyme activity is the abscission zone (AZ). To examine whether the *cel*1 and/or *cel*2 genes are expressed in AZ tissue, mature fruit were induced to abscise by either 2-chloroethylphosphonic acid spraying of the fruit or by girdling of the stem above the fruit. Abscission zones proximal and distal to the stem were collected and RNA extracted. Probes specific to *cel*1 and *cel*2 RNAs were constructed from the 3' untranslated region of each gene. RNA blot analysis of both proximal and distal AZ tissue demonstrated the presence of *cel*1 RNA but no detectable *cel*2 RNA. No detectable cellulase RNA was observed in nonabscissing control tissue. Preliminary *in situ* hybridization experiments of proximal AZ tissue localized *cel*1 transcripts in the vascular tissue above the abscission zone rather than in the abscissing cortical cells.

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1043 EVIDENCE FOR TAU-LIKE MICROTUBULE-ASSOCIATED PROTEINS IN HIGHER PLANTS. Barbara A. Triplett, Robert W. Seagull, Kim W. Daigle, and Susan M. Holland. USDA, ARS, Southern Regional Research Center, New Orleans, Louisiana 70179.

In many plant cells, a close relationship has been documented between the ordered deposition of cellulose microfibrils in the cell wall and the organization of cortical microtubules. While much is known about tubulin proteins and genes in higher plants, very little is known about associated proteins and their potential regulatory control over polymerization and stabilization of microtubules. Still less is understood about how the cytoskeleton dictates influence over the deposition of cellulose microfibrils. As part of an effort to dissect the plant cytoskeleton, we have screened commercially available antisera to microtubule-associated proteins against cytoskeletal proteins isolated from cotton suspension culture cells. Initial results indicated that these cells contained four proteins that cross-reacted with a polyclonal antibody directed against chicken tau, a cytoskeletal protein previously reported only in animal tissues. Under stringent conditions in Southern blots of total cotton genomic DNA, a bovine tau cDNA probe hybridized to a 3 KB EcoRI fragment. An antibody directed against a conserved region (tubulin-binding region) of bovine tau also cross-reacted with a 60 kDa protein from cotton suspension culture cells. Degenerate primers corresponding to the tubulin binding region were used to synthesize several PCR products that hybridized to bovine tau cDNA. The sequence and characterization of these clones and the pattern of expression in developing cotton fiber cells will be presented.

1044 AN EXTRACELLULAR LOCATION OF A SUPPOSED *ARABIDOPSIS* LIPID TRANSFER PROTEIN. Sharon Thoma[1], Yasuko Kaneko[1], and Chris Somerville[1]. [1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 and [2]Department of Botany, University of Wisconsin, Madison, WI 53706

A class of proteins named lipid transfer proteins (LTPs) have been shown to mediate transfer of phospholipids between membranes *in vitro*. However, there is no evidence that these proteins catalyze lipid transfer *in vivo*. Although these LTPs were believed to be cytosolic, our recent experiments show that the spinach LTP is cotranslationally processed and inserted into the endoplasmic reticulum[1]. This indicates that the protein is targeted to a specific organelle or is secreted. Either fate is inconsistent with the proposed role of lipid transfer between membranes. To determine the location of this protein, antibodies were raised against an *Arabidopsis* LTP:protein A fusion that had been expressed in *E. coli*. These antibodies were used for immunogold localization of the *Arabidopsis* LTP. Preliminary results indicate an extracellular location of LTP. Although the localization can provide no clue as to the *in vivo* function of this protein, it appears to preclude the proposed role in lipid transfer.

[1]Bernhard *et al.* 1991. Plant Physiol. 95:164-170.

1045 VITRONECTIN- AND FIBRONECTIN-LIKE PROTEINS IN MEMBRANE-WALL AND PROTOPLAST ADHESION IN SALT-ADAPTED TOBACCO CELLS. J.-K. Zhu[*], N.C. Carpita[*], J. Shi[*], R.A. Bressan[1], U. Singh[*] and P.M Hasegawa[1]. Purdue University, Depts. of Horticulture[1] and Botany & Plant Pathol.[*], W. Lafayette, IN 47907.

Cells of tobacco (*Nicotiana tabacum* cv. W38) adapted to grow in high concentrations of NaCl develop tight zones of adhesion between the plasma membrane and cell wall Protoplasts isolated from these tobacco cells also adhere to each other The protoplast adhesion could be disrupted specifically by the hexapeptide Gly-Arg-Gly-Asp-Ser-Pro, a sequence that represents the membrane receptor binding domain of several animal matrix proteins such as vitronectin and fibronectin Proteins antigenically cross-reactive to human vitronectin and fibronectin were greatly enriched in the salt-adapted tobacco cells. The vitronectin-like proteins were localized by immunofluorescence microscopy on cell walls and on the outer surface of protoplasts, and were localized to the plasma membrane by aqueous two-phase membrane partitioning. The fibronectin-like proteins were localized on cell walls by immunoblot analysis of purified wall proteins and by immunofluorescence microscopy Our observations suggest that plants have cell-matrix adhesion systems, similar to those of animal cells. The induction of vitronectin-like and fibronectin-like proteins in salt-adapted cells indicates that they and an adhesion system may be involved in tolerance to osmotic stress.

Supported by the McKnight Foundation and Grant 90-37280-5647 from the USDA/CRGO

POSTER ABSTRACTS

1046 SYSTEMIN, A POLYPEPTIDE INDUCER OF PROTEINASE INHIBITOR SYNTHESIS, IS DERIVED FROM A LARGER PREPROTEIN. Barry McGurl, Gregory Pearce and Clarence A. Ryan. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

An 18-amino acid polypeptide, called systemin, which was isolated from tomato leaves, is a potent inducer of proteinase inhibitor synthesis. The cDNA and gene encoding systemin have now been isolated and characterized. Systemin is encoded by a single, 4.6 Kb gene which produces a 920 base mRNA. The systemin gene is interrupted by 10 introns. Homology matrix analyses of the cDNA and genomic clones reveal that the gene evolved by at least three gene duplication-elongation events. Based on the cDNA and gene sequences it was found that systemin is located at the carboxy terminus of a 200-amino acid preprotein. Systemin is represented only once within the preprotein although other sequence elements are repeated. Northern blot analysis showed that the systemin gene is wound-inducible and is expressed throughout the aerial parts of the plant, but not in the roots. (Supported in part by the Washington State University College of Agriculture and Home Economics, the McKnight Foundation and grants from the National Science Foundation.)

1047 ANTISENSE SYSTEMIN RNA SUPPRESSES WOUND INDUCTION OF PROTEINASE INHIBITORS IN TOMATO PLANTS. Barry McGurl, Martha Orozco-Cardenas, Gregory Pearce and Clarence A. Ryan.
Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Systemin may be one of the first components of the signal transduction pathway leading to wound-induced expression of proteinase inhibitor genes in tomato leaves. Part of the systemin cDNA was placed in the antisense orientation, under the control of the constitutive CaMV 35S promoter, within the binary vector pGA643. Two groups of tomato plants were transformed, one group with the antisense construct and the other with the binary vector alone. Eighteen antisense plants and 21 controls were regenerated and assayed for their ability to synthesize proteinase inhibitors I and II in response to wounding. Two thirds of the antisense plants showed greatly reduced synthesis of both inhibitors I and II in response to wounding. In those antisense plants which showed least proteinase inhibitor synthesis the levels of inhibitors I and II were 20% and 10%, respectively, of the mean inhibitor levels in the control plants. True-breeding F2 plants, exhibiting maximal suppression of proteinase inhibitor synthesis, are being used to test models for the signal transduction pathway leading to wound-induced expression of proteinase inhibitor genes. (Supported in part by the Washington State University College of Agriculture and Home Economics, the McKnight Foundation and grants from the National Science Foundation.)

1048 ANALYSIS OF GENES AND cDNAS ENCODING PROTEIN KINASES IN ARABIDOPSIS THALIANA. Nobuaki Hayashida, Tsuyoshi Mizoguchi, Kazuko Yamaguchi-Shinozaki, Masahiro Koizumi and Kazuo Shinozaki. The Institute of Physical and Chemical Research (RIKEN) Gene Structure Laboratory, Tsukuba, 305 Japan.

Environmental stimuli affect various physiological and developmental processes via signal transduction pathways in plants. Number of protein kinases are thought to function in the signal transduction processes by phosphorylating specific proteins in plant as well as animal systems. We started to clone both cDNAs and genes encoding a variety of protein kinases to analyze function of protein kinases in signal transduction pathways. Protein kinases contain eleven short regions which are highly conserved among various protein kinase families in the catalytic domains. Oligonucleotide primers corresponding to such conserved regions were used to carry out PCR with Arabidopsis thaliana cDNA or genomic DNA as templates to identify protein kinase genes in plants. We have obtained several amplified DNA fragments which have protein kinase sequences, and screened genomic and cDNA libraries of A. thaliana with these DNA fragments as probes. In this paper we report genomic and cDNA clones which resemble cyclic nucleotide-dependent and calcium dependent protein kinases. Tissue specific expression of these protein kinase genes has been analyzed by using Northern hybridization method.

1049 THE CHARACTERISATION AND INTRACELLULAR DISTRIBUTION OF PLANT ANNEXINS.. Hugh D. Blackbourn, Alison D. McClung and Nick H. Battey. Department of Horticulture, Univ. of Reading, Berks., RG6 2AS U.K.

Calcium-dependent phospholipid-binding proteins have been partially purified from maize coleoptiles using a protocol employing their Ca^{2+}-dependent association with added phospholipids, followed by hydroxylapatite chromatography[1]. An assay for Ca^{2+}-dependent binding to artificial liposomes and analysis by SDS-PAGE reveals that in maize extracts, three proteins have annexin-like properties. Further evidence that the plant proteins are related to animal annexins is given by antibody cross-reactivity and amino acid sequence analysis. We are studying the characteristics of plant annexins in tip-growing pollen tubes to test whether they regulate vesicle fusion or associate with the cytoskeleton in a manner analogous to some of the animal annexins. Western blotting with antiserum raised to the annexins from maize reveals that immunoreactive polypeptides are present in pollen tubes of both *Tradescantia virginiana* and *Lilium speciosum*. In *Tradescantia* pollen tubes fixed with formaldehyde and then permeabilised with Triton X-100, localisation at the LM level indicates that the annexin-like proteins have a diffuse distribution. We are currently comparing the distribution of F-actin and annexins in pollen tubes prepared under a variety of conditions. Data to be presented will include further biochemical and functional characterisation of these plant annexins.

1. Blackbourn, H.D., Walker, J.H. and Battey, N.H. (1991) Planta 184, 67-73

1050 IMMUNOPHILINS-WHAT IS THEIR ROLE IN PLANTS?
Adina Breiman*, Timothy W. Fawcett and Autar K. Mattoo
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Immunophilins are a class of structurally unrelated and immunologically distinct proteins that bind either of the two immunosuppressive drugs, Cyclosporin A or FK506. Both types possess peptidyl prolyl cis-trans isomerase (PPIase) activity that catalyzes the rotation of the peptide bonds at the amino side of proline residues (rotamase). This activity, thought to be essential for protein folding in the cell, has been detected in *E. coli*, yeast, fungi, *Drosophila*, plants and mammalian thymocytes (see Schreiber, S.L., Science 251: 283, 1991). We have identified both types of immunophilins in higher plants based on their ability to either bind Cyclosporin A or cross-react with an antibody to a fungal FK506 binding protein. The latter detected its homologous protein in plant mitochondrial extracts. Cyclophilins were also found in pea leaf chloroplasts as well as in the cytosol. The presence of these proteins in plant organelles is a new finding. Furthermore, these plant immunophilins exhibited marked PPIase activity. Their possible role(s) will be discussed in relation to similar activities discovered in organisms other than plants. This protein class may prove to belong to a group of well conserved receptors, important for cytoplasmic and organellar signal transduction in prokaryotic and eukaryotic organisms.

1051 SUCROSE-INDUCTION AND POLYGALACTURONIC ACID-INDUCTION OF SPORAMIN AND β-AMYLASE GENES OF SWEET POTATO Masa-aki Ohto, Shin Takeda and Kenzo Nakamura. Lab. of Biochem Sch. of Agric., Nagoya University, Chikusa, Nagoya 464-01, Japan

Treatment of leaf-petiole cuttings from sweet potato plants with high concentrations of sucrose induce expression of genes encoding sporamin and β-amylase, two most abundant proteins of tuberous roots, concomitant with the accumulation of starch (1 2). When polygalacturonic acid (PGA) or chitosan was applied to leaf-petiole cuttings, induction of accumulation of both sporamin and β-amylase mRNAs occured in petiole and leaf parts of the cuttings. The same set of members of the sporamin multigene family seems to be induced by sucrose or PGA. Physiological significance of PGA-induction is not clear since wounding of leaves only occasionally caused local and systemic induction of sporamin and β-amylase mRNAs. Unlike sucrose, PGA did not induce starch accumulation. Although PGA may not be transported to a long distance from the cut-edge, *in situ* hybridization of sporamin mRNAs in petiole and leaf sections showed similar spatial patterns between the PGA-treated cuttings and the sucrose-treated cuttings. In both cases, the accumulation of sporamin mRNAs first occured in cells surrounding the vascular system. Neither abscisic acid nor methyl jasmonate induced the expression of sporamin and β-amylase genes significantly. Both of the induction by sucrose and by PGA of sporamin and β-amylase mRNAs were inhibited by cycloheximide, suggesting that *de novo* synthesis of proteins is required in both induction processes. Results with calmodulin inhibitors and EGTA suggest possible involvement of Ca^{2+}/calmodulins in both of the induction processes.

(1) Hattori et al Plant Cell Physiol. 32 79-86 (1991) (2) Nakamura et al Plant Physiol. in press (1991)

POSTER ABSTRACTS

1052 ISOLATION OF A PROTEIN TYROSINE KINASE (APK1) cDNA FROM *ARABIDOPSIS THALIANA*. Atsuhiro Oka and Takashi Hirayama. Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

We have previously shown that *Arabidopsis thaliana* contains a *CDC2* gene family. One of the member genes, *CDC2a*, is both structurally and functionally similar to the *S. pombe cdc2* gene. *Arabidopsis* $p34^{CDC2a}$ seems to be modulated by the level of its own phosphorylation like *S. pombe* $p34^{cdc2}$ because three out of the four major phosphorylation sites of *S. pombe* and chicken $p34^{cdc2}$ are conserved in *Arabidopsis* $p34^{CDC2a}$. Despite extensive efforts to search protein kinases which phosphorylate $p34^{cdc2}$, no candidates have been found; moreover no protein tyrosine kinase has been identified from yeasts and plants except for *wee1* and *YPK1* protein serine/tyrosine kinases, a novel type of kinases. To find plant tyrosine kinases which may relate to modification of the $p34^{CDC2a}$ activity, polymerase-chain-reaction was done on an *Arabidopsis* cDNA mixture using a set of primers each corresponding to an animal tyrosine kinase-specific amino acid sequence motif. One of the PCR fragment products could code for a polypeptide which has amino acid sequences characteristic of both tyrosine and serine/threonine kinase. Thus the translation product of the corresponding gene may belong to the above novel type of protein kinases. To confirm this, an *Arabidopsis* cDNA library was screened with this PCR fragment probe, and a nearly full length cDNA (1.4 kb) was obtained. This sequence contained an open reading frame for a 45-kDa polypeptide (APK1), in which the kinase domain was flanked by non-kinase regions. This kinase domain is similar largely to serine/threonine kinases, but limitedly also to tyrosine kinases, and the NH_2-terminal sequence implies myristylation of APK1. No proteins highly homologous to APK1 have been found in databases. To see if APK1 has actually the activity of protein tyrosine kinase, cDNA corresponding to the APK1 kinase domain was expressed in *E. coli* cells with the *tac* promoter and total proteins isolated from IPTG-induced those cells were subjected to Western blotting analysis using anti-phospho tyrosine antibody. The results showed that some *E. coli* proteins were Tyr-phosphorylated by APK1. Thus, APK1 belongs to the novel class of kinases, which phosphorylates tyrosine residues but largely resembles protein serine/threonine kinases. Since another cDNA highly homologous to the above APK1-coding cDNA was also obtained, *Arabidopsis* appears to have multiple APK1 genes.

1053 PROTEIN CHANGES DURING FLORAL DEVELOPMENT. Margaret Dietrich and Judith Verbeke. University of Arizona, Department of Plant Sciences, Tucson, AZ 85721

During normal floral development in *Catharanthus roseus*, carpel epidermal cells redifferentiate following contact of the adaxial surfaces of the two carpels. Within 9 hours of contact, approximately 400 epidermal cells in this region redifferentiate into parenchyma cells. Several cytological changes have been described, including changes in cell shape, the reorientation of the plane of division, and the loss of the cuticle. A water soluble factor is thought to be involved in cell to cell communication preceding carpel fusion and, in fact, each carpel may produce a unique but necessary component. Because we are interested in obtaining stage-specific carpel cDNAs (prefusion, fusing, and postfusion) with which to characterize this redifferentiation process, we are examining the protein profiles of carpels at several stages of bud development. Differences in the abundance of several proteins have been documented by SDS-PAGE. These differences are being further characterized using 2-D gel electrophoresis to analyze the results of *in vivo* labeling and *in vitro* translations.

1054 CELLULAR AND SUBCELLULAR LOCALIZATION OF A RECEPTOR-LIKE PROTEIN KINASE. Veronica P. Counihan and John C. Walker, Division of Biological Sciences, University of Missouri, Columbia 65211

In *Zea mays*, the gene *ZmPK1* encodes a novel, receptor-like protein kinase. Its structure is similar to that of receptor tyrosine kinases found in mammalian systems: a catalytic domain, related to the serine/threonine-specific kinases, is linked via a transmembrane domain to an extracytoplasmic domain with homology to the Self-incompatibility-locus glycoproteins (SLGs) of *Brassica*. Based on the structural similarity to mammalian receptors and the sequence conservation with the SLGs, ZmPK1 may represent a novel class of plant receptors with intrinsic protein kinase activity, and may be involved in intercellular signalling in maize.

To gain a better understanding of the function of ZmPK1, we are examining its expression in various tissues, cells and subcellular compartments. We have raised polyclonal antibodies to peptides corresponding to regions of the catalytic and extracellular domains of ZmPK1. Western blot analysis of plasma membranes prepared by phase partitioning of maize shoot and root extracts indicates that these antibodies recognize a protein of approximately 100KDa. This protein is found predominantly in plasma membrane fractions and not in other subcellular compartments. The anti-ZmPK1 antibodies have also been used in immunolocalization experiments to determine the specific tissues expressing ZmPK1. ZmPK1 appears to be expressed in the epidermis and cortex of young roots, and a variety of cell types in young shoots, including the coleoptile.

1055 THE *RLK* (RECEPTOR-LIKE PROTEIN KINASE) GENES OF *ARABIDOPSIS THALIANA*. Tom Moran and John C. Walker, Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA

Receptor protein kinases consist of an extracellular ligand binding, a single transmembrane, and a cytoplasmic domain that has intrinsic protein kinase activity. These molecules function by ligand-induced oligomerization leading to activation of the protein kinase activity and phosphorylation of cellular substrates. These receptors play a major role in cellular metabolism and developmental regulation in many eukaryotes.

Genes encoding molecules resembling receptor protein kinases have recently been described in *Zea mays*, and are thought to function as components of signal transduction pathways. To further characterize the structure and function of receptor protein kinases in higher plants, we have screened *Arabidopsis thaliana* genomic and cDNA libraries under reduced stringency conditions with a nucleic acid hybridization probe derived from the conserved serine/threonine kinase domain of the maize receptor-like protein kinase, *ZmPK1*. Several cDNA clones were identified and by cross-hybridization and restriction endonuclease mapping were determined to represent five different genes, *RLK1*, *RLK2*, *RLK3*, *RLK4*, and *RLK5*. The deduced amino acid sequence of *RLK1* and *RLK2* indicates that they are members of a novel family of receptor-like protein kinases found in plants. These molecules could be of importance in development, responses to environmental cues, or in cell-to-cell signalling. Studies on the temporal and spatial aspects of expression and mutational analysis are underway.

1056 IMMUNOLOGICAL ANALYSIS OF A RECEPTOR-LIKE PROTEIN KINASE OF *ARABIDOPSIS THALIANA*. Sara Patterson, G. Eric Schaller and Tony Bleecker, University of Wisconsin Department of Botany, Madison Wisconsin 53706

Genomic and cDNA clones encoding a novel receptor-like protein kinase have been isolated from *Arabidopsis thaliana*. The predicted protein has an intracellular kinase domain most related to the receptor tyrosine kinases but containing diagnostic serine/threonine sequences, a transmembrane domain, and an extracellular domain containing 11 copies of a leucine rich repeat. Leucine rich repeats have only been found in proteins associated with the plasma membrane and are involved in protein/protein interactions. Portions of the extracellular and intracellular domains of the kinase have been fused in frame to a glutathione-S-transferase gene on a plasmid and expressed in *E. coli*. Expressed protein was isolated and used to produce domain-specific antibodies. These polyclonal antibodies recognize the domain to which they were made and are capable of immunoprecipitating the fusion protein. In extracts of *Arabidopsis*, the antibodies specifically immunodecorate a polypeptide of M_r=110,000. This immunodecorated polypeptide has been found in all portions of the plant examined including root, stem, leaf, flower, and silique. As such the kinase appears to represent part of a general pathway of transmembrane signaling in the higher plant.

1057 BETAINE PHOSPHATE FURTHER ENHANCES THE EFFECT OF ACETOSYRINGONE ON THE EFFICIENCY OF *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF APPLE. David James[1], Sandie Uratsu, Jiasheng Cheng, Paola Negri, Peter Viss[2], Abhaya M. Dandekar, Pomology Dept., Univ. of California, Davis CA 95616 USA, [1]Horticulture Research International, East Malling, ME19 6BJ UK, [2]Plant Research Laboratories, 2737 North Avenue, Modesto CA 95351 USA.

Plant signal molecules like acetosyringone (AS) have been shown to induce the transcription of the virulence genes of *Agrobacterium tumefaciens* (At) through a two component regulatory system. It is generally assumed that this virulence induction results in increased efficiency of transformation of plant cells. We have investigated this hypothesis in apple by including AS and betaine phosphate (BP) in the growth medium for At prior to inoculation of apple leaf discs and then monitoring GUS activity fluorometrically in individual leaf discs. A disarmed strain of At carrying the binary plasmid pKIWI105 was grown in two previously reported virulence induction media (MS20 and SIM) for 5h prior to inoculation of leaf discs.

During the first 21 days after inoculation, GUS activity was consistently higher in discs infected by bacteria induced with MS20 as compared to SIM but this difference was no longer apparent 42 days after inoculation. In further experiments bacteria were grown in MS20 or MS20 supplemented with AS (0.1 mM), BP (1 mM) or both. When AS or AS+BP were present in the bacterial growth medium GUS activity in subsequently assayed leaf discs was increased approximately 3 fold and nearly 15 fold respectively on a per disc basis compared to unsupplemented MS20 medium.

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1058 **IDENTIFICATION OF A SOLUBLE SALICYLIC ACID-BINDING PROTEIN THAT MAY FUNCTION IN SIGNAL TRANSDUCTION IN THE PLANT DISEASE RESISTANCE RESPONSE.** Daniel F. Klessig and Zhixiang Chen. Waksman Institute, Rutgers, The State University of New Jersey, P.O. Box 759, Piscataway, New Jersey 08855

It has been recently demonstrated that salicylic acid (SA) may serve as an endogenous signal molecule in the induction of systemic acquired resistance in tobacco and cucumber. In addition, SA is an endogenous regulator of heat and odor production in the inflorescence of some thermogenic plants. No information, however, is currently available concerning the mode of action(s) of SA in plant signal transduction. In a search for possible cellular factors which directly interact with SA, we have detected and partially characterized a SA-binding protein in tobacco leaves. Detection of the SA-binding activity required development of a new method for studying ligand binding factors based on spin-column exclusion chromatography. The SA-binding protein is both SDS- and proteinase-sensitive and behaves as a soluble protein with an apparent molecular weight of 370 kDa. The protein has an apparent K_d of 14 μM for SA, which is consistent with the range of physiological concentrations of SA observed for the induction of plant resistance responses. Furthermore, the ability of SA analogues to compete with SA for binding to this soluble protein is strictly correlated with their biological activity to induce the expression of genes associated with disease resistance. Biologically active analogues effectively inhibit SA binding while biologically inactive analogues do not. These results collectively indicated that this SA-binding protein may play a role in perceiving and transducing the SA signal to appropriate response elements, which ultimately activate one or more of the plant disease resistance responses. Purification and further characterization of the SA-binding protein is in progress.

1059 CALCIUM-DEPENDENT PROTEIN KINASE (CDPK) FROM *Arabidopsis thaliana*. Brad M. Binder, Jeffrey F Harper* and Michael R Sussman. Univ of WI, Plant Sci. Building, 1575 Linden Dr., Madison WI 53706 and *Scripps Inst. Dept. of Mol. Biol. 10666 N Torrey Pines Rd., La Jolla CA 92037

Protein phosphorylation is known to control and modulate transduction events in many cell types. We have explored the role that a calcium-dependent protein kinase (CDPK) may play in this regulation. Specifically we have examined regions where this kinase autophosphorylates and determined whether it phosphorylates endogenous peptides in *A. thaliana*.

A full length cDNA encoding a CDPK has been isolated from *A. thaliana* and expressed as a fusion protein in *E. coli*. The CDPK portion of the fusion protein has an approximate molecular weight of 66 kDa as determined with SDS-PAGE. This is somewhat larger than the CDPK that has been previously purified from crude extracts of oats and soybean.

The *E. coli*-expressed CDPK phosphorylates histone III-S in a calcium-dependent manner. In addition when the enzyme is added to membranes from *A. thaliana*, the phosphorylation of endogenous polypeptides is enhanced. Like many protein kinases, the enzyme autophosphorylates and this activity is calcium regulated.

To localize putative phosphorylation sites on the CDPK we constructed fusion proteins with different regions of the CDPK and tested them as kinase substrates. Our evidence suggests that the *E. coli*-expressed fusion protein containing the N-terminal domain of CDPK is a good substrate for the active kinase.

1060 The differential response of two wound-related genes of tomato to oligosaccharides P.J. O'Donnell and D.J. Bowles Centre for Plant Biochemistry and Biotechnology, Department of Biochemistry and Molecular Biology, The University of Leeds, LS2 9JT, U.K.

A number of genes are known to be activated in response to-wounding. These include genes encoding proteinase inhibitors (PIs) and genes encoding proteins involved in ethylene biosynthesis and ethylene-induced events. In this study we have compared the response of two wound-related genes of tomato to a range of elicitors to define the specificity of signalling pathways involved in their induction. We show that the expression of the genes encoding PI 1 and the ethylene-forming enzyme (pTOM 13) is differentially-regulated in response to oligosaccharides of defined sizes and structures. These results, together with data on timing and cell-specificity of expression suggest that the two genes can be used as markers to study the different signals released at a local injury-site.

1061 THE OCCURRENCE OF G PROTEIN BETA-LIKE POLYPEPTIDES IN HIGHER PLANTS. Yuan-kuey Hsuen and Teng-yung Feng. Institute of Botany, Academia Sinica, Taipei, Taiwan 115, Republic of China.

GTP-binding regulatory proteins (G proteins) have been known as very important mediators for signal transduction in animal and yeast systems. In plant system there is still uncertain to identify the existence of the corresponding G proteins. A polyclonal and a monoclonal antibodies raised against animal G protein beta subunit were used to screen for the existence of G proteins in plant tissues. By using western blotting analysis, we found that all plant tissues tested contain some specific polypeptides which can crossly react with the animal G protein antibodies. The patterns of immunoblots for different plant species (Arabidopsis, maize, mung bean, rice, tobacco) reveal that some bands are common in most of plants and some are species-specific. Different parts of plant, such as immature fruits, flowers, leaves, stems and roots of mung bean plant, show identical patterns in their immunoblots. cDNA clones isolated from tobacco cDNA library by immuno-screening encodes a polypeptide that shows sequence similarity with the beta subunit of guanine nucleotide binding proteins from mamals, yeast and green algae, Chlamydomonas.

1062 EFFECTS OF ABSCISIC ACID ON INTRACELLULAR CALCIUM LEVEL AND GENE EXPRESSION IN BARLEY ALEURONE PROTOPLASTS.

R.M. van der Meulen, F. Van der Mark, B. E. Valk, F. Heidekamp & M. Wang

Center for Phytotechnology RUL/TNO, Department of Molecular Plant Biotechnology, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The plant hormone abscisic acid (ABA) plays an important role in seed development. It is also known that ABA induces the expression of a number of genes late in embryogenesis. However, the signal transduction pathway from hormone (ABA) to gene expression is not well understood. It is known that Ca^{2+} is an important secondary messenger in both plant and animal cells. Both increase and decrease of intracellular calcium (Ca_i) have been demonstrated to occur in plant cells upon the addition of (phyto) hormones. Using the calcium-sensitive fluorescent dye Indo-1, we were able to measure Ca_i and the effects of ABA on Ca_i. The expression of ABA-inducible genes has been studied. The results of our studies on the effects of different ABA analogues on Ca_i and gene expression will be presented.

1063 EFFECTS OF RED LIGHT AND FUSICOCCIN ON GUARD CELL INTRACELLULAR pH. Elba E. Serrano[1], Victor Corvalan[2], and Steven H. Young[3], [1]Biology Dept., New Mexico State Univ., Las Cruces, NM 88003, [2]Brain Research Institute, [3]Physiology Dept., UCLA, Los Angeles, CA 90024.

Guard cells respond to various stimuli with changes in turgor pressure associated with ion movement. Previous studies provide evidence that the transduction of signals such as red and blue light, and fusicoccin, involves proton transport across the plasmalemma. The aim of this study was to determine whether red light and fusicoccin cause changes in guard cell intracellular pH. *Vicia faba* guard cell protoplasts were loaded by incubation in the membrane permeant acetoxymethyl form of the pH indicator dye, BCECF (BCECF-AM; 20 μM). When BCECF fluorescence emission is measured at 535 nm, the excitation efficiency of 495 nm is enhanced by increasing pH. Fluorescence measurements were taken at 3-10 minute intervals with either a SIT camera in conjunction with a video-digital imaging system, or a photomultiplier tube. Changes in intracellular pH were expressed as fluorescence ratios obtained from excitation at 495 and 440 nm. In control experiments, we did not detect changes in fluorescence ratios when dark-adapted cells were illuminated at these two wavelengths. Stimulation with continuous 1 mmol $m^{-2}s^{-1}$ red light caused negligible change in fluorescence, and hence intracellular pH. In contrast, application of the proton pump agonist, fusicoccin (20 μM), led to increased fluorescence that reached a maximum after 40-50 min. The protonophore, CCCP (10 μM), reduced fluorescence within 5-15 min. Confocal microscopy of loaded protoplasts showed that dye fluorescence was emitted from the cytoplasmic compartment. We interpret the results obtained in this study as indicating that fusicoccin increases guard cell intracellular pH, most likely by stimulation of proton pumping. These results support the hypothesis that signal transduction events involving proton transport can affect the intracellular pH of guard cells.

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1064 PLANT GENES ENCODING HOMOLOGS OF THE YEAST SNF1 KINASE. Martine Thomas, Laurence LeGuen, Michele Bianchi, Nigel Halford* and Martin Kreis.
Université de Paris XI, Biologie du Développement des Plantes, Bât. 430, 91405 Orsay Cedex, France.; (*) Long Ashton Research Station, Bristol University, UK.

We have isolated an *Arabidopsis thaliana* gene coding for a putative protein kinase using a cDNA previously shown to complement the snf1 mutation in yeast. The encoded protein is highly homologous to the SNF1 Ser/Thr kinase of *S cerevisiae*. SNF1 is needed for the release from carbon catabolite repression of several genes including Suc2 encoding invertase.

Data on the structure and expression of the *Arabidopsis* gene, and of its homolog in barley will be presented.

(This project is partially financed by the MRT)

1065 EXPRESSION IN *E. COLI* OF THE FULL LENGTH cDNA ENCODING SOYBEAN CALCIUM-DEPENDENT PROTEIN KINASE Alice C. Harmon and Byung-Chun Yoo. University of Florida, Dept. of Botany, Gainesville, FL 32611

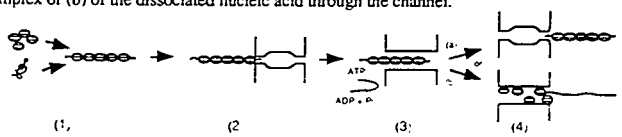
Soybean calcium-dependent protein kinase (CDPK) is the prototype for a new class of calcium-regulated protein kinases having a regulatory domain that is 40% identical to plant calmodulin (Harper, J.F. , G. Eric Schaller, M.R. Sussman, C L. Putnam-Evans, H. Charbonneau, and A.C. Harmon. 1991. Science *in press*) The full length cDNA encoding the SK5 CDPK has been expressed under control of the T7 promoter in *E. coli*. The expressed protein has a molecular weight estimated from SDS polyacrylamide gels to be about 56 kDa which agrees well with the predicted value of 57,175. The expressed protein crossreacts with antibodies directed toward pure soybean CDPK. In addition, the expressed SK5 CDPK phosphorylates histone IIIS in a calcium-dependent manner as does the native enzyme.

1066 DIFFERENTIAL ACCUMULATION OF TRANSCRIPTS ENCODING PROTEIN KINASE HOMOLOGS IN GREENING PEA SEEDLINGS. Xia Lin, Xin-Hua Feng, and John C. Watson. University of Maryland, Department of Botany and Center for Agricultural Biotechnology, College Park, MD 20742-5815.

As a first step toward understanding the role protein kinases may play in photoregulated leaf development, we have examined transcripts encoding putative protein kinases in the garden pea. Degenerate oligonucleotides, corresponding to conserved regions within the catalytic domain of known protein-serine/threonine kinases, were used as primers for the polymerase chain reaction to amplify cDNA synthesized from poly(A)+ RNA purified from the apical buds of 7 d-old pea seedlings. Five partial cDNAs were obtained and designated PsPK1 through PsPK5 in order of decreasing length. The deduced amino acid sequences show that each member of the PsPK series is different in length, and, although their sequences are quite similar overall, each has a unique sequence. Moreover, each member of the PsPK series has structural features typical of members of the protein-serine/threonine kinase family. All are equally similar to cyclic nucleotide-dependent protein kinase and protein kinase C, suggesting that the pea homologs may be involved in signal transduction. DNA gel blots show that each PsPK cDNA is likely to be encoded by a single gene within the pea genome. RNA blot analyses show that the PsPK transcripts accumulate differentially during greening of etiolated seedlings. PsPK3 and PsPK5 transcripts show a large and rapid decline during de-etiolation. In contrast, the level of PsPK4 RNA increases steadily during de-etiolation whereas PsPK1 and PsPK2 transcripts show little change during the greening period. The differential alterations in transcript abundance we observe may reflect changes in the protein kinase forms present during photoregulated leaf development in the garden pea

1067 HOW DO NUCLEIC ACIDS MOVE ACROSS MEMBRANE CHANNELS? Vitaly Citovsky, Mei Lie Wong, David Knorr and Patricia Zambryski.
Department of Plant Biology, University of California, Berkeley, CA 94720

We study transmembrane transport of nucleic acids in plants. Cell-to-cell movement of plant viruses is used as an experimental system for transport of nucleic acids across intercellular connections, the plasmodesmata. Intracellular transport of nucleic acids through nuclear pores is studied using the example of T-DNA transfer from *Agrobacterium*. In both cases, single strand (ss) nucleic acid binding proteins have been found involved in the transport process. Cell-to-cell movement proteins of two different plant viruses (an ssRNA virus, TMV; and a dsDNA pararetrovirus, CaMV) were shown to cooperatively coat ssDNA and RNA molecules to produce unfolded and thin (less than 2 nm in diameter) protein-nucleic acid complexes. Detailed analyses of protein domains involved in nucleic acid binding will be presented. Similar thin and unfolded complexes were observed following interaction of VirE2, a plant inducible ssDNA binding protein of *Agrobacterium*, with ssDNA molecules. Based on these findings, we propose that specific transport proteins function as molecular chaperones for nucleic acids in transit. A model for nucleic acid transport across membrane channels is proposed: (1) binding of transport protein to nucleic acid to form an unfolded transport complex; (2) transport protein-mediated targeting of the complex to membrane channel; (3) transport protein-induced increase in channel permeability; (4) translocation of (a) the entire complex or (b) of the dissociated nucleic acid through the channel.



1068 A PLANT PROTEIN KINASE WITH PROPERTIES SIMILAR TO MAMMALIAN CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II. Z Q. Wang, A.S.N Reddy[1], A. Bhatia[1], S Iyer[2], G. An[2], A J Czernik[2] & B W Poovaiah. Lab of Plant Mol. Biol. and Physiol. Dept. of Horticulture, [1]Institute of Biological Chemistry, Washington State University, Pullman, WA 99164; [2]Lab of Mol and Cell Neurosci, The Rockefeller University, NY 10021

The presence of multifunctional calcium/calmodulin-dependent protein kinase (CaM KII) in plants was investigated using i) immunoblotting with an affinity purified anti-peptide antibody produced against α-subunit of rat brain CaM KII and ii) specific substrates for CaM KII. The CaM KII antibodies recognized a 56 kDa protein in soluble proteins of corn roots. Immunoblots with soluble extracts from different plants and tissues detected one or two bands of similar molecular weight (56-58 kDa) Two other anti-peptide antibodies raised against CaM KII also recognized these same proteins. The molecular weight of the plant proteins are similar to the mammalian CaM KII. *In vitro* phosphorylation and immunoprecipitation studies indicate that the protein recognized by Cam KII antibody is phosphorylated in a calcium/calmodulin-dependent manner. Soluble extract from corn roots phosphorylated synapsin I, a physiological substrate for mammalian calcium/calmodulin-dependent protein kinases, and this phosphorylation of synapsin I was stimulated by calcium and calmodulin. One dimensional phosphopeptide mapping revealed that the site of enhanced phosphorylation of synapsin I was located with in the 30 kDa fragment that is the known site of phosphorylation by CaM KII. BB40, a specific peptide substrate corresponding to residues 281-291 of the α-subunit of CaM KII, was used to detect the CaM KII like kinase activity in plants. Corn root soluble extract phosphorylated BB40 in a calcium/calmodulin dependent manner. These results suggest that plants contain a homolog of multifunctional CaM KII We have isolated several cDNA clones from *Arabidopsis* and from corn by screening the expression libraries with the antibody raised against CaM KII These cDNAs are being characterized

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1069 DEVELOPMENTAL REGULATION OF CALMODULIN GENE EXPRESSION Y. J. Choi[1], G. An[1], G. M. Chowrira[2], T.-J Han[2], and B W Poovaiah[2]. [1]Institute of Biological Chemistry, [2]Lab of Plant Mol Biol. and Physiol, Dept of Horticulture, Washington State University, Pullman, WA 99164

Calmodulin, a ubiquitous calcium-binding protein, plays a central role in signal transduction. In our laboratory we are studying calmodulin gene structure and regulation of its expression We have cloned and sequenced a potato calmodulin cDNA (pPCM-1) and shown that the expression of calmodulin is developmentally regulated (Jena, Reddy and Poovaiah, Proc. Natl. Acad. Sci., Vol. 86, pp. 3644-3648, 1989). Using pPCM-1 as a probe, we have isolated several genomic clones from rice and potato. There are at least four calmodulin genes in rice and two of them have been sequenced. The genes consist of two exons and one intron at 25th amino acid from the NH_2 terminus. Deduced amino acid sequences are highly conserved between different calmodulins from plants. Our results indicate that potato also contain multiple genes of calmodulin. It is not known whether all or any one of these calmodulin genes are expressed preferentially in a tissue. We have obtained three different genomic clones of calmodulin from potato and they are being characterized. Using gene specific probes, we are investigating the developmental regulation of calmodulin gene expression in different tissues of potato plants at various stages of development. We are characterizing transgenic plants containing the calmodulin promoters fused to a reporter gene to study how the expression of the calmodulin gene is regulated by various factors

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1070 ANALYSIS OF A SITE-DIRECTED CALMODULIN MUTANT DEFECTIVE IN METHYLATION IN TRANSGENIC PLANTS. D.M. Roberts, S.-H. Oh, L. Besl, R. Masterson and G. Stacey, Center for Legume Research, Depts. Biochem. and Microbiol., Univ. of Tenn., Knoxville, TN 37996

In order to understand the function of plant calmodulin and calmodulin methylation in vivo, we produced transgenic tobacco plants transformed with a synthetic calmodulin gene (VU-1) and a site-specific mutant of this gene (VU-3, lys to arg-115) under the control of the 35S promoter. VU-3 was selected since it is resistant to posttranslational lysine methylation and thus activates NAD kinase to a higher level than endogenous plant calmodulin. The VU-1 and VU-3 proteins are stably expressed in F1 tobacco plants and accumulate to levels equal to those of the endogenous calmodulin. VU-3 calmodulin purified from transgenic plants retains its altered regulatory properties (i.e., high NAD kinase activation), while VU-1 calmodulin was methylated and was identical to the endogenous plant calmodulin in its regulatory properties. VU-1 transformed plants were similar to untransformed tobacco in most respects. However, all lines of VU-3 plants exhibited clear differences in growth, including shorter stem internode lengths, reduced seed and pollen viability, and reduced seed yields. Since these changes are only observed in the VU-3 plants, it suggests that they result from the expression of the mutant calmodulin and not from a change in calmodulin levels.

1071 THE EFFECT OF ENVIRONMENTAL NITRATE ON GENE EXPRESSION IN MAIZE ROOTS. M.G. Redinbaugh[1], J.M. Vyba[1] and W.H. Campbell[2]. [1]Crop Science Dept. North Carolina State University and USDA, ARS, Oxford, NC 27565; [2]Phytotechnology Res. Center and Dept. Biological Science, Michigan Tech. Univ., Houghton, MI 49931.

In the higher plants, nitrate reductase (NR) activity, apoprotein and transcript accumulate in response to environmental NO_3^-. Among the other physiological and biochemical responses of higher plants to NO_3^- are the induction of nitrite reductase and nitrate uptake activities, as well as localized changes in root morphology and respiration. In an effort to begin to delineate the effect of external nitrate on maize root gene expression, we examined NO_3^- induced changes in maize root gene expression with a cDNA probe to maize leaf NADH: NR. The probe did not hybridize with total RNA isolated from the roots of plants grown hydroponically on 0.1 mM $CaSO_4$, or on a nutrient solution lacking NO_3^-. The NR mRNA levels increased significantly when the hydroponically grown plants were subsequently treated with 10 mM KNO_3. Root NR mRNA levels were also induced by exposure of the plants to low concentrations of KNO_3 (10 µM to 1 mM). A 1 h pre-treatment with 50 µg/ml cycloheximide prior to KNO_3 exposure did not prevent the increase in NR mRNA levels. Thus, the NR mRNA accumulates in maize roots in response to low external concentrations of nitrate. The increase in transcript level is independent of the ion content of the hydroponic solution and is not dependent on cytoplasmic protein synthesis. We are in the process of identifying other transcripts in maize roots which resond to NO_3^- and cycloheximide in a similar manner. Supported by USDA Grant CRCR-9002408.

1072 PURIFICATION AND FURTHER CHARACTERIZATION OF AN AUXIN-STIMULATED NADH ACTIVITY OF SOYBEAN PLASMA MEMBRANES A. O. Brightman, F. L. Crane and D. J. Morre. Dept. Medicinal Chemistry and Dept. Biological Sciences, Purdue University, West Lafayette, IN 47907.

An auxin-responsive NADH oxidase is present in plasma membranes isolated from etiolated hypocotyls by aqueous two-phase partition. the activity was purified by sequential separations by ion exchange and size exclusion chromatography followed by native gell electrophoresis. The activity was stabilized during purification by the inclusion of lysophosphatidyl choline in the isolation buffers. Both the 36 kDa and the 72 kDa were purified to near homogeneity and are in the process of being sequenced. The identity of the 72 kDa protein as the catalytic subunit of the oxidase is based on analogy with the enzyme purified from rat liver and an activity stain developed to show NADH oxidase activity in non-denaturing gel electrophoresis. The relationship of the previously reported 52 kDa peptide that copurified with the NADH oxidase complex remains to be determined. The possibility remains that the catalytic portion of the oxidase is part of a larger complex in the membrane and that associated peptides are involved in regulation of the activity of the enzyme.

1073 IS THE CELL SKELETON ONE MORE MESSENGER SYSTEM ? Boris V. Sorochinsky. Institute of Cell Biology and Genetic Engineering. Ukrainian Acad. Sci. Lebedeva str. 1. Kiev-143. 252143. USSR

Cytoskeleton is the central element of the cellular organization. Cytoskeleton's structures provide for physical contact between different cell compartments and take part in the regulation of most important biochemical processes within a cell. Polymerization-depolymerization transitions of the cytoskeleton structures are effected in cells with many chemical factors and could be considerably modificated under the influence of some intracellular factors. It's known that processing with many biological active compounds including herbicides, hormones, alcaloides has a marked effects on the cytoskeleton system both in vivo and in vitro. We have shown in particular that such plant hormones as kinetin, heteroauxin and gibberellic acid can modified the microtubules assembly in vitro. It may be assumed that polymerization - depolymeryzation transitions in the cellular skeleton whole ansamble and loca. Reorganizations the fine cytoskeleton structure can play the role of a signal transmission way from intracellular ligands to the different cellular compartments. The identified principle of signal transmission consist of using the secondary mediators and enzyme induction; the function of cytoskeleton alterations is supposed to be a certain type of messenger system. Cooperative transitions of the protein molecules ansamble are the main principle of the cytoskeleton components function as a mediator and the whole cytoskeleton system may be determined as the structurated messenger.

1074 CHARACTERISATION OF PLANT ANNEXIN GENE EXPRESSION Usha Chaudhari, Margaret Smallwood, Sarah Gurr, Michael McPherson and Dianna Bowles

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Annexins are a family of well characterised proteins from animal cells, that share in common a calcium-dependent affinity for acidic phospholipids. Calcium is known to modulate a wide range of metabolic and developmental events in higher plants. This central role of Ca^{2+} in signal transduction led us to examine whether annexins also exist in higher plants. Two polypeptides (P34 and P35) were isolated from tomato suspension culture cells, which cross-reacted with antisera raised against animal annexins and bound to acidic phospholipids in a Ca^{2+} - dependent manner. Peptide sequence analysis of the two proteins revealed extensive homology with the conserved repeat region found in all annexins to date, although there were some significant differences. Using this information, amplimers for PCR were designed, and a fragment of anticipated size (96 bp) was amplified from tomato genomic DNA, and sequenced to confirm its authentic identity. This has been used as a probe in Northern analysis of root, stem and leaf tissue from tomato, potato and barley plants. In parallel, the same tissues were subjected to the annexin-isolation procedure and the products analysed on SDS/PAGE and immunoblots with antisera raised against P34. These studies revealed a differential pattern of plant annexin expression in the tissues studied.
Currently PCR is being used to generate tomato annexin gene sequences 5' and 3' of the centrally-located 96 bp fragment. This will provide the foundation for antisense studies and mutational analysis of plant annexin function.

1075 IN VITRO REGULATION OF PROTEIN PHOSPHORYLATION IN SOLUBLE PROTEIN EXTRACTS OF MAIZE COLEOPTILES. Brenda J. Biermann, Lily I. Pao, and Lewis J. Feldman. University of California, Department of Plant Biology, Berkeley, CA 94720.

Our objective is to elucidate the molecular details of second messenger-dependent signal transduction involving plant protein kinases, using maize root tropic responses as a model system. As a first step, we cloned and sequenced a maize root cDNA encoding a protein highly homologous to the conserved catalytic domain of mammalian and microbial second messenger-dependent protein kinases. Antiserum obtained from rabbits injected with the denatured protein, overexpressed in E. coli, immunoprecipitated two proteins from crude plant lysates which could be detected on immunoblots. Immune complexes did not phosphorylate exogenous histones under a variety of assay conditions, and were only weakly autophosphorylated when labelled with 32P ATP. In a complementary approach, we are determining what messengers regulate protein phosphorylation in soluble protein extracts of maize coleoptiles, by in vitro labelling with 32P ATP, then electrophoresing on denaturing polyacrylamide gels. Chromatography of protein extracts on a desalting column of Sephadex G200 removed an inhibitory factor, resulting in greatly enhanced protein kinase activity in pooled protein-containing fractions. Addition of 1/1000 volume of crude protein extract to G200-purified proteins reduced protein phosphorylation to a low level equivalent to that in the crude lysate, except for an 80 kDa protein which was detectably phosphorylated only upon addition of the crude lysate. Calcium-regulated phosphorylation of several peptides, some of which exhibited a gel mobility shift in the presence of calcium could be observed in G200-purified proteins.

POSTER ABSTRACTS

1076 IN VITRO PHOSPHORYLATION OF IMMUNOPRECIPITATED MAIZE PHYTOCHROME. Brenda J. Biermann, Lily I. Pao, and Lewis J. Feldman. University of California, Department of Plant Biology, Berkeley, CA 94720.

We are interested in studying the biochemistry of signal transduction in maize roots, particularly pathway steps which involve protein kinase activation. In maize variety Merit light is required for root curvature in response to gravitropic stimuli. Effects of different light wavelengths and fluences show that the light requirement is solely mediated by phytochrome (Feldman and Briggs, 1987. Plant Physiol. 83:241-243). The root cap, the site of both gravity and light perception, expresses the phytochrome message at a high level (Johnson et al., 1991. Plant Physiol. 95:544-550).

Protein kinase activity has been found to co-purify with phytochrome, but not to reside in the phytochrome molecule itself (Grimm et al. 1989, Planta 178:199-206; Kim et al., 1989. Photochem. and Photobiol. 49:319-323; Wong et al., 1989. Plant Physiol. 91:709 718), suggesting that phytochrome effects may be transduced or modulated by protein phosphorylation. We found that phytochrome is phosphorylated in vitro in washed immunoprecipitates from etiolated or illuminated (saturating red) maize coleoptiles, supporting the view that the protein kinase is bound to the phytochrome. Various types of histones added to immune complexes formed with phytochrome antisera were not significantly phosphorylated, indicating that the protein phosphorylation activity may be substrate-specific.

1077 LOW LEVELS OF ATMOSPHERIC METHYL JASMONATE INDUCE THE EXPRESSION OF SOYBEAN VEGETATIVE STORAGE PROTEINS, A VACUOLAR LIPOXYGENASE, AND ANTHOCYANINS. Howard D Grimes, Vincent R. Franceschi, and Timothy J. Tranbarger. Department of Botany, Washington State University, Pullman, WA 99164-4238.

Soybean seedlings were exposed to low levels of atmospheric methyl jasmonate (MJ) to determine if low levels of this compound could regulate the expression and accumulation of specific proteins, the vegetative storage proteins (VSPs), that are involved in nitrogen metabolism. Atmospheric MJ (maximum concentration approx. 10^{-7} M) induced the accumulation of three VSPs with molecular weight of 27, 29, and 94 (vsp27, vsp29, and vsp94, respectively). At this level of MJ exposure, there were no visible indications of senescence effects. Furthermore, there was an organ-specific pattern of the accumulation of these different VSPs. Vsp27 preferentially accumulated in shoot tips and primary leaves, while vsp29 preferentially accumulated in the cotyledons. Vsp94 accumulated uniformly in all above-ground organs. We now have strong evidence that vsp94 is actually a lipoxygenase (see abst. by Tranbarger, Franceschi, Hildebrand, and Grimes) that is associated with the paraveinal mesophyll layer. One product of 18:3 metabolism by lipoxygenase is methyl jasmonate and thus, a role in signal transduction is implicated. In addition to the effects of MJ on the VSPs, MJ also induced the biosynthesis of anthocyanins in light-grown seedlings. From these experiments, it is concluded that MJ (or, more generally, the jasmonates) may play an important role as either a gaseous hormone or an intracellular messenger that is involved in the regulation of nitrogen partitioning in the soybean. The organ-specific differential accumulation may reflect changes in the pattern of nitrogen partitioning between various compounds and/or organs. We are currently examining the cellular and subcellular localization of vsp94 as well as examining its molecular regulation at the mRNA level.

1078 **Immunophilins/peptidyl-prolyl *cis-trans* isomerases of higher plants.** Charles S. Gasser, Rachanee Jampates-Beale, Irene Chou, Steven M. Theg*, Sydney V. Scott*, and Veronica Lippuner. Departments of Biochemistry and Biophysics, and *Botany, U. C. Davis, Davis, CA 95616. Immunophilins are a class of ubiquitous proteins first identified in mammals as the specific targets of T-cell active immunosuppressive drugs. The mode of action of these drugs indicates that the immunophilins participate in transduction of activating signals in the induction of T-cell differentiation and proliferation. Immunophilins have additionally been shown to have peptidyl-prolyl *cis-trans* isomerase (rotamase) activity. This activity is necessary for the efficient folding of many proteins *in vitro*, and may be essential for this and other processes involving conformational alterations of proteins *in vivo*. We are studying the biochemistry and molecular biology of the immunophilins of higher plants. Initial work indicates that immunophilins are expressed in all organs of higher plants. Sequence analysis of immunophilin cDNA clones from maize, tomato, *Brassica napus*, and *Arabidopsis thaliana* demonstrate that the plant proteins are highly conserved relative to their mammalian counterparts. Genomic blotting experiments and the isolation of different classes of immunophilin clones, indicate that immunophilins are encoded by a small family of genes in *Arabidopsis thaliana*. Expression of plant immunophilins in *E. coli* has allowed us to demonstrate that these proteins have the expected rotamase activity and may therefore participate in protein folding events in plants. Studies on the structure of the gene family, the patterns of expression, and the properties of the rotamase activity of these proteins will be presented.

1079 PURIFIED CALCIUM CHANNEL BLOCKER BINDING PROTEIN FROM CARROT CELLS FORMS CA^{2+}-PERMEABLE IONS CHANNELS.

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Ca^{2+}-channels have been suggested to play an important role in signal transduction processes of higher plant cells. Recently, from membranes of carrot cell-suspension cultures, we have identified a 75-kDa polypeptide which binds calcium channel blockers of the phenylalkylamine family. The protein was purified by lectin-affinity and ion exchange chromatographies and was incorporated into phosphatidylcholine vesicles. Subsequently the small vesicles were transformed into giant liposomes by the dehydration-rehydration technique. Single-channel patch-clamp studies on these proteoliposomes show Ca^{2+}-channel activities which were not found in control experiments. One single channel conductance state was in the range of 30 pS in asymmetrical solutions with a ten-fold gradient of 90 mM to 9 mM $CaCl_2$ or calcium glutamate. The currents are inhibited by 20 µM of the calcium channel antagonist, bepridil. Therefore, it appears that the 75-kDa binding polypeptide can function as a component of a higher plant calcium channel.

1080 ISOLATION AND MOLECULAR ANALYSIS OF SMALL GTP-BINDING PROTEINS OF ARABIDOPSIS THALIANA

M. Matsui[1], T. Anai[1], K. Hasegawa[2], Y. Watanabe[3], H. Uchimiya[4] and R. Ishizaki[1] [1] Molecular Biology Laboratory, Nippon Medical School, Nakahara-k Kawasaki, 211 [2] Faculty of Science and College of Liberal-art Kagoshima University, Korimoto Kagoshima 890 [3]Department of Bioscience Teikyo University, Toyosato-Dai, Utsunomiya, 320 [4]Institute of Applied Microbiology University of Tokyo Bunkyo-Ku, Tokyo, 113 (Japan)

Many small GTP-binding proteins (small G proteins) have been identified in various species These molecules are thought to be involved in signal transduction pathways in eucaryotic cells We previously isolated a member of this small G protein gene from the genome of an Arabidopsis thaliana (A. thaliana) and named it ara (Matsui et al., gene,1989) Small G protein genes make up a supergene family in mammalian cells and they are localized in different intra-cellular membranes The amino acid sequences of their products are conserved in four regions, which are now known to be important for binding and hydrolysis of the GTP moiety Small G proteins can be classified into three sub-family, ras, rho and YPT by differences around amino acid residue 35 which is thought to be important for the binding of proteins regulating GTPase activity(GAP) and transducing this activity to subsequent unidentified molecules The structure of ara is most similar to that of YPT of Saccharomyces cerevisiae To examined whether there is also an ara gene family that functions in signal transduction pathways, we cloned ara-related sequences from a cDNA library of A. thaliana. The products of these genes, ara-2, ara-3, ara-4 and ara-5 showed conservation of amino acid residues in four regions which are conserved in all small G proteins These products were especially highly homologous with those of the YPT genes in the regions around amino acid residue 35 The products of these four genes showed charactenstic features at their C termini and end with Cys-Cys-X-X Another charactenstic of this family is serine in place of glycine in the first conserved region(Gly-12 of mammalian ras) To examine the function of these proteins these genes were expressed in yeast cells with YPT mutation When ara-5 gene was expressed transformed cells stopped growing This meaning is unclear About the behavior of transformants of another genes are now examining

1081 CHARACTERIZATION OF AN *ARABIDOPSIS* MUTANT HAVING DECREASED SENSITIVITY TO METHYL JASMONATE.

Paul E. Staswick[a], Wenpei Su,[b] Stephen H. Howell[b].

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Jasmonic acid (JA) and its methyl ester (MeJA) are newly recognized plant signalling molecules. Primary root growth of wild type *Arabidopsis thaliana* (ecotype Columbia) seedlings was inhibited 50% when seedlings were grown on agar medium containing 10 µM MeJA. An ethyl methane sulfonate mutant with decreased sensitivity to methyl jasmonate-inhibited root elongation was isolated and characterized. Roots of the mutant were about 10-fold longer after 10 days of growth on 100 µM MeJA. In the absence of MeJA, mutant plants were somewhat stunted in growth. MeJA-induced polypeptides were detected in *Arabidopsis* leaves using antiserum to a MeJA-inducible vegetative storage protein from soybean. Induction of the crossreacting proteins by MeJA was greatly diminished in the mutant, compared to wild type. In contrast, the mutant was more sensitive to ABA inhibition of germination than was wild type. These results suggest the mutation is part of a general MeJA signal transduction pathway, which is active throughout the plant and which may influence the expression of many different genes.

POSTER ABSTRACTS

1082 IS THE PHOSPHORYLATION POSSIBLE MECHANISM OF CYTOSKELETON REORGANIZATION IN PLANT CELLS? Ya. B. Blume, B. V. Mikicey, V. G. Solodushko, N. N. Ostapets. Inst. Cell Biol. & Genet. Eng., Kiev-143, 252143, USSR

The regulatory mechanisms that govern assembly-disassembly of basic components of plant cytoskeleton (microtubules, microfilaments and intermediate filaments) are obscure. As phosphorylation-dephosphorylation is involved in control of structure animal cytoskeleton, it is possible that assembly-disassembly of the structures of plant cytoskeleton might be regulated in a similar manner. As a first step towards defining this mechanism, we carried out an analysis of phosphorylation of tubulin and actin, using the permeabilized protoplasts of higher plants. After preloading with [γ-^{32}P]-ATP they were incubated with Mg^{2+}, Ca^{2+} and with activators of various protein kinases (cAMP, 1,2-diacylglycerol, calmodulin) in different combinations. The cytoplasmic proteins from the samples were resolved by SDS-PAAG electrophoresis, dried, autoradiographied and scanned by densitometry. It has been shown that α- and β-tubulins as well as actin were effectively phosphorylated during activation of Ca^{2+}-dependent, Ca^{2+}-calmodulin-dependent protein kinases and protein kinase C. In parallel we found that monoclonal antibodies MPM-2 (from Dr. P. N. Rao, Houston) not only react specifically with phosphoproteins of nuclear envelope (lamins), but recognize intermediate filaments in cytoplasm of protoplasts. This reaction was inhibited by antagonists of Ca^{2+}. Our results indicate that reversible phosphorylation of main proteins of plant cytoskeleton may be involved in mediation of its dynamic structure.

1083 ANALYSIS OF MECHANISMS OF TRANSCRIPTIONAL SUPPRESSION OF GENES FOR PHOTOSYNTHESIS IN *ARABIDOPSIS THALIANA*. Hirokazu Kobayashi and Kyoko Kobayashi. Laboratory of Plant Cell Technology, School of Food and Nutritional Sciences, University of Shizuoka, 395 Yada, Shizuoka 422, Japan

We have hypothesized the involvement of DNA methylation in reduction of the transcriptional template activity of both nuclear and plastid genes for photosynthesis in some nongreen cells (J. Ngemprasirtsiri *et al., Proc. Natl. Acad. Sci. USA*, 86, 7919-7923, 1989; H. Kobayashi *et al., EMBO J.*, 9, 307-313, 1990). To thoroughly investigate mechanisms underlying the suppression, we employed *Arabidopsis thaliana* which is most suitable for the analysis of genomic DNA without cloning due to its small size and for handling in the aspect of molecular genetics. The methylation status of DNA prepared from nonphotosynthetic organs such as roots and seeds and heterotrophically-grown calli was analyzed in comparison with that of green leaves and stems. 5-methylcytosine was unexpectedly detected in several photosynthesis genes encoded in chloroplast DNAs from the leaves and stems by digestion with methyl-sensitive and -insensitive isoschizomeric endonucleases and subsequent Southern hybridization. The nonessentiality of DNA methylation at sites recognized by the endonucleases to gene expression prompted us to analyze the methylation in promoters and their upstream regions at nucleotide sequence levels by genomic sequencing and others. To further reveal the entire steps leading to the suppression of gene expression, we have focused on several members of nuclear genes for photosynthesis. Genes for reporters and drug resistance were placed downstream of DNA fragments composed of promoters and their upstream regions of the photosynthesis genes, and tried to be introduced into *A. thaliana*. The transgenic *A. thaliana* plants are mutagenized by ethylmethanesulfonate and mutants which express the genes in calli are screened.

1084 REGULATION OF THE *ARABIDOPSIS* NITRATE REDUCTASE GENE 1 (NR1) Gregoria Acedo[1], Mark A. Conkling[1], and Chi-Lien Cheng[2]. [1]Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695. [2]Departments of Botany and Biology, University of Iowa, Iowa City, Iowa 52242.

Arabidopsis has two nitrate reductase genes, NR1 and NR2. RFLP analysis showed that NR2 is the gene of locus *chl-3*. NR1 mapped at a locus that has not been identified genetically. Both of these genes are nitrate inducible, light inducible, and exhibit circadian rhythm. The expression patterns of the two genes are coordinate but not identical. We have fused the 5' flanking region of the NR1 gene to reporter gene CAT and transformed the construct into *Arabidopsis*. The transcriptional regulation of NR1 gene in response to these environmental and metabolic factors is examined.

1085 METABOLIC DEREPRESSION OF α-AMYLASE GENE EXPRESSION IN SUSPENSION-CULTURED CELLS OF RICE. Su-May Yu, Yen-Hong Kuo and Li-Fei Liu. Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, R.O.C. 11529

We presented evidence to show that the α-amylase gene family in rice is under two different modes of regulation: 1) hormonal regulation in germinating seeds, and 2) metabolic repression in cultured cells by the levels of available carbohydrate nutrients. The level of α-amylase mRNA in suspension-cultured cells is not affected by exogenous gibberellic acid (GA_3); instead, it is induced by the deprivation of carbohydrate nutrient. A lag period of 2-4 hr is required for the induction of α-amylase mRNA in sucrose-depleted medium. The induction of α-amylase expression is extraordinarily high and levels of α-amylase mRNA can be increased 8 to 30 folds after 24 hr of sucrose starvation. The synthesis and secretion of α-amylase is also dependent upon the level of carbon source. The derepression or repression of α-amylase synthesis can be readily reversed by the deprivation or replenishment of sucrose in the medium, respectively. Glucose and fructose exert a repression on the α-amylase synthesis similar to that of sucrose. A hypothesis that explains the induction of α-amylase synthesis by carbohydrate starvation is proposed. Our data have suggested a hitherto undiscovered, potentially important control mechanism of carbonydrate metabolism in plants.

1086 REGULATION OF PEA Cab GENE PROMOTERS IN TRANSGENIC Arabidopsis. Jie Gao, Kathleen A. Marrs and Lon S. Kaufman. University of Illinois at Chicago, Laboratory for Molecular Biology, Department of Biological Sciences, Chicago, Illinois 60680

Expression of the chlorophyll a/b binding protein(Cab) gene in Arabidopsis is regulated by excitation of a blue-light receptor. This phenomena is characterized by analyzing the steady-state level of Cab gene transcripts in 6-day-old red-light grown Arabidopsis seedlings which have been irradiated with a short pulse of blue-light. Cab gene transcripts increase with increasing fluences of blue-light where tested between 10^{-2} to 10^{4} $\mu mol \cdot m^{-2}s^{-1}$. The threshold for the response is below 10^{-2} $\mu mol \cdot m^{-2}s^{-1}$. Similar blue-light regulation of the Cab gene family has been detected in Pisum sativum where blue-light regulates the rate of transcription in the absence of cytoplasmic protein synthesis.

RNAase protection assays suggest that the pea Cab gene, pAB2 (Watson and Thompson, unpublished data) is blue-light responsive while pAB8 is not. In order to identify the cis-acting DNA element(s), the regions upstream from the translation initiation site of these two Cab genes were fused to the coding region of the ß-Glucuronidase(GUS) gene. These chimeric genes were transferred into Arabidopsis via Agrobacterium tumefaciens mediated transformation system. GUS activity is observed in calli, transgenic plants and F1 seedlings. The analysis of blue-light response of the chimeric genes is in progress.

1087 PURIFICATION OF FACTORS INVOLVED IN THE ACTIVATION OF CHALCONE SYNTHASE (CHS) TRANSCRIPTION IN CELL CULTURES OF PHASEOLUS VULGARIS (P.V.). L.M. Yu[1], R.A. Dixon[2] and C.J. Lamb[2]. [1] Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and [2]The Plant Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037.

Phenylpropanoid metabolism produces compounds necessary for plant development and defense. CHS is a transcriptionally regulated enzyme located at the base of a branch path, off the central phenylpropanoid pathway, whose end products are the (iso)flavonoids found in legumes such as P. v. A variety of treatments similarly activate the transcription of certain members of the P. v. CHS gene family. The 5' region of CHS15 contains cis elements necessary for transcriptional activation as it confers responsiveness of expression to a linked reporter gene in soybean cells. The nucleotide sequence $CCTACC(N)_7CT$ occurs three times in this region. The 15mer or its 5' most hexanucleotide is found in other transcriptionally activatable defense genes and has been functionally implicated in the expression of genes from snapdragon and parsley. We are purifying factors that bind to this sequence. The core 15mer was embedded in a ds28mer without other relevant sequences such as SBF-1 or G boxes and binding to cell extracts was analyzed by a gel retardation assay. Assay validity was established by the success and failure of certain mutations within the embedded 15mer to compete for binding in the presence of a large weight excess of poly(dI-dC)·poly(dI-dC). Purification schedules for several binding activities, similar in both elicited and control cultures, will be presented.

POSTER ABSTRACTS

1088 SALICYLIC ACID AS A NATURAL SIGNAL MOLECULE IN THE DISEASE DEFENSE RESPONSE IN *ARABIDOPSIS THALIANA*

D'Maris Dempsey, John R. Cutt, Anne Simon† and Daniel F. Klessig
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Salicylic acid (SA) treatment of tobacco is known to activate the expression of the pathogenesis-related (PR) genes and to enhance resistance to tobacco mosaic virus. Recently, our laboratory demonstrated that SA may function as an endogenous signal in the resistance response of tobacco to viral infection (Malamy *et al.*, 1990). Métraux *et al* (1990) have also shown that SA plays a role in the induction of systemic acquired resistance in cucumber after pathogen attack.

We are presently examining *Arabidopsis thaliana* (*A.t.*) to determine if SA also functions in this plant as a natural signal transduction molecule during pathogen attack. For some of these studies, we are utilizing turnip crinkle virus (TCV) which is a pathogen on *A t* (Li and Simon, 1990). Preliminary results indicate that TCV infection of the resistant *A t.* ecotype, Dijon, stimulates an increase in SA levels over that observed in uninfected plants. In contrast, TCV infection of the susceptible *A t* ecotype, Columbia, does not enhance SA levels. Moreover, TCV infection and treatment with exogenous SA induce defense-related chitinase activity in *A t* The finding that exogenous SA treatment stimulates chitinase activity and that TCV infection induces both SA and chitinase activity suggests a possible role for SA as a natural signal molecule in *A.t*

Malamy *et al.*, (1990) Science 250, 1002; Métraux *et al.*, (1990) Science 250, 1004; Li and Simon, (1990) Phytopathology 80, 238.

1089 **Salicylic Acid and Disease Defense Response in Tobacco.**

Jocelyn Malamy, Jacek Hennig and Daniel F. Klessig. Waksman Institute, Rutgers, The State University of New Jersey, P.O. Box 759, Piscataway, New Jersey 08855

Some cultivars of tobacco are resistant to tobacco mosaic virus (TMV) and synthesize pathogenesis-related (PR) proteins upon infection. Recently, we reported that a large rise in endogenous levels of salicylic acid (SA) is associated with the defense response (Malamy et al. 1990). This finding, together with the earlier demonstration that treatment with exogenous SA induces PR genes and resistance, suggests that SA functions as the natural transduction signal. To further elucidate SA's role, the tobacco-TMV interaction has been further characterized. In addition, the levels of endogenous SA following infection of tobacco with other pathogens or treatment with biotic or abiotic inducers of the PR genes has been analyzed.

In tobacco, resistance to TMV is temperature sensitive - at or above 32°C restriction of viral spread and necrotic lesion formation (the hypersensitive response-HR) is lost. At 32°C the levels of SA do not rise and HR does not occur, in accord with SA's presumptive role in establishment of resistance. However, upon shifting to 22°C endogenous SA levels rapidly increase approx. 100-fold. This rise is followed within a few hours by induction of PR genes and appearance of HR.

Many pathogens and eliciting compounds induce PR genes. Of the compounds tested, only xylanase (kindly provided by Dr. J. Anderson, USDA) reproducibly enhances endogenous SA levels In contrast, preliminary data suggest that several viral and fungal pathogens of tobacco induce SA.

Malamy et al., (1990) Science 250, 1002.

1090 **24- AND 58-kDa G-PROTEINS HAVE BEEN ISOLATED AND PURIFIED FROM Avena SEEDLINGS.**

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We have observed a red/far-red photoreversible modulation of GTP binding and GTPase activity in crude extract from etiolated oat seedlings. Also, cholera toxin up-regulated the expression of Cab gene, whereas it down-regulated the expression of phyA gene (Type A phytochrome gene) in etiolated oat seedling slices in the dark, thus simulating the phytochrome-mediated red light effects on the expression of these genes (L. Romero, D. Sommer, C. Gotor and P. Song, FEBS Lett.282:341-346, 1991). These results suggested that a G-protein(s) may be involved in the light signal transduction in etiolated oat seedlings. To find the possible coupling between the red light signal and its transduction cascade via a G-protein, we isolated and purified two GTP-binding proteins of apparent molecular masses at 24- and 58-kDa from etiolated oat seedlings. These proteins were extracted from the membrane fraction of 3.5-day old etiolated oat seedlings. Following detergent solubilization, the proteins were purified by ion-exchange chromatography, GTP-agarose affinity chromatography and gel filtration. Three fractions possessing GTP-binding activity were obtained. Two of the fractions were nearly homogeneous, as determined by SDS-PAGE, with bands at about 58- and 20-kDa. The third fraction contained two bands at 23- and 24-kDa. We have further purified 24- and 58-kDa proteins from these fractions. The purified proteins were nearly homogeneous on SDS-PAGE (Coomassie blue and silver staining). Initial characterization of these proteins will be discussed.

1091 PRODUCTION OF HIGH LEVELS OF SEROTONIN IN PEGANUM CULTURES BY EXPRESSION OF A FOREIGN PLANT TRYPTOPHAN DECARBOXYLASE.

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Cell suspension cultures of *Peganum harmala* usually produce low levels of serotonin and ß-carboline alkaloids and may loose completely this initial ability. In the case of serotonin it has been shown that the lack of tryptophan decarboxylase activity (TDC) is the reason for the blockade of serotonin biosynthesis. Serotonin biosynthesis was thus restored by selection for cells expressing TDC-activity (1), but should also be repairable by expression of a foreign TDC in *Peganum* cells. A tryptophan decarboxylase gene (tdc) was cloned from *Catharanthus roseus* and integrated into the binary vector Bin19 under the control of the CaMV35S-promoter. *Agrobacterium tumefaciens LBA4404* and *C58C1pRiA4b* were used as acceptor strains. Several kanamycin-tolerant hairy root cultures were obtained expressing on the average 250 pkat TDC/mg protein during the growth period, while con-trols contained 30-fold lower activities. The root cells expressing high TDC-activity accumulated 4-6% serotonin on dry mass basis, while controls contained 0.5-1%. Similar large increases were found for transformed sus-pension cultures with TDC-activities up to 300 pkat/mg protein and 1-3% serotonin. As no tryptamine accumulated in the transformed cells, the over-synthesized tryptamine was immediately used for serotonin biosynthesis.

(1) J.Plant Physiol. 131: 225-236 (1987)

1092 EXPRESSION OF A BACTERIAL LYSINE DECARBOXYLASE GENE IN TOBACCO.

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The aim of the project is to affect metabolic pathways by the integration of foreign gene products into plant-owned biosyntheses. Here we want to study the biochemical consequences of expressing a bacterial lysine decarboxylase gene (ldc, in tobacco cells. The ldc of Hafnia alvei has been cloned into various Ti-plasmid derived plant cell expression vectors. When the gene was under the control of the promoter of rbcSl from potato fused to the coding region of the rbcS transit peptide, lysine decarboxylase activity (LDC) was detected in the chloroplasts of transgenic tobacco plants. The biochemical consequence was the accumulation of high levels of cadaverine which was, however, not channeled into derived pathways. For expression of the ldc in the cytoplasm it was brought under the control of the bidirectional Tr-promoter. Neither LDC-activity nor enhanced cadaverine levels were found in these transgenic plants despite the presence of distinct levels of LDC-mRNA. The ldc has also been cloned under the control of the CaMV35S-promoter with and without the rbcS signal sequence for transport into chloroplasts/leucoplasts or cytoplasmic expression. Data of the characterization of hairy root and suspension cultures as well as of plants derived from transformations with these constructs will be presented with respect to LDC-activity, cadaverine levels and effects on nicotine and cinnamoylputrescines biosyntnesis both of which are able to use cadaverine as substrate instead of putrescine.

1093 TARGETS FOR TRANS-ACTIVATION BY MAIZE R AND C PROTEINS ARE CONSERVED IN OTHER MONOCOTS.

Ben Bowen[1], Jeff Bennetzen[2], Chifumi Nagai[3], Sam Sun[4], and Brad Roth[1]. [1]Pioneer Hi-Bred, P.O. Box 38, Johnston, IA 50131; [2]Purdue Univ., W. Lafeyette, IN 47907; [3]HSPA, Aiea, HI 96701; [4]Univ. of Hawaii, Honolulu, HI 96822.

Anthocyanin biosynthetic genes in maize are coordinately regulated by R and C proteins, which share homology with the helix-loop-helix and myb families of transcriptional activators, respectively. Genes encoding R and/or C can be used as markers for optimizing particle gun mediated DNA delivery in a variety of maize cells. We have now found that R and C genes fused to regulatory sequences which have been optimized for expression in maize can induce anthocyanin biosynthetic genes in at least ten graminaceous and two liliaceous monocots. As in maize, both R and C are required for trans-activation in each species tested; neither R nor C alone is sufficient. This suggests that R and C proteins may form a complex which recognizes target sites that are highly conserved amongst monocots. Currently, we are testing whether promoters from maize anthocyanin biosynthetic genes can act as targets for heterologous trans-activators in those monocots in which maize R and C proteins have been shown to function.

POSTER ABSTRACTS

1094 GLYPHOSATE-TOLERANT EPSP SYNTHASES. ENZYMOLOGY AND USE IN THE DEVELOPMENT OF GLYPHOSATE-TOLERANT CROPS. Stephen R Padgette, Diane Biest Re, David A. Eichholtz, Xavier Delannay, Charles S Gasser, William Stallings, and Ganesh M. Kishore. Monsanto Agricultural Company, Chesterfield, MO 63198.

Roundup® is a broad spectrum, post-emergent herbicide, widely used for weed control. Its active ingredient, glyphosate (N-phosphonomethylglycine), is practically nontoxic to mammalian, aquatic and avian species. These properties and its rapid biodegradability make Roundup® an attractive herbicide for engineering tolerance in crop plants. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimic acid pathway, which operates only in plants and microbes. Specifically, glyphosate is a competitive inhibitor with respect to the substrate phosphoenolpyruvate (PEP). The active site of EPSPS has been probed using site-directed mutagenesis and inhibitor binding techniques. Replacement of a specific glycyl with an alanyl residue (G101A in petunia EPSPS) in a highly conserved region confers glyphosate tolerance to several bacterial and plant EPSPS enzymes, suggesting a high degree of structural conservation between the enzymes. The glycine to alanine substitution perturbs the interaction of PEP as well as glyphosate with EPSPS, the effect on glyphosate binding being significantly greater. Chemical mutagenesis was used to generate additional variants of petunia EPSPS and a selection scheme was developed in *E. coli* for expressing the petunia EPSPS cDNA. Several glyphosate tolerant variants, such as G101A G144D, have been identified which have K_m values for PEP that are significantly lower than the G101A variant. Genetically modified soybean plants expressing glyphosate-tolerant EPSPS enzymes have demonstrated high-level glyphosate tolerance in 1991 field tests. The modes of substrate and inhibitor binding to EPSPS can be further understood by study of the recently solved X-ray crystal structure of *E. coli* EPSPS

1095 CLONING OF PLASTID POLYPHENOL OXIDASE (PPO) cDNAs FROM *SOLANUM TUBEROSUM*. Michelle D. Hunt, Haifeng Yu, Sally M. Newman and John C. Steffens. Cornell University, Department of Plant Breeding and Biometry, Ithaca, New York 14853. Polyphenol oxidases (PPO; E.C. 1.10.3.2) catalyze the oxidation of phenols to quinones at the expense of O_2. The quinoid reaction products are highly reactive electrophilic molecules which covalently modify a variety of cellular constituents, contributing to oxidative browning and melanization. Two forms of PPO exist in higher plants: the 45 kD plastid and 59 kD trichome PPOs. The 59 kD trichome PPO is well established as a catalyst for the polymerization of insect entrapment exudate. However, a function for 45 kD PPO has not been elucidated. Proposed functions include participation in pseudocyclic photophosphorylation, oxygen-regulation and/or plant insect resistance. The 45 kD PPO is a ubiquitous, nuclear-encoded, copper-containing higher plant metalloenzyme. Two cDNA clones of the 45 kD PPO of potato have been isolated and sequenced (2 kb and 1.5 kb) which exhibit greater than 90 percent sequence similarity. Northern analysis of isolated poly $(A)^+$ RNA suggest that the full length transcript is approximately 2 kb. The first test of *in vivo* physiological function of PPO is pending via the analysis of potato plants transformed with vectors specifying sense and antisense expression of the 45 kD plastid PPO

1096 cDNA CLONING OF THE ABUNDANT RUBBER PARTICLE PROTEIN GENE FROM GUAYULE. R.A. Backhaus, Z. Pan and K. Cornish. Arizona State Univ., Dept. of Botany, Tempe, AZ 85287 and USDA-ARS, Albany, CA Rubber particle isolated from stembark tissues of guayule (Parthenium argentatum), a desert shrub, possess high activity of the enzyme, rubber transferase (EC 2.5.1.20). SDS-PAGE analysis of these biosynthetically active rubber particles reveals a simple protein profile and includes an abundant protein of 48.5-52 kD which has been designated RPP (Rubber Particle Protein). RPP was purified and characterized as a glycoprotein which resides on the inner surface of the rubber particle. Cleavage of RPP with CNBr resulted in at least 6 peptide fragments which could be partially sequenced. Degenerate plus and minus strand oligonucleotides corresponding to one of these sequences were used amplify a 92 bp cDNA probe which matched with the known amino acid sequence of the peptide fragment. The 92 bp probe was then used to screen a guayule stembark cDNA library. Of the 27 clones isolated, one, designated c17, contained an 823 bp insert coding for the 3' end of the RPP gene. The c17 clone contains an open reading frame of 507 bp which encodes a polypeptide of 169 amino acids, a TAA stop codon and a 3' noncoding 313 bp sequence. Although c17 does not contain full-length RPP, it does show exact homology with three of the six CNBr fragments. The open reading frame of c17 contains 2 methionines and two cysteines. It also contains two potential N-linked glycosylation sites which are consistent with the known properties of RPP. A computer search of GENBANK, EMBL and NBRF did not result in homology with any existing DNA or amino acid sequences in c17, nor do they match any of the known sequences for prenyl transferases isolated from other organisms.

1097 PURIFICATION AND cDNA CLONING OF ACETYLCOENZYME A DEACETYLVINDOLINE 4-O-ACETYLTRANSFERASE FROM *CATHARANTHUS ROSEUS* Ingeborg Roewer[1], Ruth Power[2], Normand Brisson[3] and Vincenzo De Luca[1]

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[3] University of Montreal, Department of Biochemistry, Montreal, PQ H3C 3J7, Canada

Catharanthus roseus (L.) G. Don plants contain vinblastine and vincristine, which are used as antineoplastic agents. Both these indole alkaloids are dimers composed of catharanthine and vindoline. The efforts in producing *in vitro* systems which synthesize these medicinally useful compounds have only met with partial success. Whereas callus and cell suspension cultures which accumulate catharanthine have been characterized in several laboratories, no vindoline producing cultures have been established.

Our studies with enzymes which catalyse the last three steps involved in vindoline biosynthesis have revealed that these activities are not expressed in cell suspension cultures, whereas both enzyme activities and vindoline accumulation reappear upon regeneration of shoots from these cultures. These studies as well as experiments with developing seedlings have demonstrated that the pathway for vindoline biosynthesis is developmentally regulated and explains why cell culture systems fail to produce this indole alkaloid.

We have recently succeeded in purifying acetylcoenzyme A: deacetylvindoline 4-O-acetyltransferase (DAT) which catalyses the last step in vindoline biosynthesis (Arch. Biochem. Biophys. 279, pp. 370-376, 1990). The enzyme was enriched 3300-fold and is composed of two subunits with M_r 33,000 and 21,000 respectively. Both DAT subunits were electroblotted onto nitrocellulose after SDS-PAGE and protease-treated to obtain peptide fragments. The fragments were purified by HPLC and partial amino acid sequences were obtained. Present research involves the isolation of cDNA clones for further characterization at a molecular level of the developmentally regulated expression of this pathway.

1098 PRODUCTION OF TRANSGENIC PLANTS WHICH ACCUMULATE TRYPTAMINE THROUGH EXPRESSION OF TRYPTOPHAN DECARBOXYLASE. Supa Chavadej[1], Normand Brisson[2] & Vincenzo De Luca[1]. Departments of Biology[1] and Biochemistry[2] C.P. 6128 Station A, Montreal, Quebec, Canada H3C 3J7

Recent research in our laboratory has succeeded in producing transgenic tobacco plants which overexpress tryptophan decarboxylase activity to levels 45-times greater than did control plants (Plant Physiology 94:1410-1413). These plants accumulated tryptamine, the decarboxylation product of tryptophan, to levels 260-fold over that of controls (over 1 milligram of tryptamine per gram fresh weight). These results suggest that plants can accomodate additional biosynthetic pathways without visibly affecting the modified plant.

The successfull overproduction of tryptamine in tobacco plants has encouraged us to investigate it's overproduction in other crop plants such as potato and Canola. The purpose of these investigations is to study whether these crops can accomodate this tryptamine production and whether competition for the tryptophan pool(s) will affect the levels of other tryptophan-based natural products. Transgenic tryptamine-accumulating *Brassic napus* and *Solanum tuberosum* plants have been produced in our laboratory, and results of this work will be presented.

1099 ISOLATION AND CHARACTERIZATION OF A 2-OXOGLUTARATE DEPENDENT DIOXYGENASE IN VINDOLINE BIOSYNTHESIS. Emidio De Carolis and Vincenzo De Luca. Universite Montreal, Sci. Biologiques, Montréal, Québec, Canada H3C 3J7.

Young leaves of *Catharanthus roseus* contain the enzymes which convert the monoterpenoid indole alkaloid tabersonine by three hydroxylations, two methylations and one acetylation step to vindoline. A novel direct enzyme assay has been developed for the hydroxylase which catalyzes the C-4 hydroxylation of 2,3-dihydro-3-hydroxy-*N*(1)-methyltabersonine to the 3,4-dihydroxy derivative (second to last step in vindoline biosynthesis). The enzyme showed an absolute requirement for 2-oxoglutarate and enzymatic activity was enhanced by ascorbate, establishing it as a 2-oxoglutarate-dependent dioxygenase. The simplicity and accuracy of this assay was used to study the properties of the 4-hydroxylase. The enzyme was stable in crude extracts and in partially purified preparations, whereas little enzyme activity could be recovered after several steps in purification. Recently, we identified the components required to re-activate the 4-hydroxylase and as a result have been able to further its purification. Studies with developing seedlings have demonstrated that the 4-hydroxylase is developmentally regulated and induced by light. The purification and characterization of this enzyme will contribute to our understanding of the factors which regulate monoterpenoid indole alkaloid biosynthesis in *Catharanthus roseus*.

POSTER ABSTRACTS

1100 **KEY ENZYMES IN PHYTOALEXIN BIOSYNTHESIS: THE ACTIVE SITE IN RESVERATROL AND CHALCONE SYNTHASE.** Joachim Schröder, Thomas Lanz, Susanne Tropf and Gudrun Schröder. Inst. für Biologie II, Universität, D-7800 Freiburg, FRG.

Resveratrol (RS) and chalcone (CHS) synthase are related enzymes with key functions in the biosynthesis of stilbene- and chalcone-derived phytoalexins. They perform the same type of condensing reaction, but the intermediates are folded differently to give either resveratrol or naringenin chalcone. The condensing reaction corresponds to that in fatty acid synthase. This predicts that RS and CHS contain cysteines which are essential because they covalently bind the substrates. This was investigated with site-directed mutagenesis of RS from peanut (Arachis hypogaea) and CHS from mustard (Sinapis alba). All of the six conserved cysteines were exchanged separately into alanine, and the mutants were tested for enzyme activity after expression of the proteins in E. coli. Only one cysteine (cys-169) was essential for activity with both enzymes, and studies with the site-specific inhibitor cerulenin support the proposal that cys-169 is the active site of RS and CHS. Most of the other cys->ala exchanges led to reduced enzyme activities. Differential inhibition rates with the cys-135 and cys-195 mutants indicate that these amino acids are involved in the different product specificity. The environment of cys-169 reveals no similarity with the active sites of other polyketide synthases. This indicates that RS and CHS evolved independently of other condensing enzymes.

1101 INTEGRATION AND EXPRESSION OF A CHIMERIC RABBIT LIVER CYTOCHROME P-450 GENE IN *Nicotiana tabacum*.
Kazuki Saito[1], Masaaki Noji[1] Shigeru Ohmori[1], Yoshio Imai[2] and Isamu Murakoshi[1]. [1]Faculty of Pharmaceutical Sciences, Chiba University, Chiba 260, Japan. [2]Faculty of Agriculture, Osaka Prefecture University, Sakai 591, Japan.

Cytochrome P-450 is an iron-heme containing monooxygenase. This enzyme is involved in a number of oxidative reactions including biosynthesis of plant secondary products. The objective of this research is to change the pattern of secondary metabolites of plant by expression of the gene for cytochrome P-450 from mammalian cells. We have made a chimeric construct pSN002 containing the cDNA of rabbit liver cytochrome P-450 (IIC14) under the control of the TR2' promoter for mannopine synthase of the *Agrobacterium* Ti plasmid. *Nicotiana tabacum* was transformed with *A. tumefaciens* harboring a cointegrated plasmid pSN002::PGV2260. The presence of mRNA and of the translated protein from the chimeric cytochrome P-450 gene in transgenic plants was confirmed by RNA blot hybridization and by Western blot and immunohistochemical analyses, respectively. The transformants in which the foreign cytochrome P-450 protein is expressed show marked phenotypic changes, notably a tendency rapidly to senesce. We detected 1-propenyl-pyrrolidine, a degradative metabolite of nicotine alkaloids, in transgenic tobacco showing this pronounced phenotypic change. Such metabolism is likely to be due to the effect of senescence, not directly to the presence of the cytochrome P-450.

1102 MOLECULAR CHARACTERIZATION OF TWO PLANT FLAVONOL SULFOTRANSFERASES Luc Varin[a], Vincenzo De Luca[b], Ragai K. Ibrahim[b] and Normand Brisson[c] [a]Biochemistry and [c]Biology Departments, Université de Montréal, and [b]Biology Department, Concordia University, Montréal, Canada

A number of position-specific flavonol sulfotransferases (ST) have been recently characterized from *Flaveria chloraefolia*. These enzymes exhibited strict specificity for positions 3 of flavonol aglycones and 3' and 4' of flavonol 3-sulfate. The flavonol 3-ST has been purified to apparent electrophoretic homogeneity by a five step purification procedure. The availability of purified 3-ST permitted partial determination of its amino acid sequence and the production of polyclonal anti-3-ST antibodies. The anti-3-ST antibodies were used to screen a cDNA library generated from poly(A)$^+$ mRNAs isolated from shoot tips of *F. chloraefolia*. cDNA clones coding for the 3 (pFST3) and 4' (pFST4') STs were isolated and characterized. Expression of these clones in *E. coli* led to the synthesis of ST-β-galactosidase fusion proteins having the same substrate and position specificity than the 3 and 4'-ST enzymes synthesized by the plant. The complete nucleotide sequence of pFST3 and pFST4' has been determined. Comparison of their deduced amino acid sequences revealed an overall identity of 69% in 311 amino acid residues. In addition, the two STs of *F. chloraefolia* share sequence similarities with other STs characterized from animal tissues suggesting a common ancestral origin.

1103 ISOLATION AND CHARACTERIZATION OF MUTANTS OF *TAGETES ERECTA* WITH ALTERED THIOPHENE CONTENTS
J.J.M.R.Jacobs, F. Muijs, A.M.W. Engelberts, A.F. Croes, G.J. Wullems. Novaplant Cell Biotechnology Group, Dept. of Experimental Botany, University of Nijmegen, The Netherlands

A mutant of *Tagetes erecta* with altered thiophene spectrum was isolated without selection by HPLC-analysis of thiophene contents in root samples from 316 M2 plants. The M2 population of *Tagetes erecta* was created by mutagenizing wildtype seeds with aqueous ethyl methane sulfonate and permitting the resulting plants to self-fertilize. Two new peaks were prominent in the mutant HPLC spectrum at retention times that were slightly longer than those of the known thiophenes butenenyldithienyl and α-terthienyl.
Feeding experiments with [^{35}S]sulfate showed that sulfur is incorporated in the unidentified new compounds, as well as in known thiophenes. This result supports our hypothesis that the compounds are intermediates in thiophene metabolism. Identification of the metabolites is underway.
Further analysis showed that the new compounds were also produced in sterile root cultures initiated from mutant plants in erlenmeyer snake flasks. This makes the mutants suitable for studies on thiophene metabolism in an in-vitro system with sterile root cultures. Differences in gene expression between mutant and wild-type plants are being studied with the aim to identify genes that are involved in thiophene metabolism.

1104 **MOLECULAR CLONING AND CELL-SPECIFIC EXPRESSION OF HYOSCYAMINE 6β-HYDROXYLASE.** Takashi Hashimoto, Takeshi Kanegae, Yasuhiro Amano, Asuka Hayashi, Jun Matuda and Yasuyuki Yamada, Kyoto University, Kyoto 606, Japan

Roots of several solanaceous plants synthesize tropane alkaloids such as hyoscyamine and scopolamine. Hyoscyamine 6β-hydroxylase (H6H; EC 1.14.11.11) catalyzes the 6β-hydroxylation of [*S*]-hyoscyamine in the biosynthetic pathway leading to scopolamine. The hydroxylase is a 2-oxoglutarate-dependent dioxygenase which requires dioxygen, 2-oxoglutarate, ferrous ion and ascorbate for catalysis. A full-length cDNA clone encoding H6H was isolated by screening a cDNA library made from mRNA from cultured roots of *Hyoscyamus niger* with synthetic oligonucleotide probes which encode the internal amino acid sequences of purified H6H protein. A search of protein data base revealed that H6H is homologous to two synthases involved in the biosynthesis of β-lactam antibiotics in some microorganisms, and the gene products of tomato pTOM13 cDNA and maize *A2* locus which respectively had been proposed to catalyze oxidative reactions in the biosynthesis of ethylene and anthocyan. Northern hybridization showed that H6H mRNA is abundant in cultured roots, and present in plant roots but absent in leaves, stems and cultured cells of *H. niger*. Immunohistochemical studies using an H6H-specific monoclonal antibody further revealed that H6H is localized in the pericycle of the root. A promoter fragment of a genomic H6H clone was isolated and fused with a reporter GUS gene. Results from the H6H-GUS fusion introduced into alkaloid-producing and non-producing plants will be presented

1105 THE DISTRIBUTION OF THE HYOSCYAMINE - 6β - HYDROXYLASE GENE IN THE SOLANACEAE. John Fitchen, Peter Bachmann, Takashi Hashimoto and Yasuyuki Yamada. Laboratory of Molecular and Cellular Biology, Kyoto University, Kyoto, 606 JAPAN

The tropane alkaloid scopolamine is found in only relatively few solanaceous plants. However, these plants do not all belong to the same subtribe, or even tribe, and many members of tribes containing scopolamine-producing plants do not synthesize scopolamine. We have investigated whether the genetic capacity for scopolamine biosynthesis is more widely distributed than the ability to make and accumulate this product. Our approach has been to test for the presence of a gene encoding an enzyme for scopolamine biosynthesis in a range of solanaceous plants using PCR to amplify specific sequences from genomic DNA. The sequences amplified were defined by oligonucleotides synthesized to match the sequence of the gene for hyoscyamine-6 - hydroxylase from Hyoscyamus niger. Our results suggest that this gene, coding for the penultimate enzyme in the biosynthesis of scopolamine, is more widely distributed than the occurrence of this alkaloid. Furthermore, the genes in the various scopolamine-producing plants appear to be very similar.

POSTER ABSTRACTS

1106 ISOLATION AND CHARACTERIZATION OF cDNA CLONES CORRESPONDING TO TWO ENZYMES OF THE SHIKIMATE PATHWAY OF HIGHER PLANTS. Jürg Schmid, Andreas Schaller, Urs Leibinger, and Nikolaus Amrhein. Swiss Federal Institute of Technology, Institute of Plant Sciences, 8092 Zürich, Switzerland.

We have isolated full length cDNA clones of shikimate kinase (EC 2.7.1.71) from tomato (*Lycopersicon esculentum* Mill. UC82B) and of chorismate synthase (EC 4.6.1.4) from *Corydalis sempervirens* Pers. Two degenerate oligonucleotides corresponding to highly conserved sequences of known microbial shikimate kinases were used in a polymerase chain reaction (PCR) with ss cDNA as template. The deduced amino acid sequence of a cloned PCR fragment showed high homology to the known shikimate kinase sequences and was used as a probe to screen a tomato cDNA library. A chorismate synthase specific cDNA has been isolated by screening a *C. sempervirens* expression library with an antiserum against the purified protein from a tissue culture of this plant. The isolated clone was used as a probe to rescreen the library in order to isolate full length cDNA clones. The analysis of both clones will be presented.

1107 ELICITOR-TREATED PINE CELL CULTURES - A MODEL FOR INDUCIBLE LIGNIFICATION. Malcolm M Campbell, Dept. of Chem & Biochem. University of Guelph, Guelph, Ont., N1G 2W1 and Brian E. Ellis, Dept. of Plant Science, University of British Columbia, Vancouver, BC, V6T 2A2

We have developed a model system to investigate inducible phenylpropanoid metabolism in conifer tissue, utilizing suspension cultures of *Pinus banksiana* and an elicitor derived from the ectomycorrhizal fungus *Thelephora terrestris*. Elicited pine cell cultures rapidly accumulated cell wall-bound phenolics and remained viable. On the basis of a variety of chemical analyses, we determined that the accumulated cell wall-bound phenolics had all the characteristics of gymnosperm lignin. During the initial stages of lignin accumulation, elicited pine cultures exhibited rapid, yet transient increases in the activities of a number of lignification-associated enzymes, including phenylalanine ammonia-lyase, 4-coumarate:CoA ligase, caffeic acid *O*-methyl transferase, coniferyl alcohol dehydrogenase, and a coniferin-specific β-glucosidase. A gradual increase in the amount of total peroxidase activity was documented in the pine cell cultures following elicitation and, concurrently, quantitative and qualitative changes in the peroxidase isoform pattern were observed. The value of the *Pinus banksiana* cell culture/ fungal elicitor system as a model for the study of the molecular mechanisms which underlie the process of lignification in an economically important softwood species will be discussed.

1108 PCR-BASED CLONING OF A GENE FROM PINE ENCODING PHENYLALANINE AMMONIA-LYASE. Malcolm M Campbell, Dept. of Chem & Biochem. University of Guelph, Guelph, Ont. N1G 2W1, Jack Pitel and Robert Rutledge, Petawawa National Forestry Institute, Chalk River, Ont. and Brian E. Ellis, Dept. of Plant Science, University of British Columbia, Vancouver, BC, V6T 2A2

The very large size of the conifer genome makes it difficult to clone single-copy genes by conventional library screening methods. We have taken advantage of the properties of the polymerase chain reaction (PCR) to amplify sequences encoding phenylalanine ammonia-lyase (PAL, EC.4.3.1.5) in *Pinus banksiana*. Several regions of the PAL gene from *P. banksiana* have been amplified using pairs of PAL-specific PCR primers, with both cDNA and genomic DNA as template. Sequence analysis has revealed that the pine PAL gene is similar to that from angiosperm species but displays some unique features. The analysis of these clones, as well as the pattern of inducible transcription of the *P. banksiana* PAL gene during the process of lignification in elicited pine cultures, will be presented.

1109 ENVIRONMENTAL CONTROL OF POLYISOPRENOID BIOSYNTHESIS. Rachel Goss, K. V. Venkatachalam and C. R. Benedict, Texas A&M University, Department of Biochemistry & Biophysics, College Station, TX 77843-2128

Electron photomicrographs of stem tissue of a desert shrub Parthenium Argentatum show polyisoprenoid rubber is deposited in the cytosol as discrete rubber particles in cortical parenchyma cells. Exposure of plants to the low winter temperature in the Chihuahuan Desert induces rubber particle formation from a few particles in the parietal cytoplasm to a population of particles nearly filling the cell. Rubber particles are isolated from stem homogenates by gel filtration chromatography. Treatment of isolated rubber particles with detergent followed by SDS-PAGE shows rubber particle protein is increased in plants exposed to low temperature. Poly A+RNA was isolated from plants harvested in September and February, translated in vitro using ^{35}S-methionine and the wheat germ system and the translation products immunoprecipitated with anti-rubber particle protein. The incorporation of ^{35}S into protein was 3-fold higher in plants exposed to the cold winters. Rubber particles contain an enzyme for synthesizing rubber. Adding ^{14}C-IPP, Mg^{2+} and in allylic-PP to isolated particles leads to incorporation of ^{14}C into rubber. A tightly bound rubber transferase catalyzes the repetitive addition of IPP to trans-allylic-PP to form a high mol wt rubber polymer. Using trans FPP as a allylic-PP initiator the polymerization reaction would lead to an arrangement of isoprene units along the rubber chain as: ω-terminal unit, 2 trans units, a long sequence of cis units and an α-terminal unit similar to the structure of rubber chains established by ^{1}H and ^{13}C-NMR. The polymerization reaction is highly correlated (R^2=0.96) to rubber synthesis throughout the low temperature period and accounts for the hydrocarbon content of rubber particles. The results are consistent with the conclusion that the induction of poly A+RNA coding for rubber particle protein is an important feature of low temperature control of polyisoprenoid rubber biosynthesis.

1110 ANALYSIS OF RADIATION-INDUCED MUTATIONS AT TWO FLAVONOID BIOSYNTHETIC LOCI IN ARABIDOPSIS. Brenda W. Shirley and Howard M. Goodman. Harvard Medical School, Department of Genetics and Massachusetts General Hospital, Department of Molecular Biology, Boston, MA 02114. Two genes involved in flavonoid biosynthesis, chalcone flavanone isomerase (CHI) and dihydroflavonol 4-reductase (DFR), have recently been cloned in *Arabidopsis*. Two mutants generated by ionizing radiation were found to contain large structural changes at these loci. The *tt5* allele, resulting from fast-neutron irradiation of seeds, contains an inversion within the CHI gene. A *tt3* allele, generated by X-ray treatment of seeds, consists of a deletion/insertion at the DFR locus. These genes thus provide two new contact points between the *Arabidopsis* genetic and RFLP maps. The mutations have been characterized in detail. The sequences surrounding the mutation breakpoints in *tt3* and *tt5* have been cloned and sequenced. In addition, the effects of the mutations on gene expression have been examined. One other gene which is expressed in wild-type *Arabidopsis* plants has been identified in the region covered by the deletion in *tt3*.

1111 ENZYMES OF LIGNIN BIOSYNTHESIS IN XYLEM OF LOBLOLLY PINE Ross Whetten, David O'Malley and Ronald Sederoff, Department of Forestry, North Carolina State University, Raleigh NC 27695-8008

The biosynthetic pathway for lignin monomers (monolignols) is known, but the regulation of monomer production and polymerization is not fully understood. Developing wood of loblolly pine (*P. taeda* L.) can be obtained as a relatively pure tissue composed largely of differentiating tracheids, and is an abundant source of enzymes of lignin biosynthesis. We are using biochemistry and molecular biology to address the question of how lignification is controlled in this tissue. We have isolated from differentiating wood the first (phenylalanine ammonia-lyase, PAL) and last (cinnamyl alcohol dehydrogenase, CAD) enzymes of the monolignol biosynthetic pathway, and characterized the purified proteins. No evidence was found for isozymes of PAL in pine xylem; the purified enzyme displayed a single isoelectric point (5.7) and K_m (27 μM). Pine CAD shows extensive variation in electrophoretic mobility, segregation analysis of different electromorphs indicate a single gene encodes CAD in loblolly pine. Purified CAD shows a marked preference for coniferaldehyde over sinapaldehyde as substrate. Preliminary results indicate that CAD may be inhibited by coniferin, a storage form of the CAD reaction product that accumulates to high levels in differentiating pine xylem. A cDNA clone of the xylem form of pine PAL has been isolated from an expression library, and experiments to isolate a CAD clone are in progress.

POSTER ABSTRACTS

1112 THREE GENES ENCODE HMG-CoA REDUCTASE IN HEVEA BRASILIENSIS: CHARACTERIZATION OF HMGR3. Mee-Len Chye, Chio-Tee Tan and Nam-Hai Chua*. Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511; *Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021, U.S.A. Hevea brasiliensis is the major producer of natural rubber which is cis-1,4-polyisoprene. The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) that converts HMG-CoA to mevalonate is involved in isoprenoid biosynthesis in plants. Some of these isoprenoids include natural rubber, abscisic acid, gibberellins, cytokinins, carotenoids, chlorophylls and ubiquinone. We are interested in examining the role of HMGR in isoprenoid biosynthesis, particularly that of rubber. We have previously reported the isolation of two classes of HMGR cDNA, HMGR1 and HMGR2, from Hevea brasiliensis. We now report the isolation of a third class of HMGR cDNA from Hevea. A genomic clone of HMGR3 was obtained when HMGR1 cDNA was used as a hybridization probe. Subsequently, the HMGR3 genomic clone was used to screen a Hevea cDNA leaf library for HMGR3 cDNA clones. Comparison of HMGR1 cDNA with HMGR3 cDNA shows 70% nucleotide sequence homology and 77% amino acid homology. Northern blot analysis indicated that the HMGR3 transcript of 2.4 kb is found in both leaf and laticifer. The transcription start site of HMGR3 has been mapped by primer-extension analysis. The HMGR3 promoter has been cloned on a 2kb-XbaI genomic fragment. Sequence analysis has shown that the HMGR3 promoter, unlike most plant promoters, lacks a TATA box. However, the hamster HMGR promoter, in comparison, also does not have a TATA box. Experiments are in progress to further characterise HMGR3.

1113 PRELIMINARY CHARACTERIZATION OF THE PROMOTER FOR THE FIRST ENZYME OF THE SHIKIMATE PATHWAY IN POTATO. John M. Henstrand[1], José E.B.P. Pinto[2], Lisa M. Weaver[2], Klaus M. Herrmann[2], and Avtar K. Handa[1]. Departments of Horticulture[1] and Biochemisty[2], Purdue University, W. Lafayette, IN 47907.

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase catalyzes the first reaction of the shikimate pathway that leads to the aromatic amino acids and to a number of secondary products involved in plant defense and repair. The enzyme is encoded within the *shkA* gene, part of which was cloned by probing a potato genomic library with a cDNA encoding the enzyme (Dyer *et al* (1990) J. Biol. Chem. **265**, 1608-1614). The 5' flanking region (0.9 kb) and part of the coding sequence of *shkA* were fused to the coding region of the reporter gene ß-Glucuronidase (GUS). The construct was introduced into potato line FL-1607 via *Agrobacterium*-mediated transformation. Histological examination of several transgenic plants reveals constitutive cell-specific expression within the vascular tissues of both stem and tuber. Wounding induced GUS activity in tubers within 48 h 2 to 50 fold in independent transgenic plants. Wound-induced transcripts for the fusion product reached transient maximal levels within 6 h. Interestingly, wound induction of GUS was stronger in transgenic tubers with lower uninduced enzyme levels. The data demonstrate that the 5' flanking region of *shkA* confers expression of GUS similar to that of endogenous 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase.

1114 GENETIC MODIFICATION OF CONDENSED TANNIN ACCUMULATION IN TRANSGENIC *LOTUS CORNICULATUS*. T.R. Carron, P. Morris, T.E. Evans, K.J. Webb, and M.P. Robbins. AFRC-IGER, Aberystwyth, Wales, U.K SY23 3EB.

Condensed tannins are polymeric phenolic compounds accumulated by many higher plants in various tissues. They are of agronomic importance in forage crops due to there role in the prevention of pasture bloat. *Trifolium repens*, the major forage legume in U.K. agriculture, does not contain tannins in its vegetative tissue, *Lotus corniculatus*, however, does. For this reason and for reasons of amenability to transformation techniques, we have chosen to study tannin biosynthesis in *L.corniculatus*. Condensed tannins are produced *via* the phenylpropanoid biosynthetic pathway. They share much of the pathway with anthocyanin biosynthesis, the last common enzyme being dihydroflavonol-4-reductase (DFR). We have used the DFR cDNA from *Antirrhinum majus* to produce 3 antisense constructs, and these have been introduced into *L.corniculatus via Agrobacterium rhizogenes* mediated transformation, producing over 300 'hairy root' cultures. So far 17 of the antisense transformants have been studied in detail. Northern analysis has demonstrated the presence of antisense RNA in some of the transformed lines. Root cultures of some of the antisense transformants have shown significantly reduced tannin levels compared with equivalent controls. Determination of copy number and arrangement of the introduced genes is underway and will be presented, as will data relating to developmental profiles of tannin accumulation in antisense transformants.

1115 THE GENE DEFECTIVE IN BLUE FLUORESCENT *ARABIDOPSIS* TRYPTOPHAN MUTANTS. Alan Rose, Andrea Barczak, and Robert Last. Boyce Thompson Institute, Tower Rd., Ithaca, New York, 14853.

Arabidopsis trp1-1 mutant plants lack detectable phosphoribosyl anthranilate transferase (PRAT) activity and accumulate anthranilate, so these plants are auxotrophic for tryptophan and are blue fluorescent[1]. The following lines of evidence indicate that we have isolated an *Arabidopsis* PRAT gene, which we are naming *PAT1*. The gene was selected[2] from an *Arabidopsis* cDNA library by complementation of an *E. coli* strain (*trpD*) defective for PRAT activity, demonstrating that *PAT1* encodes PRAT function. DNA sequence analysis reveals an open reading frame that is very similar to the PRATs from many other organisms. This homology (35% identical plus 40% similar over 237 residues compared to yeast *TRP4*) includes several stretches of highly conserved amino acids. The cDNA was used to isolate a genomic clone containing *PAT1*, which allowed determination of the position and sizes of introns and exons, and the endpoints of the mRNA. RFLP mapping showed that the genomic *PAT1* clone is genetically linked to the *trp1* mutation of *Arabidopsis*. Several means of transformation are being used to re-introduce the *PAT1* gene into *trp1* plants, and genetic complementation is being assessed by reversal of the auxotrophy and fluorescence. Preliminary analysis of the molecular defects in the *PAT1* gene in several different alleles of *trp1* will be presented.

[1] Last, R.L. and G.R. Fink (1988) Science 240:305-310.
[2] by John Mulligan at Stanford.

1116 MUTANTS OF *ARABIDOPSIS THALIANA* DEFECTIVE IN SINAPOYL MALATE BIOSYNTHESIS. Clint Chapple[1], Thomas Vogt[2], Brian Ellis[2], and Chris Somerville[1]. [1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing MI, 48824-1312. [2]Department of Plant Science, University of British Columbia, Vancouver B.C., V6T 2A2.

O-Sinapoyl-L-malate and other sinapic acid esters are major phenolics accumulated in leaves and seeds of *Arabidopsis thaliana*. These compounds may play a role in the plant's defense against pathogens and may mitigate damage by ultraviolet light. In an effort to determine whether these compounds have a function in the plant, an ethylmethane sulfonate-mutagenized population of *Arabidopsis* was screened for mutants with altered sinapoyl malate metabolism. Extracts of individual leaves were fractionated by TLC and sinapic acid esters were visualized with UV light. Of 4200 individuals screened, 6 mutants have been identified which show a >95% reduction in leaf sinapoyl malate levels. At least two of these mutants are allelic. The absence of sinapoyl malate in the mutants does not have a significant effect on plant growth, at least under standard laboratory conditions. One of the mutants that lacks sinapoyl malate instead accumulates the sinapoyl malate precursor, 1-*O*-sinapoyl-ß-D-glucose in leaves of the mature plant, although the presence of this compound is normally restricted to seeds and young seedlings. The biochemical lesions in the mutants are presently under investigation.

1117 BIOSYNTHESIS OF MELANINS AND SIDEROPHORES BY A TEMPERATURE SENSITIVE MUTANT OF RHIZOBIUM PHASEOLI. Guillermo C. Carrillo and Ma. Elisa C. Alvarado. Colegio de Postgraduados, Department of Genetics, Montecillo Méx. 56230, México

We have studied several strains of R. phaseoli which byosynthesize both a blackish pigment and siderophores. In all of them, the scarcity of iron in a minimal medium induced the production of siderophores - determined by their spent media growth-promoting activity on Escherichia coli RW 193. E. coli RW 193 does not synthesize siderophores and requires them to grow on the iron deficient medium - and the presence of L-tyrosine in such inducing conditions increased the levels of siderophore production. The synthesis of melanins had the same pattern of induction. At 35°C the production of pigment by the temperature sensitive mutant CPMéx. 46 was drasticaly decreased (more than 50%) in comparison with the other strains. The pattern of pigment release by the cells was affected by the temperature of incubation. We propose that the aromatic amino acid L-tyrosine is precursor for the synthesis of both the blackish pigment and siderophores in the bacterial strains studied.

POSTER ABSTRACTS

1118 TOWARDS CLONING OF CYTOCHROME *P*-450-DEPENDENT GERANIOL 10-HYDROXYLASE FROM *CATHARANTHUS ROSEUS*. A.H. Meijer, A. de Waal, R. Verpoorte, J. Memelink and J.H.C. Hoge. Biotechnology Delft Leiden, Project Group Plant Cell Biotechnology, Department of Plant Molecular Biology, Leiden University, Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

We study the regulation of terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*. Genes encoding key enzymes in this biosynthetic pathway and the mechanisms that control their expression have our prime interest. One of our targets is the membrane-bound, cytochrome *P*-450-dependent enzyme, geraniol 10-hydroxylase (G10H), which catalyses the conversion of geraniol to its 10-hydroxy derivative. G10H was purified from *C. roseus* cell suspension cultures by cholate-solubilisation and chromatography on DEAE-Sephacel, Hydroxylapatite Ultrogel, ω-aminooctyl agarose and TSK Phenyl-5PW. The G10H peak-fractions of TSK Phenyl-5PW showed a single band on SDS PAGE with silver staining, corresponding to an M_r of 56,000. On DEAE-Sephacel the cytochrome *P*-450 enzyme was separated from NADPH:cytochrome *P*-450 reductase, that functions in electron transfer from NADPH to the *P*-450 protein. The reductase was purified to homogeneity on 2'5'ADP Sepharose 4B. Screening of a *C.roseus* cDNA expression library with antisera raised against purified G10H and NADPH:cytochrome *P*-450 reductase and analysis of the cDNA clones obtained are in progress.

1119 REGULATION OF COLOUR IN APPLE SKIN. Carolyn Lister[1], Jane Lancaster[1], Kevin Sutton[1], and John Walker[2]. [1]DSIR Crop Research, Private Bag, Christchurch; [2]Dept of Plant and Microbial Sciences, University of Canterbury, Private Bag, Christchurch, New Zealand.

We are interested in the molecular and biochemical regulation of colour in apple skin. Apple skin colour is a blend of various amounts of chlorophyll, carotenoids and anthocyanins/flavanols. A variety of red colours are produced by cyanidin glycosides copigmented with flavanols and procyanidins. The quantification and identification of anthocyanins, flavanols and procyanidins is carried out on C18 reverse phase HPLC. 3 cyanidin glycosides, 8 quercetin glycosides and 4 proanthocyanidins have routinely been detected. Trace amounts of kaempferol glycosides and gallocatechin have been detected.

The developmental regulation of cyanidin and quercetin glycosides is outlined. Quercetin glycosides were very high in juvenile fruit, decreased during fruit expansion, and increased two fold during ripening. Procyanidins were high in juvenile fruit and decreased with fruit expansion. Cyanidin glycosides were present in juvenile fruit, disappeared and then increased 8 fold during ripening.

A likely biosynthetic pathway to cyanidin glycosides, procyanidins and flavanols is outlined. The pathway to leucocyanidin and procyanidins is operative in both red and green apples. Evidence is presented to show that reddening involves the induction of enzymes between leucocyanidin and cyanidin glycosides. These enzymes have not been characterised, neither have the genes been cloned.

1120 CLONING ALLIINASE, A GENE FOR FLAVOUR IN ONIONS. Jane Lancaster[1], Sonya Clark[1], Martin Shaw[1], Julie Farrant[1], and Alan Carne[2]. [1]DSIR Crop Research, Private Bag, Christchurch. [2]Dept of Biochemistry, Otago University, Private Bag, Dunedin, New Zealand.

The characteristic taste and aroma of onions and garlic occurs when the cells are disrupted. The enzyme alliinase is compartmentalized in the vacuole and upon release hydrolyses flavour precursors in the cytoplasm. Onion flavour precursors are alk(en)yl cysteine sulphoxides (non-protein sulphur amino acids) which when hydrolysed produce 20-30 volatile sulphides giving the plant its distinctive flavour and odour.

The aim of this work is to clone the gene for alliinase in order to (i) transfer anti-sense copies of the gene into onions and determine the expression of the antisense gene and its effects on flavour production, ii) isolate the promoter region of this gene. As alliinase is a constitutive, highly expressed protein, the promoter region may be of use in regulating expression of genes in transgenic monocots.

Alliinase protein was purified and the sequence of the NH_2-terminal (23) amino acids was determined. A further region of amino acid sequence has also been determined from cyanogen bromide fragments but the location of this sequence in the alliinase protein is unknown. Redundant oligonucleotide probes were derived by reverse translation of amino acid sequence. Polyclonal antibodies to alliinase have been raised. An onion cDNA library of 3.2×10^5 pfu has been constructed in lambda Zap II vector, and is being screened using both antibodies and oligonucleotide probes. Redundant oligonucleotides have also been designed as PCR primers for amplification of products from onion DNA.

The effectiveness of each approach in isolating clones coding for alliinase will be discussed.

1121 REGULATION OF TERPENOID INDOLE ALKALOID BIOSYNTHETIC GENES FROM *CATHARANTHUS ROSEUS* BY HORMONES AND ELICITORS.

J.Memelink, G.Pasquali, O.J.M.Goddijn, P.B.F.Ouwerkerk, R.A.Schilperoort and J.H.C.Hoge. Department of Plant Molecular Biology, Clusius Laboratory, Wassenaarseweg 64, 2333AL Leiden, The Netherlands.

We cloned two genes encoding enzymes acting early in a pathway of secondary metabolite biosynthesis in *Catharanthus roseus* (periwinkle). Tryptophan decarboxylase (TDC) converts tryptophan to tryptamin, and strictosidin synthase (SSS) subsequently couples tryptamin to secologanin to form strictosidin, which then serves as the precursor for numerous terpenoid indole alkaloids. TDC and SSS are encoded by single-copy genes that are coordinately regulated. In intact, healthy plants steady state mRNA levels for both genes are highest in roots. In cell suspension cultures steady state mRNA levels were found to be reduced by auxin, and induced by elicitors (yeast extract or *Pythium aphanidermatum* culture filtrate). These signal molecules modulate *tdc* and *sss* expression at the transcriptional level and independent of *de novo* protein synthesis, indicating that they activate short signal transduction pathways. Currently, *in vivo* and *in vitro* promoter studies are underway to elucidate the pathways leading to expression of both genes with emphasis on the negative regulation by auxin and the positive regulation by elicitors.

1122 PRODUCTION OF SECONDARY METABOLITES WITH INSECTICIDAL PROPERTIES BY PLANTS AND CULTURED CELLS OF CHRYSANTHEMUM CINERARIAEFOLIUM. Robert G. McDaniel. University of Arizona, Department of Plant Sciences, Tucson, Arizona 85721

Chrysanthemum cinerariaefolium, commonly called pyrethrum, is grown as a crop in several areas of the world, with the flowers harvested as a source of six insecticidal chemicals called pyrethrins. Selections of this plant have been grown in Arizona to evaluate the effects of stresses upon the synthesis of these insecticidal metabolites. These chemicals result from a mevalonic acid condensation, with subsequent esterification of pyrethric acid and chrysanthemic acid derivatives. In contrast to reports in the literature that stressing plants may result in increased synthesis of other secondary metabolites, salinity and drought stress applied to pyrethrum elicited a significant decrease in the quantity of pyrethrins synthesized. Qualitatively, judged by reverse phase HPLC analyses, with authentic Kenyan pyrethrins as standards, stress had little effect on the relative amounts of the six pyrethrins. If stresses on plants were minimized, both quantity and quality of pyrethrins varied little across locations and years of production. The genes controlling the synthesis of pyrethrins appear to be tightly regulated, with maximum expression in developing flowers (over 2 by dry weight), less than 0.1% pyrethrins in subtending leaves and stem tissues, and less than 1% in tissue cultured cell lines.

1123 BIOSYNTHESIS OF THE ISOFLAVONOID PISATIN: ELUCIDATION OF THE LATE STEPS. Gregory DiCenzo, Carol Preisig*, and Hans VanEtten. Department of Plant Pathology, University of Arizona, Tucson AZ 85721 USA. *U.S. Department of Agriculture Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia PA 19118.

Pisatin is a phytoalexin synthesized by pea (*Pisum sativum*) when challenged by microbial infection or other stress. While biosynthetic steps leading to isoflavone intermediates are understood, the later mechanisms of pterocarpan formation are presently unclear. In particular, the enzymatic basis for the penultimate step - formation of 6a-hydroxymaackiain (HMK) - is not known. Earlier work studying incorporation of ^{14}C-radiolabelled intermediates suggested that the pterocarpan maackiain is an intermediate en route to pisatin as well as the likely substrate for a 6a-hydroxylation activity. However, more recent work proves the 6a oxygen is derived from H_2O rather than O_2. This indicates that the oxygen is not derived from a monooxygenase-catalyzed reaction and appears to rule out a direct hydroxylation of maackiain at the 6a position. Identity of HMK as the immediate precursor to pisatin is confirmed, however, by the purification of highly specific HMK 3-*O*-methyltransferase. Recently, inducible NADPH-dependent 2',7'-Dihydroxy-4',5'-methylenedioxyisoflavone (DMI) reductase, catalyzing formation of the isoflavone sophorol, a likely precursor of HMK, has been purified from pea. Using radiolabelled sophorol as a substrate, we are accumulating labelled intermediates for identification and subsequent ordering of the biosynthetic pathway. Preliminary data suggests 3 products other than HMK and pisatin are produced in a NADPH-dependent manner. Therefore, the formation of HMK from sophorol most likely involves several steps. We are continuing to characterize these products and relevant enzymic activity.

POSTER ABSTRACTS

1124 cDNA CLONING, CHARACTERIZATION, AND MANIPULATION OF ISOFLAVONE REDUCTASE, A KEY ENZYME IN PHYTOALEXIN BIOSYNTHESIS IN ALFALFA. Nancy L. Paiva, Jack W. Blount, Robert Edwards, Richard A. Dixon, Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73401; Yuejin Sun, Geza Hrazdina, Institute of Food Science, Cornell University, Geneva, New York 14456.

One response of alfalfa (Medicago sativa) to attack by fungal pathogens is the production of the isoflavonoid phytoalexin medicarpin. Isoflavone reductase (IFR) stereospecifically reduces 2'-hydroxyformononetin to one isomer of vestitone, and a second reductase (pterocarpan synthase) stereospecifically reduces vestitone to form (6aR, 11aR)-medicarpin. We have isolated a cDNA clone from alfalfa which expresses active, stereospecific IFR in Escherichia coli. The transcript is highly induced in elicited alfalfa cell cultures, and the kinetics of induction are consistent with the appearance of IFR activity, medicarpin accumulation, and the induction of other enzymes in the pathway. Medicarpin-3-O-glucoside-6"-O-malonate (MGM) accumulates constitutively at low levels in roots and nodules but not leaves of healthy alfalfa, while medicarpin has been shown to accumulate in leaves of infected plants. In an attempt to assess the role of medicarpin in the anti-fungal defense response, anti-sense IFR constructs are currently being introduced into alfalfa plants to block phytoalexin biosynthesis. These transgenic plants will be examined for altered secondary metabolite accumulation and changes in resistance to pathogens. In conjunction with these studies, the pathway intermediates which may accumulate (e.g., 2'-hydroxyformononetin) are being assessed for antimicrobial activity relative to medicarpin.

1125 PRODUCT ENHANCEMENT AND RELEASE FROM TRANSFORMED ROOT CULTURES OF *Nicotiana glauca*. K.D. Green, N.H. Thomas. University of Birmingham, Dept of Chemical Engineering, Birmingham, B152TT, UK.

Transformed roots are becoming the vehicle of choice for the production of valuable secondary metabolites. Transformed root productivities are comparable to the parent plants and are not associated with the genetic and biochemical instabilities which continue to plague cell cultures. Root cultures also release a proportion of their secondary products into the surrounding medium. The influence of additional media nitrate levels and types on growth and productivity were investigated. Secondary product (nicotine and anabasine) release was greatly increased in the later growth stages when potassium or calcium nitrate is added (0.5%). Delicate nitrate additions can also be used to enhance growth (0.1%) or intracellular alkaloids (0.01%). Heat treatment can be used to permeabilise transformed root tissue. After 5 hours of heat treatment(60°C), 60% of the total alkaloids were released into the medium. Root viability was maintained at temperatures up to 50°C after 5 hours of treatment.

1126 ISOLATION AND POLYPEPTIDE COMPOSITION OF 1,3-ß-GLUCAN SYNTHASE FROM PLANT PLASMA MEMBRANES. Per O. Kiellbom, Karin Fredrikson and Christer Larsson.Plant Biochemistry, Univ. of Lund. Sweden.

The 1,3-ß-glucan synthase (callose synthase)was solubilized from cauliflower plasma membranes with digitonin, and patially purified by FPLC ion exchange chromatography and gel filtration using CHAPS in the elution buffers. These initial steps were necessary to obtain specific precipitation of the enzyme during product entrapment, the final purification step. Five polypeptides of 32, 35, 57, 65 and 66 kDa were highly enriched in the final preparation and are thus likely components of the callose synthase complex. The purified enzyme was activated by Ca^{2+}, spermine and cellobiose in the same way as the enzyme in situ, indicating that no essential subunits were missing. The polyglucan produced by the purified enzyme contained mainly 1,3-linked glucose.However, 8% of the glucose residues were 4-linked, which may indicate that the enzyme has the ability to make both 1,3- and 1,4-polyglucan. Our efforts in cloning the genes corresponding to the five polypeptides using oligonucleotides as probes and primers will be accounted for, as well as the regulation of the enzyme activity in barley plasma membranes in response to infection by the fungal pathogen powdery mildew.

1127 SINAPINE SYNTHASE FROM BRASSICA NAPUS. Thomas Vogt and Brian Ellis. University of British Columbia, Department of Plant Science, 2357 Main Mall, Vancouver, B.C. V6T 2A2, Canada

Sinapine synthase (SCT) is the key enzyme responsible for the synthesis and the accumulation of sinapoylcholine (sinapine) in seeds of B. napus (rape seed, canola) as well as other members of the Brassicaceae. One approach to reducing the undesirably high level of sinapine in rape seed meal, which is used as an animal feed supplement, is to genetically engineer existing canola varieties by introducing an antisense RNA-construct for sinapine synthase.

SCT from B. napus has been purified to apparent homogenity by various chromatographic techniques. The enzyme has been characterized and compared to SCT of related Brassicaceae. Polyclonal antibodies have been raised and a partial amino acid sequence established, in order to screen a B. napus seed expression library and identify possible oligonucleotide primers for the polymerase chain reaction.

1128 REGULATION AND OVEREXPRESSION OF THE TRYPTOPHAN DECARBOXYLASE GENE IN CATHARANTHUS ROSEUS. O.J.M. Goddijn, P.J.L. van der Helm, R.J. de Kam, R.A.Schilperoort and J.H.C. Hoge. Biotechnology Delft-Leiden, Project Group Plant Cell Biotechnology, Department of Plant Molecular Biology, Clusius laboratory, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

One of the key enzymes in the biosynthesis of secondary metabolites in Catharanthus roseus is tryptophan decarboxylase (TDC:EC 4.1.1.28) as it links primary metabolism to terpenoid indole alkaloid (TIA) production by converting tryptophan into tryptamine. In suspension cultures tdc mRNA levels increase 5 to 10-fold upon transfer to auxin-free medium. Addition of 10^{-5}M NAA to auxin-starved suspension cultures results in very rapid (within 15 min.) transcriptional downregulation of the tdc gene, independent of de novo protein biosynthesis. A tdc cDNA clone was overexpressed under control of the 35S CaMV promoter in wild type Agrobacterium tumefaciens transformed C. roseus tissue. Enhanced tdc activity and tryptamine levels but no significant increase in TIA production could be found in tdc transgenic C. roseus crown gall tissues. Currently we are studying the regulation of the tdc gene in transgenic N. tabacum plants containing fusions between the tdc promoter and the GUS reporter gene.

1129 SESQUITERPENE BIOSYNTHESIS IN *LYCOPERSICON* TRICHOMES. C.D. Carter, J. Gershenzon, J.N. Sacalis, and T.J. Gianfagna. South Dakota State Univ., Dept. of Plant Science, Brookings, SD 57007; Washington State Univ., Inst. of Biological Chemistry, Pullman, WA 99164; Rutgers Univ., Dept. of Horticulture, New Brunswick, NJ 08903

The sesquiterpene zingiberene accumulates in trichomes of *Lycopersicon hirsutum* f. *hirsutum* and provides resistance to the Colorado potato beetle (Carter et al., J Agric Food Chem 37: 1425). Zingiberene is controlled by multiple alleles at a single gene locus designated *Z*, where Z^1 codes for presence of zingiberene and is codominant to Z^3 and recessive to Z^2 (Carter et al., J Cell Biochem Supple 15A: 65). Labelled precursors were incorporated into zingiberene in the trichomes but not in the leaf matrix. Preliminary results indicate the presence of sesquiterpene cyclase activity in extracts from isolated trichomes. Zingiberene levels were 10 times greater under short days and cool temperatures than under long days and warm temperatures. Few genes associated with sesquiterpene biosynthesis have been identified. Leaf and trichome RNA did not hybridize to cDNA probes for hamster or tomato HMGR. To identify genes controlling sesquiterpene biosynthesis in trichomes, we are constructing cDNA libraries from leaf and trichome poly(A+) RNA of Z^1Z^1 and Z^3Z^3 plants. Results of differential screening among these libraries will be presented.

POSTER ABSTRACTS

1130 NON-COORDINATE REGULATED EXPRESSION OF BZ1 AND BZ2 PROMOTERS IN MAIZE PROTOPLASTS John P. Bodeau and Virginia Walbot. Stanford University, Dept. Bio. Sci., Stanford, CA, 94305

The maize regulatory genes R and C1 both encode putative DNA-binding proteins required for transcription of several genes encoding enzymes required for anthocyanin biosynthesis. Electroporation-mediated transfection of colorless maize protoplasts with plasmid vectors constitutively expressing R and C1 (p35S:R and p35S:C1) activated the endogenous anthocyanin biosynthetic genes, resulting in pink protoplasts within 24 hr. Chimeric constructs with anthocyanin structural gene promoters driving luciferase expression were also induced: a 689 bp upstream promoter/leader region from Bz2 (in pBz2:l:Luc) was activated over 800-fold by p35S:R and p35S:C1, while up to a 90-fold activation of Bz1 and A1 promoters occurred. Absolute luciferase expression from pBz2:l:Luc was approximately four times that of p35S:l:L, ranking the Bz2 promoter among the most active yet tested in maize cells. Reporter gene expression p35S:l:Luc preceeds expression from pBz2:l:Luc co-electroporated with p35S:R and p35S.C1, consistent with the R and C1 proteins being required for Bz2 transcription. A ten-fold excess of p35S R to p35S:C1 reduces Bz2 promoter activity but activates Bz1, while a 50-fold excess of p35S'R reduces both Bz2 and Bz1 expression. These results suggest a direct interaction between the R and C1 proteins but a differential impact on target promoters

1131 REGULATION OF CELL-AND TISSUE-SPECIFIC EXPRESSION OF THE PARSLEY 4CL-1 GENE. Carl J.Douglas, Gopal Subramaniam, and Karl D.Hauffe, University of British Columbia, Department of Botany, Vancouver, B.C. V6T 2B1 Canada.

The enzyme 4-coumarate:CoA ligase (4CL) catalyzes the formation of activated thiol esters of cinnamic acid derivatives, the last step of general phenylpropanoid metabolism. These esters are required as substrates for the the synthesis of many phenylpropanoid compounds, including flavonoids and lignin. Expression of the parsley 4CL-1 gene is activated in parsley cells by elicitor and light, and in transgenic tobacco plants by elicitor, light, and wounding. In addition, the 4CL-1 promoter specifies a complex developmentally-regulated pattern of cell- and tissue-specific expression in transgenic tobacco and *Arabidopsis* plants. The ability to specify expression in developing xylem, root tips, nectaries, pollen, and in the epidermal cells of stigmas, ovules, and petals, was localized to a 210-bp 4CL-1 promoter fragment, within which several sites of protein-DNA interaction in parsley tissue-cultured cells had been identified by *in vivo* footprint analysis. A detailed investigation of the 210-bp region was undertaken using site-directed mutagenesis, internal deletions, fusion of the 35S enhancer to 5'-truncated promoters, fusion of potential *cis*-acting elements to a minimal heterologous promoter, and gel retardation assays. Expression of gene constructions was assayed in transgenic tobacco plants and in parsley protoplasts. These experiments demonstrate that functionally-redundant *cis*-acting elements regulating developmental expression are located upstream of -210 bp, that several *cis*-acting elements located between -78 and -180 bp regulate developmental expression in transgenic tobacco plants, and that these elements act to enhance expression from a minimal promoter in parsley protoplasts. The combinatorial functions of the *cis*-acting elements in specifying cell-type specific expression, and their interactions with factors in nuclear extracts of parsley tissue culture cells and tobacco floral and vegetative tissues will be discussed.

1132 CHANGING THE COLOUR OF DENDRANTHEMA (CHRYSANTHEMUM) FLOWERS

Simon Deroles, Kathy Slindee, Kevin Davies, Marie Bradley, Susan Ledger, Robyn Miller and Nigel Given, MAF Technology, Levin, New Zealand and Ken Markham, DSIR, Chemistry, Gracefield, New Zealand.

The red colour in Dendranthema flowers is due to the accumulation of cyanidin derivatives. In order to produce a novel shade of red in Dendranthema flowers we have inserted the dihydroflavonol-4-reductase (DFR) gene from Antirrhinum into the cultivar D. indicum. The DFR gene from Antirrhinum is capable of using the monohydroxylated substrate dihydrokaempferol to produce leucopelargonidin. Plants from these experiments are due to flower shortly.

We have developed an efficient transformation system for D. indicum (Plant Cell Reports, in Press). In addition, to aid in our understanding of flavonoid biosynthesis in Dendranthema, we identified the flavonoids in 40 cultivars, and conducted supplementation experiments on a selected few.

1133 GENOME CHARACTERIZATION OF THE RICE TUNGRO BACILLIFORM VIRUS. R. Qu, M. Bhattacharyya, G.S. Laco, A de Kochko, B.L. Subba Rao, M.B. Kaniewska, J.S. Elmer*, D.E. Rochester, C.E. Smith* and R.N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130, and *Monsanto Company, Chesterfield, MO 63198

Rice tungro disease is caused by double infection of rice tungro spherical virus, a (+) sense RNA virus, and rice tungro bacilliform virus (RTBV), with a genome of circular double-stranded DNA. The RTBV genome was cloned, sequenced and found to be 8000 bp in length, containing four open reading frames (ORFs) on only one strand of the genome. Determination of both termini of the genome-length major transcript indicates that it is terminally redundant, with an overlap of 265-268 nucleotides. Purified virus particles contain two major proteins with molecular masses of 37 kDa and 33 kDa, respectively. Only the 37 kDa protein was detected in the infected rice tissues. The amino acid sequence of the amino terminus of the 33 kDa protein was determined and its coding region identified on the RTBV genome. The unusually long ORF 3 of RTBV is predicted to encode a polyprotein (194.1 kDa) that includes the coat protein(s), a viral proteinase, a reverse transcriptase, and a ribonuclease H-like protein. Selected regions of ORF 3 show varying degrees of similarity to the counterparts of *Commelina* yellow mottle virus (CoYMV), a badnavirus, and several caulimoviruses. RTBV is very likely a plant pararetrovirus

1134 SYSTEMIC EXPRESSION OF A BACTERIAL GENE BY A TOBACCO MOSAIC VIRUS-BASED VECTOR. J. Donson, C.M. Kearney, M.E. Hilf & W.O. Dawson. Dept. of Plant Pathology, University of California, Riverside, CA92521

A hybrid viral RNA (TB2) was constructed, containing sequences from two tobamoviruses (TMV-U1 and odontoglossum ringspot virus; ORSV). Two bacterial sequences inserted independently into TB2 moved systemically in Nicotiana benthamiana, though they differed in their relative stability on serial passage. Systemic expression of the bacterial protein neomycin phosphotransferase (NPTII) was demonstrated. Hybrid RNAs containing both TMV-U1 and the inserted bacterial gene sequences were encapsidated by the ORSV coat protein, facilitating their transmission and amplification on passaging to subsequent plants. cDNA was made to hybrid viral RNA populations that had undergone 10 passages (170 days in planta) in N. benthamiana. The cDNA was cloned in E. coli and sequenced in order to examine the stability of the foreign gene inserts compared to native TMV sequence. Additional hybrid viral RNAs have been constructed to both extend the host range and the level of expression of foreign genes inserted into TMV-based vectors. These vectors provide a rapid means of expressing genes and gene variants in plants.

1135 cDNA CLONING AND SEQUENCING OF RICE RAGGED STUNT REOVIRUS Naravana M Upadhyaya, Jacqueline A Gellatly, *Wichai Kositratana, Wayne L. Gerlach and Peter M. Waternouse. CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia. *Plant Genetic Engineering Laboratory, Kasetsart University, Kampaeng Saen Nakhon Pathom 73140, Thailand

Rice ragged stunt virus (RRSV) is a member of the plant-infecting Reoviridae and causes severe disease and economic loss in rice cultivation. The viral genome consists of 10 dsRNA segments with a total size of ~15 Md. The virus particles have at least five structural protein subunits. An RNA dependent RNA polymerase also seems to be a component of the particle. Our main objectives are to analyse RRSV at the molecular level and to produce synthetic resistance genes for introduction into rice plant.

We have isolated and purified the dsRNA from plants infected with RRSV using combination of CF-11 and Sephacryl-400 chromatography with yields of dsRNA up to 10 ug per gram of shoot tissue. We have produced ELISA and cDNA probes for sensitive viral detection.

By random priming of heat denatured dsRNA template we have produced cDNAs, which were then adaptor (*EcoRI*) ligated and cloned into pUC119. Hybridization analysis of ~1500 cDNA clones using partially-hydrolysed and end-labelled dsRNA segments suggest that these clones cover the RRSV genome with five-fold redundancy. The biggest clone obtained was ~3 kb representing 70% of the largest genome segment. Available sequence data will be presented

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1136 DIGITARIA STREAK GEMINIVIRUS REPLICATES IN S-PHASE CELLS OF DIGITARIA SETIGERA. Gian-Paolo Accotto*, Philip M. Mullineaux**, Spencer C. Brown*** and Vera Masenga*.
*Istituto di Fitovirologia Applicata, CNR, Torino, Italy **Dept. of Applied Genetics, John Innes Institute, Norwich, UK ***Cytométrie, Institut des Sciences Végétales, CNRS, Gif-sur-Yvette, France.

Geminiviruses are small geminate plant viruses with a genome composed of circular ssDNA. Digitaria streak virus (DSV) can infect some Gramineae and its genome is composed of one DNA molecule of 2701 nucleotides. In thin sections of infected Digitaria setigera tissues large crystals of virus particles accumulate in the nucleus and long and winding ribbon-like arrays of ordered particles are visible in the cytoplasm and in the vacuolar space. Nuclei were sorted using a flow cytometer and fractions corresponding to G0-G1, S and G2 phases of the cell cycle were collected. DNA was analyzed. Southern blots with a DSV-specific probe showed that the viral double-stranded RFs are absent in G0-G1 phase, abundant in S phase, still present but in much lower concentration in G2. In contrast ssDNA was present in all nuclei preparations. Since the residual presence of RFs in G2 is likely to be a contamination from sorted nuclei at the end of the S phase due to the statistical overlap with G2, we conclude that the replication of DSV is strictly synchronized with the replication of cellular genomic DNA. The involvement and the interaction of host encoded factors with polypeptides encoded by the virus itself will be discussed.

1137 REGULATION OF A TOBACCO β-1,3-GLUCANASE GENE PROMOTER BY TMV INFECTION, SALICYLIC ACID AND DEVELOPMENTAL SIGNALS.
Jacek Hennig, Ralph E. Dewey, John R. Cutt and Daniel F. Klessig.
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The tobacco cultivar Xanthi-nc is resistant to infection by tobacco mosaic virus (TMV). TMV infection of this cultivar results in the *de novo* synthesis of a number host, pathogen-related (PR) proteins. Among the PR proteins are the hydrolytic β-1,3-glucanase enzymes.

We have isolated a DNA clone containing the promoter for a tobacco PR-2, β-1,3-glucanase gene. The PR-2 promoter DNA sequence and a series of promoter deletion clones have been fused to the GUS reporter gene. The chimeric *PR-2::GUS* fusion genes were then used in the construction of transgenic Xanthi-nc tobacco plants. TMV infection of the *PR-2.GUS* transgenic tobacco plants activated expression of the *PR-2::GUS* gene. Histological visualization of GUS activity in TMV-infected tissue indicated that the GUS activity was first seen adjacent to the TMV-infection sites. Salicylic acid, which is believed to be an endogenous signal in the induction of viral resistance (Malamy *et al.*, 1990), also activated the *PR-2::GUS* gene in these transgenic plants when exogenously applied to leaf tissue. The identification of *cis*-acting elements controlling the regulation of this promoter is in progress.

During normal development the *PR-2::GUS* fusion gene exhibited a specific pattern of gene expression in multiple floral organs. Our data suggests that the PR-2 β-1,3-glucanase genes may play a role in both in plant development and in the defense response against pathogen infection.

Malamy J., Carr J P., Klessig D.F. and Raskin I. Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. Science 250, 1002-1004 (1990).

1138 STUDIES ON RNA-RNA RECOMBINATION IN BROME MOSAIC VIRUS
Peter D. Nagy, Stanislaw Flasinski, and Jozef J. Bujarski
Northern Illinois University, Plant Molecular Biology Center and Dept. of Biological Sciences, DeKalb, IL 60115

We have investigated the phenomenon of the RNA-RNA recombination in both noncoding and coding regions of brome mosaic virus (BMV) RNAs. A series of the RNA-3 constructs that incorporated a duplication of the 3' end noncoding replication promoter region contained a deletion (designated M4) at either of the duplicated sequences. This arrangement allowed for the separation of replication and recombination functions. When tested in whole plant or protoplast infections, all the mutants bearing the 3' terminal M4 deletion generated new recombinants whereas when the mutation was positioned internally, no effect on recombination was observed. A series of both legitimate and illegitimate 3' recombinants have been characterized. To study the recombination in coding regions, frame-shift mutations were introduced in the coat proteins or 3a protein BMV RNA-3 ORFs. When inoculated on a local lesion host (Chenopodium hybridum), most of these mutants were not infectious. Co-inoculation with two RNA-3 variants, one defective in the 3a and another in the coat proteins, restored the infectivity. An RT-PCR analysis revealed the occurrence of complementation. However, a reinoculation on a systemic host (barley) proved the presence of recombinant wt RNA3 molecules in several local lesions. Based on the analyses of sequences at the junction points we propose a recombination mechanism in which two recombining RNA molecules are juxtaposed by a negative complementary RNA strand.

1139 CHARACTERIZATION OF DEFECTIVE RNAS IN BROAD BEAN MOTTLE VIRUS STRAINS, Javier Romero, Qi Huang, Judy Pogany, Jozef J. Bujarski
Plant Molecular Biology Center, Northern Illinois University DeKalb, IL 60115

Several strains of BBMV had been described showing different symptoms in their plant hosts. We have found that two of them contained electrophoretically distinguishable additional RNA components. Northern blots with an RNA-2 specific probes proved that this defective particle was RNA-2 related. Using the RNA-2 specific primers and the RT-PCR reactions, we have obtained, in addition to the full length cDNA-2, smaller cDNA products. Sequence analysis of the cloned cDNAs revealed that defective RNAs contained internal deletions in frame that could be translated in vitro into a distinct protein. Defective RNAs in BBMV seem to be associated with the broad bean host, and possibly regulating symptoms severity.

1140 VIROID STRUCTURE AND INTERACTION WITH HOST CELL COMPONENTS.
Cynthia P. Paul*, Andrea D. Branch*, and Hugh D. Robertson*.
*Cornell University Medical College and *The Rockefeller University, New York, NY 10021.

Viroids are composed solely of RNA and do not code for any proteins; therefore, they rely on host cell components to complete their life cycle. Host components may be required for several steps in replication and for viroid transport. The viroid central conserved region (CCR) is nearly identical in many viroids and thus, is likely to be important in viroid-host interactions. This region of the viroid RNA contains an unusual tertiary structural element, identified by the crosslinking of two nonadjacent bases upon exposure to UV light, followed by direct RNA analysis to determine the crosslinked bases. The minus strand of the viroid RNA, which serves as a template in the replication of plus strand viroid, also undergoes crosslinking upon exposure to UV light, indicating the presence of a tertiary structural element(s). RNA structure is important in RNA-protein interactions and these structures, identified by UV crosslinking, are likely candidates in the search for regions of the viroid RNA which interact with host cell components. An interaction between viroid and host cell components is suggested by the similarities between the viroid CCR and a region of 5S ribosomal RNA (rRNA) (Branch et al. 1985. PNAS 82:6590-6594.). This region of 5S rRNA is the binding site for transcription factor IIIA (TFIIIA). The binding of TFIIIA to 5S ribosomal DNA (rDNA) is required for the transcription of the 5S gene by RNA polymerase III. 5S rRNA acts as a negative regulatory factor by binding to TFIIIA itself. The possibility that viroid RNA may also interact with TFIIIA is being investigated.

1141 STRUCTURE AND FORMATION OF THE DELTA "AXEHEAD" SELF-CLEAVAGE SITE: STUDIES OF TRANS CLEAVAGE. Andrea D. Branch, Umberto Pace, Bonnie J. Levine, Olivia D. Neel, and Hugh D. Robertson.
The Rockefeller University and the Cornell University Medical College, Center for Studies of the Biological Correlates of Addiction, New York, New York 10021, U.S.A.

The RNA to RNA rolling circle replication cycle of viroids and circular satellite RNAs requires an intact circular template. In many of the small infectious RNAs, maintenance of the circular template demands regulation of a potential self-cleavage structure. Appropriately timed conformational switching results in the refolding of the RNA needed to generate the active ribozyme. This process may depend upon the binding of host proteins. We previously described a potential ribozyme control element in the genomic RNA of the delta agent (Branch et al. Science: 243, 649), a small infectious circular RNA with many structural similarities to viroids and the circular satellite RNAs of certain plant viruses, and have now split the delta genomic RNA ribozyme domain into two parts. The two parts can be associated in vitro to give trans cleavage. The trans cleavage reaction is accelerated by the addition of a nuclear extract from tomato plants. We are characterizing the active host factor supplied by the extract of this viroid host plant.

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1142 POTYVIRAL ANTISENSE RNA OR COAT PROTEINS IN TRANSGENIC NICOTIANA BENTHAMIANA. John Hammond and Kathryn K. Kamo. USDA-ARS, FNCL, BARC, Beltsville, Maryland 20705

Agrobacterium-mediated transformation was used to introduce either the coat protein (CP) gene from bean yellow mosaic virus (BYMV), or an antisense construct derived from the C-terminal half of the CP gene and the 3' untranslated region, into Nicotiana benthamiana. One CP construct utilized the nos leader sequence, initiation codon and fourteen amino acids fused in-frame to the BYMV CP sequence, while the other gained a short leader and an initiation codon from an oligonucleotide. In addition two chimeric CP genes with the N-terminal portion of the BYMV gene and the C-terminal domains from pepper mottle virus (PeMV) and zucchini yellow mosaic virus (ZYMV) were separately used to produce transgenic plants. Regenerated plants were analyzed for the presence of the introduced sequences by PCR, and by indirect ELISA for CP expression where appropriate. R1 plants with either of the BYMV-CP constructs expressed up to 20% of the level of CP in a BYMV-infected plant, as estimated from indirect ELISA. Additional transformants were obtained in which no BYMV-CP was detected by ELISA in PCR-positive plants; possible reasons are being examined. Levels of protection against BYMV and heterologous potyviruses are being examined in homozygous R2 populations of both CP and antisense plants.

1143 ANALYSIS OF COAT PROTEIN-MEDIATED PROTECTION IN TRANSGENIC TOBACCO PLANTS WHICH EXPRESS ASSEMBLY DEFECTIVE COAT PROTEIN GENES OF TOBACCO MOSAIC VIRUS W. G. Clark and R.N. Beachy. Washington University, Department of Biology, St. Louis, MO 63130

During the last five years, our and other laboratories have effected virus resistance against a variety of plant viruses using the coat protein-mediated protection strategy. This strategy involves expressing the coat protein (CP) gene of a particular RNA plant virus in a plant to afford protection for that plant against subsequent infection by the particular virus or a related virus. To further characterize the molecular events of coat protein-mediated protection, mutant coat protein (CP) genes of the U1 strain of tobacco mosaic virus (TMV) were expressed in tobacco plants. The mutants were constructed using oligonucleotide mutagenesis and were based on previously isolated TMV coat protein mutants and X-ray fiber diffraction data. The mutants were designed to create CPs that would be unable to assemble on viral RNA, precluding the formation of virus rods. In this way we could determine if the assembly function of the CP was essential for virus protection. A CP mutant with changes at amino acid 28 and 95 was constructed along with single mutants with changes at each of these positions. Subsequently, these mutants were expressed in local lesion and systemic tobacco plant lines. Plant lines expressing the single point mutants exhibited protection against the U1 strain of TMV when compared with the the negative control line. Data will be presented showing how the protection observed in the plant lines expressing mutant CP genes compares with that in lines expressing the wild type gene. The data will be discussed with regard to structure-function relationships between the CP, viral disassembly and coat protein-mediated protection.

1144 STUDIES OF INTERFERENCE IN BROME MOSAIC VIRUS GIVE INSIGHT TO VIRAL REPLICATION. Clayton C. Huntley and Timothy C. Hall.

Texas A&M University, Department of Biology, College Station,Tx. 77843

Brome mosaic virus (BMV) is a well characterized tripartate (+)-strand RNA virus. RNAs 1 and 2 encode replicase subunits, while RNA-3 is dicistronic and gives rise to a coat protein mRNA via a subgenomically produced RNA-4. The subgenomic promoter region of RNA-3 has recently been shown to be the element primarily responsible for the 100:1 (+):(-) strand asymmetry characteristic of infections with RNAs 1+2+3. A transcriptional vector has been made for the production of an anti-sense RNA-4 including the subgenomic promoter region, but not containing the region complementary to the tRNA-like region common to the 3' region of each genomic RNA. Cotransfection of small amounts of this (-) RNA-4 with capped wild type RNAs 1+2 (each transcribed *in vitro*) to barley protoplasts decreased the replication of each RNA by 90% when compared with transfection using RNAs 1+2 alone. Similarly, accumulation of RNAs 1+2+3 in the presence of (-) RNA-4 was reduced by 85%. Because (-) RNA-4 has no regions in common with RNAs 1 and 2, anti-sense hybridization has been ruled out as a causative process for the observed interference with replication. Contribution of the subgenomic promoter region to interference will be discussed.

1145 STUDIES ON THE FUNCTION OF TWO TMV (TOBACCO MOSAIC VIRUS) ENCODED PROTEINS USING TISSUE SPECIFIC EXPRESSION IN TRANSGENIC TOBACCO PLANTS.

U. Reimann-Philipp, C Holt & R.N. Beachy. Dept. of Biology, Washington University, St. Louis, MO 63130, USA

Two promoter fragments with tissue specific activity have been used to control the expression of the TMV 30 kD "movement" protein (MP) and coat protein (CP) genes in transgenic tobacco plants cvs. Xanthi Sx (systemic symptoms upon TMV infection) and Xanthi NN (formation of local necrotic lesions). Expression in the epidermis of young leaves and living xylem cells (PAL-promoter from *Phaseolus vulgaris*) and in the phloem (rol c promoter from *Agrobacterium rhizogenes*) is demonstrated by staining for activity of the reporter gene GUS and tissue-print immunostaining.

The expression of TMV CP from the CaMV 35S promoter in transgenic tobacco confers protection against infection by TMV. This promoter drives strong constitutive expression in most tissues. Epidermis-specific CP expression results in a reduction of the number of local lesions in the transgenic NN host and in a reduced number of infected plants in the systemically susceptible host A delay in the formation of systemic symptoms has not yet been shown in plants expressing CP in the phloem. This suggests that the interference with the initial infection event is of major importance in CP-mediated protection.

Transgenic tobacco plants expressing the TMV MP from the 35S promoter can provide the MP function for defective TMV mutants. MP expression under the control of the PAL promoter is used to investigate whether TMV can spread between epidermal cells and whether this is sufficient to initiate the hypersensitive response of the NN host

1146 MUTATION IN THE 3' PSEUDOKNOT OF BMV RNA-3 INTERFERES WITH (+) STRAND ACCUMULATION

A. L. N. Rao and Timothy C. Hall. Department of Biology, Texas A&M University, College Station, Texas 77843-3258.

RNA pseudoknotting permits formation of the aminoacyl acceptor stem of the tRNA-like structure at the 3' end of the three genomic RNAs of brome mosaic virus (BMV). To establish the importance of the RNA pseudoknot, we have introduced mutations into this region and examined their effects on viral replication in barley protoplasts and plants RNA-3 mutant 5'+3' Psk, in which three base-pairs in the pseudoknot region are inverted, exhibited near wild-type levels of minus-strand synthesis and tyrosylation *in vitro* Northern hybridization analysis of progeny RNAs from barley protoplasts transfected with wild-type RNAs -1 and -2 and 5'+3'Psk RNA-3 revealed the almost complete absence of RNA-4, despite efficient generation of (-) RNA-3 *in vivo*. Furthermore, the 5'+3'Psk mutation also interfered with amplification and accumulation of progeny (+)-sense RNA-3 Inocula containing wild type RNAs -1 and -2 and 5'+3'Psk RNA-3 did not induce any local lesions on *Chenopodium hybridum* and failed to establish a systemic infection in barley plants The possible interaction between the 3' pseudoknot and the intercistronic region of BMV RNA-3 will be discussed

1147 PEPPER ISOLATES OF POTATO VIRUS Y AND THEIR INTERACTION WITH SUSCEPTIBLE AND RESISTANT HOSTS. M.J. Soto, R. Arroyo, N. Sánchez, P. Perez, A. Fereres, V. Torres and F. Ponz. CIT-INIA, Ctra La Coruña km. 7.5, 28040 Madrid, Spain

Potato virus Y, the type member of the potyvirus group, is an important pathogen of Solanaceous crops, such as potato, pepper, tomato or tobacco. The molecular and biological characterization of potato strains of the virus are far more advanced than for strains from other crops. We have undertaken the characterization of pepper isolates by analyzing their behaviour with monoclonal antibodies and by nucleotide sequence. The biological characterization has also included host range, cross protection, and transmission studies on the activity of aphid vectors and their propensity to transmit the virus. Pepper-infecting isolates have previously been classified into different pathotypes according to their ability to overcome certain resistance genes. Using several immunocytochemical techniques we have analyzed the interaction of different pathotypes of the virus with susceptible and resistant hosts, at the cellular level. Our results show that pepper plants that bear the y^a resistance gene allow replication of the virus in a few localized cells in the inoculated leaf, to which the viral infection is confined. Possible implications of this result will be discussed.

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1148 INTERACTIONS OF RNA VIRUSES AND VIROIDS WITH ARABIDOPSIS PLANTS. D. Martinez-Herrera, J. Romero, J.M. Martinez-Zapater and F. Ponz. CIT-INIA. Ctra de la Coruña Km 7,5. 28040-Madrid. Spain.

Opposite to the rapid progress in the molecular characterization of viral factors or viroidal domains involved in the induction of plant disease, there has been little success in the identification of host factors engaged in the interaction. Probably one of the major obstacles is the difficulty that plants commonly used as hosts in the laboratories show for genetical and molecular analysis. *Arabidopsis thaliana* is a crucifer plant whose special biological and genetical characteristics make it an excellent model system for the analysis of plant physiological functions difficult to study in other systems. We have identified several RNA viruses able to infect Arabidopsis. Using these viruses we are screening different Arabidopsis genotypes for variants in steps of the virus infection process. Another approach to identify variants in the evolution of viral disease is the screening of a chemically induced population searching for plants not showing symptoms after inoculation. No resistant mutant has been found. We are screening for variability in a transgenic Arabidopsis collection where some plant genes involved in the plant-virus interaction could have been damaged in the transformation process. Results in this direction will be shown. Our attempts to infect Arabidopsis with a viroid by means of mechanical inoculation were unsuccesful. Therefore we have introduced a PSTVd dimer cDNA in Arabidopsis by an Agrobacterium mediated tranformation. The viroid sequences were ligated in both orientations into the untranslated leader sequence of β-glucuronidase, and the hybrid gene was constitutively expresed from a CaMV 35S promoter. These RNA molecules, resembling intermediary molecules in a viroid replication cycle, are able in vitro to be translated yielding GUS protein. In Arabidopsis we have found differential GUS activities for different constructions, possibly due to some reaction of the plant cell recognizing the hybrid RNA as an intermediate in the viroid replication cycle. Possible implications will be discused.

1149 DUPLICATED SEQUENCE AT THE 3.-TERMINAL REGION OF BOTH GENOMIC RNAs OF CHERRY LEAFROLL VIRUS AND ITS USE IN PCR DETECTION OF THE VIRUS.

M.José Botia [1], Florentina Sanchez[1], Adib Rowhani[2], Danny Alexander[3], Angel Frias[1], George Bruening[2], and Fernando Ponz[1,2] 1- Dpto. Proteccion Vegetal. CIT-INIA. Apartado 8111. 28080 Madrid. SPAIN.2: Dpt. Plant Pathology, U.C. Davis, Davis, CA 95616. USA.3: Calgene Inc., 1920 Fifth St., Davis, CA 95616. USA.

Cherry Leaf Roll Virus (CLRV) is considered a member of the nepovirus group. The walnut strain of CLRV causes a lethal disease in walnuts known as blackline. We have found that a sequence of 1572 nucleotides at the 3' -terminal region of the viral genomic RNAs is present in both of them, showing >95% of identity. The sequence of this fragment shows that is a non-coding region immediately upstream the poly (A) track. The homology between the sequenced 3 end untranslated regions of the nepovirus group has been analyzed. The STAR program has been used to predict the secondary structure of the sequenced 3' end untranslated regions of the nepovirus group. Two oligonucleotides from this region were chosen for the detection of CLRV by reverse transcription and polymerase chain reaction amplification (RT-PCR). RT-PCR can detect CLRV in 50 µg of infected walnuts buds, even without previous RNA purification.

1150 MOLECULAR CLONING OF TURNIP MOSAIC POTYVIRUS GENOMIC RNA AND PROTEOLYTIC ACTIVITY OF THE NIa PROTEIN IN ESCHERICHIA COLI. Jean-François Laliberté, Olivier Nicolas and Anne Charbonneau. Institut Armand-Frappier, Centre de recherche en virologie, Ville de Laval, Québec, Canada, H7N 4Z3.

Turnip mosaic potyvirus (TuMV) genome is made up of one molecule of RNA of 10 000 nucleotides in length and codes for a single polyprotein. We have isolated a TuMV strain from a rutabaga from L'Assomption, Québec exhibiting severe mosaic symptoms and stunting. At present, 5.5 kb of the viral genome have been cloned using an adapted method based on the polymerisation chain reaction (PCR). The 3' NCR and the genes coding for the capsid, the nuclear inclusion a and b (NIa and NIb), the 6 kd and part of the cytoplasmic inclusion (CI) proteins were located by comparison with other potyviruses and animal viruses (e.g. picornaviruses). In the NIb gene, we have identified the regions coding for the amino acid sequences GNNSGQPSTVVNLVMx-xA and GDD which are conserved in proteins with replicase activity and, in the CI gene, the nucleotide triphosphate-binding motif (NTBM) which is associated with helicase activity. The specific cleavage sites of the NIa protease were identified and, interestingly, one site was found to diverge from the consensus sequence, a glutamic acid residue replacing the usual glutamine. The different cDNA fragments were assembled together and inserted in the pET11 plasmid and we have shown that the NIa protease is functional in E. coli, cleaving with a high efficiency at the CI-6 kd and the NIb-capsid junctions. We are continuing with the cloning of the entire genome and characterizing the different viral proteins.

1151 THE STUDIES ON RICE RAGGED STUNT VIRUS.

Gong Zuxun, Lu Huihua.
Shanghai Institute of Biochemistry, Academia Sinica.

Rice Ragged Stunt Virus (RRSV) was first reported by Hibino et al in 1977. As a unique member of the subgroup 3 of the family of Phytoreoviridae, it is of great interest to know whether it contains the RNA polymerase activity or not. We reported here that in an in vitro transcription system RRSV could direct the synthesis of an acid-insoluble material. This indicated that the virus might contain RNA polymerase activity, with an opumum range of activity at 30-40 C, maximal at 35 C. Divalent cations were essential for the activity too. We also showed that the 10 genomic ds-RNAs of RRSV could translate viral proteins efficiently in the in vitro translation system. By means of 1% agarose gel electrophoresis and hexadecyl-tri-methyl-ammonium-bromide mediated phase-transferring extraction, the viral genome could be divided into 5 fractions which could act as translation templates after being denatured by 0.5% hydromethylmercury. The genome coding assignment of the virus was elucidated by analysing the in vitro translation products of the viral ds-RNA fractions.

1152 EXPRESSION OF POTATO VIRUS Y PROTEASE IN ESCHERICHIA COLI.

Makoto Hidaka, Yukiko Yoshida, Haruhiko Masaki and Takeshi Uozumi. The University of Tokyo, Department of Biotechnology, Faculty of Agriculture, Bunkyo-ku, Tokyo, 113 Japan

The genome of Potato virus Y (PVY) is a 10-kb single-stranded RNA and it is supposed to be translated into a large polyprotein, which is then processed to various viral proteins by its own protease activity. We cloned a 5-kb cDNA of PVY genomic RNA. Based on its nucleotide sequence, we presumed that this cDNA encoded the C-terminal half of PVY polyprotein and that a 432-amino acid polypeptide portion of the polyprotein was the self-processing protease. To test the possibility, the cDNA was modified to express the protease-polymerase-coat protein (CP) portion of PVY polyprotein under the control of the lac promoter in E. coli. Western blotting analysis of the total proteins expressed in the E. coli using anti-PVY CP antibodies revealed the presence of a 32-kD protein, whose N-terminal amino acid sequence was identical to that of PVY CP. A large deletion in the polymerase-coding region of the cDNA did not affect the expression of 32-kD CP, whereas a deletion of 14 amino acids in the protease portion resulted in disappearance of the CP and appearance of a larger protein, corresponding to the non-processed polyprotein, which were detected by Western analysis. These results indicated that the expressed PVY polyprotein was processed correctly by its own protease activity in E. coli. Furthermore, the protease lost the activity by the site-directed point mutations of His234, Asp269 or Cys339 of the polypeptide. These three amino acids must constitute the catalytic triad of the PVY protease.

1153 GENETICALLY ENGINEERED RICE RESISTANT TO RICE STRIPE VIRUS, AN INSECT TRANSMITTED VIRUS. T.Hayakawa, Y.Zhu, K.Itoh, Y Kimura, T Izawa, K.Shimamoto, and S Toriyama Plantech Research Institute, 1000, Kamosnida, Midoriku,Yokohama,227 Japan -National Institute of Agro-Environmental Sciences, 1-1 Kannondai 3-chome, Tsukuba, 305, Japan

The coat protein (CP) gene of rice stripe virus, which is a type member of Tenuivirus, was cloned and sequenced, and introduced into japonica varieties of rice by electroporation of protoplasts. The resultant plants expressed the CP at high levels (up to 0.5% of total soluble protein) and exhibited a significant level of resistance to virus infection. The high level of the CP expression could be attributed to two factors: the presence of the first intron of castor bean catalase in the expression vector and of the leader sequence of the CP gene itself functioning like the Ω-seqence of tobacco mosaic virus. Seedlings derived from selfed progeny of the primary transformants also expressed the CP and showed virus resistance, indicating stable transmission of the CP gene to the next generation. These studies demonstrated that the CP mediated protection against virus infection can be extended to other cereals and to other viruses transmitted by insect vectors.

POSTER ABSTRACTS

1154 EXPRESSION OF THE TOBACCO VEIN MOTTLING VIRUS COAT PROTEIN (CP) AND CYLINDRICAL INCLUSION PROTEIN (CI) GENES IN TOBACCO. Indu B. Maiti[1], John Murphy[2], John G. Shaw[2], and Arthur G Hunt[1], Departments of Agronomy[1] and Plant Pathology[2], University of Kentucky, Lexington, KY 40546-0091

The tobacco vein mottling virus (TVMV) coat protein (CP) and cylindrical inclusion protein (CI) genes were introduced into tobacco (*Nicotiana tabacum* cv burley 21) under control of a modified CaMV 35S promoter and a polyadenylation signal from a pea *rbcS* gene. In one of the CP constructs, a 39 nt 5' untranslated leader sequence of alfalfa mosaic virus (AlMV) RNA 4 was inserted upstream from the TVMV-CP reading frame. More than 30 independent transgenic lines carrying each CP construction were generated. Western blot analysis showed that expression levels in these lines varied greatly, ranging from .01% to 0.15% of total soluble leaf protein. Expression levels in lines with the 5'AlMV leader-CP construct were not significantly different from levels in lines with the 35S[2] promoter-CP lines. Seedlings from self-fertilized transgenic plants were inoculated with tobacco etch virus (TEV) or TVMV and observed for developing disease symptoms. Some CP-containing lines were resistant to TEV. However, TEV-resistant lines were invariably normally sensitive to TVMV. Resistance did not seem to be correlated with expression levels, since low- and high-expressing lines had similar resistance characteristics. These studies corroborate previous observations made in our group (J. Murphy *et al.*, submitted) and suggest that the TVMV CP can be used to obtain protection against TEV, but not TVMV, in transgenic plants. In twelve independent lines carrying the CI gene, expression levels were found to vary greatly, with CI polypeptide levels ranging between 1 to 10% what is seen in TVMV infected plants. The responses of CI transgenic lines to TEV were indistinguishable from controls. However, some of the lines showed a degree of resistance to TVMV. These preliminary studies indicate that expression of CI sequences can affect the response of plants to potyviruses.

1155 FINE SCANNING DELETION ANALYSIS OF TOBACCO MOSAIC VIRUS MOVEMENT PROTEIN. Moshe Lapidot, Ron Gafny, and Roger N. Beachy. Washington University, Department of Biology, St.Louis, MO 63130.

The initial entry and replication of a plant virus in its host is followed by cell-to-cell movement of progeny virus into neighbouring healthy cells. The tobacco mosaic virus (TMV) 30 kDa movement protein (MP: 268 amino acids) is essential for virus cell-to-cell movement, and is therefore an important component in virulence and host range of TMV.

In order to identify and map structural and/or functionally important regions of the TMV-MP gene, fine structure deletion mutagenesis was carried out. This procedure involves the introduction of small deletions at intervals throughout the gene, i.e., at every 10 amino acids a deletion of 3 amino acids was made. Each mutated MP gene was cloned into an infectious full-length cDNA clone of TMV, and under the regulatory control of the bacteriophage T7 promoter, *in vitro* transcripts were produced. Xanthi and Xanthi-N/N tobacco plants, as well as transgenic tobacco plants expressing the MP gene, were inoculated with the different transcripts. The movement function as well as the intracellular localization of the mutated proteins was determined and compared with wild type MP. The results of these experiments and implications for function of the MP will be discussed.

1156 GENETIC AND BIOLOGICAL BASIS OF RESISTANCE TO MAIZE DWARF MOSAIC VIRUS (MDMV) IN MAIZE. Michael D. McMullen[1,2], Raymond Louie[1,3] and Richard C. Pratt[2]. Corn and Soybean Research Unit, USDA-Agricultural Research Service[1] and the Departments of Agronomy[2] and Plant Pathology[3], The Ohio State Univ., OARDC, Wooster, Ohio, 44691 USA.

We reported (*Mol. Pl-Microbe Inter.* 2:309-314) the identification of a gene, *Mdm1*, that controls resistance to MDMV-strain A in maize inbred Pa405. Restriction fragment length polymorphism analysis was used to map the locus for *Mdm1* to the short arm of maize chromosome 6. The genetic basis of resistance to MDMV-A was also examined in resistant maize inbreds B68, CI44, Ky61:2335, and Pa32. After inoculation with MDMV-A, the symptom responses of F_2 and backcross plants derived from crosses of these lines with Pa405 and the MDMV-A susceptible inbreds yM14 and H55 were consistent with the hypothesis that resistance to generalized mosaic symptoms was controlled by a single locus, that is allelic with *Mdm1* of Pa405. However, many of the F_2 and backcross plants that did not develop generalized mosaic symptoms exhibited delayed, limited virus symptoms (narrow chlorotic streaks on upper leaves). The number of plants exhibiting limited symptoms was dependent on the genotype of both the resistant and susceptible parental line. ELISA was used to determine the extent of virus replication within resistant and susceptible plants. MDMV-A replication occurred in the inoculated apical tips of leaves of both resistant and susceptible plants, however virus was only present in the uninoculated basal region of these same leaves of plants that lacked *Mdm1*. These results were consistent with the hypothesis that resistance to MDMV-A is due to a block in systemic virus movement.

1157 CROSS PROTECTION STUDIES USING PLANTS EXPRESSING ALFALFA MOSAIC VIRUS REPLICASE. Peter E.M. Taschner, Guido van Marle, and John F. Bol. Leiden University, Gorlaeus Laboratories, Einsteinweg 5, 2333 CC Leiden, The Netherlands.

Alfalfa mosaic virus (AlMV) is a plant virus with a tripartite genome consisting of 3 RNAs of plus strand polarity. Transgenic plants (P12 plants) expressing cDNA genes corresponding to RNAs 1 and 2 were susceptible to AlMV infection. The P12 plants replicate infectious transcripts made in vitro from cDNA3 of AlMV and they produce subgenomic messenger RNA4, indicating that they express the viral replicase encoded by cDNAs 1 and 2. P12 plants preinoculated with RNA3 transcripts of strain 425 were challenged with virulent strain YSMV. These P12 plants were resistant to YSMV infection, whereas buffer-inoculated plants were not. This resistance, which resembles classical cross protection and is called "semi-classical" cross protection, probably is the result of the production of coat protein at wild type level in the P12 plants. Also, only a single genome segment is replicated at wild type level. This places "semi-classical" cross protection between classical cross protection and coat protein mediated resistance. Semi-classical cross protection offers a new approach to the elucidation of the mechanisms of classical cross protection and coat protein mediated resistance: The cross protection ability of mutated RNA3 transcripts can rapidly be tested on P12 plants.

1158 TRANSCRIPT MAPPING OF ABUTILON MOSAIC VIRUS, A GEMINIVIRUS. Sabine Frischmuth, Thomas Frischmuth and Holger Jeske, Institut für Allgemeine Botanik, AMP, Universitat Hamburg, Ohnhorststr. 18, 2000 Hamburg 52, Germany

The transcripts of Abutilon mosaic virus, a geminivirus with a bipartite genome (DNA A and DNA B), were characterized by Northern blot hybridization, S1 nuclease assay, primer extension analysis, and sequencing the 3'termini of cDNA clones. It was shown that transcription is bidirectional and that the transcripts are polyadenylated. Two overlapping transcripts of 1.6 kb and 0.7 kb were mapped to the complementary strand of DNA A and two of 1.3 kb and 1.1 kb to the complementary strand of DNA B. One transcript of 0.9 kb was mapped to the viral sense of DNA A and one of 1.0 kb to the viral sense of DNA B. The complementary and viral mRNA overlap in each genomic DNA in termination regions rich in polyadenylation signals.

1159 AFRICAN CASSAVA MOSAIC VIRUS DI DNA INTERFERES WITH THE REPLICATION OF BOTH GENOMIC COMPONENTS. Thomas Fnschmuth and John Stanley, Department of Virus Research, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK

Natural infections of African cassava mosaic virus (ACMV), a geminivirus with a bipartite genome (DNA A and B) are known to be associated with low levels of defective interfering (DI) DNAs Recently it has been demonstrated that extrachromosomal copies of the DI DNA, mobilised and amplified from an integrated DI DNA dimer, can ameliorate ACMV symptoms in transformed *Nicotina benthamina*, providing a possible means for the control of cassava mosaic disease. To further understand the molecular basis of the interference phenomenon, we have compared the ability of ACMV and tomato golden mosaic virus (TGMV) genomic components to replicate in leaf discs derived from DI DNA-transformed and control plants. Results indicates that the ACMV DI DNA interferes with the replication of both genomic components of ACMV to a similar extent. TGMV DNA A replicates to normal levels in transformed leaf discs and plants because it is unable to mobilise and amplify ACMV DI DNA Differences in the relative levels of ACMV genomic components in transformed leaf discs and plants are discussed in terms of DNA replication and the availability of the genomic components for spread throughout the plant.

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1160 THE USE OF A MOLECULAR PROBE TO STUDY THE INTERACTION TYLCV WITH ITS VECTOR BEMISIA TABACI. Yeheskel Antignus, O. Adler and S. -Cohen, Dept. of Virol., Volcani Cen., Bet Dagan 50250, Israel

Tomato yellow leaf curl virus (TYLCV) was detected in its whitefly vector using the virus full-length c-DNA clone as a template for the production of a highly radioactive riboprobe. Acquistion of the virus by the vector and by a non vector whitefly, Trialeurodes vaporariorum was found to be in correlation with the length of the acquisition access period. Viral nucleic acids were detectable in the whitefly vector during a period of at least 16 days following an acquistion access of 48 h on TYLCV infected source plants. However, the maximum biological retention time of TYLCV in Bemisia tabaci is limited to about 14 days.

Using the membrane feeding procedure it was shown that the vector whitefly B. tabaci could acquire and transmit purified intact virions of TYLCV but failed to do so after acquisition feeding on UV irradiated virions, naked virion ssDNA or TYLCV cloned dsDNA. The implication of these findings on the virus transmission mechanism will be discussed.

1161 AGROINFECTION WITH A SINGLE DNA COMPONENT OF THE WHITEFLY-BORNE TOMATO YELLOW LEAF CURL GEMINI VIRUS. Yeheskel Antignus and S. Cohen, Dept. of Virol., Volcani Cen., Bet Dagan 50250, Israel

Tomato yellow leaf curl virus (TYLCV) single-stranded DNA served as a template for the synthesis of its double-stranded form in vitro. A viral DNA clone (ca. 2.8 kbp) hybridized to component A of bean golden mosaic virus but failed to hybridize to component B of this virus, indicating the possibility that it represents the putative component A of TYLCV. A head-to-tail construct of the 2.8 kbp component was mobilized into Agrobacterium tumefaciens which served to inoculate host plants of TYLCV. Systemic viral infection to tomato, Nicotiana benthamiana and Datura stramonium was accomplished by agroinoculation with the proposed A DNA. The resulting symptoms in the tested plants were similar to those obtained by whitefly infection of the native virus. The presence of the viral nucleic acid and the viral coat protein in the agroinfected plants was demonstrated by dot spot hybridization and immunoblotting respectively. Virions purified for agroinfected plants have the same shape and size as the native virus when analyzed by electron microscopy. Whiteflies were able to acquire the virus through membranes and to transmit it to healthy test plants. Our results indicate that the putative component - of TYLCV can support systemic infection of plants (via agroinoculation), yielding infective whitefly transmissible progeny virus.

1162 INFECTIOUS IN VITRO RNA TRANSCRIPTS DERIVED FROM CLONED cDNA OF ZUCCHINI YELLOW MOSAIC VIRUS. Amit Gal-On, Y. Antignus, A. Rosner and B. Raccah. Dept of Virology, Volcani Center, Israel

A full length cDNA clone of the cucurbit potyvirus zucchini yellow mosaic virus (ZYMV) RNA genome was constructed downstream from a bacteriophage T7 RNA polymerase promoter. An extra guanosine residues which are not originally present in the ZYMV RNA was added to both the 5' and 3' ends. Capped (m^7GpppG) ZYMV RNA transcripts were shown to be infectious in 10/91 of the Cucurbita pepo test plants. Uncapped RNA transcripts were not infectious. Symptoms appearance in plants inoculated with the infectious transcript were delayed by more than a week compared to plants inoculated by the native viral RNA. The progeny virions recovered from the infected plants maintained the same biological properties (non-aphid transmissibilty and typcial symptoms) as did the parental virus. It also reacted positively to ZYMV antiserum and to ZYMV specific probes by dot blot hybridization. The authenticity of the progeny virus was verified by identifying the specific molecular marker (C substituted for T at the 3' noncoding region) using sequence analysis.

1163 CLONING OF POPLAR MOSAIC CARLAVIRUS COAT PROTEIN GENE
Janey Henderson*+, Mary Lou Edwards+, Valerie Clarke and Jillian Cooper+

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The strategy of virus resistance associated with the expression of a carlavirus coat protein gene has been demonstrated in transgenic potatoes [MacKenzie et al., 1991].

Poplar mosaic virus, also a carlavirus, is ubiquitous in commercial stocks of poplar trees and cultivars, and diminishes tree growth by up to 30%. The genome composition and gene organisation of a representative poplar mosaic viral isolate is being determined to see whether the virus coat protein, expressed in cells of transgenic black poplar hybrids, protects trees from the damaging effects of this pathogen.

The virions contain a single species of ssRNA (c.6kbp) enveloped in a capsid of 36,000 daltons. A number of cDNA clones have been obtained encoding the poplar mosaic virus RNA. These range in size from 0.5kbp to 5kbp. Larger clones are being sequenced to identify the coat protein gene.

Reference*
D.J. MacKenzie, J.H. Tremaine and J.McPherson (1991)*
Genetically Engineered Resistance to Potato Virus S in Potato Cultivar Russet Burbank.
Molecular Plant-Microbe Interactions. Vol 4, No 1, pp 95-102.

1164 ENGINEERING RESISTANCE TO POTATO LEAFROLL VIRUS IN RUSSET BURBANK POTATO. Nilgun E. Tumer, Cliff Lawson, Cindy Hemenway, Jim Weiss, Wojciech Kaniewski, Debbie Nida, Jerry Anderson and Bernie Sammons. Monsanto Company, St. Louis, Missouri 63198

Potato leafroll virus (PLRV) infects potato plants world-wide and causes significant economic losses. Previous research has shown that expression of viral coat protein (CP) genes in transgenic plants protects these plants from infection by the virus from which the CP gene is isolated. We have cloned the CP gene of PLRV from a Washington State field isolate. This gene was modified to increase its expression, engineered into plant expression vectors and transformed into Russet Burbank potato. Expression of the CP gene was confirmed in transgenic potato. Plants were propagated from the primary transformant and challenged with PLRV using viruliferous aphids. The leaves were sampled at various times after inoculation and virus levels were determined by ELISA. Transgenic Russet Burbank showed significant resistance to PLRV compared to the controls.

1165 ENGINEERED RESISTANCE TO TOMATO SPOTTED WILT VIRUS, AN ENVELOPED, NEGATIVE STRAND RNA VIRUS. J.J.L. Gielen[1], A.J. Kool[1], P.T. de Haan[2], R.W. Goldbach[2] and M.Q.J.M. van Grinsven[1]. [1]Zaadunie B.V., P.O. Box 26, 1600 AA Enkhuizen, [2]Agricultural University, Dept. of Virology, P.O. Box 8046, 6700 EM Wageningen, The Netherlands.

Tomato spotted wilt virus (TSWV) causes serious diseases worldwide in many economically important crops and ornamentals. The host range of TSWV includes at least 370 plant species in 50 botanical families. Among plant viruses, TSWV is unique in its particle morphology and genome structure and thusfar TSWV is the only plant virus known to be exclusively transmitted by thrips. The viral genome consists of three single stranded RNA segments, denoted S RNA (2.9 kb), M RNA (ca. 5 kb) and L RNA (8.9 kb). The genomic RNAs are tightly associated with nucleocapsid protein to form nucleocapsids, which are surrounded by a lipoprotein envelope. Recently, the nucleotide sequence of the S and L RNA has been determined and the gene encoding the nucleocapsid protein identified. The nucleocapsid gene is located on the viral complementary strand of the S RNA. The L RNA harbours only one large open reading frame, also located on the viral complementary strand, thus illustrating the negative polarity of the genomic RNA segments. Based on its genome organization and its virion morphology TSWV is considered as a member of the Bunyaviridae, a large family of arthropod-borne animal viruses, being unique in its property to infect plants. For the moment limited progress has been made in identifying sources for natural resistance to TSWV Therefore, the nucleocapsid gene has been transformed to tobacco in order to obtain engineered resistance to TSWV infections. By means of ELISA and Western blot analysis nucleocapsid protein could be detected in transgenic tobacco, ranging in accumulation level to almost 2% of the total soluble leaf protein. Upon mechanical inoculation, progeny lines accumulating N protein showed a reduced susceptibility to TSWV infection. However, a correlation between the level of accumulation of the nucleocapsid protein and the level of resistance could not be observed. This summer the observed resistance to TSWV will be assessed under natural field conditions.

POSTER ABSTRACTS

1166 A CONDITIONAL LETHAL PHENOTYPE CAUSED IN N GENE TOBACCO (NICOTIANA TABACUM CV. XANTHI/NC) BY TRANSFECTION FROM CHROMOSOMALLY INTEGRATED TOBACCO MOSAIC VIRUS cDNA. Thomas H. Turpen[1,2], Ann M Turpen[1] and W. O. Dawson[2]. [1]Biosource Genetics Corp., 3333 Vaca Valley Pkwy., Vacaville, CA 95688 and [2]University of California, Dept. of Plant Pathology, Riverside CA 92521

Alleles of the N gene locus of the genus *Nicotiana* in combination with tobacco mosaic virus (TMV) comprise one of several model systems for the molecular analysis of symptom induction in plants. When leaf surfaces of N gene containing plants are mechanically inoculated with any viable strain or mutant of TMV characterized to date, either as virion particles or naked RNA, necrotic lesions form at sites of infection, localizing the virus to inoculated leaves. Whereas in plants containing the recessive allele n, systemic movement of wild type (wt) and many mutant strains of TMV is observed. We have engineered biologically active cDNA of wt and a coat protein minus (cp-) mutant of TMV into the transformation vector *Agrobacterium tumefaciens* for expression in plant cells. During regeneration of n gene tobacco (cv. Xanthi), wtTMV transfection events were frequent enough that the first true leaves of the developing plantlets had typical mosaic symptoms and contained wt virus in the primary transformants (T0 generation). The naked RNA virus was detected by Northern analysis in cp-TMV/n plants and was typically associated with a mild mosaic symptom distinct from that induced by wtTMV. In the absence of coat protein synthesis, a single viral transcript accumulated to ~5% of the total leaf mRNA. To introduce the N gene, selected individuals of the T1 generation were crossed with cv. Xanthi/nc. Observations of TMV induced necrosis in developing F1 progeny indicate that all phases of plant development are competent for TMV replication and N gene function including tissues of the juvenile and adult leaves, the calyx, pericarp and corolla. In the earliest case observed all individuals of the genotype cp-TMV/N became necrotic as 2-8 week seedlings. Because the N gene of cultivar Xanthi/nc is temperature sensitive, individuals with this genotype can be rescued by culture at the permissive temperature (>30°C).

1167 AGROBACTERIUM-MEDIATED TRANSFORMATION OF PLUM (PRUNUS DOMESTICA L.) WITH THE COAT PROTEIN GENE OF PAPAYA RINGSPOT VIRUS

R. Scorza[1], J.M. Cordts[1], S. Mante[2], D. Gonsalves[3], V.D. Damsteegt[4], L. Lepes[4] and J.L. Slightom[5]
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The potential for papaya ringspot virus (PRV) coat protein-mediated cross protection of plum against plumpox virus (PPV), a major plum pathogen, is being tested. Both PRV and PPV are potyviruses. The transformation system utilized hypocotyl slices from ungerminated plum seeds. These were co-cultivated with A. tumefaciens EHA101 (Hood et al., 1986. J. Bact. 168:1291-1301) containing the plasmid pGA482GG/cpPRV-4. This plasmid carries the papaya ringspot virus (PRV) coat protein gene construct and chimeric NPTII and GUS genes (Fitch et al., 1990, Plant Cell Repts. 9:189-194). Shoots were regenerated from hypocotyl slices cultured on shoot regeneration medium consisting of Murashige and Skoog (MS) salts, vitamins, 2% sucrose, 2.5 µM indolebutyric acid (IBA), 7.5 µM thidiazuron, and appropriate antibiotics for selection. Regenerated plants tested positive for both NPTII and GUS activity. Polymerase chain reaction (PCR) amplification identified the presence of the PRV coat protein and NPTII genes in DNA isolated from young leaves. Southern blot analysis is being used to obtain information concerning the organization of the PRV cp gene in transgenic clones. The reaction of transgenic plants to plum pox virus is under test.

1168 CLONAL LINES WITH HIGH COPY NUMBER OF REPLICATING RECOMBINANT TGMV VECTORS IN *N TABACUM*. Ivan E. Kancevski, Sanjay Thakur, Laurey Cosowski, *Garry Sunter, *Clare Brough, *David Bisaro and Pal Maliga. Waksman Institute, Rutgers University, Piscataway, NJ 08855 and *Biotechnology Center, Ohio State University, Columbus, OH 43210

The replicating vectors are based on a tomato golden mosaic virus (TGMV) A genome in which the coat protein sequence was replaced by the bacterial neomycin phosphotransferase type II (NPTII) enzyme coding region (pTGA32), or by a chimeric gene in which the NPTII enzyme is under the control of the *Agrobacterium* nopaline synthase (nos) gene promoter (pST31). 1.5 mers of the recombinant TGMV A derivatives were cloned in plasmid pUC118. Reconstitution in protoplasts of a unit-length recombinant TGMV A genome was about as efficient as of a wild type TGMV A genome from plasmid pTGA26. *N tabacum* suspension culture cells were bombarded with the recombinant TGMV A constructs, and selected for kanamycin resistance conferred by the expression of the NPTII enzyme. The average number of kanamycin resistant clones per bombardment was the same when using the recombinant TGMV A vectors, or a non-replicating control plasmid. Replicating recombinant TGMV A derivatives were found in about 10% of the kanamycin resistant clones as the result of the reconstitution of a unit-length recombinant TGMV A genome. The highest number of copies was 1,000 and 100 per diploid nucleus after bombardment with pST31 and pTGA32 constructs, respectively. Lack of increase in the number of kanamycin resistant clones after biolistic transformation in spite of the efficient reconstitution of the replicating viral genome in the short-term protoplast system suggests that the replicating TGMV A genomes in the kanamycin resistant lines derive from an integrated copy.

1169 COAT PROTEIN MEDIATED PROTECTION FOR GRAPEVINE CHROME MOSAIC NEPOVIRUS (GCMV). T. CANDRESSE, V. BRAULT, R.P. DELBOS, M. LANNEAU, L. HIBRAND, O. LE GALL and J. DUNEZ, Station de Pathologie Végétale, INRA, BP 81, 33883 Villenave d'Ornon, France.

The coat protein (CP) gene of GCMV has been mapped to the 3' terminal third of genomic RNA2. The CP is freed from RNA2 polyprotein by cleavage at an R/A dipeptide. An hybrid gene with the CP coding sequence downstream of an AUG codon and of TMV genomic RNA leader (Ω') has been constructed and its expression confirmed by *in vitro* translation of RNAs transcribed from the DNA construct. This gene has been transferred in both orientations and under the control of CaMV 35S promoter in the genome of two species of tobacco. High-level expression of the transgene has been detected in *N. tabacum* transformants. Inoculation experiments revealed that CP expressing F1 progenies are protected against infection by GCMV particles but that at best only marginal protection is observed against tomato black ring virus (TBRV), a closely related nepovirus against which GCMV shows a natural cross protection phenomenon. No protection has been observed so far in plants transformed to express the CP gene in antisense orientation. No GCMV empty particles (top component) or transcapsidation of TBRV RNAs by GCMV coat protein could be detected in CP transgenics. Work is in progress to obtain transgenics expressing TBRV CP as well as other RNA2 encoded proteins.

1170 TRANSIENT EXPRESSION OF HETEROLOGOUS CAPSID PROTEINS IN A TMV-DERIVED VECTOR: A TEST FOR TRANS-ENCAPSIDATION AND SYSTEMIC SPREAD. Carlos A. Malpica, Wei Liu, Curtis A. Holt, Roger N. Beachy. Washington University. Department of Biology. Campus Box 1137. St. Louis MO 63130, U.S.A.

cDNAs encoding the coat proteins (CP) of sweet potato feathery mottle potyvirus (SPFMV) and cucumber mosaic cucumovirus (CMV) were engineered to introduce plant consensus initiation sequences and, in the case of SPFMV, a consensus stop context. The modified CP genes were subsequently introduced into an infectious cDNA clone of tobacco mosaic tobamovirus in place of either the TMV movement protein (MP) gene or the CP gene.

Transcripts derived *in vitro* from the four constructs are infectious when inoculated onto transgenic tobacco (*N. tabacum* cvs. Xanthi and Xanthi NN) plants that express a TMV MP gene. CP accumulation was detected by western blot analysis of infected tissue. To date, however, we have not recovered infectious particles, or detected systemic spread of the virus in which TMV-CP was replaced by the heterologous CP.

1171 IDENTIFICATION OF VIROID BINDING PROTEINS. Anastasia Prombona, Martin Tabler and Mina Tsagris. Institute of Molecular Biology and Biotechnology, P.O.Box 1527, GR-71110 Heraklion/Crete GREECE.

Viroids are autonomously replicating subviral pathogens that are found in certain higher plants. Their genome consists of a single-stranded, covalently closed circular RNA molecule of only 246-375 nucleotides. There is no indication that viroid RNA codes for a peptide and, therefore, viroids are believed to express their genetic function, like replicability, host specificity or pathogenicity by interacting of the viroid RNA with pre-existing or induced factors of the host. Since little is known about the nature of viroid-interacting proteins, we have modified a technique that had been developed for DNA binding proteins [Vinson et al., (1988), Genes & Dev.,2, 801]. In order to isolate some viroid interacting host factors we screened a cDNA expression library from tomato using a labeled probe of potato spindle tuber viroid (PSTVd). Several clones that express a protein which binds to PSTVd RNA have been identified. The corresponding clones are currently analyzed.

POSTER ABSTRACTS

1172 BEET PSEUDO YELLOWS VIRUS AN INITIAL MOLECULAR CHARACTERISATION Robert S Coffin and Robert H A. Coutts Biology Department, Imperial College, London, United Kingdom

Beet pseudo yellows virus (BPYV) is the first whitefly transmitted infectious agent to be identified in Europe where it causes severe economic losses to greenhouse growers of cucurbits and other similar crops It has been tentatively identified as a closterovirus due to it's mode of transmission and particle morphology We have identified a single species of dsRNA in infected plants, assumed to be the RF, purified it to homogeneity and produced a series of virus specific cDNA clones for diagnosis by hybridisation and PCR Sequence analysis has allowed identification of the coat protein gene and production of a synthetic anti-serum, while a novel PCR has allowed the generation of clones to the 3' and 5' genomic termini and thus the sequence of terminal ORFs Sequence obtained has shown no homology with beet yellows virus or apple chlorotic leafspot virus, the only two closter0-grouped viruses for which whole or partial sequence is available, and no significant homology with any sequences stored in the GEN-EMBL database

1173 GENETIC ANALYSIS OF BEET CURLY TOP VIRUS. Sheriar G. Hormuzdi and David M. Bisaro. Biotechnology Center and Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210.

Beet curly top virus (BCTV, strain Logan) is a monopartite geminivirus transmitted by the the beet leafhopper, *Circulifer tenellis*. The DNA genome of BCTV has three complementary sense ORFs (L1, L2 and L3) that are conserved with respect to size, sequence and relative location with the complementary sense ORFs of the A components of bipartite geminiviruses. However, like monopartite geminiviruses that infect monocots, it has multiple viral sense ORFs (R1 and R2; R1 encodes the coat protein). In bipartite viruses, genes necessary for systemic virus spread are located on the B genome component. In order to identify genes required for spread of the monopartite BCTV, we have initiated mutational analysis of BCTV ORFs. Preliminary experiments have shown that genomes containing mutations in ORF R1 (29.6 kD) or ORF R2 (12.1 kD) are not infectious, although both types of mutants are capable of replicating DNA in tobacco protoplasts. The R1 and R2 mutants accumulate reduced amounts of ssDNA relative to wild type controls. Surprisingly, a mutant containing a mutation in a third viral sense ORF (R3, which can encode a protein of 10.1 kD) produces wild type levels of viral ssDNA in protoplasts but also is not infectious. Western blot analysis suggests that mutations in R2 and R3 do not hinder the expression of capsid protein. Further studies of these and the complementary sense BCTV ORFs L2 and L3 are in progress.

1174 EVIDENCE FOR ROLLING CIRCLE REPLICATION OF GEMINIVIRUS DNA. Drake C. Stenger, Gwen N. Revington, Matthew C. Stevenson, and David M. Bisaro. Biotechnology Center and Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210.

Agroinoculation of *Nicotiana benthamiana* plants with Ti plasmids bearing tandem genome repeats derived from different, distinguishable strains of the geminivirus beet curly top virus (BCTV) resulted in the production of unit-length, recombinant progeny genomes in systemically infected plants. When two putative plus strand origins of replication were present in constructs used as inocula, a replicational release mechanism was favored which gave rise to progeny genomes of a single predominant genotype. Sequencing across the junction between parental strains in the recombinant progeny allowed mapping of the plus strand origin of replication to a 20 bp sequence containing the conserved geminivirus hairpin. The conserved hairpin has previously been identified as an essential *cis*-element required for viral DNA synthesis, and the replication of DNA sequences located between two plus strand origins present in the same molecule is an established property of rolling circle replication. In contrast, several progeny genotypes were observed when the inoculum contained a partial tandem repeat with only a single conserved hairpin, a result expected if progeny genomes were generated by random intramolecular recombination events. In another study, we have found that inversion of a tomato golden mosaic virus (TGMV) DNA fragment that contains the conserved hairpin results in the accumulation of free complementary sense single-stranded DNA, rather than the viral sense which accumulates in wild-type infections. These data and other considerations suggest that geminivirus DNA replication occurs by a rolling circle mechanism.

1175 TOMATO GOLDEN MOSAIC VIRUS GENE FUNCTION. Garry Sunter, Marcos D. Hartitz, Sheriar G. Hormuzdi, Clare L. Brough, and David M. Bisaro. Biotechnology Center and Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210.

Tomato golden mosaic virus (TGMV) is a whitefly-borne agent belonging to the geminivirus group. The viral genome is divided between two DNA components, designated A and B, which are both required for infectivity. We have previously shown that only one of the six viral open reading frames, namely AL1, is absolutely required for viral DNA synthesis. Mutants carrying lesions within the remaining TGMV open reading frames (ORFs) have been constructed, and their ability to replicate in protoplasts have been examined. We have found that coat protein (AR1) deletion mutants, which are capable of infecting plants, accumulate reduced amounts of ssDNA. AL2 mutants display a similar phenotype in protoplasts, although AL2 mutants are not infectious. Further analysis has revealed that the AL2 gene product transactivates expression of the coat protein gene (AR1) and is also required for expression of BR1, a gene necessary for movement of the virus between cells. Both the AR1 and BR1 proteins appear to expressed late in the multiplication cycle. AL3 mutants, which produce attenuated infections, accumulate reduced amounts of all viral DNA forms. In contrast, mutations in B component ORFs (BL1 and BR1) have no effect on the synthesis or accumulation of viral ssDNA or dsDNA, although these mutants cannot infect plants. Therefore, BR1 and BL1 proteins appear to be directly involved in spread of the virus through the infected plant, while the role of AL2 in movement is indirect.

1176 EVOLUTION OF NUCLEOTIDE SEQUENCES OF CAULIFLOWER MOSAIC VIRUS ISOLATES. Kelly D. Hooper and Ulrich Melcher Oklahoma State University, Department of Biochemistry, Stillwater, Oklahoma 74078 USA Cauliflower mosaic virus (CaMV) is the type member of the caulimoviruses, a group of plant viruses with double-stranded DNA as their genetic material. Many isolates are known. The isolates vary in the symptoms they induce in host plants, in the range of plants that are hosts, and in their ability to compete with one another. We approach an understanding of the evolution of viral isolates by nucleotide sequence analysis. Complete or partial nucleotide sequences are available for several isolates. We are determining the complete sequences of three additional isolates: NY8153, CMV-1, and BBC. BBC is an isolate recovered from Pak Choi plants in California. We will use the sequences to derive a CaMV DNA consensus sequence. The consensus should serve as reference for testing evolutionary hypothesis. The DNAs of sequenced isolates have diverged at about 5% of the positions Most of the deviations from the consensus sequence are unique to single isolates Recombination between DNAs of isolates, intentionally co-inoculated, occurs The distribution of informative sequence alleles among the natural isolates will be examined to test whether recombination played a role in the evolution of the isolates. Research supported by the Rapp Foundation, the Oklahoma Research Program, and the Oklahoma Agricultural Experiment Station.

1177 ACID PROTEINASES AND A STRUCTURAL MODEL OF VIRAL MOVEMENT PROTEINS. Ulrich Melcher, Department of Biochemistry Oklahoma State University, Stillwater, Oklahoma 74078 USA

Intercellular spread of viral infections requires proteins encoded by the genomes of plant viruses. For some viruses, these proteins, dubbed "movement proteins, locate near plasmodesmata.. The amino acid sequences of the proteins are diverse, but significant relationships between proteins of different viral groups exist. The relationships permitted alignment of amino acid sequences The alignment identified a leu.x.asp motif and a gly as almost invariant. Invariant leu.x.asp and gly positions are also features of aspartic acid proteinases. Anchoring alignment of lentiviral aspartic acid proteinases with movement proteins at the almost invariant positions produced a significant alignment. The alignment required only three insertions/deletions, whereas alignment of lentiviral with oncoviral proteinases required five. The sequence similarity led to the prediction of a three-dimensional structure of the "movement proteins.' In this structure, a dimeric 'movement' protein has a protein binding domain on one side and a nucleic acid binding region made of N- and C-terminal domains on the opposite surface. The model was consistent with several experimental observations. The sequence similarity also led to a hypothesis for the evolutionary origin for 'movement protein genes in the proteinases encoded by retroid elements of host genomes. Research supported by the Oklahoma Health Research Program and the Oklahoma Agricultural Experiment Station.

POSTER ABSTRACTS

1178 A TOBAMOVIRUS CAUSES SYMPTOMS IN TURNIP AND TOBACCO. R. T. Lartey, S. Hartson, R. Pennington and Ulrich Melcher. Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma 74078 USA We have identified a tobamovirus replicating in turnip plants Two preparations, one of cauliflower mosaic virions, and the other a turnip mosaic virus infected leaf contained a contaminating virus. The two contaminants are the same strain or very closely related strains. The contamination was identified by the presence of an unexpected nucleic acid species in agarose gel electrophoresis. The nucleic acid species was a single-stranded RNA, 6.4 knt in length. The virion coat protein of 17kd (determined by SDS-PAGE) was just slightly smaller than common strain TMV coat protein. Electron micrographs of the preparations showed rod-shaped viral particles which in dimension and appearance were similar to TMV. The virion preparation reacted (partial identity) in Ouchterlony double diffusion with an antiserum against the common strain of TMV. Hybridization probes made from one of the suspect strains hybridized at high stringency with RNA of the other strain on northern blots. The unknown virus replicated in high levels in turnips but caused only mild vein clearing on late emerging leaves. The strain caused typical TMV-mosaic on tobacco Partial nucleotide sequences analysis of cDNA clones revealed that the virus contained a tobamoviral RNA not previously sequenced. Future experiments will determine what features of this tobamovirus allow replication in turnips. Research was supported by the Oklahoma Health Research Program and the Oklahoma Agricultural Experiment Station

1179 MOLECULAR BIOLOGY OF THE BADNAVIRUSES. Neil Olszewski, Mohammed Bouhida, Ben Lockhart and Scott Medberry. Departments of Plant Biology and Plant Pathology, University of Minnesota, St. Paul, MN, 55108

The badnaviruses contain a circular, doubled-stranded, 7.5 to 8.0 kb DNA genome. We have begun a molecular characterization of three of these viruses: commelina yellow mottle virus (CoYMV),sugarcane bacilliform virus (ScBV) and kalanchoe top spot virus (KTSV). CoYMV and KTSV each encode a single transcript that is greater than genome length. Mapping experiments indicate that the CoYMV transcript is approximately genome length plus 120 bp The sequences of an infectious CoYMV genome and a ScBV genome have been determined. The transcript of both viruses encodes, in a polycistronic manner, three polypeptides of approximately 23, 15 and 216 kd. In both viruses, the ORFs encoding these proteins are arranged similarly. Similarities between the polypeptide encoded by the 216 kd ORF and CaMV ORFs IV and V suggest that this ORF encodes a polyprotein consisting of the a protein of unknown function, the coat protein, a protease that processes the polyprotein and the viral replicase (reverse transcriptase/RNase H). These observations, together with the presence of a $tRNA^{met}$ binding site located near the 5'-end of the transcript of all three viruses, suggest that these viruses are pararetroviruses. The nature of the proteins encoded by the other ORFs is unclear. We attempting to ascertain the function of these proteins by mutational analysis and by determining their subcellular localization.

1180 IN VITRO TRANSLATION OF RICE YELLOW MOTTLE VIRUS RNA. Martin Ngon a Yassi, Christophe Ritzenthaler, Claude Fauquet and Roger N. Beachy. Washington University, Department of Biology, One Brookings Drive, CB 1137, St Louis, MO 63130

Rice yellow mottle virus (RYMV) has been proposed as a member of the sobemovirus group and is recognized as an important endemic rice viral disease in Africa. RNA encapsidated in particles of RYMV was used as template for proteins synthesis in mRNA-dependant rabbit reticulocyte lysates or wheat germ translation systems following dialysis of virions against mildly alkaline buffer. Purified genomic RNA was also used as template. Five principal products were synthesized, with estimated molecular weights at 105, 68, 29, 17.5 and 13 kD. Time course experiments showed that 13 and 17.5 kD proteins appear first, followed by 68 kD and then 105 kD polypeptides The 13 and 17.5 kD proteins were immuno-precipitated by the antibodies raised against RYMV. The 29 kD protein (putative coat protein) is synthesized *in vitro* in small amounts perhaps due to the coat protein being synthesized *in vivo* via subgenomic RNA. These results are similar to the characteristics of the members of the sobemovirus group. Further work in RNA sequencing and genome analysis will help us to understand better the genomic organization of RYMV RNA and confirm its classification in the sobemovirus group.

1181 TRANSGENIC PLANTS THAT EXPRESS THE COAT PROTEIN OF PLUM POX POTYVIRUS. Marie Monsion, Michel Ravelonandro, Pierre-Yves Teycheney, René Delbos, Ann Callahan*, John Cordt*, Ralph Scorza*, Dennis Gonsalves** and Jean Dunez. Station de Pathologie Végétale, INRA, Centre de Recherches de Bordeaux, BP 81, 33883 Villenave d'Ornon, France. * Appalachian Fruit Research Station, USDA, Kearneysville, West-Virginia. **Cornell University, Department of Plant Pathology, Geneva, New York-State. The cDNA encoding the coat protein cistron of plum pox virus (PPV) was engineered with an ATG codon and inserted into plant expression vector, pBI121. The recombinant plasmid was then introduced into *Agrobacterium tumefaciens* and leaf discs of tobaccos: *Nicotiana xanthii*, *benthamiana* and *clevelandii* were infected with it and regenerated into plant. Genomic southern analysis confirmed the presence of coat protein gene in DNA from different transgenic plants. A protein band with similar molecular weight to that of PPV coat protein was detected in leaf extracts of transformed plants by western-blot analysis with the anti-PPV antibody. Transformed plants with high levels of PPV coat protein expression (0.2% of the total leaf soluble proteins) showed significant delay in symptom expression after inoculation with Potato virus Y (PVY) and PPV. These data support the opportunity to transfer PPV coat protein in woody plants.

1182 GENOMIC ORGANIZATION OF APPLE CHLOROTIC LEAF SPOT CLOSTEROVIRUS. S. GERMAN, T. CANDRESSE, M. LANNEAU and J. DUNEZ, Station de Pathologie Végétale, INRA, BP 81, 33883 Villenave d'Ornon, FRANCE.

Apple chlorotic leaf spot closterovirus (ACLSV) is known to infect most fruit tree species. It is responsible for serious diseases in stone fruits and induces severe graft incompatibilities in some *Prunus* combinations, causing economically important problems in nurseries.
ACLSV has an elongated, flexuous particle of 720 X 12 nm, encapsidating a single-stranded, positive sense genomic RNA of 2.5 x 10^6 Da. The complete 7555-nucleotide long sequence of ACLSV genomic RNA has been determined. ACLSV RNA is polyadenylated and harbors three overlapping open reading frames coding for proteins of 216, 50 and 28 kDa. The 216 kDa protein has significant homology (helicase and polymerase signature sequences) with the replication-associated proteins of other "alpha-like" RNA viruses, specially potex- and carlaviruses. Translation of RNA transcripts obtained from cDNA clones shows that the 22 kDa coat protein is produced by initiation on the second ATG codon of the 28 kDa ORF. Analysis of the viral dsRNAs shows that in addition to molecules corresponding to the full-length genome and to putative subgenomic RNAs for the 50 and 28 kDa ORFs, two major species, 5' co-terminal with the genomic RNA, are found. A model for the production of these unusual molecules will be presented.

1183 TRANSFORMATION OF NICOTIANA BENTHAMIANA PLANTS WITH FULL-LENGTH cDNA TO CYMBIDIUM RINGSPOT VIRUS SATELLITE RNA. L. Rubino[1], J.C. Carrington[2] and M. Russo[1,2]. [1]Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Bari, Italy and [2]Department of Biology, Texas A&M University, College Station, Texas, USA.

A full-length DNA copy of CymRSV satellite RNA was cloned downstream of the phage T7 promoter. In vitro transcripts had no extra nucleotides at the 5' end and 7 extra nucleotides at the 3' end. Transcripts were replicated in Nicotiana benthamiana and N. clevelandii plants upon inoculation either with the helper virus or in vitro transcripts of CymRSV genomic RNA. RNA sequencing of the 3' end of the satellite progeny indicated that this end was restored. Test plants inoculated with CymRSV in the presence of in vitro transcripts of satellite RNA clearly showed symptom attenuation as compared with infection with CymRSV alone. Full-length clones of CymRSV satellite RNA under control of the CaMV 35S promoter and terminated by a poly(A) signal were inserted in the binary vector pGA482. Plants were transformed by the leaf disk transformation method. Rooted plants were tested for the presence of CymRSV satellite sequence in DNA extracts amplified by PCR, and in RNA preparations for the presence of RNA transcripts of the predicted size. A number of transformed plants were transferred to soil, leaves were detached, inoculated with CymRSV and mantained in moist chambers. After 7 days Northern blot analysis of RNA extracted from such leaves showed that satellite RNA of the same size as the natural molecule was actively replicated only in transformed plants with integrated satellite sequence.

POSTER ABSTRACTS

1184 CORRELATION OF HOST RESISTANCE PATTERNS WITH SEQUENCE-BASED PHYLOGENIES IN THE POTYVIRIDAE. Molly Kyle and E.P. Rybicki
Dept. of Plant Breeding, Cornell University, Ithaca, NY 14853, and Dept. of Microbiology, University of Cape Town, PB Rondebosch 7700, South Africa.

Determination of relationships among potyviruses has been problematic, despite accumulation of partial nucleotide sequence data from a number of group members. The biological relevance of relationships based solely upon sequence comparisons has been unclear. We present evidence that a phylogeny based on coat protein sequence correlates well with groupings based on resistance in a number of plant species to infection by various potyviruses. Genes and gene clusters in host species have been identified that confer resistance to two or more clearly distinct potyviruses. The genetic basis for simply inherited multiple virus resistance may be a single allele, an allelic series at a single locus, or a tightly linked series of loci that show identical inheritance and phenotypically similar resistance, each to a distinct virus or viral pathotype. The *resistance spectrum* is defined as the set of potyviruses whose pathogenic interaction with the host is determined by that gene or gene cluster. One measure of relationship between viral isolates is a function of how much divergence has occurred in the host. We assume that a set defined by a single host allele is more closely related than if defined by a series of linked distinct genes. Comparisons of resistance spectra compiled from a number of host species delineate overlapping sets of viruses. The various resistance genes from diverse host species clearly have completely different genetic bases and different mechanisms of action. Nevertheless, consensus related groupings of viruses emerge. There is a striking correlation between groups determined by host genes, and the most parsimonious phylogenetic trees calculated from the aligned sequences of potyviral coat proteins. Based on this correlation, predictions of relationships based on host reactions and sequence similarity can be generated and tested.

1185 DELETION OF THE RED CLOVER NECROTIC MOSAIC VIRUS CAPSID PROTEIN IMPAIRS SYSTEMIC INFECTION AND ALTERS SYMPTOMATOLOGY Z Xiong, and S A Lommel. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695

The genome of the red clover necrotic mosaic dianthovirus (RCNMV) consists of two non-homologous RNAs. RNA-1 encodes replicase and a 37 kDa capsid protein (CP), and RNA-2 encodes a 35 kDa cell-to-cell movement protein. A series of deletion and insertion mutations were constructed within the capsid protein gene. RNA-1 infectious transcripts containing capsid protein mutations were inoculated along with RNA-2 transcripts onto the systemic host *Nicotiana benthamiana*. The capsid protein deletion mutants replicated as free RNA and moved cell-to-cell throughout the inoculated leaves. All deletions within the capsid protein cistron rendered the capsid protein unstable and undetectable *in vivo*. Systemic infection also occurred but was delayed and limited to a few adjacent leaves, forming an unusual necrotic "oak-leaf" pattern symptom. These data suggest that capsid protein contains determinants for viral long distant transport and symptoms in a systemic host. The ability of the mutant virus to spread within the inoculated leaves indicates that capsid protein is not required for cell-to-cell movement. We speculate that the limited systemic infection in adjacent non-inoculated leaves occurred as a result of cell-to-cell movement through stem tissue.

1186 TRANSFORMATION OF PLANT CELLS USING INSECT PICORNAVIRUS CAPSID SEQUENCES. Susan A Marlow, Jason M Pickering, Catherine L Trapnell and Linda A King, School of Biological and Molecular Sciences, Oxford Polytechnic, Gipsy Lane, Headington, Oxford, OX3 0BP.

Growing concern about the use of chemical insecticides has lead to increased interest in the development of biological control agents. One area which has received attention is the use of invertebrate specific viruses, in particular the baculoviruses to control insect pests such as pine beauty moth, a pest of lodge pole pine. Other potential candidates for use as biological control agents are the entomopoxviruses and the insect picornaviruses. The current work uses Cricket paralysis virus (CrPV), an insect picornavirus (8kb; single stranded RNA). With recent advances in plant molecular biology, alternative methods of integrated plant protection against pests are emerging. We are investigating the use of transgenic plants as a delivery system for insect specific viruses.
Initial experiments will assess the expression of coat protein sequences of CrPV cDNA in plant cells. CrPV cDNA has been prepared and the sequence is currently being completed. The sequence coding for CrPV coat protein will be identified and cloned into a binary expression vector (pROK1 kindly given by Dr J I Cooper, IOV Oxford). Two transformation systems have been established, a transient protoplast system using Nicotiana tabacum and Agrobacterium mediated transformation of Brassica species using cotyledon and stem explants.

1187 TWO PROTEINS ENCODED BY BNYVV RNA 3 INFLUENCE SYMPTOM PHENOTYPE Isabelle JUPIN*, H. GUILLEY, K. RICHARDS & G. JONARD
Institut de Biologie Moléculaire des Plantes, CNRS, 12 rue du Général Zimmer, 67084 Strasbourg, France. * present address : Institut des Sciences Vegetales, CNRS, Avenue de la Terrasse, 91198 Gif sur Yvette, France.

Beet necrotic yellow vein virus (BNYVV) causative agent of sugarbeet rhizomania has a multipartite genome consisting of four single-strand plus-strand RNA. RNA 1 and 2 are necessary for replication and packaging of the virus, while RNA 3 and 4 are involved in the transmission of the virus to sugarbeet roots by its fungal vector. RNA 3 of BNYVV is not essential for virus multiplication on leaves of local lesions host such as *Tetragonia expansa* but has striking effects on symptom expression. Virus isolates containingRNA 3 produce bright yellow local lesions while isolates from which RNA 3 is missing produce much milder symptoms. Using directed mutagenesis of RNA 3 cDNA clones followed by *in vitro* synthesis of biologically active transcripts we determined that a 25 kD open reading frame was responsible for the yellow local lesion phenotype. In addition, we observed that deletion mutants of RNA 3 elicit the appearance of severe necrotic local lesions. Analysis of one of these mutants revealed that necrosis was due to the overexpression of a second ORF, N, overlapping the 3'terminal portion of the 25 kD ORF. As shown by gene fusion studies, gene N is normally silent on full-length RNA 3 but is translationally activated by deletion of upstream sequences. Introduction of Gene N into the genome of the unrelated virus cauliflower mosaic virus (CaMV) elicits a necrotic response instead of the typical mosaic symptoms, demonstrating that gene N can induce necrosis outside of the context of a beet necrotic yellow vein virus infection.

1188 SYSTEMIC SPREAD OF A GEMINIVIRUS IS INHIBITED BY SPREAD GENES FROM OTHER VIRUSES. Albrecht von Arnim and John Stanley, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, U.K.

Systemic spread of plant viruses through the host organism is mediated by viral gene products, which are generally referred to as transport proteins or spread proteins. African cassava mosaic virus (ACMV) belongs to the geminivirus group of plant viruses. Its DNA genome is split between two genomic components DNA A and DNA B. In ACMV and the related geminivirus tomato golden mosaic virus (TGMV) replication functions reside on DNA A while two genes on DNA B have been implicated in mediating systemic spread.
In order to further define the functions of geminiviral DNA B genes we sought to identify functional counterparts for ACMV DNA B genes among spread genes from other viruses. Several ACMV recombinants harbouring foreign spread genes were constructed and tested for infectivity on *Nicotiana benthamiana*. Two such genes, namely the cell-to-cell spread gene of an unrelated RNA virus (tobacco mosaic virus) and the putative spread gene BC1 of TGMV, inhibited systemic spread but not replication of ACMV even in the presence of functional ACMV spread genes. Possible mechanisms to explain inhibition of systemic spread and its implications will be discussed.

1189 ISOLATION OF MUTANTS OF ARABIDOPSIS THALIANA IN WHICH ACCUMULATION OF TOBACCO MOSAIC VIRUS COAT PROTEIN IS REDUCED TO LOW LEVELS. Masayuki Ishikawa, Fumie Obata, Tsuneko Kumagai and Takeshi Ohno, Hokkaido University, Faculty of Agriculture, Sapporo 060, Japan.

We found that Arabidopsis thaliana was susceptible to infection with a crucifer strain of tobacco mosaic virus (TMV-Cg); the coat protein of TMV-Cg accumulated to a high level in uninoculated rosette leaves several days after inoculation. For the first step to search for host-coded factors that are involved in virus multiplication, we tried to isolate mutants of A. thaliana in which the multiplication of TMV-Cg was impaired. Of six thousand M2 plants descended from ethyl methanesulfonate-treated seeds, two lines (PD114 and PD378) in which the accumulation of TMV-Cg coat protein was reduced to low levels were isolated. Genetic analyses suggested that this phenotype of PD114 was caused by a single nuclear recessive mutation, and PD114 and PD378 belonged to the same complementation group. The coat protein accumulation of a tomato strain of TMV (TMV-L) was also reduced in PD114 plants compared to that in the wild-type plants. In contrast, in PD114 plants infected with turnip crinkle or turnip yellow mosaic viruses which belong to taxonomic groups other than Tobamovirus, the levels of their coat protein accumulation were similar to those in the wild-type plants.

POSTER ABSTRACTS

1190 OBSERVING VIROID EVOLUTION: RECOMBINATION IS STILL IN PROGRESS BETWEEN COLEUS VIROIDS
H. L. Sänger and R. Spieker, Max-Planck-Institut für Biochemie, Abteilung Viroidforschung, D-8033 Martinsried, F.R.G.

In various *Coleus blumei* cultivars three new viroids have been found which are named CbVd 1, CbVd 2 and CbVd 3. Sequence analysis revealed that their prototypes from the cultivar "Bienvenue" are 250, 302 and 362 nucleotides long, respectively, and that they all contain an identical conserved central region (CCR) the sequence of which differs strikingly from that of the CCRs of all other presently known viroids. Except for the sequence of their CCR, CbVd 1 and CbVd 3 are completely unrelated. Surprisingly, CbVd 2 proved to be a recombinant viroid that has arisen by fusion of the right-hand part and the CCR of CbVd 1 with the left-hand part of CbVd 3. The right-hand part of CbVd 1 carries sequence motifs also present in hop latent viroid, whereas in the left-hand part of CbVd 3 certain sequences are found which are characteristic for grapevine viroid 1B. The analysis of the distribution of the three *Coleus* viroids in individual "Bienvenue" plants obtained by biological cloning via cuttings revealed that CbVd 1 and CbVd 3 were present in 82% of them, 6% harboured all three viroids and in 12% of the plants only CbVd 1 and CbVd 2 were found. This situation renders the *Coleus* viroid system a kind of naturally occuring missing link which demonstrates that RNA recombination is an important factor of viroid evolution.

1191 INVESTIGATION OF MOVEMENT MECHANISM OF POTATO VIRUS Y IN SOLANUM BREVIDENS AT MOLECULAR LEVEL. Tuula Pehu, Jari Valkonen, Eija Pehu and Kirsi Lehto. University of Helsinki, Department of Crop Husbandry, SF-00710 Helsinki, Finland.

In our research we investigate the molecular basis of the virus resistance in Solanum brevidens. According to our preliminary results the general virus resistance in S. brevidens is related to inhibition of cell-to-cell movement of viruses rather than their replication. Based on the sequence homology studies of the potyviruses the first gene of the 5' end of the PVY genome codes for the putative movement protein (31 Kd). To investigate the role of the 31 Kd protein in the movement process we are cloning and expressing the movement protein gene in order to study its location in the resistant S. brevidens and susceptible S. tuberosum tissue. The protein product will also be used to study its binding activity to viral RNA. Results on the cloning and characterization of the cloned sequence will be presented in this poster.

1192 THE EFFECT OF SPECIES SPECIFIC REPETITIVE SEQUENCES ON THE RATE OF DNA INTEGRATION IN PARTIAL GENOME TRANSFER EXPERIMENTS IN POTATO

S. Suomensaari, Y-S Xu, T. Pehu and E. Pehu Dept. of Crop Husbandry, University of Helsinki, SF-00710 Helsinki, Finland

The objective of our research program is to identify and transfer DNA-sequences coding for broad virus resistance in Solanum brevidens to potato germplasm through partial genome transfer. Therefore maximization of transformation frequency is of great importance. There are a few reports on the use of "booster" sequences, i.e. repetitive sequences to enhance the transformation rate in other species (1,2). In this poster results will be presented of the effect of species specific sequences on the electroporation mediated transformation efficiency of potato protoplasts. The "booster" sequences will be introduced in a plasmid containing a sequence fusion construct of the species specific sequence and a marker gene as well as in cotransformation experiments with two plasmid populations.

Ref.1: Marchesi ML., Castiglione S., Sala F. (1989) Theor Appl Genet 78:113-118
Ref.2: Meyer P., Kartzke S., Niedenhof I., Heidmann I., Bussmann K., Saedler H. (1988) Proc Natl Acad Sci USA 85:8568-8572

1193 PRODUCTION AND CHARACTERIZATION OF ASYMMETRIC HYBRIDS OF Solanum tuberosum and irradiated Solanum brevidens.
Murto, M., Xu, Y-S., Valkonen, J., Jones, M.G.K.* and E. Pehu. Dept. of Crop Husbandry, University of Helsinki, SF-007100 Helsinki, Finland. *School of Biological and Environmental Sciences, Murdoch University, Perth, Western Australia, 6150.

Solanum brevidens is a wild, diploid Solanum species that is resistant to PLRV, PVY and PVX. Due to sexual incompatibility the resistance has been transferred to potato germplasm by somatic fusion (Fish et al. 1988, TAG 76:260-6). To reduce the transfer of undesirable characteristics we have used 20-30 krad gamma irradiation of the S. brevidens protoplasts to fractionate the donor genome prior to fusion. Of five thousand fusion calli, 100 regenerated into plantlets. The nuclear hybridity of ten regenerants has been confirmed in slot blot hybridization experiments by species specific sequences. Results of the characterization regarding general morphology, chromosome number, virus resistance and chromosome identification using linkage group specific RFLP markers (kindly supplied by S. Tanksley) will be presented.

1194 BIOLOGICALLY ACTIVE cDNA CLONES OF PANICUM MOSAIC VIRUS SATELLITES.
J. Monis, D. S. Sopher, and A. O. Jackson. Department of Plant Pathology, University of California, Berkeley, CA 94720

Two strains of panicum mosaic virus (PMV) naturally found infecting centipede (PMV-C) and Saint Augustine grass (PMV-S) are associated with two distinct classes of satellite agents: a satellite virus (826 nt) that encodes its own capsid protein and satellite RNAs (ca. 380 - 440 nt) that encode no protein. Nucleic acid hybridization analyses revealed extensive relatedness between the satellite RNAs associated with these two strains, but no cross hybridization was evident with the respective satellite or helper viruses. We have initiated studies to determine the structure-relationships between the helper, the satellite virus and the satellite RNAs. For these investigations, the satellite RNAs from the PMV-S strain (ca. 380 nt) and the PMV-C strain (ca. 380 nt and 440 nt) have been cloned and sequenced. The sequence data reveals that the these satellite RNAs have extensive relatedness at the 5' ends of their genomes but have no significant relatedness with the satellite virus. Full length cDNA clones of PMV-C (ca. 440 nt) and PMV-S (ca. 380 nt) satellite RNAs were constructed in a transcription vector. These plasmids produced *in vitro* transcripts of the expected size for PMV-C and PMV-S satellite RNAs. These RNA transcripts were infectious when inoculated onto pearl millet plants in combination with helper and satellite virus inoculum free of satellite RNAs. These assays demonstrated that the satellite virus is not required for sat-RNA infectivity or its movement *in planta*. Results of ongoing molecular genetic analyses will be presented.

1195 REPLICATION OF BARLEY STRIPE MOSAIC VIRUS RNA.
A. O. Jackson[1], R. G. K. Donald[1], H. Zhou[1], I. T. D. Petty[2] and R. C. French[3]. [1]Department of Plant Pathology, University of California, Berkeley, CA 94720. [2]Department of Microbiology, North Carolina State University, Raleigh, NC 27695 and [3]Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

Barley stripe mosaic virus (BSMV) has a genome comprised of RNAs α, β, and γ which encode seven polypeptides, designated αa, βa, βb, βc, βd, γa and γb, respectively. Infectivity experiments in protoplasts with in vitro transcripts derived from biologically active cDNA clones demonstrate that the αa and γa genes encode the essential replicase components of the genome. In order to investigate the requirement of cis-acting elements for replication, a series of deletions were constructed in RNAs β and γ. Infectivity tests with these mutants reveal that the 5' leader sequences and the 3' terminal tRNA-like domain are necessary for replication. In contrast, extensive deletions within RNAβ have little effect on the replicative capacity of the RNA. RNAγ mutants containing deletions within the γa and γb genes will also replicate when protoplasts are coinoculated with wild type RNAα and RNAγ. The results of these experiments suggest that the essential cis-acting elements are located at the extreme 3' termini of the genomic RNAs and that only a small amount of additional internal sequence may be required for spacer or sequence context purposes. Additional experiments to assess the biochemical properties of the βb and γb genes have been conducted with protein expressed in E. coli. Preliminary results of these experiments indicate that the βb protein preferentially binds double-stranded RNA over single-stranded RNA and in vitro and in vivo analyses suggest that both the βb and γb products have RNA binding activity. Mutagenesis experiments are being carried out to more fully evaluate the functional requirements of different motifs within these proteins.

POSTER ABSTRACTS

1196 THE cDNA SNYTHESIS, CLONING AND EXPRESSION OF RICE STRIPE VIRUS COAT PROTEIN GENE.* Qiu B.S., Wang J.F., Zhao S.Z., Yan Y.T., and Tien P., Institute of Microbiology, Academia Sinica, Beijing 100080 China

The total RNA was extracted from purified RSV by treating with SDS-phenol and proteinase K. Catalyzed by poly A polymerase, about 63 AMPs were added to the 3'-terminal of RSV-RNA. The 0.3-3kb cDNA to RSV-RNA was synthesized with BRL cDNA synthesis kit, cloned with plasmid pUC19 and transformed into E.coli DH 5α. Hybridization test in situ with RSV-RNA probe showed that 50 recombinants were from RSV-RNA. Immumological screening of colonies with pUC19 showed that the No.146 clone had a strong reaction with RSV-antiserum. It was revealed by western blot that the No.146 clone had a same molecular weight band as the coat protein of RSV. The coat protein gene was inserted into plant expression vector ROK-II. The recombinant plasmid was then introduced into Agrobacterium tumefaciens containing Ti-plasmid. Leaf discs of tobacco were infected with the Agrobacterium tumefaciens. The transgenic tobacco plants expressed RSV coat protein. Electroporation and PEG-mediated protoplast transformation methods were used for generating transgenic rice plant. The construct harbouring RSV coat protein gene was introduced into rice protoplasts via electroporation and PEG-mediated. The transfomed rice calli were obtained. Transgenic Rice plant harbouring RSV coat protein gene will be regenarated.

* This work is supported by Rockefeller Foundation in International Program on Rice Biotechnology.

1197 TYLCV PRESENCE AND REPLICATION IN TOMATO PROTOPLASTS AND CALLUS CULTURES. Dan Pelah, Mara Reichenbach-Dekel, Arie Altman and Hanokh Czosnek. The Otto Warburg Center for Biotechnology in Agriculture, Faculty of Agriculture, The Hebrew University of Jerusalem, Renovot, Israel 76-100.

The Tomato Yellow Leaf Curl Virus (TYLCV), a whitefly-transmitted geminivirus, causes heavy damages to tomato crops world wide. Protoplasts of virus-resistant, tolerant and sensitive genotypes were inoculated with a cloned dimer of the TYLCV genome, using PEG and liposomes as a tool for gene delivery. TYLCV replicative form appeared 8 and 3 days post-inoculation in the sensitive and tolerant protoplasts, respectively. TYLCV replicative form was not found in the resistant protoplasts. Viral single-stranded DNA did not appear in any of the treated protoplasts. Viral presence in callus cultures over an 8 month period, was investigated using TYLCV genomic probe. It was found that viral levels declined throughout the entire subculture period, until complete elimination. Virus concentration within the callus varied considerably. Virus "dilution" in callus cultures was probably due to a lower viral replication rate, as compared to that of the callus. Therefore, the virus was not spread uniformly throughout the callus.

1198 THE USE OF MAIZE PROTOPLASTS TO STUDY MAIZE STREAK VIRUS GENE FUNCTIONS. Margaret I. Boulton, Charles K. Pallaghy and Jeffrey W Davies. Department of Virus Research. John Innes Institute, Colney Lane, Norwich NR4 7UH., UK.

The geminivirus maize streak virus (MSV) infects cereals and grasses. Agroinoculation of cloned viral DNA has allowed preliminary examination of MSV gene function in plants Mutagenesis studies have shown that the virion-sense genes, V1 and V2, are necessary for systemic infection of maize plants, but are not required for virus replication. To further study the effect of mutations within V1 and V2, without the limitations of cell-to-cell spread, we have established a maize protoplast system which is capable of supporting MSV replication. Virus replication was concomitant with the onset of cell division, and both double-stranded (ds) and single-stranded (ss) MSV DNA forms were produced. The immunodetection of coat protein and virus particles showed that the virus replication cycle may be completed in protoplasts, making them an ideal system for the study of mutant MSV genomes. Inoculation of V1 deletion mutants resulted in an infection identical to that obtained with the wild-type inoculum, whereas in V2 mutant infections ss DNA was not detected, although ds DNA reached wild-type levels. Possible roles for the virion-sense gene products in the spread of the monopartite geminiviruses in cereals will be discussed.

1199 MOLECULAR CHARACTERIZATION OF RICE YELLOW STUNT VIRUS AND THE VIRUS-INDUCIBLE GENES IN RICE. R.X.Fang 1, Z. Pang 1, D.M.Gao 2, K.Q.Mang 1, and Nam-Hai Chua 3. 1: Dept. of Virology, Inst. of Microbiol., Academia Sinica, Beijing 100080, China; 2: Inst. of Plant Protec -tion, Zhejiang Academy of Agr. sci., Hangzhou 310021, China; 3: Lab. of Plant Mol. Biol., The Rockefeller Univ., 1230 York Avenue, New York, NY 10021, USA.

Rice Yellow Stunt Virus, a plant rhabdovirus, was partially purified from infected rice stalk. At least five viral proteins, L(170K), G(84K), N(60K), M1(31K) and M2(29K) were identified by SDS-PAGE. The N-terminal amino acid sequence of 15 residues for G protein has been determined by automated Edman degradation method. The size of RYSV RNA genome was estimated as about 12kb. A cDNA library was constructed from the purified genomic RNA. In order to study the interaction between the virus and the host plant, a Lambda ZAP II based cDNA library was constructed from the poly(A)+RNA of RYSV-infected rice and differentially screened for RYSV infection-specific clones. So far five clones have been isolated and four of them were completely sequenced. Northern blot analysis indicated that the expreesion of the three genes were stimulated by RYSV infection. They are glycine-rich-protein(GRP) gene, phenylalanine-ammonia-lyase(PAL) gene and heat-shock 70 protein(HS70)gene. The GRP comprises an N-terminal 27 amino acid signal peptide followed by a gly-rich sequence of 156 amino acids and 66% of which are gly, in which regular repeats,G-X-G-X,exist, where in most cases X is gly,ser or tyr.

1200 CARROT EXTENSIN PROMOTER CONTROLLED EXPRESSION OF PVX COAT PROTEIN GENE CONFERS VIRUS RESISTANCE IN TRANSGENIC POTATO PLANTS A Fehér[1], KG Skryabin[2], E Balázs[3], J. Preiszner[1], O Shulga[2], VM Zakharyev[2] and Dudits[1]

[1] Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521, Hungary [2] Institute of Molecular Biology, USSR Academy of Sciences, Moscow USSR. [3] Agricultural Biotechnology Center, H-2101, P.O.B. 170, Hungary. Tuber discs of minitubers of potato cultivars Desiree and Gracia have been infected with Agrobacterium tumefaciens cells carrying a binary vector. The modified T-DNA of this vector contained the neomycine posphotransferase gene and the coat protein gene of PVX under the control of the NOS and the carrot extensin gene long-transcript promoters, respectively As the result of the transformation mediated by Agrobacterium cells several transgenic potato plants have been obtained after a one step regeneration of shoots in the presence of kanamycin. The presence of the coat protein gene has been proved in several randomly chosen transformants by Southern analysis Most of these plants exhibited some degree of resistance following virus inoculation as determined by comparison the accumulation of virus RNA in the infecter leaves of control and transgenic plants The low expression level of the introduced virus gene has been proved at the mRNA level in one Gracia and one Desirée transgenic clones In the case of this later clone it was confirmed by Western analysis Ethrel treatment resulted in an around five times increase in the level of the coat protein MRNA which suggests the possibility that virus infection itself can enhance PVX coat protein gene expression through the induction of ethylene generation in plant tissues

1201 A GEMINIVIRUS REPLICATION PROTEIN IS A SEQUENCE-SPECIFIC DNA BINDING PROTEIN. Elizabeth Fontes*, Heather Gladfelter*, Verne A Luckow†, and Linda Hanley-Bowdoin*. *Department of Biochemistry, North Carolina State University, Box 7622, Raleigh, NC 27695. †Monsanto Corporate Research, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

Tomato golden mosaic virus (TGMV), a plant DNA virus, is a member of the geminivirus family. Its genome consists of two components, A and B, that are packaged as single-stranded DNAs in virions and exist as single and double-stranded DNAs in infected plant cells. The only DNA sequence that is conserved between the two components is a 230 bp region, the common region, that contains promoter elements for left and rightward transcription and the probable origin of replication. The TGMV A component encodes the only viral protein, AL1, that is required for viral DNA replication. As a first step towards understanding the function of AL1, we have asked if the replication protein interacts specifically with TGMV DNA by using an immunoprecipitation assay for protein:DNA complex formation We have shown in binding reactions containing a 460 bp fragment of TGMV A DNA that a monoclonal antibody against AL1 precipitates AL1:DNA complexes while an unrelated antibody fails to precipitate the complex. This TGMV A fragment includes common region and flanking 5' gene sequences. Competition assays with homologous and heterologous DNA sequences established the specificity of the binding. AL1 produced by transgenic tobacco plants and by baculovirus-infected insect cells exhibited similar DNA binding activity. This is the first demonstration of specific DNA binding by a geminivirus replication protein and has important implication for its role in viral replication. Experiments are currently underway to precisely define the AL1 DNA binding site and to determine the catalytic role for AL1 in virus replication.

POSTER ABSTRACTS

1202 ORGANISATION AND FUNCTIONAL ANALYSIS OF BARLEY YELLOW MOSAIC VIRUS GENOME. Andrew Davidson, Joachim Bendiek, Ellen Peerenboom, Peer Schenk, Jeff Schell and Hans-Henning Steinbiß. Max-Planck-Inst. für Züchtungsforschung, D-5000 Köln F.R.G. // Barley yellow mosaic virus (BaYMV), a fungally transmitted virus, is responsible for a serious disease of winter barley cultivars in European and Asiatic countries. BaYMV has a bipartite, positive sense, single-stranded RNA genome.

We have prepared full length cDNA clones corresponding to the genome of a German isolate of BaYMV and the complete nucleotide sequence of both RNAs has been determined (Prols et al., 1990 J. Phytopath. 130:249; Davidson et al., 1991 J. Gen. Virol. 72:989). RNA 1 and 2 are 7647 and 3585 nucleotides in length respectively, excluding their poly (A) tails. Each RNA contains only one long, single ORF, encoding proteins of 270K and 98K for respectively, RNA1 and 2. Comparison of the 270K protein with protein databases revealed that with the exception of an amino terminal 45K region, the 270K protein corresponds to the carboxy three quarters of polyproteins encoded by potyviruses in organization and sequence homologies. It appears that the 270K protein is a polyprotein that is processed into at least 5 proteins, a 45K protein, an RNA helicase, a proteinase, an RNA polymerase and the coat protein (in order from the amino terminus). By contrast, the 98K protein has homology to potyviruses only in an amino terminal region corresponding to the proteolytic domain of the helper component-proteinase. Deletion analysis combined with *in vitro* translation studies of RNA2 demonstrated that the 98K protein is a polyprotein which is processed to give a 28K amino terminal and a 70K protein. Antibodies are currently being prepared against these proteins to determine their localization and possible functions in the virus/host interaction. RNA transcripts have been produced from the cDNA clones and experiments are currently being performed to determine if the *in vitro* transcripts are able to infect barley suspension protoplasts and plants.

Recently, a resistance breaking' strain of BaYMV was isolated from previously resistant barley plants and the RNA used to prepare cDNA clones. These cDNA clones are being analyzed using restriction mapping, DNA sequencing and genome replacement experiments with the aim to localize differences between these two isolates of BaYMV responsible for the altered virulence.

1203 MOLECULAR CHARACTERIZATION OF A NEW GEMINIVIRUS INFECTING PEPPERS IN NORTHERN MEXICO. R. F. Rivera-Bustamante, J. A. Garzón, I. Torres, B. Jimenez and L. Herrera-Estrella. Centro de Investigaciones y Estudios Avanzados. Departamento de Ingeniería Genética. Irapuato Gto. México

Peppers and tomatoes are two of the most important horticultural crops in México. Before 1988, TEV, CMV and TMV were the most devastating viruses affecting these crops. In the last three years, however, they have been replaced by several white-fly transmitted pathogens reported in almost all horticultural areas in the country. A white fly transmitted geminivirus was reported as the causal agent for the "Rizado Amarillo" disease of peppers. We isolated and cloned dsDNA molecules associated only with infected tissue and proceeded to its characterization. The differences in the host range, the lack of mechanical transmission and the restriction map of the dsDNA suggested that this geminivirus has not been reported before. Sequencing of the fragments cloned apparently suggests that the "Rizado Amarillo" disease is caused by more than one geminivirus. Analysis of the sequences of one component showed high degree of homology with the bipartite geminiviruses: TGMV and BGMV. Hybridization analysis with dsDNA from infected tissue from other horticultural areas suggested the presence of another unrelated viruses. A full comparison, including DNA sequences, with previously reported geminiviruses will be presented as well as a discussion about the probable origin of these pathogens in México.

1204 ~~MOLECULAR ANALYSIS OF AN INFECTIOUS CLONE OF RICE TUNGRO~~ BACILLIFORM VIRUS. I.M.Hay, I.Dasgupta, B.Yiming, M.C.Jones, M.L.Blakebrough, J.W.Davies, R.Hull. John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

A complex of two viruses, rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV), is responsible for the most important viral disease of rice, rice tungro disease. RTBV has a circular, double-stranded DNA and is thought to be a member of the recently recognised group of viruses characterised by bacilliform particles containing DNA, *Commelina* yellow mottle virus (CoYMV) being the type member. Neither RTBV nor its DNA is transmissable into rice plants and infectivity of a RTBV clone has been shown by agroinoculation. The nucleotide sequence of this infectious clone indicates the genome is 8002 bp and that the transcribed strand potentially contains four open reading frames (ORF). The first ORF is capable of encoding a protein of 24 kd, but has no initiation (ATG) codon. The other three ORFs potentially encode proteins of 12 kd, 194 kd and 46 kd (P12, P194 and P46) respectively. Although the functions of P24, P12 and P46 are unknown, comparitive analyses with retroviruses and CoYMV suggest the large 194 kd ORF encodes a polyprotein that is proteolytically cleaved to yield the virion coat protein, a protease and replicase (reverse transcriptase and RNase H) characteristic of retroelements. These ORFs are currently being expressed in *E. coli* using the expression vector pGex. The RTBV genome is interrupted by two discontinuities which map at specific sites, one in each strand, and the structure of the DNA at these gaps is being determined. A 14-nucleotide sequence located at one of the discontinuities has complementarity to the tRNA $^{met}_{i}$, and by analogy with caulimoviruses and CoYMV is considered to be the minus-strand DNA primer. A purine-rich sequence near the site of the second discontinuity is postulated as the priming site for synthesising the plus-strand DNA The position of likely transcriptional signals suggests a greater-than-genome-length viral transcript is produced and this is consistent with replication by reverse transcription. The transcripts are currently being mapped.

1205 LONG DISTANCE MOVEMENT OF CAULIFLOWER MOSAIC VIRUS IN *ARABIDOPSIS THALIANA*. Scott Leisner and Stephen H. Howell. Boyce Thompson Institute, Ithaca, NY 14853

We are studying long distance movement of cauliflower mosaic virus (CaMV) in *Arabidopsis thaliana* and host factors that confer resistance by interfering with long distance movement. We have screened various *A. thaliana* ecotypes for resistance to four different CaMV strains. The viral isolates examined were CM4-184, CM1841, W260 and D4 that gave on turnip mild, moderate, severe chlorotic vein clearing and necrotic flecking, respectively. Several *A. thaliana* ecotypes showed varying resistance with specific viral isolates, but no ecotype was found resistant to all four isolates. For example, the Ct-1 ecotype appeared to be resistant to isolate CM4-184 while symptoms were delayed when the Ws-0 ecotype was inoculated with isolate CM4-184. Through the use of a "whole plant" *in situ* hybridization technique, we have been able to examine the distribution of CaMV DNA in infected plants. CaMV is present in all organs of *Arabidopsis thaliana* Col-0 (Columbia ecotype) such as rosette and cauline leaves, roots, the flower stalk, flowers and seeds. The distribution pattern CaMV DNA suggests that the virus moves from inoculated leaves following a sink-to-source flow of photoassimilates. The distribution pattern of CaMV was also examined in several other *A. thaliana* ecotypes that exhibited differences in symptoms, compared to the standard Col-0 ecotype. For example, when the Nd-0 ecotype was inoculated with CaMV isolate D4, the hybridization pattern showed that the virus was confined to the flower stalk. The genetic properties of the ecotype differences are under study.

1206 IDENTIFICATION OF POTYVIRAL PROTEINS INVOLVED IN VIRAL RNA REPLICATION. Patricia Gamble Klein, Emilio Rodriquez-Cerezo and John G. Shaw. University of Kentucky, Department of Plant Pathology, Lexington, KY 40546.

Tobacco vein mottling virus (TVMV) is a member of the potyvirus group of plant RNA viruses. TVMV has a monopartite positive-sense genome which encodes a single open reading frame coding for a polyprotein of approximately 340 kDa. Upon proteolytic processing, the polyprotein yields the viral coat protein (CP) and at least six non-structural proteins: 34 kDa, HC, 42 kDa, CI, NIa and NIb. To investigate the role of the individual TVMV gene products in viral RNA replication, we constructed a series of linker insertion mutants bearing four-codon insertions throughout the TVMV genome using a full-length TVMV cDNA from which infectious transcripts can be transcribed in vitro. Inoculation of tobacco seedlings or protoplasts with RNA from cDNA clones with mutations in the 42 kDa, CI, NIa and NIb proteins abolished viral activity suggesting that these are replication-associated proteins. Mutations in the 34 kDa, HC and coat proteins did not block viral replication. Complementation between CI, NIa and NIb mutants did not restore viral activity suggesting that these proteins can not act in trans during RNA replication. Complementation with additional replication-defective mutants is being tested in an attempt to restore viral activity.

1207 MOLECULAR TAGGING OF THE TOBACCO CHROMOSOME CARRYING THE N GENE BY *AGROBACTERIUM*-MEDIATED TRANSFORMATION. George W Bates, Department of Biological Science, Florida State University, Tallahassee, Florida, 32306

The hypersensitive response of tobacco to inoculation with Tobacco Mosaic Virus (TMV) is controlled by a single dominant gene, the N gene. As a first step in localizing the N gene, we have prepared a line of tobacco plants in which the kanamycin-resistance (KmR) gene is closely linked to the N gene. *Nicotiana tabacum* plants heterozygous for the N gene were transformed to KmR by *Agrobacterium* carrying pMON200. Eighty-six independent transformed clones were regenerated and were backcrossed with nontransformed, TMV-sensitive plants. Progeny from these crosses were screened first for KmR: then the KmR progeny were inoculated with TMV and scored for the hypersensitive response. Of the initial 86 clones, 69 appeared to have integrated a single functional KmR gene. Initial tests for TMV resistance indicated possible linkage between KmR and the N gene in 8 plants. With further testing, linkage has been established for two of these plant lines. In one of these lines, the two genes were 40 map units apart, but in the second, linkage between KmR and the N gene was tight, with only 5% recombination between the genes in this case. Southern hybridization revealed that this plant contained only a single copy of the KmR gene. Linkage between KmR and the N gene in this plant line has been verified in each of two additional backcross generations We have stabilized linkage by making these plants homozygous for both KmR and the N gene Work is planned to use this plant line to map further the chromosomal location of the N gene in tobacco.

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1208 CHANGES INDUCED BY WHEAT STREAK MOSAIC VIRUS
Annette Nassuth, Sandy Kofalvi and Jian-guo Gao. University of Guelph, Department of Botany, Guelph, Ont. N1G 2W1.

Microscopical observations of wheat, barley and triticale leaf tissues revealed several wheat streak mosaic virus-induced cytological changes which were further studied at the protein and nucleic acid level to investigate the role of these changes in host defence and/or virus replication. (1) The deposition of a layer seemingly containing polyphenolics and the appearance of holes in the cell walls, which at late infection stages collapse. We are investigating whether this indicates the induction of enzymes similar to those involved in the defence mechanism shown for dicot plants. We did not detect the induction of typical pathogenesis-related proteins. (2) Cytoplasmic and nuclear inclusions, including membraneous structures. In vivo labelled, virus-encoded protein was detected in the cytoplasmic inclusion body fraction as early as 2 days after inoculation. Microautoradiography on tissue incubated in the presence of a host RNA synthesis inhibitor and ^{3}H Uridine support the hypothesis of nuclei as viral RNA replication site. (3) Disruption of all cell organelles at a later infection stage. Results from experiments to confirm and extend our results will be reported. (Supported by NSERC Grant 36698).

1210 TRANSGENIC PLANTS EXPRESSING THE COAT PROTEIN OF TOBACCO MILD GREEN MOSAIC VIRUS ARE RESISTANT TO VIRAL INFECTION.
Ann Pizanis Sturtevant, Ali Nejidat and Roger N. Beachy. Department of Biology, Washington University, St Louis, MO 63130.

Tobacco Mild Green Mosaic Virus (TMGMV or U2) is a member of the tobamovirus family. TMGMV differs from Tobacco Mosaic Virus (TMV) in producing less severe symptoms on tobacco and in having a different host range. The TMGMV coat protein gene under control of the CaMV 35S promoter was introduced into tobacco and into tomato, a nonhost. Although only low levels of TMGMV coat protein were detected, transgenic tobacco plants infected with TMGMV exhibited a high degree of resistance against TMGMV infection. Results will be presented on the activity of TMGMV coat protein mediated protection against the U1 strain of TMV (69% homology), Tomato Mosaic Virus (67% homology), Odontoglossom Ringspot Virus (60% homology) and Ribgrass Mosaic Virus (44% homology).

1211 PRODUCTION OF INFECTIOUS CLONES OF TOBACCO NECROSIS VIRUS AND MAPPING OF SUB-GENOMIC RNA SPECIES.
Robert H.A. Coutts and Samuel K. Offei. Imperial College, Department of Biology, London, U.K.

The complete sequence of the RNA genome of tobacco necrosis virus strain D (TNV-D) consisting of 3759 nucleotides has been determined. The positive strand contains five open reading frames (ORFs). The 5' proximal ORF encodes a 22K protein (p22) terminating with an amber codon which may be read through to produce a 82K protein (p82). Two small centrally located ORFs each encode two out of frame 7K proteins (p7a and p7b). The 3' proximal ORF encodes the 29K (p29) coat protein (CP) whose N-terminus has been sequenced directly. The genomic organisation of TNV-D is very similar to that of TNV strain A (TNV-A) but differs in the placement of the p7a ORF, which does not overlap the p82 ORF in TNV-D, and in the absence of an ORF downstream of the CP gene in TNV-D. The p82 ORF shows extensive sequence similarity with the putative polymerases of the carmovirus group. The amino sequence of the TNV-D CP gene is similar to both the TNV-A and southern bean mosaic virus CP genes. Of the two p7 ORFs, p7a exhibits amino acid sequence similarity with a corresponding protein from TNV-A and some other tombus- and carmoviruses, whereas the p7b ORF appears to be unique to TNV-A and TNV-D. Our efforts at producing full-length infectious transcripts of TNV-D RNA using PCR amplification will be reported. Mapping of the two sub-genomic RNAs found in vivo (one of 1.6 kb, the putative mRNA for the p7a ORF, the other, of 1.3 kb, acting as the mRNA for the CP gene, ORF 5) and their promotors will also be described.

1212 NUCLEOTIDE SEQUENCE OF BEET YELLOWS VIRUS AND EXPRESSION OF THE COAT PROTEIN GENE IN E. COLI AND IN PLANTS
Janne Brunstedt and Roger Hull*, Danisco A/S, Biotechnology Research Division, Copenhagen, Denmark and *John Innes Institute, Norwich, UK

Beet yellows virus (BYV), the type member of the closteroviruses causes considerable losses of sugar beet yields. BYV consists of long flexous rod-shaped particles containing one single-stranded messenger-sense RNA of about 14500 nucleotides. Molecular cloning of the virus RNA resulted in a cDNA clone which expressed the coat protein gene in E. coli. The sequence of the 2724 nucleotide BYV insert revealed three open reading frames. Subcloning of fragments of the insert in expression vectors showed that the most 3' ORF was the coat protein cistron. The BYV coat protein gene has been inserted into a plant transformation vector between a CaMV 35S promotor and terminator. Transgenic plants containing the BYV coat protein gene have been produced by Agrobacterium mediated transformation and the expression of the coat protein gene is being studied.

1213 USING RECOMBINANT VIRUSES TO MAP FUNCTIONAL DOMAINS OF CUCUMBER MOSAIC VIRUS RNA 1.
Marilyn J Roossinck, The Noble Foundation Plant Biology Division Ardmore, OK 73402; and Peter Palukaitis Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Cucumber mosaic virus (CMV) is a tripartite, plus-stranded RNA virus with a broad host range. Numerous strains of CMV have been isolated, which induce widely different symptoms in host plants. Two strains of CMV, Fny-CMV and Sny-CMV, are closely related at the nucleotide level as assessed by heterogeneity assays using *in vitro* transcripts, but have several phenotypic differences: time of appearance and severity of symptoms when inoculated onto squash, efficiency of replication of satellite RNAs, and temperature-sensitive replication in muskmelon. We have mapped these differences to RNA 1 with pseudorecombinant viruses. Using full length cDNA clones of the three Fny-CMV RNAs and a full length cDNA clone of RNA 1 of Sny-CMV we have constructed a series of recombinant viruses, which are being used to map precise domains of RNA 1 responsible for the phenotypic differences between these strains. RNA 1 is required for viral replication, and its single open reading frame contains the conserved motifs found in all known helicases, as well as sequences consistent with NTP-binding properties, and the conserved methyltransferase domain of Sindbis-like viruses. However, the precise role of RNA 1 in viral replication and/or virus-host interactions is unknown. We have found that the phenotypic differences between Fny- and Sny-CMV map to different regions of RNA 1. Results will be presented which delineate some of the functional domains of RNA 1 and their possible role in the observed phenotypic differences will be discussed.

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1214 CLONING OF PEANUT STRIPE VIRUS (PStV) COAT PROTEIN AND ITS EXPRESSION IN E. COLI. B.G. Cassidy[1], J. Sherwood[2], R.S. Nelson[1]. [1]The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and [2]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

The 3' terminus of PStV was cloned and sequenced. The sequence included 300 nucleotides from the 3' end of the NIb region, 843 nucleotides encoding the coat protein (CP) gene and 256 nucleotides of 3' nontranslated sequence. The PStV CP and portions of the CP were expressed in E. coli. Viral CP was detected by Western, dot blot, and ELISA with polyclonal and monoclonal antibodies. The antigenicity of different portions of the CP were determined. Transgenic tobacco plants expressing the CP are to be tested for resistance to potyviruses.

1215 THE "SENSE" STRATEGY CONFERS PROTECTION AGAINST TURNIP YELLOW MOSAIC VIRUS (TYMV) IN TRANSGENIC *BRASSICA* PLANTS.

Francoise Cellier[1], Bruno Zaccomer[1], Jean-Christophe Boyer[2], Marie-Dominique Morch[2], Anne-Lise Haenni[2] and Mark Tepfer[1]

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Turnip yellow mosaic virus (TYMV) is the type member of the tymovirus group; its genome is composed of a single molecule of (+) sense RNA, with a tRNA-like structure at the 3' end. The tRNA-like region is recognized by the TYMV replicase in initiating the synthesis of (-) strand RNA from the (+) sense template. Morch *et al.* (Nucl. Acids Res. 15, 1987, 4123-4129) have shown that RNAs bearing the tRNA-like region are competitive inhibitors of the replication of TYMV genomic RNA in an acellular replicase assay. We have introduced into the genome of *Brassica napus* several genes that interfere with replication of TYMV. "Sense" genes whose transcripts include a CAT coding sequence and the 3' region of the viral genome confer partial protection against TYMV infection. In order to improve the level of competition, we have constructed genes in which a GUS coding region is flanked by sites of initiation of replication: on infection by TYMV this should lead to amplification of the competitor molecule (an artificial DI RNA) by the viral replicase. Results obtained with several artificial DI RNAs will be discussed.

1216 REPLICATION AND RECOMBINATION PROPERTIES OF THE DNA1 COMPONENT OF CASSAVA LATENT VIRUS.

Uta Paszkowski, S.B. Zhang, J. Masson, I. Potrykus and J. Paszkowski. Inst. of Plant Sci., ETH Zürich, CH-8092 Zürich. Switzerland.

Cassava Latent Virus (CLV) belongs to the group of whitefly transmitted bipartite geminiviruses which comprise two circular singlestrand (ss) DNA molecules and infect dicotyledonous plants. Taken individually the CLV components DNA 1 (2779nt) and DNA 2 (2724nt) are not infectious, however, DNA 1 is capable of autonomous replication in actively dividing Nicotiana plumbaginifolia protoplasts since it supplies all required virus-encoded functions (Townsend et al. Nucl. Acid. Res. 14, 1253, 986). DNA 1 and 2 share little homology besides a strongly conserved region of 200 bp. Since this "common region" is not only present on both DNA components of CLV but represents a feature of all bipartite geminiviruses it has been widely proposed to participate in DNA replication (reviewed by Davies and Stanley, TiG 5, 77, 1989). It is not known in how far the replication is determined by the host plant cells. We have studied replication properties of DNA 1 of CLV in host and non-host cellular systems.

It was shown that DNA 1 and DNA 2 can undergo recombination (Etessami et al. Nucl. Acid Res. 16, 4811, 1988). We examined the ability of DNA 1 to recombine with the plant genome by homologous and illegitimate recombination.

1217 SYNERGISTIC INTERACTIONS OF POTATO VIRUS X AND UNRELATED VIRUSES IN TOBACCO. Vicki Bowman Vance. University of South Carolina, Department of Biological Sciences, Columbia, South Carolina 29208

Potato virus X (PVX) and potato virus Y (PVY) may co-infect tobacco to cause a classic synergistic disease. In the acute stage the disease is characterized by a dramatic increase in the accumulation of infectious PVX particles, with no corresponding increase or decrease in the accumulation of PVY. The accumulation of PVX genomic RNA and coat protein has been examined in doubly versus singly infected tobacco leaves. These experiments indicate that the steady state levels of both viral components increase in doubly infected plants to about the same extent as the level of infectious PVX particles. The level of PVX subgenomic coat protein mRNA found associated with polyribosomes of synergistically infected plants is also increased to a similar extent. Pulse labelling experiments suggest that the increase in PVX coat protein is due to an increased rate of synthesis. The level of PVX (-) strand RNA template increases disproportionately in doubly infected tissue, to a level three times higher than that of the virion or its component parts. This result suggests that PVX/PVY synergism involves an alteration in the normal regulation of the relative levels of PVX (+) and (-) strand RNAs during viral replication. The results of similar experiments involving the interaction of PVX with each of several related potyviruses and with tobacco mosaic virus are presented.

1218 EVALUATION OF CP MEDIATED RESISTANCE TO CASSAVA COMMON MOSAIC VIRUS (CCMV) IN *Nicotiana benthamiana*.

Claude Fauquet[1], Didier Bogusz[1], Claudine Franche[1], Christian Schopke[1] Paul Chavarriaga[1], Lee Calvert[2], and Roger Beachy[1] 1: Washington University, St. Louis,MO, USA. 2: CIAT, PoBox 6713, Cali, Colombia.

The cassava common mosaic virus (CCMV) is the most widespread virus on cassava in South-America. Several strains of this potexvirus have been isolated from different countries, and the coding region of the coat protein has been sequence for the Brazilian strain. *Nicotiana benthamiana*, which is also host for CCMV has been transformed with *Agrobacterium tumefasciens* containing different constructs made with CCMV coat protein and many different lines have been regenerated. Many lines are expressing at a very high level, up to 1.6% of the total soluble protein content and are highly resistant to CCMV. The type and level of resistance of some of these lines has been evaluated with virus and RNA and will be described. Other strains of CCMV isolated in Colombia and Paraguay have been used for challenging the transgenic tobacco plants as well as other potexviruses like Cassava virus X and PVX have been also tested and results will be reported.

1219 Mutation of *Arabidopsis thaliana* for tolerance to tymovirus infection. Mary Skotnicki, Anne Mackenzie and Adrian Gibbs, Research School of Biological Sciences, Australian National University, Canberra, Australia.

Arabidopsis thaliana ecotype Columbia is highly susceptible to infection by an Australian isolate of turnip yellow mosaic virus, TYMV-BL. This virus causes necrotic lesions, strong yellow mosaic of systemically infected leaves, flower sterility, and finally plant death. After EMS mutagenesis of Columbia seeds, the M2 generation was screened for survival after TYMV-BL inoculation. Two further rounds of selection on surviving M3 and M4 generations enabled isolation of about twenty individual mutants reproducibly tolerant to TYMV-BL infection. These mutants exhibit a range of phenotypes, from symptomless to strong yellow mosaic with reduced fertility, but all survive infection and most allow replication of the virus. Further characterization is in progress. Columbia seeds have also been mutated by Km^R tagging via *Agrobacterium* seed transformation. The T2 and T3 generations are currently being screened for tolerance to TYMV-BL infection, as tagged mutations should facilitate the identification of plant genes involved in the host-virus infection response.

POSTER ABSTRACTS

1220 ANALYSIS OF HOST-RANGE LIMITATIONS OF TOBACCO MOSAIC VIRUS USING AN INFECTIOUS cDNA CLONE. Curtis A. Holt, Andrew A. Vaewhongs, and Roger N. Beachy. Research Institute of Scripps Clinic, Department of Cell Biology, La Jolla, CA 92037

An infectious cDNA clone of TMV has been modified to permit the introduction of foreign open reading frames in place of the viral movement protein (MP) gene. Approximately 450 nucleotides of the MP gene were deleted and replaced with a unique *Eco*RV restriction site. Transcripts generated *in vitro* from this clone were fully infectious when inoculated onto transgenic host plants that express a TMV MP gene. Insertion of the ß-glucuronidase (GUS) reporter gene into the *Eco*RV cloning site of the TMV vector allowed us to monitor viral infection in both MP-expressing transgenic plants as well as in nontransgenic hosts GUS expression was observed in single cells of all nontransgenic plant species tested, including tobacco, pepper, and *Chenopodium* (all natural hosts of TMV), as well as soybean, corn, barley, and rice (all considered non-hosts of TMV). This result suggests that the inability of TMV to infect many non-host plants involves a lack of cell-to-cell movement rather than a block in viral replication. To test this hypothesis, we have utilized PCR techniques to isolate MP genes from plant viruses that exhibit host ranges differing from TMV. These genes were introduced into the *Eco*RV cloning site of the TMV vector, and the resulting progeny were analyzed for host-range characteristics.

1221 INTERACTION OF POTATO VIRUS X WITH THE POTATO RESISTANCE GENE N_X. Simon Santa Cruz and David C. Baulcombe, Sainsbury Laboratory, Norwich Research Park, Colney, Norwich NR4 7UH UK.

Isolates of Potato Virus X (PVX) differ in their ability to infect potato plants carrying the resistance gene N_X. cDNAs representing different isolates of PVX have been used to construct hybrid viral genomes. Infection of potato plants with in vitro transcripts synthesized from hybrid clones has allowed the viral determinants of virus/N_X interactions to be mapped precisely.

1222 THE CHARACTERISATION AND MANIPULATION OF BANANA BUNCHY TOP VIRUS. Sarah L. Roberts-Oehlschlager, Thomas M. Burns, Ralf G. Dietzgen, Robert M. Harding, Mirko Karan, Karen E. Low, Moira W. Wood and James L. Dale. Centre for Molecular Biotechnology, Queensland University of Technology, Brisbane, 4000. Australia.

Banana bunchy top disease is the most serious virus disease affecting bananas. Associated with the disease are 18-20 nm virus like particles (VLP) which contain circular, single-stranded DNA of about 1kb. These VLP's are similar to those of subterranean clover stunt virus. We have cloned and sequenced one circle of the BBTV genome and will report on work currently in progress to:

1. identify the number of components which comprise BBTV, with the aim of isolating the coat protein gene.

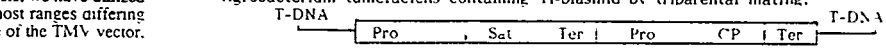
2. infect banana tissue both *in vivo* and *in vitro* with purified BBTV using a particle accelerator. This will help to determine if a second virus is required for the development of symptoms.

3. develop a regenerative tissue culture system for *Musa* spp. which can be used in transformation studies.

1223 TRANSGENIC TOBACCO PLANT WITH HIGH RESISTANCE TO CUCUMBER MOSAIC VIRUS BY EXPRESSING SATELLITE RNA AND COAT PROTEIN. Ye Y., Zhao F., Zhao S.Z., Liu Y.Z., and Tien P., Institute of Microbiology, Academia Sinica, Beijing 100080 China

In order to produce practically applicable transgenic plant with high resistance to virus disease, it is necessary to combine different strategies of virus resistance, that is to say to construct chimeric genes involving several approaches. Coat protein (CP) approach is effective in protection only in the early stage of infection, on the contrary, satellite RNA approach is valid in the later stage of infection. Higher resistance in all stage of growth will be obtained by combination of sat-gene and CP gene.

The cDNA to CMV sat-RNA-1 was synthesized and the sequence showed that the cDNA contain the full length sequence of 335 nucleotides(Zhang et al. 1989). The coat protein gene of CMV-6 isolate was synthesized and the sequence contain 657 nucleotides. A chimeric gene containing monomeric sat-gene and CP gene was constructed (Fig.), inserted into expression vector ROK-II and introduced into Agrobacterium tumefaciens containing Ti-plasmid by triparental mating.



Tobacco leaf discs were transformed via A. tumefaciens. Gene product was detected by northern blot and gel electrophoresis for sat-RNA and by northern and western blot for coat protein. The transgenic tobacco plants expressing both sat-RNA and coat protein showed high resistance to CMV infection. Eighty percent of the transformed plants was symptomless and the other showed mosaic two months after inoculation with a virulent CMV, while the non-transformed plants produced severe mosaic, stunting and fern leaf symptoms.

1224 REQUIREMENTS FOR REPLICATION IN THE BIPARTITE GEMINIVIRUSES. Sondra G. Lazarowitz[1], Leeju C. Wu[1] and Scott C. Elmer[2] [1]University of Illinois, Department of Microbiology, Urbana, Illinois 61801; and [2]The Monsanto Company, Plant Molecular Biology Section, St. Louis, Missouri

Modulation of both replication and movement functions have been shown to be important in determining the host range properties of the bipartite geminiviruses. Although the analysis of deletion mutants has suggested that the bipartite viral common region contains the viral replication origin, the actual origin(s) for single-strand and double-strand DNA replication as well as the sequences recognized by the viral replicase have not been identified. To more precisely define the viral replication origin(s) and sequence elements interacting with the viral replicase, we have constructed hybrid viral common regions containing sequences from both squash leaf curl virus (SqLCV-E) and tomato golden mosaic virus (TGMV) and utilized reporter gene constructs to directly test replication of these hybrid viral common regions by either the replicase of SqLCV-E or TGMV. Our results have identified a ~70 basepair fragment of the viral common region which is recognized by the viral replicase in a sequence specific manner and required for both double-strand and single-strand DNA replication. This fragment of the viral common region includes the conserved geminivirus sequence element which can potentially form a hairpin structure as well as short motifs of direct and inverted repeat elements. Through site-directed mutagenesis we are further defining those sequence elements essential for specificity in recognition by the viral replicase and for initiation of double-strand and single-strand DNA replication.

1225 MOLECULAR STUDY OF RESISTANCE IN *SOLANUM TUVEROSUM cv.* CARA AND POTATO VIRUS X (PVX). Bärbel Köhm, Simon Santa Cruz, Matthew Goulden*, Tony Kavanagh* and David Baulcombe. The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK and *Trinity College, Dublin, Eire.

Several potato (*Solanum tuberosum*) cultivars posess resistance genes to potato virus X (PVX). These can confer either a hypersensitive response(*Nx, Nb*) or an immune response (*Rx*) which affects virus replication. We are interested in the molecular biology of the recognition and resistance response involving these genes.

The mechanism of action of *Rx* is being studied by inoculation of plants and protoplasts with avirulent and resistance breaking strains of PVX. This work indicates that *Rx* has an effect on viral RNA accumulation.

The recognition reaction between host and viral genes is being studied at the level of the virus by analysis of hybrid viral genomes incorporating elements of virulent and avirulent strains of PVX. The hybrid viral genomes were constructed at the cDNA level and the viral phenotype analysed by inoculation of cDNA transcripts to tobacco and potato. This work indicates that sequences within the coat protein gene affect the interaction between the virus and the resistance gene product.

Eventually, this information will complement attempts to isolate the resistance gene *Rx* by transposon directed mutagenesis. The maize transposable element Ac has been transformed into *Rx* plants and plants are being screened for transposon mediated mutation to PVX susceptibility

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1226 EXPRESSION OF A GENE ENCODING TRICHOSANTHIN , CHINESE HERBAL MEDICINE, IN TRANSGENIC TOBACCO PLANT. Jinhua Han, Naisui Pan and Zhangliang Chen. National Laboratory of Protein Engineering and Plant Genetic Engineering, Biology Department, Peking University, Beijing 100871, China

Trichosanthin, a protein prepared from the root tuber of Trichosanthes Kirilowii (Cucurbitaceae), has been used as a medicine for reestablishing mensturation and as an abortifacient for centuries in China. Functional studies showed that trichosanthin exhibits the function as ribosome inhibitory protein (RIP), as well as some other functions. Recently, more interests are put on its inhibitory function of hepatitis virus, herpes simplex virus, polio virus and human immunodeficiency virus (HIV). We have cloned the gene encoding trichosanthin and expressed in E.coli. The gene has also been transferred to tobacco by using an Agrobacterium-mediated gene transfer system driven with CaMV 35S promoter. The tobacco plants were regenerated and the expression of trichosanthin was detected in Western blot with antibody against trichosanthin. Studies of localization of tricosanthin in leaf cells and its activity against virus infection are underway.

1227 FUNCTIONAL ANALYSIS OF THE POTEX VIRUS TRIPLE BLOCK SEQUENCE. Pantea Etessami, Christopher Davies, Konstantin Kanyuka and David Baulcombe. Sainsbury Laboratory, John Innes IPSR, Colney, Norwich NR4 7UH, UK.

The triple block sequences of potato virus X (PVX) which are comprised of three overlapping open reading frames, have the capacity to encode polypeptides of 25, 12 and 8 kilodaltons and carry homologies with hordei-, furo- and carlaviral groups. Translation *in vitro* of a synthetic RNA from the triple block sequences of PVX or the homologous region of potato carlavirus M (PVM) leads to the synthesis of all three triple block polypeptides, suggesting that expression of these proteins *in vivo* may be from a single, polycistronic mRNA. This idea is reinforced by the failure to detect multiple sub-genomic RNAs corresponding to mRNAs of the putative 12 kd and 8 kd polypeptides. The results from *in vitro* translation and the effects of various mutations on the products of the triple block sequences as well as the absence *in vivo* of proteins larger than 25 kd reacting with anti-sera raised against this protein suggests that this region is not expressed as a single product of translational fusion. The functional role of the triple block polypeptides during viral replication is currently being investigated in electroporated protoplasts and on inoculated leaves by analysis of mutant forms of the viral genome and the sub-cellular location of triple block polypeptides. The 25 kd polypeptide is found to be essential for infection of tobacco plants but its function is dispensable for viral replication in protoplasts. However, this polypeptide could only be implicated in the viral movement within the experimental host indirectly since antibodies raised against the 25 kd polypeptide have shown it to be principally associated with intracellular X-bodies commonly observed during PVX infections

1228 *ARABIDOPSIS* MUTANTS WITH ALTERED RESISTANCE TO *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. Barbara N. Kunkel, Andrew F. Bent, Roger W. Innes and Brian J. Staskawicz. Department of Plant Pathology, University of California, Berkeley, CA 94720.

We are using a genetic approach to identify and characterize plant genes that control disease resistance in *Arabidopsis thaliana* to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) (Whalen *et al.* 1991. The Plant Cell 3:49-59). We have initiated a screen for *Arabidopsis* mutants that have lost the ability to express disease resistance to *Pst* carrying the bacterial avirulence gene *avrRpt2*. 5,000 M2 plants derived from diepoxybutane (DEB)-mutagenized Colombia (Col-0) seed have been screened to date. Two susceptible mutants and several other potentially susceptible mutants have been isolated. Preliminary results indicate that one of these mutants is altered specifically in its ability to recognize bacteria containing *avrRpt2*, as it retains resistance to bacteria carrying other avirulence genes. Further genetic analysis of this mutant and our progress towards isolating and characterizing additional mutants will be reported.

1229 MOLECULAR GENETIC ANALYSIS OF ARABIDOPSIS RESISTANCE TO PSEUDOMONAS SYRINGAE PATHOGENS. Andrew F. Bent, Roger W. Innes, Barbara N. Kunkel, and Brian J. Staskawicz. Department of Plant Pathology, University of California, Berkeley, CA, 94720.

We are developing the interaction between *Arabidopsis thaliana* and *Pseudomonas syringae* pv. *tomato* (*Pst*) as a model system to study the molecular basis of plant disease resistance (The Plant Cell 3:49-59, 1991). In many plant-pathogen interactions, resistance requires active recognition mediated by single pathogen avirulence genes and single plant resistance genes. We have identified bacterial avirulence genes that allow functional recognition of *Pst* by certain *Arabidopsis* ecotypes. Some of these genes also allow recognition by bean and soybean, suggesting that these plants use similar resistance mechanisms. *Pst* strains that differ only by the presence or absence of single avirulence genes are being used to analyze the genetics of resistance in the plant host. F2 populations from crosses between resistant and susceptible ecotypes of *Arabidopsis* (Col-0 x Po-1) are being scored for resistance to *Pst* carrying the avirulence gene *avrRpt2*. Segregation ratios suggest that this resistance is controlled by more than one plant locus. We are now testing for single gene determination of resistance in a subset of the F3 populations derived from these crosses, and anticipate use of RFLP markers to genetically map these loci. We are also characterizing *Arabidopsis* mutants that have lost resistance to bacteria that carry *avrRpt2* (see poster from B. Kunkel *et al.*). In a separate project, virulent and avirulent *Pst* are being used together with ethylene-insensitive mutants of *Arabidopsis* to examine the role of ethylene in plant responses to infection. The ethylene mutants tested are fully resistant to avirulent *Pst*, but *ein2* plants show altered responses to virulent *Pst* strains.

1230 GENETIC DISSECTION OF BACTERIAL SPECK DISEASE RESISTANCE IN TOMATO. John M. Salmeron, Francine M. Carland, Susan J. Barker and Brian J. Staskawicz. Department of Plant Pathology, University of California, Berkeley, CA, USA 94720.

Many plants resist disease by recognizing specific pathogen signals determined by pathogen "avirulence" genes. Subsequent induction of a successful defense response is thought to involve localized cell death and defense gene activation, resulting in the accumulation of a variety of antimicrobial factors. Recognition of avirulence determinants depends on specific functions encoded by plant "resistance" genes. Using the cloned bacterial avirulence gene *avrPto*, recognized by tomato in resistance to *Pseudomonas syringae* pv. *tomato* (bacterial speck disease), we are taking a mutational approach to identification and cloning of genes involved in disease resistance. We have mutagenized tomato plants homozygous for the *Pto* resistance gene that recognizes *avrPto* and are screening M2 seed for loss of bacterial speck resistance using a rapid and sensitive inoculation procedure. Our goal is to isolate mutations both in the *Pto* resistance gene and in other tomato genes required for disease resistance. The four mutagens we are employing in our studies, diepoxybutane (DEB), fast neutrons, gamma rays, and the transposable element *Activator* (*Ac*), were selected because they create alleles amenable to tagging and genomic subtraction cloning strategies. To date we have screened over 65,000 *Ac*-containing R2 plants for loss of resistance and have identified three as candidate heterozygote (*Pto/pto*) mutants. We have screened over 10,000 progeny from DEB-treated plants and have not identified a mutant. These results indicate that in contrast to other plant resistance genes, *Pto* displays a low spontaneous mutation rate ($< 10^{-4}$). Our results of attempts to confirm and characterize the nature of the three plants identified in the *Ac* screen will be reported.

1231 CONSTRUCTION OF A GENETIC MAP OF THE REGION CONTAINING THE BACTERIAL RESISTANCE GENE, *PTO* IN TOMATO. Francine M. Carland and Brian J. Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA USA 94720

Pseudomonas syringae pathovar *tomato* (*Pst*) is the causal agent of bacterial speck disease of tomato. Resistance to the pathogen is governed by the single semi-dominant gene, *Pto* that specifically corresponds to the dominant avirulence gene *avrPto* in the pathogen in a gene-for-gene manner. In an effort to gain a better understanding of the molecular genetic basis of the disease resistance response, we are attempting to clone *Pto* by different strategies, one of which is a physical isolation approach. In this approach, it is useful to determine the map position of the gene on both the classical and molecular genetic maps We have repositioned *Pto* on the classical genetic map using the chromosome five morphological markers macrocalyx (*mc*), anthocyanin free (*af*), and trifoliate (*tf*), and found the gene to reside at map position 25 The molecular marker, CD31, was mapped with respect to *Pto* as well as the morphological markers enabling us to superimpose the molecular and the classical genetic maps. In order to construct a well-detailed physical map of the *Pto* region, we are generating more molecular markers via the RAPD (Random Amplified Polymorphic DNA) technique. Presently, four amplified products distinguish the near-isogenic lines, 76R (resistant to *Pst*) and 76S (susceptible to *Pst*) and are being mapped. We are continuing to analyze new RAPD primers for their ability to differentiate 76R and 76S.

POSTER ABSTRACTS

1232

Synthetic hordothionin genes for bacterial disease resistance breeding in Solanaceae

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The Netherlands

Bacterial diseases in plants cause dramatic losses worldwide. A decrease in yield of 25% due to bacterial infections is not unusual. Since only few resistances are available for classical breeding programs, we have chosen for a molecular approach. Several (semi-) synthetic genes were made which code for hordothionins. These small, highly basic proteins generally occur in the endosperm and leaves of monocots. Toxicity assays with thionins from wheat and barley endosperm on a number of bacteria infectious to Solanaceae indicated that Xanthomonas campestris pv. vesicatoria, Clavibacter michiganensis ssp. michiganensis and C.michiganensis ssp. aepedonicus were sensitive. In barley cells these thionins are processed from a larger precursor peptide, consisting of a signal-peptide, the mature toxic thionin and a C-terminal acidic protein. Constructs were made that code for the complete prepropeptide, the mature toxin fused to the signal-peptide and the mature toxin only. To study their performance these were introduced in tobacco. Transgenics were evaluated by Northern and Western dot blot analysis. These experiments indicated expression levels from 0 to 3% of total protein, depending on the construct introduced. SDS-PAGE indicated correct processing of the prepropeptide in tobacco cells. The prepropeptide coding sequence was also introduced in tomato. These transgenics also exhibited thionin expression levels up to 3% and are being evaluated for bacterial disease resistance.

1233 CHARATERIZATION OF THREE ANTI-RICE BACTERIAL BLIGHT PROTEINS. Jin-Yuan Liu, Nai-Sui Pan and Zhang-Liang Chen. National Laboratory of Protein Engineering and Plant Genetic Engineering Biology Department, Peking University, Beijing 100871, China

Rice blight disease, caused by the bacterium Xanthomonas campestris pv. oryzea, is one of the most severe diseases that cause great loss in rice yield every year. An antagonistic bacterium against X. campestris was recently screened and classified as Bacillus subtilis A014. Total proteins were precipitated with $(NH_4)_2SO_4$ from over night culture and showed to have the strong activity in inhibiting growth of several plant bacterial pathogens, including xanthomonads, pseudomonads and some others. This crude extract was applied to CM-5 2 column and active proteins were purified from these peaks by Mono S and PepRPC column on FPLC and named as antibacterial polypeptide LCI, LCIIB and LCIII with 5.5KD, 22.5kD and 26.9kD, respectively. These proteins were sequenced, giving a total of 47 amino acid residues for full-length LCI, 24 residues for LCIIB and 28 residues for LCIII, which represent about 12% of their expected length. Computer analysis showed no significant homology to the proteins derived from Bacillus or any other reported sequences. Work is in progress to obtain transgenic rice which can protect from the bacterial infection. (This work was supported by the Rockefeller Foundation)

1234 CHARACTERIZATION OF AVRRXV: ITS ROLE IN RESISTANCE OF TOMATO TO XANTHOMONAS CAMPESTRIS PV. VESICATORIA. M. Whalen, P. Antall, A. Toms, M. Heiskeli, S. Conover, F. Carland, D. Dahlbeck, and B. Staskawicz. Department of Biology, Colby College, Waterville, ME 04901.

Resistance against many plant pathogens occurs when a plant carries a dominant resistance gene that specifically corresponds to a dominant avirulence gene in a pathogen. Isolation and characterization of avirulence genes that serve to induce resistance reactions will help us to understand the molecular basis of disease resistance. Jones and Scott have described the hypersensitive resistance response (HR) of tomato line Hawaii 7998 to Xanthomonas campestris pv. vesicatoria (XcvT) (Plant Dis. 70:337-9, 1986). When carried by a normally virulent XcvT strain, the avirulence gene avrRxv isolated from XcvT 75-3 induces HR in Hawaii 7998. This avrRxv-specific resistance is temperature sensitive. We will discuss results from sequence analysis of avrRxv, and from experiments addressing the regulation of expression of avrRxv.

1235 GENETIC EVIDENCE FOR GLOBAL REGULATION OF EXTRACELLULAR ENZYME PRODUCTION IN THE SOFT-ROT BACTERIUM, *ERWINIA CAROTOVORA* SUBSP. *CAROTOVORA*. H. Murata, A. Chatterjee and A.K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia, MO 65211

Erwinia carotovora subsp. *carotovora* strain 71 (Ecc71) produces several extracellular enzymes, including pectate lyase (Pel), cellulase (Cel) and protease (Prt), whose activities are crucial in the development of soft-rot disease. We have isolated mutants that are pleiotropically defective in the production of these enzymes. Subsequently, we identified cosmids designated as *aepA*$^+$ and *aepB*$^+$ (*aep* = activator of extracellular protein production), which stimulated enzyme production in the Aep$^-$ parent strains, but could be differentiated by their abilities to complement pleiotropic mutants. The *aepA* gene was localized within a 1.5 kb DNA segment. AepA activated transcription of *pel-1*, the gene specifying an extracellular Pel. Nucleotide sequence analysis of *aepA* in progress should help assess its homology with genes specifying transcriptional activators including members of the two-component regulatory systems. Thus far, our data support the hypotheses that multiple gene products are involved in the expression of the genes specifying extracellular enzymes and that the intracellular pool of AepA may be a critical factor determining the levels of enzymes produced.

1236 MOLECULAR ANALYSIS OF THE REGULATORY CIRCUIT CONTROLLING PECTIN LYASE (PNL) PRODUCTION IN THE SOFT-ROT BACTERIUM, *ERWINIA CAROTOVORA* SUBSP. *CAROTOVORA* (Ecc). J.L. McEvoy, A. Chatterjee, and A.K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia. MO 65211

recA function is required in the induction of PNL, carotovoricin (CTV), and cellular lysis of Ecc strain 71 (Ecc71) in response to mitomycin C (MC) and other DNA damaging agents. The findings that *pnlA*, the structural gene for PNL, was expressed poorly and was not inducible in a RecA$^-$ LexA$^-$ *E. coli* strain, implicated a positive regulator. Mutants defective in the expression of *pnlA*, Ctv production and cellular lysis were complemented by a 6.7 kb *Eco*RI fragment of Ecc71 DNA when present in a low copy vector. The gene, designated *digA* for activator of damage-inducible genes, was localized to a 2.6 kb subsegment. A low copy *digA*$^+$ plasmid, when present with a *pnlA*$^+$ or a *pnlA-lacZ* plasmid, allowed a RecA$^+$ *E. coli* strain to produce Pnl or β-galactosidase in response to MC. A *digA-lacZ* operon fusion was inducible by MC in RecA$^+$ but not RecA$^-$ strains of *E. coli* and Ecc71. The expression of *digA-lacZ* was inhibited by multiple copies of the 6.7 kb *digA*$^+$ fragment. The degree of inhibition was positively correlated with increasing copy number. Thus, the expression of *pnlA* requires a transcriptional activator, DigA, whose production in turn is controlled by a function of RecA and possibly another regulator.

1237 CHARACTERIZATION OF PSEUDOMONAS SYRINGAE PV. TOMATO HRP REGION DNA WHICH CAUSES P. S. GLYCINEA TO ELICIT THE HYPERSENSITIVE RESPONSE ON SOYBEAN. Jennifer M. Lorang and Noel T. Keen. University of California, Department of Plant Pathology, Riverside, California 92521.

Cosmid clone pPT10E9, previously cloned from P.s. pv tomato PT23 (Kobayashi et al, PNAS 86: 157, 1989), caused P.s. pv glycinea Race 4, normally virulent on soybean plants, to elicit the hypersensitive response (HR) on all of nine soybean cultivars tested. Restriction enzyme map comparisons and Southern blot analysis of pPT10E9 with P.s. pv tomato DC3000 hrp cluster DNA (supplied by B. Staskawicz) revealed that clone pPT10E9 contains DNA highly homologous to the hrp region of DC3000, known to be important in pathogenicity and HR induction. The avirulence phenotype of pPT10E9 has been localized to an 11.3 kb DNA fragment at one end of the hrp cluster. Tn1737 mutagenesis of the 11.3 kb subclone indicates that approximately 6 kb of DNA is essential for the avirulence phenotype in P.s. pv glycinea. The promoterless lacZ reporter gene of Tn1737 is been used to determine the transcriptional organization and regulation of this 6 kb DNA fragment. In addition, the surrounding DNA is being evaluated to determine its importance in hrp function and HR elicitation.

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1238 PHYSICAL CHARACTERIZATION OF A NATIVE PLASMID FROM PSEUDOMONAS SYRINGAE PV TOMATO. Jesús Murillo, Arun K. Sharma, Hao Shen, Dave Gerhold, and Noel T. Keen. University of California, Department of Plant Pathology, Riverside, California 92521.

Strains of P.s. pv tomato harbor a native plasmid of about 80 kb which has been shown to contain avirulence gene D (avrD). We have begun to characterize this plasmid from strain PT23.2 (pPT23B) using a derivative containing Tn5 inserted into the avrD gene. pPT23B::Tn5 was electroporated into P.s. syringae 51, which contains no plasmids, and purified from this strain for further studies. The fragments resulting from BamHI digestion of pPT23B::Tn5 (4.5, 5.5, 6.7, 19 and 43 kb) have been cloned and were used to establish a restriction map with the enzymes BamHI, BglII, EcoRI, NotI, PacI, SacII and XhoI. We are currently trying to identify the origin of replication of pPT23B using pBR325 as a shuttle vector. Given its widespread distribution, this native plasmid may carry genes of importance for the pathogenicity or fitness of P.s. pv tomato. Using the levan sucrase genes for counterselection, we have constructed a derivative of strain PT23.2 devoid of the plasmid pPT23B. However, we were not able to detect changes in the virulence of this strain.

1239 A FLOWING HELIUM DEVICE FOR THE ACCELERATION OF DNA-COATED MICROPROJECTILES AND ITS USE TO TRANSFORM CELLS IN INTACT PLANT TISSUES. Y. Takeuchi, M. Dotson and N.T. Keen. University of California, Department of Plant Pathology, Riverside, CA 92521

Previously described particle acceleration devices were either not available or failed to provide efficient transient DNA transformation of intact, immature soybean hypocotyls or cotyledons. We therefore developed a simple device to accelerate DNA-coated microprojectiles. It involves the use of a 1.3 cm Millipore stainless steel filter holder with only the stainless steel screen inserted, but worked very well with intact soybean hypocotyls and cotyledons. Ethanol suspensions of DNA-coated tungsten beads were dried onto the stainless steel screen and the assembled filter holder was then connected by laboratory tubing to a helium tank with conventional regulator. The line was purged with helium at low velocity to remove air and, following positioning of the target soybean tissues below the outlet of the filter holder, helium at ca. 45 pounds per square inch was run through the line for 1-2 seconds. After 24 hr incubation, more than 100 GUS-staining cells were observed in each transformed hypocotyl or cotyledon. The flowing helium device has several advantages over current particle acceleration equipment, including low expense, ease of operation, speed, flexibility of targeting, high transformation efficiency and minimal tissue damage.

1240 NUCLEOTIDE SEQUENCE OF THE *PSEUDOMONAS SYRINGAE* PV *SYRINGAE* IAA BIOSYNTHETIC GENES. M. Mazzola and F. F. White. Kansas State Univ., Dept. of Plant Pathology, Manhattan, KS 66506.

Pseudomonas syringae pv. *syringae* is the causal agent of brown spot of bean. Many plant-associated bacteria, including *P.s.* pv. *syringae*, have the ability to synthesize indole acetic acid (IAA). Tumor induction by *P.s.* pv. *savastanoi* on olive and oleander requires bacterial production of IAA. However, *P.s.* pv. *syringae* does not incite tissue hyperplasia on bean and the function of IAA in this disease complex is unknown. A DNA fragment containing the *iaa*M and *iaa*H genes from *P.s.* pv. *savastanoi* was used as a probe to identify IAA biosynthetic genes from *P.s.* pv. *syringae*. Nucleotide sequence analysis of the tryptophan-2-monooxygenase (*iaa*M) and indoleacetamide hydrolase (*iaa*H) genes of *P.s.* pv. *syringae* was conducted. The predicted amino acid sequence for the *iaa*M and *iaa*H genes of *P.s.* pv. *syringae* have approximately 90% identity with the predicted amino acid sequence of their respective homologues in *P.s.* pv. *savastanoi*. In contrast, the region upstream from the *iaa*M structural gene of *P.s.* pv. *syringae* does not possess significant sequence similarity with the promoter region of the *iaa* operon from *P.s.* pv. *savastanoi*. Likewise, there is an absence of sequence similarity in the region 3'of the respective *iaa*H structural genes.

1241 **Molecular analysis of systemic acquired resistance in *Arabidopsis*.** Scott Uknes, Eric B Ward, Sandra Dincher, Shericca Williams, Mary Moyer, Deanna Wiederhold, and John Ryals. CIBA-GEIGY Agricultural Biotechnology Unit, P.O. Box 12257, Research Triangle Park, NC 27709 USA

Systemic acquired resistance (SAR) has been best described in tobacco and cucumber, where infection of even a single leaf by any necrotrophic pathogen causes the remainder of the plant to become resistant to a broad range of pathogens. We will describe our documentation of an SAR response in *Arabidopsis*. Specifically, we will present biological assay systems for SAR and show the molecular effects of various biological and chemical inducers of SAR in *Arabidopsis*. Several proteins and RNAs which are induced during SAR have been identified and cloned. At least one of these RNAs is induced rapidly by salicylic acid, a probable endogenous inducer of SAR. Several strategies for the isolation of mutants in the signal transduction pathway that activates SAR will be discussed.

1242 **MOLECULAR GENETIC CHARACTERIZATION OF THE REGION CONTAINING THE *PTO* RESISTANCE GENE IN TOMATO.** Gregory B. Martin, Martin Ganal, and Steven D. Tanksley. Department of Plant Breeding, Cornell University, Ithaca, New York 14853

Although plant resistance to necrotrophic bacteria is of great fundamental and commercial interest very little is known about the molecular basis of interactions between these bacteria and resistant plants. We are attempting to isolate by map-based cloning a gene from tomato, *Pto*, that confers resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*). The tomato - *Pseudomonas* interaction provides a particularly attractive experimental system for several reasons. The bacterial pathogen is amenable to molecular analysis and already has been the subject of numerous physiological and epidemiological studies. Resistance to *Pst* in tomato is encoded by a single gene (*Pto*) which has been located on chromosome 5 in relation to morphological markers and molecular markers and the resistance allele displays dominant gene action allowing for easy screening.

We are developing several techniques directed at isolating the *Pto* gene. First, using near-isogenic lines we are identifying RFLP and RAPD markers that are tightly linked to the gene (1). Second, in order to accurately place the *Pto* gene with respect to the linked markers we have developed several F_2 populations that are segregating for *Pseudomonas* resistance which we will use in creating a fine-structure genetic map of the *Pto* region. Third, the physical distance between markers flanking the *Pto* gene will be analyzed using pulsed field gel electrophoresis (PFGE) in order to determine the distance in kilobases that will need to be covered in a chromosome walk. Finally, we have constructed a three-genome equivalent yeast artificial chromosome (YAC) library which we will use in chromosome walking experiments to isolate the *Pto* gene. We will report our progress in applying these approaches to the isolation of the *Pto* gene.

Martin, G., J. Williams, and S. Tanksley (1991) Proc Natl Acad. Sci. USA 80:2336-2340

1243 PEROXIDASE ACTIVITY IN INCOMPATIBLE INTERACTIONS BETWEEN *XANTHOMONAS ORYZAE* PV. *ORYZAE* AND RICE. A. Guo, C.M. Hopkins, S.-H. Choi, P.J. Reimers, F.F. White, and J.E. Leach. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

In incompatible interactions between rice cultivar Cas 209 (carrying resistance gene *Xa-10*) and race 2 strains of *Xanthomonas oryzae* pv. *oryzae*, a hypersensitive response (HR), characterized by lignin accumulation, was observed at 24-36 h after seedling leaves were infiltrated with bacteria. An increase in a cationic peroxidase (pI 8.5) activity occurred earlier in incompatible (16-24 h) than in compatible (48-72 h) interactions. The cationic peroxidase utilizes coniferyl alcohol, a precursor of lignin in rice, as a substrate. A cosmid clone from a library of race 2 conferred incompatibility to a virulent race 6 strain of *X. o.* pv. *oryzae*; this response was characterized by an increase in cationic peroxidase activity. The cationic peroxidase activity was also observed in incompatible interactions involving near-isogenic lines IR-BB10 (*Xa-10*), IR-BB7 (*Xa-7*), and IR-BB5 (*xa-5*). Exposure of plants to different periods of light after infiltration with bacteria affected the development of the HR and the accumulation of the cationic peroxidase.

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1244 MOLECULAR ANALYSIS OF AN H.R. INDUCED IN ALFALFA BY PSEUDOMONAS SYRINGAE. Robert Esnault, Dominique Buffard, Joumana El Turk, Colette Breda et A. Kondorosi, Institut Sciences Végetales, CNRS, 91198 Gif/Yvette

Plant-pathogen interactions result in the induction of host defense response that includes the production of phenylpropanoid compounds. Among the key enzymes involved are PAL, CHS and CHI. In many plant species, PAL and CHS are encoded by multigene families, individual members of which are diferentially regulated and utilise different signals for induction. The work, presented in this abstract, focus on the expression of the CHS and CHI during early stages of alfalfa/Pseudomonas syringae pv. pisi interaction. A high induction of expression of CHS and CHI was observed 4 h after infection of the alfalfa leaves and several clones were isolated from a cDNA library prepared from the HR maximum. Two clones of CHS were particularly analysed and their sequence determined; the deduced aminoacid sequences show a strong homology between the two clones (more than 90 %) and with other CHS (more than 80 %). To analyse the diferential expression of these two clones, three oligonucleotides were used: one corresponding to an highly conserved region (present in all the so far known CHSs) and two others individualized from the 3' non-coding region of each alfalfa clone, despite the overall and rather high homology (around 82 %) of these regions. We have shown that these 3' oligonucleotides can specifically detect the corresponding sequence and that allowed to analyse the differential expression of the CHS gene during the alfalfa HR.

1245 INDUCTION OF PHENYLPROPANOID METABOLISM DURING THE INTERACTION OF *ARABIDOPSIS THALIANA* WITH PHYTOPATHOGENIC PSEUDOMONADS. Farida Shaheen and Keith R. Davis, The Ohio State University, Department of Plant Biology and the Biotechnology Center, Columbus, Ohio 43210

We are using the infection of *Arabidopsis thaliana* with phytopathogenic pseudomonads as a model system for studying plant disease resistance. Infiltration of *A. thaliana* leaves with avirulent *Pseudomonas* strains is associated with a larger, more rapid induction of phenylalanine ammonia-lyase (PAL) mRNA when compared to infiltration with virulent strains. Introduction of a putative *avr* gene from avirulent *P. syringae* pv. *tomato* strain 1065 into the virulent *P. s.* pv. *maculicola* strain 4326 causes the transconjugant to induce significantly higher levels of PAL mRNA when compared to *Psm* 4326. This suggests that PAL is regulated by a signal pathway coupled to the recognition of *avr* gene products. We have extended these studies by examining the effects of introducing other cloned bacterial *avr* genes from *P. s.* pv. *tomato*, *P. s.* pv. *glycinea*, and *Xanthomonas campestris* pv. *malvacearum* into the virulent strains *Psm* 4326 and *Pst* DC3000. Our results show that some *avr* genes induce significantly higher levels of PAL and peroxidase mRNAs compared to wild type strains. Current studies are focused on the characterization of three distinct *A. thaliana* PAL genes, determining if specific members of this PAL gene family are differentially induced by bacterial *avr* genes, and characterizing the promoter elements responsible for this induction. These results will be discussed in the context of utilizing genetic approaches for identifying factors involved in activating plant defense responses.

1246 GENETIC INTERACTION BETWEEN TOMATO AND *PSEUDOMONAS SYRINGAE* PV. TOMATO. Stockinger, Eric J. and Linda L. Walling University of California, Department of Botany and Plant Sciences, Riverside CA 92521

We have been developing a system to better characterize the interaction between the bacterial speck causing organism, *Pseudomonas syringae* pv. tomato and its host plant, tomato. *P.* tomato collections have been screened against various tomato germplasm for the ability to cause speck. Genetic analysis of tomato germplasm resistant to *P.* tomato infection is being conducted. Two putative new resistance genes, Pto 2 and Pto 3, have been identified. These genes were found in populations of *Lycopersicon pimpinellifolium* PI 126430 and *L. hirsutum* PI 134417. DNA blot analysis with RFLP markers of the F2 and backcross populations will be used to place these potentially new loci on the current tomato molecular map. As part of the current effort to clone genes by chromosome walking from a RFLP marker, a chromosome jumping library of tomato is being constructed. This library should augment other strategies for gene isolation, such as YAC and cosmid libraries.

1247 RELATION OF PEROXIDASE TO RESISTANCE IN SOME THAI RICE VARIEITES. Lolita Meksongsee,* Poontariga Harinasut,* Kasem Soontarajarn,** and Narong Singburaudom. Kasetsart University* and Department of Agriculture,** Bangkok, Thailand

The Thai rice breeders have been improving the rice plant to increase the resistance to diseases and pests. The peroxidase system in plant is widely documented to be implicated in the defense mechanism to stresses, including diseases and insect pests, so we studied the relation of rice peroxidase to resistance The sample of improved ordinary varieties include RD 1, RD 7, RD 23 and SPR 60. The aromatic varieties are composed of the improved variety PTT 60 and the traditional variety KDML 105. The roots of the improved varieties had about the same high peroxidase activities with guaiacol as substrate, but RD 23 and SPR 60 exhibited the highest. With gallic acid as substrate, the peroxidase activities of all the rice varieties were about the same. The peroxidases of RD 1, RD 7, RD 23, SPR 60 and KDML 105 oxidized pyrogallol almost to the same extent, but that of PTT 60 was the slowest. The peroxidases of the first and second leaves of all the rice plants had about the same rates of oxidation of pyrogallol, guaiacol and gallic acid. The polyacrylamide gel electropherograms of the roots and leaves showed similar isozyme patterns as that of southern bean leaves, reported by Farkas and Stahmann. Most interesting is the presence in the rice patterns of bands II and III, which were found by the above authors to be present only in southern bean mosaic virus-infected bean leaves and to be most active with guaiacol. It is worth observing that the two improved varieties, RD 23 and SPR 60 which are the most resistant to diseases and insects, had the most active guaiacol-oxidizing activities.

1248 A COMPATIBLE BACTERIUM SUPPRESSES DEFENSE GENE TRANSCRIPT ACCUMULATION. Judy L. Jakobek, Jennifer A. Smith and Peter B. Lindgren. North Carolina State Univ., Dept. of Plant Pathology, Raleigh, NC 27695

We have previously shown that transcripts for phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), and chitinase accumulate very rapidly in bean after inoculation with the incompatible bacterium, Pseudomonas syringae pv. tabaci Ptl1528. The temporal pattern of accumulation varies for each transcript and all are detectable between 4-8 hours post-inoculation. A visible hypersensitive response occurs 12-14 hours post-inoculation during this interaction. In contrast, the compatible bacterium Pseudomonas syringae pv. phaseolicola NPS3121 induces the accumulation of chitinase transcript 20 hours post-inoculation, while transcripts for PAL, CHS, and CHI are not detected until after 48 hours. These temporal differences in transcript accumulation are correlated to similar differences in the accumulation of isoflavonoid compounds. When bean plants are inoculated with NPS3121 six hours prior to inoculation with Ptl1528, defense transcript accumulation is significantly reduced when compared to plants inoculated with Ptl1528 alone. These experiments suggest that P. s. pv. phaseolicola inhibits the accumulation of certain defense gene transcripts during the initial stages of infection. We are continuing to investigate this phenomenon, and its relationship to the activation of defense responses by bacterial pathogens.

1249 *hrp* MUTANTS OF *Pseudomonas syringae* pv. *tabaci* INDUCE DEFENSE RESPONSES. Judy L. Jakobek and Peter B. Lindgren. North Carolina State Univ., Dept. of Plant Pathology, Raleigh, NC 27695

We have been studying the activation of defense responses in intact bean plants (*Phaseolus vulgaris* L.) after infiltration with bacterial pathogens. Transcripts for phenylalanine ammonia-lyase, chalcone synthase, chalcone isomerase, and chitinase accumulate after inoculation with *hrp* mutants of *P. s.* pv. *tabaci* (*Pst*), even though a hypersensitive response does not occur. The temporal pattern of transcript accumulation parallels that seen in bean after inoculation with wild-type *Pst*, which causes a hypersensitive response. These transcripts also accumulate in bean after inoculation with *Eschericia coli*, *P. fluorescens*, heat-killed *Pst* cells, or *Pst* cells treated with protein synthesis inhibitors. Defense transcripts also accumulate in bean after inoculation with the compatible bacterium *P. s.* pv. *phaseolicola*, but the pattern of accumulation is different than that seen after inoculation with wild-type *Pst*. Antifungal activity was detected by *Cladosporium* TLC bioassay in ethyl-acetate soluable fractions of ethanol extracts of bean inoculated with either wild-type or *hrp Pst* strains, but not in plants infiltrated with water.

POSTER ABSTRACTS

1250 CECROPIN SB37 TOXICITY TOWARDS PLANT PROTOPLASTS AND PLANT PATHOGENIC BACTERIA R.O.Nordeen, S.L.Sinden, J.L. Jaynes and L.D. Owens Plant Mol. Bio. Lab (R.O.N L.D.O.) and Veg. Lab (S.L.S.), USDA, ARS, Beltsville, MD 20705 and Dept. of Biochem. (J.L.J), Louisiana State University, Baton Rouge, LA 70803 Cecropins are a family of small basic polypeptides (~4 kD). They possess potent antibacterial activity and are important in the immune response of insects. We have investigated the feasibility of introducing a cecropin gene into plants to protect them from bacterial disease. To investigate host toxicity, the lethal concentration of chemically synthesized cecropin SB37 was determined for protoplasts from eleven plant species. Lethal concentrations were also determined for nine bacterial species pathogenic to these plants. Lethal concentrations ranged from 4.5 µM for tomato (*Lycopersicon esculentum* c.v. Red Cherry) to 55 µM for sugarbeet (*Beta vulgaris* cv. REL 1). Lethal concentrations for the bacteria ranged from 0.3 µM for *Pseudomonas syringae* pv. *glycinea* strain Pg4 to 3.1 µM for *P. syringae* pv. *tomato* strain 0683-23. A PCR-modified cecropin gene has been introduced into tobacco (*Nicotiana tabacum* cv. Bright Yellow) by transformation with *A. tumefaciens*, and expression of the gene is being analyzed.

1251 TRANSGENIC TOBACCO PLANTS RESISTANT TO PHASEOLOTOXIN De la Fuente-Martínez, J.M., Mosqueda-Cano, G., Alvarez-Morales, A. and Herrera-Estrella, L. CINVESTAV-IPN, Departamento. de Ingeniería Genética, Apdo. Postal 629, Irapuato, Gto. 36500 MEXICO.
Phaseolotoxin is a potent inhibitor of the enzyme Ornithine Carbamoyltransferase (OCTase) from a variety of sources. The gene coding for the phaseolotoxin-insensitive OCTase from Pseudomonas syringae pv phaseolicola has been cloned and sequenced in our laboratory. This gene (we propose be named arg K) was cloned in pKAH5, which carries a selectable marker neomycin phosphotransferase II under the nos promoter and a peptide transit from RubisCO small subunit (under the CaMV 35 S promoter) to obtain a translation fused product to direct the OCTase to the chloroplast. This construction was transferred into Nicotiana tabacum L. cv. Xanthi using the Agrobacterium tumefaciens cointegrative vector pGV3850. OCTase assays performed in transgenic (T) and wild type (WT) plants resulted in activity ratios T/WT ranging from 1 to 10. Results comprising the resistance to the bacterial toxin (in vitro OCTase crude extract inhibition), its phenotypic effect when is applied to leaves, and the inoculation response to a compatible P. s. pv. phaseolicola will be discussed.

1252 ACTIVATION OF DEFENSE-RELATED GENES UPON INFECTION OF PARSLEY LEAVES WITH *ERWINIA CHRYSANTHEMI*. Frederique Van Gijsegem, Imre E. Somssich and Dierk Scheel Max-Planck-Institut fur Zuchtungsforschung, Abteilung Biochemie, Carl-von-Linne-Weg 10, D-5000 Koln 30, FRG
Activation of defense-related genes is part of a multi-component defense response of plants towards pathogen attack. Non-host resistance of parsley to the fungal soybean pathogen *Phytophthora megasperma* involves the activation of a whole set of genes such as those encoding enzymes of the phenylpropanoid pathway, pathogenesis-related proteins, peroxidases, hydroxyproline-rich glycoproteins, and enzymes involved in the activated methyl cycle. Transcription of these genes was also induced upon infection of parsley leaves with the bacterial pathogen *Erwinia chrysanthemi* (*Ech*) but not following inoculation of *Escherichia coli*. Symptoms of infection of parsley leaves with wild type *Ech* included formation of a necrotic area around the inoculation site and, less frequently, spreading and petiole maceration. Several *Ech* mutants altered in pathogenicity factors, however, caused different symptoms. Mutants defective in one of the major pectate lyases or affected in the regulation of pectate lyase synthesis were found to be more aggressive, causing maceration of the infected leaf and the petiole. Infection with secretory mutants or mutants with a defective cell envelope, on the other hand, led only to the development of necrosis of the inoculated leaf or did not result in any symptoms. While aggressive mutants always caused rapid activation of the defense-related genes, the less aggressive mutants induced variable levels of activation.

1253 INFECTION OF *ARABIDOPSIS THALIANA* WITH *XANTHOMONAS CAMPESTRIS*. J.E. Parker, C.E. Barber, M.J. Fan and M.J. Daniels. The Sainsbury Laboratory, John Innes Centre, Norwich, U.K.
The interaction of *A. thaliana* and the crucifer pathogen *X. campestris* is being studied as a model patho-system to isolate bacterial avirulence genes and genes controlling resistance from the plant. Representative strains of *X. campestris* pathovars *campestris*, *raphani*, *aberrans* and *armoraciae* grow and cause disease symptoms when infiltrated into *A. thaliana* leaves. A search for differential responses of *A. thaliana* ecotypes to *X. campestris* strains revealed two types of resistance reactions. In the first, bacterial growth and disease symptoms are limited at moderate inoculum concentrations but symptoms are evident at high bacterial doses. Analysis of a gene derived from one strain which confers this type of avirulence on a normally pathogenic strain of *X.c. campestris* will be described. The second resistance reaction also severely attenuates pathogen growth and symptom development but inoculation with high bacterial concentrations leads to a rapid localized necrosis characteristic of a hypersensitive response. Analysis of susceptible plants derived from natural or mutagenized populations will be used to characterize genetically the resistance genes involved as a means to their isolation.

1254 HOST RESPONSES OF TURNIP TO PATHOVARS OF *XANTHOMONAS CAMPESTRIS*. Max Dow, Jutta Conrads-Strauch and Michael Daniels. Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH UK
Inoculation of mature leaves of turnip with the incompatible *Xanthomonas campestris* pv. *vitians (Xcv)* results in the induction of β-1,3-glucanase (βG) and chitinase/lysozyme (CHL) activities. One major basic βG and two major basic CHL enzymes have been purified from both healthy and inoculated leaves. The pattern of induction of CHL isoforms by *E. coli* and heat-killed pathogens was different to that seen with *Xcv*. These former treatments did not induce βG. The induction of βG in response to the compatible pathogen *X. c.* pv. *campestris* was slower than that seen with *Xcv*. Antisera to βG were used to screen a cDNA library from *Xcv*-inoculated plants constructed in lambda-Zap. Several positive clones were identified and sequenced. All the clones share the same sequence; the predicted polypeptide has good amino acid sequence homology to βGs from other sources. As antisera to CHL failed to detect any positive clones in the library, PCR methods were employed to amplify the appropriate DNA from the library. Primers were based on two conserved regions in a number of other plant chitinases. These cDNA probes have been used to follow the changes in gene expression in turnip leaves in response to compatible and incompatible pathovars of *Xanthomonas campestris*, non-pathogens and killed bacteria. In addition, immuno-electron microscopy with antisera to βG has localised this basic enzyme in the vacuole of both challenged and healthy leaf cells.

1255 ROLE OF *hrpX* IN THE INTERACTION BETWEEN *Xanthomonas campestris* AND CRUCIFERS.
Sophien Kamoun, Elisabetta Tola, Haresh V. Kamdar, and Clarence I. Kado. Department of Plant Pathology, Univ. of Calif., Davis, CA 95616

Xanthomonas campestris pv. campestris (*Xcc*) causes the black rot disease on crucifers, whereas *X.c.* pv. armoraciae (Xca) induces leaf spots on crucifers. The *hrpXc* gene of *Xcc*, a member of the *hrp* family, is required for pathogenicity as well as for hypersensitive response on nonhost plants. HrpXc mutants were shown to be complemented by coinoculation with wild-type strains suggesting that this gene may encode for an exocellular component (S. Kamoun and C.I. Kado, J. Bactenol. 172:5165-5172, 1990). The molecular analysis of *hrpXc* has been conducted and the *hrpXc* promoter localized. Southern blot hybridizations and PCR amplification with primers internal to *hrpXc* indicated that the *hrpXc* locus is highly conserved among all the examined *X. campestris* pathovars including pv. armoraciae. Using marker-integration mutagenesis, the *Xca* homolg of *hrpXc* was mutagenized, cloned, and named *hrpXa*. HrpXa mutants were unable to cause leaf spots on crucifers and HR on nonhosts, thus bearing a typical *hrp* phenotype. Chromosomal transcriptional fusions of *hrpXc* and *hrpXa* to the *gusA* reporter were constructed and used to demonstrate that the expression of these promoters is induced in, respectively, crucifer xylem and crucifer mesophyll. In conclusion, *hrpX* is required for pathogenicity of *X. campestris* on crucifers regardless of the genetic background of the strain

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1256 ANALYSIS OF DIVERSITY OF A HOST AND PATHOGEN IN A TRADITIONAL AGROECOSYSTEM. R Nelson, C.M. Vera Cruz, E Ardales, A. Bordeos, H. Leung[1] and T.W. Mew. Div. of Plant Pathology, International Rice Research Institute, Los Banos, Philippines. [1]current address: Dept. of Plant Pathology, Washington State University, Pullman, Washington, U.S.A.

The diversities of rice and the bacterial blight pathogen were assessed for the traditional agroecosystem in Ifugao Province, Luzon, Philippines. Race 5 of *Xanthomonas oryzae* pv. *oryzae* (Xoo) is endemic in this region. Analysis of DNA polymorphisms revealed considerable diversity among race 5 strains, even within single fields. Numerical analysis of RFLP data based on pTnX1, a putative transposable element probe, indicated that the race 5 stains analyzed belonged to two distinct lineages. Traditional cultivars from Ifugao were inoculated with strains representing some of the diversity of race 5. When three strains were used to test 361 cultivars, a range of reaction types was observed. A few of the cultivars showed differential reactions to the different strains. Subsequent experiments on one of these cultivars, accession 8106, confirmed that some lines derived from this accession were resistant to race 5 strains from one of the Xoo lineages, and susceptible to strains from the other Xoo lineage. The same differential reaction was seen in rice cultivars containing the *Xa-14* gene for resistance; these cultivars differentiate the two race 5 lineages observed by RFLP typing into two distinct races. Other lines derived from acc. 8106 were susceptible to both pathogen lineages. Thus, rice cultivars from this area of the Philippines show diverse reactions to endemic strains of Xoo, and even plants derived from a single accession can show heterogeneity with respect to resistance. RFLP analysis of the pathogen population indicated substantial variation among bacterial strains and was useful in revealing a new race of Xoo.

1257 STRUCTURAL DIVERSITY AMONG *ERWINIA CAROTOVORA* PECTATE LYASES. Lyudmil S. Antonov, Verlyn K. Stromberg, and George H. Lacy, Laboratory for Molecular Biology of Plant Stress, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330.

The soft rot bacterium *Erwinia carotovora* subsp. *carotovora* strain EC14 contains several genes coding for pectate lyases. Genes *pel9* and *pel10*, coding for extracellular basic pectate lyases (pIs 9.5 and 10.5), are located close to each other on the Ecc chromosome, and were cloned on a single plasmid. *Escherichia coli*-expressed pectate lyases degrade polygalacturonic acid (PGA). A third pectate lyase (pI 7.8), expressed in the periplasm and/or cytoplasm of *E. carotovora* subsp. *carotovora*, is also expressed in *E. coli* from a second plasmid. For most of their sequences, *pel9* and *pel10* are homologous to each other and to the *pelBC* family of *Erwinia* extracellular enzymes (Hinton *et al.*, Molec. Microbiol. 3:1785, 1989) and distinct from *pel153* (Trollinger *et al.*, Molec. Plant-Microbe Interact. 2:17, 1989). However, *pel10* is most similar to *palX* (Itoh *et al.*, Agric. Biol. Chem. 52:479, 1988). This represents the second *palX*-like gene described. The X pectate lyases may be different enough to be considered as a subgroup of the pelBC family. Likewise, *pel7* is the third *pel153*-like (or *pelY*-like) pectate lyase described from *E. carotovora* subspecies supporting the existence of a *pelY* group of intracellular enzymes. We constructed *pel9-10* and *pel10-9* gene fusions by joining the 5' part of *pel9* with the 3' part of *pel10* and the 5' part of *pel10* with the 3' part of *pel9*. These fusion proteins degrade PGA. Partial polypeptides expressed from *pel10* deletion mutants also degrade PGA. We compare *E. carotovora* and *Erwinia chrysanthemi* consensus sequences for *pelBC* pectate lyases. Structural diversity may be important in specificity for plant host substrates and pathogenesis.

1258 GENE CLONING AND EXPRESSION OF ANTAGONISTIC PROTEIN OF B826 STRAIN TO Xanthomonas campestris pv. oryzae. Xiong F. Chen, Yong P. Zhang, Wei L. Chen, De B. Li. Biotechnical Institute, Zhejiang Agricultural Univ. Hangzhou, Zhejiang 310029, P.R.China

One protein(B826-I) has been purified from Bacillius subtilis B826 strain, which inhibits the growth of X. campestris strongly. After the cultural medium is precipitated by ammonium sulfate, the proteins are separated on Sephadex G-75 column and the activity fractions are pooled, then the pure protein is isolated on HPLC by DEAE-5PW column and Pak-300SW column. B826-I protein has antagonistic activity to X. campestris, its molecular weight is 37kd.

B826-I protein is used as an antigen, three hybridoma cell lines that secrete specific monoclonal antibodies have been prepared. To screen antagonistic protein genes, the gene library of B826 DNA is constructed on the vector of pBS plasmid. Using the McAb as probes, 12 clones which express fusion protein are screened out from the library by Immunolgical screening, among them, CLONE 9 is more active than others and has 2.1 kb insertion fragment. Finally, the fragment is reinserted into pKK233-2 vector, and expresses active antagonistic protein in E.coli, which is identified by Western blot.

1259 Transgenic Tobacco Expressing the Phaseolotoxin Tolerant OCTase Gene from the Bean Halo Blight Pathogen. E.Hatziloukas[1] and N.J.Panopoulos. Department of Plant Pathology, University of California, Berkeley, CA 94720, USA and [1]Department of Biology, University of Crete, Heraklio, Greece

Pseudomonas syringae pv. *phaseolicola* is the only known producer of phaseolotoxin, a virulence factor in bean halo blight and a potent inhibitor of ornithine carbamoyl-transferases (OCTs) from various organisms. The bacterium possesses two different OCT-genes, *argF* and *argK*, encoding the isozymes sOCT and rOCT respectively. sOCT is sensitive, while rOCT is tolerant to the toxin. We sequenced both genes, identified their promoter regions and transcripts and studied their regulation. Both genes are transcribed constitutively, probably both from their own promoters and as part of polycistronic messages. rOCT biosynthesis is post-transcriptionally regulated. We transferred the *argK* in tobacco plants via a Ti vector by fusing its coding region to appropriate cis-acting sequences that allowed its expression in plants and the targeting of its product to the chlorplast. We identified transgenic plants transcribing the chimeric gene, by using Northern blot hybridization analysis. We also detected in protein extracts from their leaves an OCT-activity which is more tolerant to the toxin, than the activity (ies) in control extracts. These transgenic plants will allow us to study the pathophysiological consequences of OCT inhibition, which is the primary biochemical lesion associated with toxin action in halo blight infested plants.

1260 GENETIC INTERRELATIONSHIPS AMONG *AGROBACTERIUM* INFECTION PROCESSES. Stanton B. Gelvin, Todd Steck, M. Krishnan and Lillian L. Hadeck. Purdue University, Department of Biological Sciences, West Lafayette, Indiana 47907. We have previously shown that, using acetosyringone as an inducer, we can hyperinduce the *vir* genes of the *Agrobacterium* Ti-plasmid using octopine. This hyperinduction requires only *virA*, *virG*, and genes on the chromosome. We now show that this potentiation of *vir* gene induction does not involve the periplasmic domain of VirA. In addition, the extent of hyperinduction using tobacco protoplasts depends upon the amount of exogenous octopine added to the medium. Low concentrations of octopine (less than 0.2 mM) hyperinduce the *vir* genes, but higher concentrations repress this induction. *Agrobacterium* strains mutant in the octopine synthase gene are less virulent than are wild-type bacteria. The *vir* genes of these mutant bacteria cannot be induced to the same extent by normal tobacco protoplasts as can the *vir* genes of the corresponding wild-type bacteria. However, when transgenic tobacco protoplasts expressing the octopine synthase gene are used for induction, the *vir* genes of both bacterial strains are induced to the same extent. We speculate that *Agrobacterium* senses the amount of opines in the wound environment and uses this information to determine the state of growth (induction of *vir* genes or vegetative growth). Because opines can affect the induction of *vir* genes in a complex way, we tested the possibility that *vir* genes may influence a process induced by some opines, conjugal Ti-plasmid transfer. We found that, under limiting opine induction conditions, conjugal transfer can be diminished by up to four orders of magnitude by mutations in certain *vir* genes. We shall also present data suggesting that the single-stranded form of the T-DNA is the form that is transferred to plant cells.

1261 INDUCTION AND CHARACTERIZATION OF THE HYPERSENSITIVE RESPONSE IN *ARABIDOPSIS THALIANA* L. UPON ELICITATION BY A PATHOGEN *XANTHOMONAS CAMPESTRIS* pv. *CAMPESTRIS* AND A CHEMICAL ELICITOR LEAD NITRATE. M. Lummerzheim[*,1], M. Sandroni[*], F. Costa Migueis[2], C. Castresana[3], E. Louzada[*], D. De Oliveira[*], M. Van Montagu[1], and B. Timmerman[*]. [*]Laboratório de Genética Molecular Vegetal UFRJ and [2]Laboratorio de Microscopia Electronica e Ultrastructura Celular UFRJ, Rio de Janeiro, Brazil, [1]Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

Conditions have been established to induce the hypersensitive response in flowering *Arabidopsis thaliana* plants by spray inoculation. Among the several bacterial strains and the biotic and abiotic elicitors tested, *Xanthomonas campestris* pv. *campestris* and lead nitrate were found to be effective inducers. Scanning electron microscopy of the characteristic necrotic lesions revealed the potential bacterial penetration sites. *In planta* growth curve indicated the limited proliferation of the bacteria. Accumulation of poly-phenolic compounds and callose was monitored using fluorescence and confocal microscopy. The detection patterns of ß(1,3)-glucanases, chitinases, and peroxidases were followed in function of the used elicitor. This mass inoculation method allows us to currently screen thousands of M2 plants for mutants responding abnormally when challenged by *X. campestris* pv. *campestris* and lead nitrate.

POSTER ABSTRACTS

1262 LOSS OF T-DNA INTERMEDIATES UPON COCULTIVATION OF PRE-INDUCED *AGROBACTERIUM TUMEFACIENS* WITH TOBACCO PROTOPLASTS. Todd R. Steck and Stanton B. Gelvin. Purdue University, Dept. Biological Sciences, W. Lafayette, IN 47907

The form of T-DNA that is transferred from *A. tumefaciens* to recipient plant cells has yet to be identified definitively. Evidence to date has been based mainly on determining whether the form of T-DNA found in induced *A. tumefaciens* cells is single- or double-stranded, linear or circular. This strategy is based on the assumption that the most abundant species of T-DNA is the transferred form. Although results from a number of labs indicate linear single-stranded DNA (T-strands) predominates in induced cells there is also evidence for the presence of double-stranded molecules. We have determined the form of processed T-DNA that is lost from pre-induced bacteria upon cocultivation with tobacco protoplasts. We have obtained results that allow a more definitive determination of transferred T-DNA forms as well as allowing direct compansons of the relative amounts of both single- and double-stranded T-DNA products. To maximize *vir* gene induction and virulence a plasmid carrying pTiA6 *virG* was introduced into octopine-type *A. tumefaciens* strains carrying a ß-lac fusion in a *pinF* gene. The characteristics of the T-DNAs found in these induced strains were analyzed by Southern blotting and both intact linear single- and double-stranded forms were identified on the same gel. A pre-induced *pinF* mutant was cocultivated with tobacco W38 protoplasts and at various times bacterial cells bound to the protoplasts and unbound were separated and the bacterial T-DNA products analyzed. Single-stranded T-DNA was preferentially lost from the bound bacteria in as little as one-half hour after cocultivation.

1263 ENHANCED TRANSIENT GUS EXPRESSION AFTER TRANSFORMATION OF CELERIAC CALLI BY *AGROBACTERIUM* STRAINS CONTAINING AN EXTRA COPY OF *VIRG*. C.-N Lin, X.-Q. Li, T. K Hodges and S B. Gelvin Dept of Biological Sciences (C-NL, SBG), and Dept of Botany and Plant Pathology (X-OL, TKH), Purdue University, West Lafayette, IN 47907

The literature contains both positive and negative results regarding whether the presence of an extra Vir-region (including the *virG* gene) in *Agrobacterium* enhances transformation frequency In an effort to investigate transformation efficiency by monitonng transient GUS expression, *Agrobacterium* strains with or without a second copy of *virG* (cloned into the binary vector) were employed to infect celeriac (*Apium graveolens var. rapaceum (Mill) Gaud.-Beaup*) calli Two different *virG* genes (one from the agropine type TI-plasmid pTiBo542 and the other from the octopine type Ti-plasmid pTiA6) were cloned into a binary vector derived from pBin19 This binary vector harbors a modified 35S-GUS fusion gene that does not express in *Agrobacterium* (Janssen and Gardner, Plant Mol. Biol 14, 61-72). All three binary vectors (with or without a second copy of *virG*) were introduced into an *Agrobactenum* strain containing a C58 chromosomal background and the supervirulent Ti-plasmid pTiBo542. Four days after *Agrobactenum* infection, GUS assays were carried out to estimate the efficiency of transformation Blue spots were detected in most of celeriac calli transformed with either strain containing a second copy of *virG* after 5 hours of incubation in X-GLUC, whereas only a small proportion of calli were stained blue from the transformation with the strain containing only one copy of *virG* There was no significant difference in transformation efficiency between the two strains containing extra copies of the *virG* genes under conditions of this experiment No blue spots were detected from the calli infected with strains containing either no Ti-plasmid or the *gusA* gene In summary, this is the first report, as we know, of transient transformation of celeriac callus by *Agrobacterium tumefaciens*, and that additional copies of *virG* enhanced transformation efficiency

1264 ISOLATION AND GENETIC ANALYSIS OF A NEW AGROBACTERIUM TUMEFACIENS CHROMOSOMAL LOCUS THAT PLAYS A ROLE IN VIR GENE EXPRESSION. John Gray, Juan Wang and Stanton B. Gelvin. Purdue University, West Lafayette, Indiana 47907.

We report the isolation of a new chromosomal locus in Agrobacterium tumefaciens that plays a role in vir gene expression. Two individual Tn5 insertions in this locus resulted in a 2 to 4 fold reduction in the induced expression of a virB::lacZ fusion in response to 100µm acetosyringone. This reduction occurred in medium containing either sucrose or glucose at pH 5.5 and in the presence of either high or low phosphate concentration. A similar effect was seen in a reconstructed mutant that carried virD, virE or pinF::lacZ fusions. Probes from other known Agrobacterium chromosomal loci involved in virulence did not hybridize with the Tn5 disrupted locus. This locus was cloned and used to isolate a wild-type 5kb DNA fragment. This fragment partially restored virB induction in the original mutants while exhibiting no additive effect on the wild-type strain. This wild-type fragment was mutagenized with Tn5seq-1 and used in complementation studies to define further a 1.5kb region responsible for the vir gene induction phenotype. This region is currently being sequenced. This region was found to hybridize to unique DNA fragments in Agrobacterium rhizogenes and Rhizobium meliloti. The function of this locus and its effect on other vir genes is under further investigation. Preliminary experiments with the original mutants indicate that these mutants exhibit an attenuated virulence Kalanchoe stems and tobacco leaf-discs.

1265 GENETIC AND MOLECULAR ANALYSES OF *picA*, A PLANT-INDUCIBLE LOCUS ON THE *AGROBACTERIUM TUMEFACIENS* CHROMOSOME. Lijun Rong, Susan J. Karcher, and Stanton B. Gelvin. Purdue University, Dept. of Biological Sciences, West Lafayette, IN 47907. *picA* is a plant-inducible chromosomal locus we identified in *A. tumefaciens* A136 by transposon Mu dI1681 mutagenesis. The inducing compound in the carrot root extract is an acidic polysaccharide, most likely derived from the pectic portion of the plant cell wall. The induction of *picA* represents a possible new signaling system between *Agrobacterium* and its host plants. In bacteria harboring the *picA* mutation, the binding properties of bacteria in the presence of plant cells were altered: the mutant cells self-aggregated to form long "ropes", suggesting that the *picA* locus is involved in determining bacterial cell surface properties. Consistant with this hypothesis is the fact that A *tumefaciens* mutant in *picA* are more virulent than are wild-type bacteria, possibly reflecting a tighter association of the mutant bacteria with plant cells. The plant-inducible promoter region of *picA* was defined by DNA subcloning. The promoter region as well as the DNA region downstream of the *picA* promoter was sequenced. The sequence data suggest that there are at least two open-reading frames (ORFs) controlled by the *picA* promoter. One ORF is a potential membrane protein. Computer analysis of these ORFs did not reveal significant homology to proteins in the databases. However, sequencing upstream of the *picA* locus revealed an ORF that showed significant homology with polygalacturonases from bacteria and plants, suggesting that this ORF encodes a polygalacturonase. We shall also present genetic analyses of *picA* regulation.

1266 NOPALINE Vir D1,D2 ENDONUCLEASE OF *AGROBACTERIUM TUMEFACIENS* DIRECTS THE GENERATION OF T-STRANDS FROM OCTOPINE BORDERS ON A HIGH COPY NUMBER REPLICON IN *E.COLI*. Sergei A. Filichkin, Zoya V. Avramova, and Stanton B. Gelvin, Purdue University, Department of Biological Sciences, West Lafayette, Indiana 47907

The *Agrobacterium tumefaciens*-mediated transfer of T-DNA into the plant cell and possibly its integration into the host genome requires *virD* gene activity. T-DNA processing in *Agrobacterium* cells involves the generation of double- and single-stranded T-DNA molecules tightly associated with VirD2 protein. *virD1* and *virD2* gene expression is essential for the generation of T-strands in *E.coli*. The traditional approach to the production of T-strands in *E.coli* includes an introduction into the cell of two compatible replicons, one of which is designed for the inducible expression of VirD1 and VirD2 proteins, and another (usually a low copy number replicon) that carries the substrate T-DNA borders. We have used a new approach in which both the *virC* and *virD* genes, under the control of their native promoters, and the T-DNA borders are on the same high copy number plasmid. These constructs facilitate the quantitative estimation of the susceptibility of the different types of T-DNA borders to VirD1,D2 endonuclease activity. By using techniques that selectively precipitate covalently bound DNA-protein complexes, followed by T-strand purification by ion-exchange chromatography, we show that the pTiC58 *virD1,D2* and *virC1,C2* genes under the control of their native promoters are capable of processing octopine-type borders and generating high levels of T-strands.

1267 GENES FROM XANTHOMONAS CAMPESTRIS PV. VESICATORIA THAT DETERMINE THE SPECIFIC INTERACTION WITH PEPPER AND TOMATO

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Xanthomonas campestris pv. vesicatoria (Xcv) is the causal agent of bacterial spot disease in pepper and tomato. In susceptible plants the infection gives rise to watersoaked lesions whereas in a resistant plant a hypersensitive response (HR) is induced when the particular race of the pathogen carries a corresponding avirulence gene. The avirulence gene avrBs3 from Xcv race 1 constitutively expresses a 122 kDa protein the internal portion of which contains a 34 amino acid motif that is repeated 17.5 times in tandem When repeat units were deleted in most cases gene function was lost. Interestingly, several deletion derivatives now induced an HR on the susceptible cultivar ECW and/or on tomato, i. e. gained novel specificities. This finding suggests that the repeats are important for protein function and its specificity, and may play a structural role in the interaction with the plant. This is supported by results of a sequence comparison between avrBs3 and a homologous avirulence gene, avrBsP, that induces HR on tomato but not on pepper.

Hrp genes from Xcv are necessary for both, the induction of HR in resistant plants and for pathogenicity in susceptible plants. They are organized in at least six complementation groups in a 25 kb region. The function of these genes is as yet unknown. To study hrp-gene expression we used β-glucuronidase as a reporter gene. There was no detectable GUS-activity after growth of the cells in minimal or rich medium. However, GUS-activity was strongly induced (up to 10.000 fold) after growth of the bacteria in pepper leaves We are currently trying to identify the nature of the inducing compound: it is small, hydrophilic, heat stable.

POSTER ABSTRACTS

1268 *AGROBACTERIUM* ONCOGENE 6b INCREASES WOUND-INDUCED CELL DIVISION AND MODIFIES SHOOT INITIATION. Bruno Tinland, Pascal Fournier, Thierry Heckel and Léon Otten. Louis Pasteur University, Institute of Plant Molecular Biology, Strasbourg, France

The *Agrobacterium* T-DNA gene 6b is present in a large number of different strains. The 6b gene induces self-limiting cell growth on a number of plant species. We have placed the 6b gene of strain Tm4 under control of the *Drosophila* heat shock promoter hsp70. *Nicotiana rustica* plants carrying this gene and a GUS gene were regenerated.
The growth of seedlings of hsp70-T-6b transformants was hardly different from the growth of untransformed seedlings under non-induced conditions. However, hsp70-T-6b seedling development was strongly modified upon heat shock : the meristems of the primary leaves were inhibited and new meristems appeared along the hypocotyl. Root growth was also affected : roots increased in diameter.
Grafting experiments with the hsp70-T-6b plants showed that 6b effects were not transmitted to neighboring normal cells, indicating that the 6b gene product does not cause a change in hormone levels but rather affects sensitivity levels.
To determine at which point of the cell division the 6b gene product acts, we have started a study on cell division of protoplasts from hsp70-T-6b transformants. Hsp70-T-6b protoplasts showed a pattern of division which differed from that of control protoplasts : although hsp70-T-6b cells and non-transformed cells grow similarly under normal culture conditions, the 6b cells were able to maintain their competence for division in the absence of added auxins and cytokinins, for at least 5 days after protoplast isolation, whereas normal cells rapidly lost their capacity to respond.
On the basis of these data, we present a model on the mode of action of the 6b gene product, and on its role in *Agrobacterium*-induced tumorigenesis.

1269 SPONTANEOUS MUTATION TO MANNOPINE CATABOLISM IN *AGROBACTERIUM TUMEFACIENS* G LaPointe[1] C S Nautiyal[1] S K Farrand[2] W S Chilton[3] and P Dion[1] (1) Universite Laval Departement de phytologie Ste-Foy Quebec Canada G1K 7P4 (2) University of Illinois at Urbana-Champaign Department of Plant Pathology, Urbana IL 61801 (3) North Carolina State University Department of Botany Raleigh NC 27695

Upon prolonged incubation in the presence of mannopine as the sole carbon source two nopaline-type strains of *Agrobacterium tumefaciens* C58 and T37 yielded spontaneous mutants with an acquired capacity for mannopine catabolism Strain A136 a derivative of strain C58 cured of the Ti plasmid also produced spontaneous mutants capable of mannopine catabolism Generation time of the mutants on mannopine was 25 h and this substrate was observed to disappear from culture supernatants The mutants also degraded mannopinic acid but not agropine or agropinic acid Total DNA from the mannopine-utilizing mutants did not hybridize to probes derived from the Ti plasmid-encoded region of mannopine catabolism The spontaneous mutant LM136 but not the parent strain A136 had the ability to catabolize many mannityl opine analogs A clone conferring the ability to utilize mannopine on a recipient pseudomonad was selected from a genomic library from both the mutant LM136 and its parent strain In Southern analysis with the LM136-derived catabolic clone as a probe hybridization was obtained with the total DNA from strains C58 T37 and A136 but not various octopine- or agropine-type strains of *Agrobacterium* Pregrowth on mannopine mannopinic acid, glutamine and glutamate but not glucose or mannose allowed cells of LM136 to utilize [^{14}C]mannopine within 60 min After incubation on [^{14}C]mannopine cells of LM136 contained mannose glutamine and agropine as catabolic intermediates A cell free extract prepared from mannopine-grown LM136 demonstrated mannopine lactonizing activity similar to the mannopine cyclase activity of octopine strains

1270 ANTI-BACTERIAL RESISTANCE OF TRANSGENIC POTATO PLANTS PRODUCING T4 LYSOZYME

Klaus During, Matthias Fladung[1)] and Horst Lorz
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Potato plants have been stably transformed with a foreign chimeric gene consisting of a plant signal peptide and the bacteriophage T4 lysozyme gene under the control of a promoter and a polyadenylation signal active in plants The same fusion gene has already proven to enable secretion of the foreign protein from transgenic tobacco cells to the intercellular spaces in earlier studies
The host - pathogen - interaction *Solanum tuberosum - Erwinia carotovora* leads to severe damage of infected plants in the field and of tubers in the field as well as during storage. No suitable chemical means are known for disease control. Breeders are not able to provide resistant cultivars. Genetechnology enables us to set up a biological resistance mechanism against phytopathogenic bacteria in transgenic plants by introducing T4 lysozyme as an antibacterial enzyme. Endogenous lysozyme activities have already been detected in several plant species Imitation of a natural resistance mechanism by introducing a highly active lysozyme provides a resistance capacity of potato tissue against *Erwinia carotovora*

1271 SUPPRESSION OF CROWN GALL DISEASE IN TOBACCO TRANSFORMED BY THE iaaM GENE

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In order to suppress crown gall disease, a part of either the iaaM or iaaH gene was introduced into tobacco plants. A dramatic inhibition of gall formation by A. tumefaciens was found in transformed plants which contained four copies of the chimeric iaaM gene in the sense orientation. However, the amount of transcript was lower than in transformed, but non-resistant, plants. The four integrated iaaM loci were highly methylated, and apparently had the same methylation pattern. Under certain circumstances, a few organized shoots and teratomas were found to have developed from infected regions of resistant plants. These contained intact integrated T-DNA; of which the iaaM gene loci were hypermethylated but the ipt gene loci were not. Thus, in the resistant plants, auxin synthesis may have been inhibited by the pre-existing iaaM gene, thus preventing further expression of the invading iaaM gene of Agrobacterium.

1272 *ZEA MAYS*, A "SILENT HOST" FOR *AGROBACTERIUM TUMEFACIENS*

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The monocotyledonous plant *Zea mays* does not show tumour formation upon inoculation with *A. tumefaciens* and by this criterion is a non-host. We showed, however, that with *Agrobacterium*-mediated delivery of a maize streak virus-dimer (Agroinfection), there is extensive interaction between *Zea mays* and *A. tumefaciens* including transfer of the T-DNA to the plant. Therefore we propose the term silent host.

Using Agroinfection we found a big variation in the extent of this interaction between different strains of *A.tumefaciens*. Whereas the virulence regions of both the octopine strain A6(pTiA6) and the nopaline strain C58(pTiC58) are induced by some compound released from a wounded maize plant, T-DNA transfer occurs only with the nopaline strain. Since an *A. tumefaciens* strain carrying a nopaline Ti-plasmid in an octopine chromosomal background, A6(pTiC58), can agroinfect maize, we conclude that either the nopaline Ti-plasmid pTiC58 carries some promoting function(s) or that the octopine Ti-plasmid codes for some inhibitory function(s). Data on a virulence gene that plays an important role in the interaction of *A. tumefaciens* and Zea mays will be presented.

1273 CELLULAR LOCALIZATION OF *VIRB* GENE PRODUCTS OF *AGROBACTERIUM TUMEFACIENS*.

Ken Shirasu and Clarence I. Kado.
University of California-Davis, Dept. of Plant Pathology, California 95616

The virulence regulon of the *A. tumefaciens* TiC58 plasmid is comprised of six operons *virA, virB, virG, virC, virD,* and *virE,* which direct the transfer of T-DNA into plant cells The 9.5 kb *virB* operon is the largest of these operons and contains eleven open reading frames (ORFs) Hydrophathic analysis of these peptide sequences revealed nine proteins that contain either a signal-peptide-like sequence or significant hydrophobic spanning regions. The other two, VirB4 and VirB11, possess a putative ATP binding site. The VirB4 and VirB9 proteins were overexpressed under the control of a T7 promoter in *E.coli*. Specific antibodies against each protein were raised and purified. Both proteins were localized in membrane fractions of the acetosyringone induced *A. tumefaciens* cells by immuno-blot analysis. These data suggest that the majority of the VirB proteins may associate with bacterial cell membranes. The possible function of the *virB* gene products in T-DNA transport will be discussed.

POSTER ABSTRACTS

1274 AGROBACTERIUM RHIZOGENES AS A TRANSFORMATION SYSTEM FOR STUDYING GENE EXPRESSION IN ROOT BORDER CELLS OF PEA. M Nicoll, L. Brigham and M C. Hawes. University of Arizona, Departments of Plant Pathology and Molecular & Cellular Biology, Tucson, Arizona 85721

Many plant species release thousands of root "border" (BRD) cells from the root cap region daily. The function of this process is unknown. We hypothesize that the cells act to provide a "timed release" delivery of specific chemicals that help to regulate microbial populations near the root. To study the function of BRD cells, Agrobacterium rhizogenes was utilized to transform roots of Pisum sativum. Transformation was optimized with respect to several variables including A. rhizogenes strain, pea genotype, inoculum concentration, and site of inoculation. Developmental regulation of BRD cell release from hairy roots was compared to that of normal roots to assess the feasibility of using A. rhizogenes induced roots as a model system for studying gene expression in BRD cells. Preliminary studies using beta-glucuronidase (GUS) as a reporter gene indicate that expression of transferred marker genes in pea hairy roots is comparable to that in transgenic tobacco. BRD cell release in transgenic hairy roots is normal.

1275 PCR ISOLATION OF PLANT/T-DNA JUNCTIONS IN *Nicotiana plumbaginifolia*. Keith Kombrinck and Daniela Sciaky, University of Cincinnati, Department of Biological Sciences, Cincinnati, OH . 45221

In nature, *Agrobacterium tumefaciens* infects dicotyledonous plants and transfers DNA from its Ti plasmid to the plant cell, where it is integrated into plant chromosomes. Primary elements required for transformation are the *vir* region of the Ti plasmid, and 25 base pair direct repeats that are located at each end of the transferred DNA

We are determining whether *A. tumefaciens* foreign DNA insertion is nonspecific along a plant chromosome, or do certain DNA sequences signal the insertion point. We are sequencing several integration sites in *Nicotiana plumbaginifolia*, that were transformed by the same T-DNA construct, in order to answer the question of T-DNA target site specificity

First, we isolated T-DNA/plant junctions in transformants, by using inverse polymerase chain reaction (IPCR). The critical requirement is that sequences flanking the target must be known in order to produce DNA primers that initiate and maintain the reaction By using a restriction enzyme that cuts within the T-DNA and nearby in the plant DNA, the junction can be isolated on a relatively small fragment. The digest is then ligated to produce small circular DNA molecules. This brings the unknown plant sequence at the junction, within the known T-DNA. Outward directed primers that are complementary to the T-DNA are used to amplify and isolate junctions. After sequencing T-DNA/plant junctions, primers are made that are specific to the plant sequences. With these new primers, the integration site, prior to the transformation event, can be isolated through PCR amplification of that site in untransformed plants

Presently, five junctions from four different transformants have been isolated Preliminary sequence from the left and right junctions of plant 50d, shows a 72% and 67% AT rich region respectively. Southern blots that involve probing normal plant DNA with the isolated junctions, are presently underway. These will identify which integrations occurred in unique DNA as opposed to repeated DNA We will then concentrate on PCR amplifying and sequencing the integration sites that were derived from unique insertions We will compare our data to a single target site that has already been determined by another group, where it was concluded that integration was random, and involved a multistep process of recombination and repair.

1276 MUTATIONAL ANALYSIS OF VirA IN AGROBACTERIUM
Gail McLean, Elizabeth Greene, and Patricia Zambryski.
Department of Plant Biology, University of California, Berkeley, CA 94720

The VirA/VirG regulatory system in Agrobacterium enables the bacteria to link recognition of plant phenolics and other plant-derived inducing compounds to expression of vir specific transcripts, resulting in T-DNA complex formation and subsequent transformation of plant cells by the bacteria. Based on both homology with other bacterial two-component regulatory systems and recent experimental results, VirA functions as a sensor protein by transmitting information about the environment to VirG, the transcriptional regulator protein. Although the general mechanism for signal transduction has been found to be phosphorylation/dephosphorylation reactions, the specific mechanisms involved in the transcriptional activation of vir loci are not known. These steps are being characterized through a mutational analysis of VirA and VirG; the use of VirA and VirG mutants that allow constitutive expression of vir loci will simplify the characterization of transcriptional activation by separating VirA and VirG functions. At present, we have isolated and partially characterized three mutations in VirA which exhibit constitutive expression of vir loci in the absence of acetosyringone, a plant phenolic. In the presence of acetosyringone, the mutants are induced above these constitutive levels In addition, one mutant requires the presence of an inducing sugar in the media for constitutive expression of the virB::lacZ translational fusion used to assay vir gene expression

1277 TRANSFORMATION OF THE GRAMINAE BY *AGROBACTERIUM TUMEFACIENS*. J. H Gould[1], M E. Devey[2], O Hasegawa[3], E. C. Ulian[1], G Peterson[1], R. H Smith[1] [1]Texas A&M University, Department of Soil and Crop Sciences, College Station, TX 77843 [2]Institute of Forest Genetics, Placerville, CA 95667, [3]Nisshinbo Industries, Tokyo, Japan

To find a more efficient method of regenerating plants from tissues transformed by *Agrobacterium*, we re-examined meristem and shoot apex culture methods developed by Georges Morel for virus eradication In doing so, we tested a notion that meristematic tissues could not be transformed by *Agrobacterium* (Ulian et al., 1988, In Vitro Cell. Devel. Biol. 24:951-955). In the course of this study, we began to question the idea that members of the Graminae could not be transformed by *Agrobacterium*. June 1, 1988, we initiated a study involving wheat (*Triticum aestivum* L., Chinese Spring), corn (*Zea mays* L., Funk's G90) and *A. tumefaciens* EHA 101 harboring the plasmid pGUS3 containing GUS and NPT II reporter genes. The development of intact plants from inoculated shoot tissues was rapid, 3-4 weeks, for both corn and wheat. Approximately 20 to 30% of the plants appeared to be transformed. Later molecular analysis demonstrated the transferred genes present in regenerated plants of wheat and corn and in the F1 generation (Gould *et al.*, 1991, Plant Physiology 95:426-434) The data from these studies will be presented

1278 A POSSIBLE PLANT GENE REGULATORY PROTEIN ENCODED BY THE virD REGION OF THE OCTOPINE TYPE Ti PLASMID pTiA6 OF AGROBACTERIUM tumefaciens. Thomas H. Alton and Ping Hu. Department of Biological Sciences, Western Illinois University, Macomb, IL 61455

The sequence of open reading frame (ORF 6) immediately downstream from the virD operon of the Ti plasmid pTiA6 was completed. The ORF potentially encodes a protein of 478 amino acids, with a predicted molecular size of 53,700 daltons and an isoelectric point of 5.56 if there are no post-translational modifications. The predicted amino acid sequence has several features which suggest that this protein interacts with nucleic acids and with other proteins in ways suggestive of eukaryotic transcription factors. Two possible helix-turn-helix DNA binding segments are present, as is a helix-loop-helix protein-protein interaction segment. In addition, a sequence near the amino terminus has acidic amino acids every third or fourth residue, which is very suggestive of the acid activating patch found in many eukaryotic transcription activator proteins. These findings are consistent with the notion that during the process of transfer of the T-DNA into the plant cell a number of bacterially encoded proteins are also transferred, and that these proteins carry out or stimulate specific processes of T-DNA integration. Specifically this ORF6 protein could act to modify gene expression in the infected plant cell. Experiments are underway to test the predictions derived from the amino acid sequence of this protein, and specifically to test the ability of this protein to affect transcription in eukaryotic systems.

1279 Mutational Analysis of *Agrobacterium tumefaciens* virD Operon. Ann Vogel and Anath Das, Department of Biochemistry and Plant Molecular Genetics Institute, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108.

Agrobacterium tumefaciens causes crown gall tumors on most dicotyledonous plants. Tumor formation is the result of transfer and integration of a segment of bacterial plasmid DNA into plant cells. A single-stranded DNA (T-strand DNA) is believed to be an intermediate in this process Two enzymes of the *virD* operon, VirD1 and VirD2, catalyze the formation of T-strand DNA by introducing a site- and strand-specific nick at the T-DNA border sequences. Of these two proteins, VirD1 is a DNA relaxing enzyme All DNA relaxing enzymes known to date are found to contain a tyrosine residue at the active site To determine if a similar mechanism is functional in VirD1 we introduced tyrosine to phenylalanine substitutions in VirD1. The effect of these substitutions on VirD1-VirD2 catalyzed nicking reaction will be discussed.

POSTER ABSTRACTS

1280 ROLE OF POLYGALACTURONASE IN VIRULENCE OF PSEUDOMONAS SOLANACEARUM. Caitilyn Allen, Merelee Atkinson, and Luis Sequeira. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Pseudomonas solanacearum produces at least three extracellular polygalacturonases (PG). The major PG produced in culture, encoded by pehA, is produced constitutively and plays only a minor role in virulence. The two other PGs, however, have activity that increases in response to plant extracts, nutritional stress, and galacturonic acid. Two Tn5 mutants, each lacking one of the inducible PGs, are substantially reduced in virulence on eggplant although they grow normally in planta. We have cloned a trans-acting postive regulator of PG production, pehR. Reporter gene studies indicate that pehR affects pehA expression at the transcriptional level. The pehR locus has homology to a conserved region of the Agrobacterium virA locus, suggesting that pehR may belong to a family of bacterial two-component regulatory systems.

1281 INCREASED TURNOVER OF PHOSPHATIDYLINOSITOL DURING THE HR OF TOBACCO TO PSEUDOMONAS SYRINGAE. Merelee Atkinson, James Bina and Luis Sequeira. University of Wisconsin, Madison, WI 53706

An early event in the hypersensitive response (HR) of tobacco to P. syringae pathovars is the initiation of a K+/H+ response characterized by K+ efflux, extracellular alkalinization, and intracellular acidification. This response is also accompanied by an abrupt and prolonged increase in calcium influx into tobacco cells. Lanthanum, which blocks known calcium channels, prevents calcium influx, the K+/H+ response, and subsequent cell death. On the basis of these data, we have hypothesized that the HR is initiated via a plasma membrane signal transduction pathway. To further test this hypothesis, we are studying lipid turnover during the HR. Suspension-cultured tobacco cells were incubated with ^{3}H- or ^{14}C-labelled fatty acids to radiolabel cellular lipids. Prelabelled tobacco cells were inoculated with P.s. pv.syringae, or a mutant strain unable to induce the HR. Tobacco lipids were extracted at hourly intervals and later analyzed by TLC. During the HR, radiolabelled phosphatidylinositol levels decreased 20 - 30 percent within 4 h after inoculation beginning at the onset of the K+/H+ response. In contrast, radiolabelled triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine levels remained constant or increased over this time period. These results suggest an HR-associated stimulation of a phosphatidylinositol-specific phospholipase, possibly phospholipase C.

1282 **CHARACTERIZATION OF A NEW AGROBACTERIUM TUMEFACIENS STRAIN AND ITS USE FOR GENE TRANSFER.**
Lise Jouanin, Pierre Berthomieu and Ana Cristina Miranda Brasileiro.
Laboratoire de Biologie Cellulaire, INRA 78026 VERSAILLES Cedex FRANCE

The *A. tumefaciens* strain 82.139 was isolated from a wild cherry tree tumor in a forest tree nursery in Orléans (France).
Tumors induced on several plants by this strain possess the ability to develop shoots spontaneously having a normal phenotype and are able to root.

This nopaline-type strain belongs to biovar 2 and carries a Ti plasmid similar to pTiC58, except in the right part of its T-DNA where the oncogenes are located. This different region was cloned and characterized in details in order to identify the pTi 82.139 oncogenes and to understand the basis of its ability to induce shoots on tumors.

This "shooty" ability was used to establish a new transformation procedure which avoids regeneration problems. The method is based on the inoculation of stem internodes with an *Agrobacterium* suspension containing both the oncogenic 82.139 strain that induces shoot differentiation and a disarmed strain that provide the suitable genes in a binary vector. Poplar and cabbage were used as model plants to test this new strategy of transformation.

1283 CLONING AND STUDYING OF THE GENES FOR IAA SYNTHESIS FROM ERWINIA HERBICOLA pv. GYPSOPHILAE.
Y. Ophir, E. Clark, L. Valinsky, S. Manulis, I. Barash & Y. Gafni
A.R.O., The Volcani Center, Bet Dagan 50 250, Israel

Genes for Indole-3-acetic acid (IAA) biosynthesis in Erwinia herbicola pv.gypsophilae, the causal agent of crown and root galls in Gypsophila paniculata, were identified and cloned by using a DNA fragment from Pseudomonas syringe pv. savastanoi
In P. savastanoi, a pathogenic knot-forming bacterium, the IAA synthesis was shown to occur via the indoleacetamide pathway. The DNA fragment from P. savastanoi contains genes for the two enzymes in the indoleacetamide biosynthetic route (*iaaM* and *iaaH* for tryptophan monooxygenase and indolacetamide hydrolase respectively). A DNA fragment containing the cross-hybridizing sequence from E. gypsophilae was cloned from plasmid DNA and sequenced, and two open reading frames were found. Significant amino acid sequence homology was found with gene expression products of the genes *tms1* and *tms2* from A.tumefaciens; and *iaaM* and. *iaaH* from P. savastanoi. The auxin synthesis genes from E. gypsophilae (designated *ehgM* and *ehgH*) were found to be located on a 78Md plasmid in pathogenic isolate I and on a 100 Md plasmid in pathogenic isolate II. All strains of E. gypsophilae pathogenic and non-pathogenic secreted IAA in culture. Two pathways for IAA biosynthesis from tryptophan were identified in E. gypsophilae, one involved the indoleacetamide route and the other the indolepyruvate route. In the pathogenic strains, both pathways were detected, on the other hand, only the latter route was detected in non-pathogens. A DNA probe containing the genes *ehgM* and *ehgH* was used to distinguish among pathogenic and non-pathogenic strains of E. gypsophilae.

1284 HIGH-LEVEL TRANSIENT EXPRESSION OF FORIGN GENES IN TOBACCO CELLS AFTER *AGROBACTERIUM*-INFECTION
Ken Matsuoka and Kenzo Nakamura
Lab. Biochem. Sch. Agric. Nagoya Univ., Chikusa-ku, Nagoya 464-01 JAPAN

The events that occur in plant cells during the *Agrobacterium*-mediated transformation are not well understood. Recently, transient expression of genes carried on T-DNA in plant cells after *Agrobacterium*-infection has been suggested Although this phenomenon is useful for the analysis of the early events of the T-DNA transfer into plant cells no systems that is suitable for the biochemical analysis of this phenomenon has been developed We developed a high-level transient expression system using an intron-GUS gene containing an intron within the GUS coding sequence* or sporamin cDNA** and tobacco cell line BY-2 When BY-2 cells were co-cultured with *A. tumefaciens* EHA101 harboring a binary plasmid carrying the CaMV35S^{P} intron-GUS fusion gene on its T-DNA GUS activity in BY-2 cells was detected 30 h after the start of co-culture, and at 48 h, the level of activity reached to a plateau. X-Gluc staining of the BY-2 cells which were co-cultured for 48 h revealed that more than half of the cells were GUS positives. By contrast, only less than 1/1000th the number of these GUS positive cells formed Km-resistant colonies on selective plates The rate of synthesis of GUS protein analyzed by pulse labeling of the co-cultured cell with ^{35}S-Met followed by immunoprecipitation showed a peak at 36 to 42 h after the start of co-culture, and decreased about 1/10th of the maximum level at 72 h Similar results were obtained when the 35SP:sporamin cDNA fusion gene was used. In this case, the rate of the synthesis of sporamin at the peak was comparable to that of the stable transformant cells The decline of the rate of the synthesis of GUS protein was greatly accelerated when agrobacteria were removed from the BY-2 culture medium although many agrobacterial cells still attached on BY-2 cells These observations suggest that once an agrobacterial cell introduce T-DNA into a plant cell, the bacteria lose the ability of the transport of T-DNA into plant cell, although the bacteria binds on the plant cell * Ohta et al *Plant Cell Physiol* 31 805 (1990) ** Matsuoka and Nakamura *Proc Natl Acad Sci U S A* 88 834 (1991) Nakamura and Matsuoka Concurrent Symposia 5 Session 1 on Monday, this congress

1285 PLASMID pSa ENCODES SUPPRESSION OF AGROBACTERIUM TUMEFACIENS ONCOGENICITY AND NUCLEASE ACTIVITY.
Sheila M. Close and Clarence I. Kado. Dept. of Plant Pathology, University of California, Davis, CA 95616.

The oncogenic suppressive (OS) activity of the IncW plasmid pSa was localized, by Tn5 mutagenesis and deletion analysis, to a 3.1 kb region adjacent to the origin of replication. The nucleotide sequence of this region revealed four major ORFs. A 1.1 kb subclone from this region contained a 570 bp ORF with a coding capacity for a protein of 21.1 kDa, and exhibited OS activity. The effect of the OS sequence on two important virulence processes was examined. Neither induction of the virulence operons by acetosyringone, nor cleavage of the T-DNA border sequences were altered by the presence of the OS region of pSa. Additionally, bacterial IAA synthesis does not seem to be the target of OS action. A second ORF of interest (nuc) was found within the 3.1 kb fragment. This 525 bp ORF encodes a 174 amino acid potential polypeptide of 19.7 kDa which has significant amino acid sequence homology to staphylococcal nuclease and to the parB gene of plasmid RP4. Expression of the nuc gene was confirmed using in-vitro transcription-translation assays in an E. coli S30 extract. The deduced amino acid sequence revealed a signal sequence, and TnphoA mutagenesis confirmed that the protein is transported across the bacterial membrane. Nuclease activity was demonstrated using TB-D agar plates.

POSTER ABSTRACTS

1286 DIVERSITY AND SPECIFICITY OF *PSEUDOMONAS* GENES OF OCTOPINE CATABOLISM. S. S. Gill G. Champagne, L. Miville and P. Dion. Département de phytologie, Université Laval, Sainte-Foy, Quebec, Canada G1K 7P4

The soil microbe *Agrobacterium tumefaciens* genetically transforms plant cells through DNA transfer. Some DNA of bacterial origin becomes integrated into the chromosomes of the plant cell, where it directs the synthesis of opines, which are amino acid conjugates or phosphorylated sugars absent from normal plant tissue. The agrobacteria which have mediated the genetic transformation utilize opines as growth substrates, hence establishing a specific nutritional relationship with their host. Other bacteria share with agrobacteria the ability to utilize opines. Of particularly common occurrence are pseudomonads capable of utilizing octopine, an opine synthesized through the reductive condensation of arginine with pyruvic acid.

The genes of octopine catabolism from *Pseudomonas putida* CH183 were cloned on a cosmid vector and a promoter specifically inducible by octopine was subcloned. This promoter was only activated in the presence of octopine, but not arginine, as the sole nitrogen source. Transposon Tn*V* mutagenesis was used to specifically inactivate the *P. putida* CH183 genes of octopine catabolism. The DNA sequences flanking the Tn*V* insertion (about 6 kb of *Pseudomonas* DNA) were cloned and used as a probe in Southern analysis of the total DNA from various bacteria. Hybridization was seen for several strains, including strains of *Pseudomonas* spp. and *A. tumefaciens*, but irrespectively of their capacity to utilize octopine. In contrast with this, cloned genes of octopine catabolism from three different fluorescent pseudomonads did not cross-hybridize

These results suggest that, in different pseudomonads, various genes of octopine catabolism exist within unrelated genetic domains distributed among octopine utilizers as well as non utilizers. At least some of the various systems of octopine catabolism harbored by pseudomonads respond specifically to the opine substrate, as was shown previously at the phenotypic level and in the present study at the level of promoter activation.

1287 A TN5-BASED ICE NUCLEATION REPORTER GENE TRANSPOSON FOR MONITORING GENE EXPRESSION IN PLANT-ASSOCIATED GRAM NEGATIVE BACTERIA. Krista Gates, S. Celeste Posey, and Thomas Gaffney. Ciba-Geigy Biotechnology, P.O. Box 12257, Research Triangle Park, NC 27709-2257.

As a step toward monitoring *in situ* gene expression in root-colonizing pseudomonads, a Tn5-based transposon designated TnCIB3330, which carries a promoterless ice nucleation gene adjacent to one end, was constructed. This was accomplished by replacing the promoterless *lacZ* gene of a promoter-probe transposon harbored by pCIB116 (Lam et al. 1990. Plant and Soil. 129:11-18) with the promoterless *inaZ* gene of pTn3 Spice (Lindgren et al. 1989. EMBO J. 8:1291-1301). Copies of TnCIB3330 were detected at different locations in random *Pseudomonas fluorescens* transconjugants. Various levels of ice nucleation activity were observed when random *P. fluorescens* mutants were assayed after growth either in liquid culture or on wheat roots. In the latter case, colonized root samples were used directly in ice nucleation assays. In principle, it should be possible to mutagenize with TnCIB3330 any bacterium which will conjugate with an *Escherichia coli* donor and in which Tn5 will transpose.

1288 IDENTIFICATION OF A REGION OF THE Ti PLASMID WHICH MEDIATES THE TRANSFER OF MSV DNA TO MAIZE. Deanna M. Raineri[1], Margaret I. Boulton[2], Jeffrey, W. Davies[2], Eugene G. Nester[1]. [1]University of Washington, Department of Microbiology, Seattle, WA 98195, and [2]John Innes Institute Department of Virus Research, Norwich, U.K.

Agrobacterium-mediated transfer of maize streak virus DNA to monocots has been shown to be highly strain specific (Boulton *et al.* 1989, Plant Mol. Biol., 12:31-40). Introduction of MSV genomes into *Agrobacterium* strains carrying nopaline Ti plasmids resulted in virus infection whereas the use of octopine Ti plasmids did not. Octopine and nopaline Ti plasmids have been shown to differ in a number of loci. In order to determine the region responsible for the strain specific transfer, overlapping fragments of the nopaline Ti plasmid were introduced into octopine backgrounds and tested for their ability to mediate agroinfection. We observed that an 11Kb region of the nopaline Ti plasmid conferred agroinfection ability on octopine strains. Studies are underway to further delineate the complementing region and the results of these investigations will be presented. It is envisaged that this region could be used in the construction of vectors to enhance T-DNA transfer to monocots.

1289 CELLULAR SPECIFICITY CONFERRED BY THE VARIOUS SUBDOMAINS OF THE OCTOPINE SYNTHASE TRANSCRIPTIONAL ACTIVATING ELEMENT. Halina Kononowicz, Eureka Wang, Lillian L. Habeck, and Stanton B. Gelvin. Purdue University, Departments of Botany and Plant Pathology (HK) and Biological Sciences (EW, LLH, SBG), West Lafayette, Indiana 47907. We have investigated the cellular specificity conferred upon the octopine synthase (*ocs*) promoter by various subdomains of the *ocs* transcriptional activating element. Inhibitors of the octopine synthase assay in various parts of tobacco plants render this assay unreliable. Northern blot analysis of RNA from various tissues and organs of transgenic tobacco plants containing the *ocs* gene linked to various subdomains of the *ocs* activating element indicate that this gene is differentially expressed. However, no differences in expression were detected among the various *ocs* activator element subdomains. To obtain a finer definition of *ocs* activator element specificity, we made translational fusions of the *ocs* protein with β-galactosidase. These fusions were preceded by various subdomains of the *ocs* activating element, including the 16 bp palindrome essential for activity, the full element, and various partial elements containing the 16 bp palindrome and surrounding sequences. The 16 bp palindrome gave the widest range of expression, directing activity in the mesophyll and palisade leaf cells, guard cells, the tips of trichomes, and the central vascular cells of leaves. The other constructions did not express well in the leaf vascular bundle cells. The palindrome expressed well in the meristematic and vascular regions of the shoot tip, whereas the activity of the other constructions was restricted to the meristematic regions. All constructions expressed well in the root vascular tissue, but not in the epidermis or root cap. Sequences surrounding the 16 bp palindrome in the *ocs* activator therefore restrict the cells in which the *ocs* promoter functions.

1290 EFFECTS OF EXPOSURE OF EMBRYONIC AND JUVENILE ORCHID TISSUE TO AGROBACTERIUM TUMEFACIENS. H. L. Saxon, D. Tzakis, C. M. Hutchinson, C. N. Vann. Ball State University, Department of Biology, Muncie, Indiana 47306-0440.

Agrobacterium tumefaciens, a plant pathogen, is widely used as a vector to transform dicots but has not been believed to infect most monocots. Undifferentiated and dedifferentiated monocot tissue of Cattleya-alliance orchids exposed under various regimes to A. tumefaciens showed altered growth and development consistent with infection. Optimal exposure time was found to be one hour. Acetosyringone, a dicot wound signal molecule that initiates the transfer of T-DNA from A. tumefaciens was found to be effective in increasing the percentage of tissues transformed. However, host tissue exposed for more than one hour to acetosyringone generally did not survive.

1291 EXPRESSION OF MUTATED COLEOPTERA SPECIFIC -ENDOTOXIN GENE IN PLANTS.

Seong-Lyul Rhim and Tae-Young Chung
Hallym University, Department of Genetic Engineering, Chun-cheon, Korea. R.D.A. Department of Genetic Engineering, Su-weon, Korea

An insecticidal -endotoxin gene was cloned from Bacillus thuringiensis subsp. tenebrionis(BTT), which produce a coleopteran specific -endotoxin. The gene was altered by site directed mutagenesis at its 5'-end and adapted for general cloning and expression purposes with a linker including a start codon and new restriction sites. The altered toxin gene was inserted into a modified binary vector plasmid pBinAR with CaMV35S-promoter, called pBinAR-BTT. Transfer of this recombinant plasmid into tobacco plants was accomplished with the binary vector system via plant leaf disk infection. Toxin-containing protein from the transgenic plants was identified by western blot analysis. The expression of the -endotoxin gene in F2-tobacco plants was also incentified, and the population of the F2-plants was followed to Mende's law. The pBinAR-BTT was also transferred into potato plants via the leaf disk submerge-culture infection. The F2-generation of the potato plants showed the expression of the -endotoxin gene. The toxin activity tests of the potato plants against colorado potato beetle larvae are under study.

POSTER ABSTRACTS

1292 DETOXIFICATION OF α-TOMATINE BY FUNGAL PATHOGENS OF TOMATO Robert W. Sandrock and Hans D. VanEtten. Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721 USA.

The exact role preformed inhibitory compounds play in disease resistance is poorly understood. In order for preformed compounds to be considered a mechanism for disease resistance, they must be present both in the tissue invaded by the pathogen and in potentially inhibitory concentrations. One compound that meets these requirements is the glycoalkaloid a-tomatine, present in *Lycopersicon* species. High concentrations (up to 1mM) of a-tomatine are present in leaves, stems, roots, and green fruit suggesting that the glycoalkaloid may be important in resistance to potential pathogens; therefore, detoxification or other modes of resistance may be required in order for a pathogen to successfully infect tomato. Previous studies have shown that tomato pathogens are less sensitive to α-tomatine than are most non-pathogenic fungi (Arneson and Durbin, 1968, Phytopath 58:536). We have confirmed their results and also demonstrated that the enzymatic degradation products of a-tomatine are similar to those reported previously (Arneson and Durbin, 1967, Phytopath 57:1358; Schlosser, 1977, Current Topics in Plant Path 77-87). The tomato pathogen, *Alternaria solani*, possesses enzyme(s) that detoxify a-tomatine by hydrolyzing the sugar units into separate entities while another tomato pathogen, *Septoria lycopersici*, produces an enzyme that cleaves a single glucose unit from a-tomatine to yield a non-toxic product. Our research objective is to determine the importance these enzymes play in the pathogens' ability to parasitize the tomato plant

1293 MOLECULAR CLONING OF THE GENE SPECIFYING 7,2'-DIHYDROXY-4',5'-METHYLENEDIOXYISOFLAVONE OXIDOREDUCTASE FROM CUCL2 TREATED PEA (*Pisum sativum* L) SEEDLINGS

Yuejin Sun[1], Nancy L. Paiva[2], Richard A. Dixon[2], Hans D. Van Etten[3], and Geza Hrazdina[1]

[1] Institute of Food Science, Cornell University, Geneva, N.Y 14456, [2]Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and [3]Department of plant Pathology, University of Arizona, Tucson, Arizona 85721

Pterocarpan phytoalexins are produced by pea (*pisum sativum* L) in response to microbial infection. 7,2'-Dihydroxy-4',5-methylenedioxyisoflavone oxidoreductase (DMIRase) is the enzyme which introduces the first chiral center into the molecule and determines the (+) stereoisomerism in pisatin. DMIRase has been purified approximately 7000 fold by the use of $(NH_4)_2SO_4$ fractionation, gel filtration on AcA 44, ion-exchange chromatography on DEAE-biogel, hydrophobic interaction chromatography on Phenyl-Sepharose CL-4B, affinity chromatography on Reactive Red 120 agarose and preparative Na-dodecyl sulfate polyacrylamide gel electrophoresis. The enzyme has been characterized for its substrate specificity, pH optimum, PI, Km, molecular mass and product configuration. Polyclonal antibodies against this enzyme were obtained A cDNA clone responsible for the production of DMIRase in pea has been obtained and its characteristics will be discussed.

1294 TOWARDS CLONING OF AN AVIRULENCE GENE FROM OAT STEM RUST.

Joachim Sock, Roland Rohringer, and Jim A. Kolmer
Agriculture Canada Research Station, Winnipeg, MB R3T 2M9

Maternal inheritance of avirulence gene AV-3 in oat stem rust (OSR) has been demonstrated in reciprocal crosses of race 3 and race 8A. DNA from parental cultures and 14 F_2 progenies was analyzed. Plasmids were not detected indicating that AV-3 may be located in the mitochondrial genome. To identify mitochondrial (mt) restriction fragments we used mtDNA-probes from *Schizophyllum commune*, however, these were not homologous to OSR-DNA. Bisbenzimide/CsCl-gradient centrifugation failed to separate nuclear and mtDNA from OSR. Restricted total OSR-DNA and mtDNA from axenically grown wheat stem rust was used to probe restricted OSR-DNA. The mitochondrial genome size was determined to be approximately 65 kb. By "shotgun"-cloning of Hind III-restricted, total OSR-DNA we obtained 70 clones containing a total of 150 kb OSR-DNA. Ten percent of the clones tested so far revealed RFLP between the parents and Mendelian segregation in the F_2 progenies.

1295 A LIPOXYGENASE PATHWAY FOR PLANT DEFENSE. Daisuke Shibata, Yumiko Shirano, You-Liang Peng, Mitsui Plant Biotechnology Research Institute, TCI-A10, Sengen 2-1-6, Tsukuba, Ibaraki 305, JAPAN

Evidence is accumulating that fatty acids play a role in plant defense. Derivatives of linoleic or linolenic acids, hydroxy- or epoxy-fatty acids, have previously been isolated from rice leaves as antifungal compounds. Increased activity of lipoxygenases which might be involved in the production of the oxidized fatty acids was observed in rice leaves in an incompatible combination of rice and blast fungus, but not in a compatible one (Ohta et al. 5th Int. Congress Plant Physiol. p250, 1988). We are working on the regulatory mechanism of rice lipoxygenase gene expression. We isolated a cDNA clone (2.9 kbp) from a library made from rice leaves 30 h after inoculation of blast fungus. Lipoxygenase activity of the gene product was demonstrated by expressing the cDNA in E. coli using a low temperature cultivation method (Shirano and Shibata FEBS let. 271, p128-130, 1990). The amino acid sequence deduced from the nucleotide sequence shared low homology (30-40%) with those of previously identified lipoxygenases. This lipoxygenase has an extra amino acid sequence in the N terminal region, which may be a targeting signal for the chloroplast. Northern blot experiments showed rapid induction of gene expression when an incompatible race of the fungus was used for inoculation on rice leaves, but only slight induction when a compatible race was used. These results suggest a new plant defense mechanism in which a lipoxygenase pathway is involved.

1296 MUTATIONAL ANALYSIS OF USTILAGO HORDEI MATING. John E. Sherwood and Alfredo D. Martinez-Espinoza. Montana State University, Department of Plant Pathology, Bozeman, MT 59717.

Ustilago hordei is the causal agent of barley covered smut. Mating in U. hordei, which is controlled by a single locus with two alleles, results in the conversion of haploid, non-pathogenic, yeast-like sporidia to dikaryotic, pathogenic mycelium. With the long term goal of developing novel disease control strategies based on inhibition of the sexual cycle, we have initiated a mutational analysis of U. hordei mating in order to characterize the complexity of that process. To date, more than twenty mutants of U. hordei that fail to form dikaryotic mycelium (Fuz-) have been isolated from strains of both mating type. Mutants incapable of forming normal mycelium were selected by spraying cells of the opposite mating type onto colonies arising from mutagenized sporidia. Fuz- mutants were further divided into subclasses: those that continue to grow in the presence of cells of the opposite mating type, those that fail to form conjugation bridges, those that form conjugation bridges but fail to grow as mycelium, those that form greatly reduced amounts of mycelium, and those that produce a halo when grown on a lawn of the opposite mating type. Based on these phenotypes, we have developed a model for the U. hordei mating process.

1297 CUTINASE OF THE PATHOGENIC FUNGUS *NECTRIA HAEMATOCOCCA* IS NOT REQUIRED FOR PATHOGENICITY ON PEA Dietmar J. Stahl and Willi Schafer. IGF Berlin GmbH, Berlin, Germany.

The involvement of cutinase in the breakdown of the outer part of the plant barrier, the cuticle, has been discussed as being the crucial point of plant infection by many fungi. This hypothesis was based mainly on experiments concerning the cutinase of the ascomycete *Nectria haematococca*, a pea pathogen. We constructed a cutinase deficient mutant of *Nectria haematococca* by a one step gene disruption of the wild type cutinase gene. Neither a cutinase transcript nor enzymatic activity was detected in the transformed strain in contrast to the wild type strain. Using different bioassays the mutant strain showed no difference in pathogenicity and virulence on pea plants compared to the wild type and a control transformant without a disrupted cutinase gene. Therefore we conclude that the cutinase of *Nectria haematococca* is not essentially involved in the infection of pea.

POSTER ABSTRACTS

1298 HOMOLOGOUS INTEGRATION OF EXOGENOUS DNA IN PHYTOPATHOGENIC FUNGUS *ALTERNARIA ALTERNATA*. Hiroshi SHIOTANI, Hirokazu KOBAYASHI[1] and Takashi TSUGE. Faculty of Agriculture, Nagoya University, Nagoya 464-01, [1]School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka 422, Japan

In plant diseases caused by the fungus *A. alternata*, there are now at least seven host-parasite combinations, in which host-specific toxins are responsible for determining host-specific pathogenicity. For cloning and experimental manipulation of genes involved in the fungal pathogenicity, we have developed an efficient transformation system for the fungus through homologous integration of exogenous DNA. Two segments (1.9 and 2.8 kb in length) of the highly repetitive ribosomal RNA gene cluster of the fungus were inserted into a plasmid vector, pDH25, which carries the *E. coli* hygromycin B phosphotransferase gene (*hph*) as the selectable marker. Use of the vectors dramatically increased transformation efficiency: the best vector gave rise to transformants at a frequency 20 times higher than pDH25. A 3.0-kb DNA fragment, which was isolated as a single-copy sequence in the fungal chromosome, was also inserted into pDH25. The vector also improved transformation efficiency: it produced about 7 times more transformants than pDH25. Transformation events in the *Alternaria* with vectors carrying chromosomal DNA segments occurred by homologous recombination as a single crossover between the plasmid-borne chromosomal segments and their homologues in the chromosome. Homologous integration of exogenous DNA was successfully applied to gene disruption analysis in the fungus. This technique provides a powerful tool for the analysis of the molecular basis of the fungal pathogenicity.

1299 TOMATO RECOMBINANT INBREDS DIRECT A CHROMOSOME WALK TOWARDS A FUSARIUM RESISTANCE GENE.

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A major objective in molecular biology is the cloning of genes that can only be recognized by their phenotype and pattern of inheritance. The *I2* gene which confers resistance against *Fusarium oxysporum* f. sp *lycopersici* race 2 is tightly linked to the RFLP marker *TG105* on the euchromatic region of chromosome 11. *TG105* was found to be 3.5 cM away from the closest marker *TG26*. The maximum physical distance between the two RFLP markers, as estimated by pulsed field gel electrophoresis, was 195 Kb. A recombinant inbred (RI) population was made between *Lycopersicon cheesmanii* (susceptible to race 2) and *L. esculentum* (resistant). We progressed in a single seed descent from an F2 of 300 plants through F7; the F7 lines were scored for resistance to Fusarium race 2 and the RFLP markers of chromosome 11. A high frequency of recombinations was detected between *I2* and the linked markers. A "step" from *TG105* was achieved with a genomic lambda library yielding a clone whose ends were mapped using the RI population and provided unequivocal evidence for the direction of the walk towards the resistance gene. A 15 Kb "step" covered 10 recombination events in the RI population. The RI population constitutes an invaluable resource for high resolution mapping of the tomato genome.

1300 CLONING AND ANALYSIS OF A SPORULATION-SPECIFIC mRNA FROM PUCCINIA GRAMINIS. Les J. Szabo and Zhanjiang Liu. USDA-ARS, Cereal Rust Lab., Univ. of Minnesota, St. Paul, MN 55108

Highly expressed mRNAs were cloned from germinated uredospores of Puccinia graminis f. sp. tritici. Sixty-eight cDNA clones representing abundant mRNA species were isolated. The most abundant group accounted for 2.4% of the poly(A) RNA in germinated uredospores and designated usp. Southern analysis indicated that usp was a small gene family containing four members. Of the different stages of the uredial cycle examined, the expression of usp gene family was sporulation stage-specific. Primer extension and DNA sequencing indicated that expression of usp1 and usp2 accounted for 90% of the mRNA produced from this gene family. The usp1 gene encodes a small polypeptide of 113 amino acids with an unusual amino acid composition; alanine, glycine, leucine and proline represents 48% of the protein. The amino-terminal end of the polypeptide contains a highly hydrophobic region of twenty amino acids followed by two charged amino acids which is characteristic of a signal sequence found in most secreted proteins. Three members (usp1, usp3, usp4) of the family encode identical polypeptide, while one member (usp2) encodes a polypeptide that differs at 15 amino acids.

1301 ELECTROPHORETIC KARYOTYPES AND GENETIC ANALYSIS INDICATE THAT TILLETIA CONTROVERSA AND T. CARIES ARE RELATED AT THE SPECIES LEVEL. B. W. Russell[^] and D. Mills[*†] Oregon State University, Genetics Program[*], Dept of Botany and Plant Pathology[†], Corvallis, OR, 97331-2902

Tilletia controversa and T. caries are the causal agents of dwarf and common bunt of wheat, respectively. Morphological features of the teliospore and its germination properties are used to distinguish these pathogens. Although these organisms are sexually compatible and their race structures are defined by the same plant resistance genes, they remain classified as separate species. To assess their genetic relatedness, genetic (Trail and Mills, 1990, Phytopathology 80:367-370) and molecular genetic analyses have been performed using defined strains of each fungus. Electrophoretic karyotypes of T. controversa, T. caries, and progeny obtained by crossing compatible strains of these fungi, reveal close genetic relatedness. Probes made from highly conserved fungal genes and DNA fragments from either organism identified linkage groups common to both pathogens and their progeny. The number of chromosome-size DNA bands in four strains of T. controversa varied from 13 to 18 and ranged in size from ca. 680 kilobases (kb) to ca. 5,000 kb, whereas in the T. caries strains, 13 to 15 bands were detected that ranged in size from ca. 370 to 4,500 kb. Among five F1 progeny, 16 to 18 bands of ca. 830 to 3,700 kb were detected. The haploid genome size for these strains is ca. 30 megabases (mb), although strains assumed to be aneuploid may have genomes approaching 40 mb. Evidence for the occurrence of recombinant chromosomes among F1 progeny will be presented. Our data suggest that T. controversa and T. caries should be reclassified as variants of a single species.

1302 MOLECULAR GENETICS OF *PHYTOPHTHORA MEGASPERMA*. Brett Tyler*, Yuxin Mao*, Areelak Kashemsanta*, Barney Ward*, Richard Michelmore# and Howard Judelson#. Departments of Plant Pathology* and Vegetable Crops#, University of California, Davis, CA 95616, USA.

The oomycetes, including *Phytophthora*, are a major group of fungal plant pathogens infecting a very wide range of hosts. *Phytophthora megasperma* f.sp. *glycinea* (*Pmg*) is a major pathogen of soybeans causing damping-off of seedlings and a root and stem rot of mature plants. There are at least 10 different soybean major resistance genes against *Pmg* and these are dominant. In the fungus, which is diploid, there is some genetic and biochemical evidence to indicate the presence of avirulence genes which correspond gene-for-gene with the host major resistance genes. In preparation for trying to clone these presumptive avirulence genes we have characterized the genome of *Pmg* by C_0t analysis and chromosome electrophoresis. The *Pmg* genome has a complexity of 63 Mb, divided among 11 chromosomes, and contains 50% moderately repeated sequences. We have characterized the degree of polymorphism among the single copy and repetitive sequences of different races of *Pmg*. We have identified an RFLP which correlates with a presumptive avirulence gene, and are attempting to identify others by genomic subtraction hybridization. We are also developing a transformation system for introducing cloned DNA into *Pmg* based on a stable transformation system we have developed for *P. infestans*. The *P. infestans* system uses vectors containing bacterial genes for resistance to hygromycin and G418 fused to transcriptional elements from the oomycete *Bremia lactucae*. DNA is introduced into protoplasts using $CaCl_2$ and polyethylene glycol, and integrates stably into the genome.

1303 GENE CLUSTER FOR MELANIN BIOSYNTHESIS IN PHYTOPATHOGENIC FUNGUS *ALTERNARIA ALTERNATA*. Norio KIMURA and Takashi TSUGE. Faculty of Agriculture, Nagoya University, Nagoya 464-01, Japan

Alternaria alternata is a ubiquitous fungus surviving on various dead materials in the field. The fungal species, however, includes several phytopathogenic variations categorized into pathotypes which are only identifiable in host-specific pathogenicity. The walls of the fungal mycelia and conidia contain a pigment melanin that protects the fungus from natural stresses. Melanin-deficient mutants of the fungus are unable to survive under field conditions, indicating that the pigment may be involved in fitness of the pathotypes. Three types of mutants, whose deficiencies in the melanin metabolism were different from one another, were obtained by MNNG treatment. Among the mutants, mutant Brm-1 was subjected to genetic complementation with a cosmid library containing nuclear DNA inserts from the wild-type strain. We isolated a cosmid clone, designated as pMBR1, complementing the Brm-1's mutation. When mutants Alm and Brm-2 were transformed with pMBR1, both mutants also regained wild-type pigmentation, indicating that three genes involved in melanin biosynthesis were present as a cluster in clone pMBR1. These genes complementing mutations of mutants Alm, Brm-1 and Brm-2 were designated as *ALM*, *BRM1* and *BRM2*, respectively. By gene disruption and gene transcript analyses, these genes were mapped in about 35-kb region of the chromosomal DNA with the gene order being *ALM-BRM1-BRM2*. Based on metabolic analysis of the mutants, we suggest that *ALM* product is involved in pentaketide cyclization, and that *BRM1* and *BRM2* may encode scytalone dehydratase and 1,3,8-trihydroxynaphthalene reductase, respectively.

POSTER ABSTRACTS

1304 MOLECULAR GENETIC AND CHEMICAL CHARACTERIZATION OF THE RAPESEED "BLACKLEG" CAUSAL ORGANISM. Janet L. Taylor, M. Soledade C. Pedras and Victor M. Morales. NRC Plant Biotechnology Institute, Saskatoon, Sask. Canada, S7N 0W9. Leptosphaeria maculans (Desm.) Cas. et de Not. (anamorph: Phoma lingam (Tode ex Fr.) Desm.) is a heterothallic ascomycete that causes "blackleg" disease in several cruciferous species. Until recently isolates of the fungus were categorized in one of two strains based on pathogenicity. We used electrophoretic karyotyping to characterize isolates of both strains. The results indicated that, in actuality, the two strains are distinct species. This data interpreted phylogenetically will be presented. In addition, data relating to the high degree of plasticity in the fungal genome will be reported.
Highly virulent isolates of L. maculans were shown to rapidly and very specifically metabolize the Brassica phytoalexin brassinin compared to weakly virulent isolates. The "detoxification" pathway and structures of the intermediates will be shown

1305 DEFENSE GENE EXPRESSION IN SHEATH BLIGHT INFECTED RICE PLANTS C. S. Anuratha, Byron Candole*, Tom Mew*, and S. Muthukrishnan. Department of Biochemistry, Kansas State University, Manhattan, Kansas 66506 and International Rice Research Institute, Los Banos, The Philippines.
The expression of the genes for the antifungal proteins chitinases and β-glucanases has been followed using cDNA and antibody probes. These enzymes are encoded by small multigene families in rice. Analysis of extracts of rice tissues including seeds has revealed the presence of multiple forms of these enzymes differing in molecular weight and isoelectric points. While some of the isozymes are present constitutively at low levels in uninfected plants, others are induced upon infection of sheaths and leaves by the pathogen, *Rhizoctonia solani*. Extracts prepared from seeds or infected plants have antifungal activity. The antifungal activity is correlated with the levels of chitinases and β-glucanases in these extracts. A 35 kD chitinase has been purified from rice bran. The purified enzyme inhibits the growth of several fungi, including *Rhizoctonia solani* at 1 μg level. The changes in levels of chitinases and β-glucanases during infection of rice plants that are susceptible or moderately resistant to sheath blight will be presented.

1306 INDUCTION OF CDC2-LIKE KINASE LEVELS DURING THIGMOTROPIC DIFFERENTIATION IN *UROMYCES*. Srirama M.Bhairi Boyce Thompson Institute Tower Road, Ithaca, NY 14853

The plant pathogen, bean rust fungus (*Uromyces appendiculatus*), differentiates to form infection structures such as appressoria solely in response to contact stimuli. In nature, differentiation occurs when the tip of the germ tube comes in contact with the lip of the stomatal guard cells. In the laboratory, differentiation is routinely induced on oil-collodion membranes or on plastic surfaces with ridges of 0.5 micrometer height. How the touch stimulus induces differentiation has been the focus of our research for the past several years. Thigmotropic differentiation is accompanied by mitosis and differential expression of at least six genes. Since mitosis occurs only in differentiated germlings, I have examined whether the mitosis-specific factor, cdc2-like kinase, is induced in response to the touch stimulus. Using anti-cdc2 antibodies (a generous gift from Dr. David Beach, CSH), I have observed by western blot analysis that the cdc2-like protein is present only in differentiated germlings. Presently, work is underway to clone the rust homologue of the cdc2 gene and to examine the mRNA levels during thigmotropic differentiation

1307 STRUCTURE AND CHARACTERIZATION OF A CUCUMBER CLASS III CHITINASE GENE. James Beck, Leslie Friedrich, Kay Lawton, Mary Moyer, Eric Ward and John Ryals. CIBA-GEIGY Biotechnology Research, PO Box 12257, RTP, NC 27709-2257

A 12.2 kb EcoR1 fragment encoding a class III chitinase protein was isolated from Cucumis sativus. Sequence analysis revealed three highly homologous coding regions with divergence in the 5' and 3' flanking regions. Ten clones isolated from a cDNA library corresponded to the middle coding region, suggesting the other two coding regions are not expressed. Gene specific oligonucleotides are being used, however, to determine possible expression of the other two coding regions. Active chitinase protein was produced at high levels in the transgenic tobacco plants with this cDNA. Homologies to other known plant chitinase sequences were also determined

1308 ANALYSIS OF THE MATING-TYPE SYSTEM IN THE PHYTOPATHOGENIC SMUT FUNGI. Guus Bakkeren, Ben Gibbard and Jim Kronstad. Biotechnology Laboratory, Dpt.of Plant Science and Microbiology, University of British Columbia, Vancouver,B.C., Canada V6T 1Z1

Ustilago hordei causes covered smut of barley (Hordeum vulgare) and oats (Avena sativa) and is closely related to other species like U.nuda, U.kolleri and U.nigra, all pathogenic on small grain cereals. The life cycle of U.hordei like that of many smut fungi, involves hybridization of 2 haploid, non-pathogenic basidiospores of opposite mating type (bipolar mating). Fusions of haploids yields a stable, dikaryotic mycelium that represents the pathogenic cell type. U.hordei infects seedlings, grows asymptomatically with the apical meristem and sporulates primarily in the head of the plant, thereby replacing all of the developing seeds with black masses of spores. This life cycle can be contrasted with that of the corn smut fungus, U.maydis, which can infect and sporulate in any aerial part of the plant. U.maydis also has a more complex mating system in which 2 separate genetic loci are involved in sexual fusion and pathogenicity (tetrapolar mating). The a locus of U.maydis has 2 alternate alleles and may be equivalent to the mating type locus in U.hordei. The second locus in U.maydis, called b, possesses at least 25 alleles and appears to control pathogenicity and sexual development in the plant. We have extended our analysis of the b locus by surveying the cereal smut fungi for b-homologues. Although no genetically defined b locus has been described, we find hybridizing sequences in all of the smut fungi tested and we have been able to clone and sequence 2 b-like genes from U.hordei. The overall organization of these genes is much like for the U.maydis b alleles with conserved 3'regions and variable 5'regions. The 3'regions of b genes from U.maydis and U.hordei share 65% homology. These findings raise interesting evolutionary questions about the differences between bi- and tetra-polar mating systems.

1309 CORDYCEPIN INHIBITS THE HYPERSENSITIVE RESPONSE IN POWDERY MILDEW OF BARLEY. W.R. Bushnell and Zhanjiang Liu. USDA-ARS Cereal Rust Laboratory, University of Minnesota, St. Paul, Minnesota 55108

Cordycepin, an inhibitor of mRNA synthesis, was applied to barley tissues at various times after inoculation with Erysiphe graminis f. sp. hordei to determine its effect on the hypersensitive response (HR) to powdery mildew. Treatments were applied to epidermal tissues from coleoptiles of an Algerian barley line with the Mla gene, which confers resistance that is normally expressed 18-30 hr after inoculation. Cordycepin at 10-31 μM reduced the proportion of infection sites expressing HR to 6-56% of that in untreated tissues. Treatments were effective when started 0-4 hr after inoculation but ineffective if started 8 hr or later. The cordycepin was equally effective if left in place 30 or more hr or removed 4-8 hr after inoculation. Cordycepin had no effect on the number or size of haustoria in the resistant host or in a near-isogenic susceptible line without Mla. The results indicate that mRNAs required for HR are synthesized in the first 4-8 hr after inoculation and that mRNA synthesis after 8 hr is not required for expression of HR at 18-30 hr.

POSTER ABSTRACTS

1310 THE CLONING AND CHROMOSOMAL LOCATION OF MAACKIAIN DETOXIFICATION GENES FROM *NECTRIA HAEMATOCOCCA*
Sarah F. Covert and Hans D. VanEtten. Department of Plant Pathology. University of Arizona. Tucson. AZ 85721 U.S.A.
Nectria haematococca is a fungal pathogen of many plants including chickpea and pea. On these plants, the pathogenicity of *N. haematococca* is correlated with its ability to detoxify specific, plant-produced phytoalexins: maackiain and pisatin respectively. From a collection of field and laboratory isolates, three genes for maackiain detoxification (*Mak* genes) and six genes for pisatin detoxification (*Pda* genes) have been identified in this fungus. Our goal is to determine if the pathogenicity genes that encode maackiain detoxification in *N. haematococca* are carried on small, meiotically unstable chromosomes as many of the genes involved in pisatin detoxification appear to be. Our first step towards this goal is to clone the *Mak1* gene and to use it to identify homologous genes. Classic genetic analysis previously indicated that *Mak1* is closely linked to a pisatin degrading gene, *Pda6-1*, which resides on an unstable chromosome. Because detoxification of both maackiain and pisatin is catalyzed by mono-oxygenases, the possibility remained that *Mak1* and *Pda6-1* were the same gene. However, expression of *Pda6-1* in a Mak- background did not confer the Mak+ phenotype, thus demonstrating that *Mak1* and *Pda6-1* are distinct loci. We are currently constructing a chromosome-specific library of the *Mak1 Pda6-1* chromosome. A Mak- isolate of *N. haematococca* will be transformed with this library and *Mak1* clones identified by screening transformants for the Mak+ phenotype. In order to do so, we have developed a protocol that yields up to twenty-five transformants per microgram of DNA.

1311 DIVERSITY OF RICE BLAST FUNGUS POPULATIONS IN THE PHILIPPINES. E. S. Borromeo, M. A. Bernardo, J. M. Bonman, H. Leung[1] and R. Nelson. Division of Plant Pathology, International Rice Research Institute, Los Baños, Philippines. [1]Current address: Dept. of Plant Pathology, Washington State University, Pullman, Washington, U.S.A.
Molecular markers were generated and used to analyze populations of *Pyricularia grisea*, the fungus which causes the rice-blast disease, and which also infects common rice-field weeds. Using repeated DNA sequences as molecular probes, populations of the fungus infecting different host species were clearly differentiated from one another, suggesting that populations infecting weed hosts do not provide inoculum for the rice crop. From a general survey of rice-infecting isolates of the blast fungus across the Philippines, RFLP analysis revealed a high degree of genetic variability. Fifty six restriction fragment banding patterns were detected by the repeated DNA probe PGR 613 from 146 isolates collected from 17 provinces. A high level of genetic heterogeneity was also observed within pathogen populations at two screening sites used by rice breeders for assessing resistance to rice blast disease. Preliminary results revealed 20 restriction haplotypes among 70 isolates collected from Cavinti, Laguna, and 6 haplotypes among 16 isolates from the Blast Nursery at the International Rice Research Institute in Los Baños.

1312 POTATO VIRUS X AS A MODEL SYSTEM TO STUDY VIRAL REPLICATION. Carl J. Braun, James D. Weiss, Nilgun E. Tumer and Cythnia L. Hemenway. Monsanto Co., Plant Protection and Improvement, 700 Chesterfield Village Parkway, Mail Zone AA3E, St. Louis, MO 63198. We have investigated plant viral replication using potato virus X (PVX) as a model system. PVX is a single-stranded, plus-sense RNA virus of 6.436 nucleotides. PVX contains five open reading frames; the first two may be involved in viral replication due to the presence of conserved domains that are commonly found in other known replicases. We have cloned and expressed these two non-structural genes from PVX together, and separately in tobacco (var. samsun). These transgenic tobacco will be used in complementation tests with defective infectious clones.

1313 RESISTANCE TO RHIZOCTONIA SOLANI IN TRANSGENIC TOBACCO EXPRESSING THE SERRATIA MARCESCENS CHITINASE GENE. Pamela Dunsmuir, William Howie, Ed Newbigin and Trevor Suslow. DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608. We are attempting to develop strategies which provide significant fungal disease tolerance to plants. The first step in this process is the transformation of tobacco with a gene which encodes the lytic enzyme chitinase. The Serratia marcescens chiA was engineered for expression in plant cells such that the protein was targeted extracellularly, or intracellularly. Transgenic plants were regenerated which expressed high levels of the ChiA protein in each of these compartments. Progeny from these transformants were compared with (chiA-) control plants in Rhizoctonia solani infection assays. Populations of seedlings were planted in infested soil, then stem lesions were quantitated after 10-14 days. Significant reductions in both lesion number and disease severity were associated with the expression of ChiA protein in these plants. A similar level of disease resistance was observed when the ChiA protein was expressed intra- or extracellularly.

1314 ACTIVATION OF DEFENSE-RELATED GENES IN ONION. Alex. F.Dmitriev, Galya Y.Perkovskaya and Yulia V.Dychok. Cell Biology Institute, Lebedev st. I, 252650 Kiev.
An example of rapid gene expression in the defense response of plant to pathogen is observed in the interaction of onion (Allium cepa L.) with Botrytis cinerea. Among the rapidly activated genes are those encoding hydrolytic enzymes, whereas the genes encoding phytoalexins respond more slowly. For the first time two phytoalexins were discovered in onion. We called them tsibulins Id and 2d (from Ukrainian "tsibulya"=onion). The analysis by HPLC, plus UV, IR, NMR and mass-spectra revealed they as 1,3-dion-5-octyl-cyclopentane and 1,3-dion-5-hexyl-cyclopentane. In respond to infection both phytoalexins were accumulated with much higher speed in limited lesions (incompatible interaction) than in spreading lesions (compatible interaction). Several pathogenesis-related (PR) proteins were shown by SDS-PAGE accumulated earlier in incompatible interaction than in compatible ones. Two of the PR-proteins were purified and used to raise polyclonal antibodies. One of the proteins (M_ 27,000 D) showed chitinase activity, while the other (M_ 33,000 D)was able to degrade laminarin (1,3-B-glucanase activity). The early accumulation in incompatible interaction of both chitinase and glucanase was also visualized by Western blotting. The rapid gene expression and accumulation of these enzymes, potentially capable of degrading the hyphal walls of B. cinerea at the site of penetration, might play an important role as an active plant defense reactions.

1315 **PrB-1, A PROTEINASE INVOLVED IN PATHOGEN CONTROL BY THE MYCOPARASITE *Trichoderma harzianum*.** R. Geremia[1], G. Goldman[1], D. Jacobs[3], M. Van Montagu[1] and A. Herrera-Estrella[1,2]. [1]Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Ghent, Belgium; [2]CINVESTAV Irapuato, AP 629 Irapuato Gto., Mexico; [3]State Research Station for Phytopathology, B-9820 Merelbeke, Belgium.
Hydrolytic enzymes secreted by *Trichoderma harzianum* are believed to play an important role in the biocontrol process of phytopathogenic fungi. Among these enzymes we have identified a basic proteinase (PrB-1) that is induced in the presence of either autoclaved mycelia or cell walls of phytopathogenic fungi. The proteinase has been biochemically characterized and identified as a serine proteinase. By using synthetic oligonucleotides deduced from peptide microsequence data, we have been able to clone the corresponding cDNA from a library generated using poly(A)$^+$-RNA from induced mycelia.

The gene encoding for PrB-1 has been mapped to chromosome 6 by Southern analysis of chromosomes separated by pulse field electrophoresis. Northern analysis has shown that induction by cell walls occurs at the RNA level. In addition, preliminary immunofluorescence experiments indicate that this proteinase is produced only in the areas of direct physical contact with the pathogen when they are confronted *in vitro*.

Further experiments will be carried out to characterize the regulatory signals present in the promoter region of the gene coding for PrB-1.

POSTER ABSTRACTS

1316 REGULATION OF ERGOT PEPTIDE LACTAM SYNTHETASE FROM THE ERGOT FUNGUS CLAVICEPS PURPUREA.
Ulrich Keller, Norbert Quandt and Brigitte Riederer Technische Universitat Berlin, Institut fur Biochemie und Molekulare Biologie, Franklinstraße 29 D 1000 Berlin 10, Germany

The ergot fungus *Claviceps purpurea* produces the therapeutically important ergot peptide alkaloids It grows parasitically on a number of cereals, mostly rye The alkaloid production phase is coupled to the development of the sclerotium on the plant. In saprophytic culture, culture conditions are required which induce the formation of so-called sclerotia-like cells. Only in these morphological conditions alkaloid production occurs. Ergot peptide alkaloid synthesis is under phosphate limitation and is induced by exogenously added tryptophan We have isolated from *C. purpurea* ergot peptide lactam synthetase, a multifunctional enzyme catalysing the synthesis of ergotamam, the immediate precursor of ergotamine. The enzyme is a single polypeptide chain of M_r 265,000 - 280,000 It activates all four constituent acids of the peptide, i e D-lysergic acid, alanine, phenylalanine and proline as thioesters via the corresponding adenylates The enzyme contains 4'-phosphopantheteine as prosthetic group, and thus is similar to other peptide synthetases of microbial origin. Measurements of the enzyme's activity at various stages of cultivation in alkaloid production medium revealed its constitutive expression Also the presence of phosphate at various concentrations did not affect the level of enzyme in the cells significantly whereas the amount of alkaloid formed declined drastically We conclude that target gene(s) of phosphate repression and tryptophan induction may be involved in earlier steps of the biosynthetic pathway or in the step of peptide lactam processing Purification and properties of ergot peptide lactam synthetase, data on the cloning of its gene and transcriptional analysis will be presented

1317 ROLE OF ANTIBIOTIC PRODUCTION BY PSEUDOMONAS FLUORESCENS IN COLONIZATION OF THE RHIZOSPHERE.
J. S. Hill, J. M. Lucas and J. M. Ligon, Ciba-Geigy Corporation, Agricultural Biotechnology Research Unit, P.O. Box 12257, Research Triangle Park, NC 27709, USA

Antibiotic production by rhizosphere bacteria has been demonstrated to play a role in the biological control of soil-borne fungal plant pathogens. We report the isolation of the genes from a strain of Pseudomonas fluorescens originating from cotton roots that encode the biosynthesis of a antibiotic with activity against Rhizoctonia solani, a pathogen of cotton. In biological disease control tests in the greenhouse, this strain is an effective biocontrol agent for R. solani and Phythium ultimum. The antibiotic compound is known to be effective against other fungi and some bacteria. An antibiotic minus mutant was constructed and its ability to compete against indigenous microflora on cotton roots was compared to the antibiotic producing wild-type strain. The results indicate that the antibiotic produced by this Pseudomonas strain does have a positive effect on the bacteria's ability to compete in the rhizosphere.

1318 UNUSUAL SEGREGATION PATTERNS OF GENES FOR PATHOGENICITY IN LABORATORY CROSSES OF *NECTRIA HAEMATOCOCCA*.
D. L. Funnell-Baerg, P.S. Matthews and H. D. VanEtten, Department of Plant Pathology, University of Arizona, Tucson, Arizona 85721 (DFB and HDV) and Department of Plant Pathology, Cornell University, Ithaca, New York 14853 (PSM).

Conventional genetic studies with the broad-host-range fungus, *Nectria haematococca*, have facilitated the analysis of putative traits involved in its pathogenicity on pea. However, natural isolates of this ascomycete that are highly virulent on pea also tend to produce crosses of low fertility, while isolates from other sources can produce highly fertile crosses. In order to make the fungus more amenable to genetic analysis, a breeding project was undertaken that successfully combined high fertility and high virulence in individual strains. These strains were also able to demethylate the pea phytoalexin, pisatin (pisatin demethylating ability, Pda), a trait previously suggested to be required for pathogenicity. When one of these isolates (34-18) was crossed to nonpathogenic pda$^-$ strains, tetrad analysis gave ratios that could be interpreted as if 34-18 might have zero, one or two *Pda* loci. Additionally, most of the progeny, regardless of Pda phenotype, had relatively low virulence on pea. Recent Southern analysis of 34-18 has shown that there are three DNA fragments hybridizing to a *pda* gene probe: the hybridization pattern in most likely due to the presence of two active *Pda* genes and one non-functional gene. The unusual segregation patterns and loss of virulence observed in 34-B may result from conventional segregation events occurring together with the loss of a large portion of a chromosome(s) during meiosis, as previously observed for this fungus (Miao and VanEtten, unpublished). The lack of virulence in the progeny isolates may also be explained by the loss of a putative meiotically unstable chromosome carrying a *pda* gene and, perhaps, other genes for pathogenicity.

1319 FUNGAL PATHOGENS OF PEA PRODUCE BIOCHEMICALLY DIFFERENT PISATIN DEMETHYLASES.
H. L. George, and H. D. VanEtten, Department of Plant Pathology University of Arizona, Tucson, Arizona 85721

Many fungal pea pathogens have the ability to demethylate and thus detoxify the phytoalexin pisatin produced by pea. In *Nectria haematococca*, the enzyme responsible for this demethylation is an inducible microsomal cytochrome P450, whose gene (*Pda*) has been cloned. When the DNAs from other pea pathogens were probed with *Pda*, little or no homology was detected (Delserone, unpublished). In order to further compare the pisatin detoxification system in other pea pathogens to that in *N. haematococca*, we have examined the induction of whole cell activity and the biochemical properties of microsomal preparations from the pea pathogens *Fusarium oxysporum* f. sp. *pisi*, *Mycosphaerella pinodes*, and 2 isolates of *Ascochyta pisi*. All are cytochrome P450s, but they differ in their whole cell regulation and sensitivity to P450 inhibitors. Enzyme preparations from *N. haematococca* and *F. oxysporum* had similar patterns of sensitivity to inhibitors, and both were subject to strong glucose repression. Preparations from *M. pinodes* and *A. pisi* isolate 422 responded similarly to a different pattern of inhibitors and showed less induction specificity and glucose repression than those of *N. haematococca* and *F. oxysporum*. The enzyme activity from isolate 422 of *A. pisi* was sensitive to the largest number of inhibitors and showed the least specificity of induction. These biochemical differences suggests that these organisms have evolved separate cytochrome P450s to detoxify pisatin.

1320 CHARACTERIZATION OF PISATIN DEMETHYLASE GENES IN THE PATHOGENIC FUNGUS *NECTRIA HAEMATOCOCCA MPVI*.
K. Hirschi, C. Reimmann*, A. Malonev**, and H. D. VanEtten. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721 USA. *Mikrobiologisches Institut ETH Zurich, CH-8092, Zurich, Switz. **Department of Plant Pathology, Cornell University, Ithaca, NY 14853 USA.

The pea pathogens *Nectria haematococca* can detoxify pisatin, an isoflavonoid phytoalexin produced by pea. Detoxification is accomplished by pisatin demethylation (Pda) an inducible microsomal cytochrome P-450. Previous genetic analyses have revealed at least six loci for *Pda* genes in *N. haematococca*. The genes encoded by these loci confer three distinct Pda phenotypes characterized by the lag time from exposure of the fungus to pisatin until 50% of the pisatin is demethyleted and by the maximal level of enzyme obtained. The three phenotypes (Pda^{SH}, short lag, high activity; Pda^{SM}, short lag, moderate activity; and pda^{LL}, long lag, low activity) appear to be correlated with different levels of virulence on pea. Recently, *Pda* genes representing all three phenotypes have been cloned and sequenced. We report here comparisons among the three different *Pda* structural genes, and their 5' regulatory regions. Restriction fragment length polymorphism analysis of *N. haematococca* field isolated is being completed to determine the natural diversity of this cytochrome P450 subfamily. We propose to correlate the different Pda phenotypes with geographic regions, biological habitats and host specificity. Additional studies will involve the construction of chimeric genes to identify domains involved in regulation, biochemical properties, and pathogenicity functions.

1321 REGULATION OF CUTINASE GENE EXPRESSION IN THE PHYTOPATHOGENIC FUNGUS *FUSARIUM SOLANI*.
Jorg T. Kamper, Ute Kamper, Linda M. Rogers and Pappachan E Kolattukudy, Ohio State Biotechnology Center, The Ohio State University, Columbus, OH 43210, USA

During early states of infection some phytopathogenic fungi penetrate the cuticular barrier of their hosts by secretion of cutinase, a cutin hydrolyzing enzyme. In *Fusarium solani f. sp. pisi* the expression of the cutinase gene was found to be regulated by cutin monomers and glucose concentration of the medium. We used both *in vivo* and *in vitro* systems to analyze different promoter elements of this gene. Plasmids harboring different promoter regions linked to the CAT gene were used for transformation of *Fusarium solani pisi* and CAT expression of the transformants was studied under varying conditions. The -225 to -360 upstream region was found to be involved in regulation of the gene: deletion analysis of a cAMP responsive element and an Sp1 transcription factor binding element, contained in this segment, indicate that cAMP dependant transacting factors are involved in glucose repression. The same promoter elements were also analyzed by gel retardation assays to identify the binding regions of these transcription factors. Experiments are under way to study the mechanism of signal transduction and cutinase gene regulation, including the role of phosphorylation in this process

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1322 ISOLATION AND CHARACTERIZATION OF HMG-COA REDUCTASE IN RICE Amy J Nelson and Christopher J. Lamb, The Salk Institute, Plant Biology Lab, San Diego, CA 92037

An IR36 rice genomic library (gift of N.-H. Chua) was screened using a heterologous Arabidopsis HMGR probe. The probe was a cDNA fragment, 439 bases in length, from the conserved catalytic region of the gene. 20 positive clones were purified and these could be grouped into 3 classes based on digestion and hybridization analysis with a rice HMGR probe However, hybridization analysis with lambda DNA indicated that the classes were probably overlapping clones from the same gene and not clones from different genes. Genomic Southern analysis suggested that there are 2 HMGR genes in rice. The cloned HMGR gene has been mapped and sequenced. In the catalytic domain, it is ~70% identical to the Arabidopsis HMGR sequence at the nucleotide level and ~90% identical at the amino acid level. The tissue-specific pattern of expression of the gene, as revealed by Northern analysis, is discussed. Also, an elicitor fraction prepared from the cell walls of Pyricularia oryzae, a rice pathogen and causal agent of rice blast disease, is used to examine elicitor-inducibility of the HMGR gene.

1323 PROTEINS WITH ANTI-FUNGAL ACTIVITY TOWARDS PHYTOPHTHORA INFESTANS. L.S. Melchers, C.P. Woloshuk, E.J.S. Meulenhoff, M.B. Sela-Buurlage, A.A. Vloemans, J.S.C. van Roekel, P.J.M. van den Elzen and B.J.C. Cornelissen. MOGEN Int. NV, Einsteinweg 97, 2333 CB Leiden, The Netherlands.

The phenomenon of induced resistance in plants, triggered by a prior infection, is interesting because of its broad spectrum of effectiveness. Resistance against Oomycetes (e.g. Phytophthora infestans) is inducible in several plant species. We have isolated a protein from extracts of pathogen-inoculated tobacco and tomato plants with anti-fungal activity towards P.infestans. A 24 kDa protein (AP24) causing lysis of sporangia and growth inhibition of P.infestans was purified and characterized. The tobacco AP24 protein was identified as the thaumatin-like protein osmotin II. While the tomato AP24 protein was similar to osmotin II and the NP24 protein of tomato. We will present data concerning, expression of AP24 in transgenic plants and the anti-fungal activity of AP24.

Reference:

Woloshuk, C.P., Meulenhoff, E.J.S., Sela-Buurlage, M., van den Elzen, P.J.M. and Cornelissen, B.J.C. (1991). Pathogen-induced proteins with inhibitory activity toward Phytophthora infestans. The Plant Cell (in press).

1324 MATING TYPE REGULATED GENES IN THE CORN PATHOGEN *Ustilago maydis* Scott Gold, Denise Pierre and James Kronstad. Biotechnology Laboratory, University of British Columbia, Vancouver, B.C. Canada V6T 1Z3

Several approaches are being employed to clone and analyse genes specifically expressed in cells of *U maydis* which are heterozygous at the b mating type locus. Diploid cells heterozygous for b express a filamentous phenotype, while haploids or diploids homozygous at b form yeast like colonies. A heterozygous condition at b is a critical requirement for pathogenicity of the fungus on its host *Zea mays*. We are presently screening genomic libraries constructed as translational fusions with a promoterless *lacZ* gene on an autonomously replicating vector. To date 500 promoter fragments have been identified in a diploid strain heterozygous at b. Gene fusions with *lacZ*, which are transcribed only when the b locus is heterozygous or in later stages of filamentous growth, should yield colorless colonies in haploid transformants. A similar approach, but utilizing genetic selection rather than screening, is being developed using complementation of pyr6 auxotrophic mutants. Genes specifically expressed in transformants heterozygous at b should confer pyr6 prototrophy when translationally fused to the yeast URA3 gene (equivalent to *U maydis* pyr6). In contrast, these gene fusions will not confer prototrophy in haploids or in diploids homozygous at b. In conjunction with the above approaches we are presently determining, via northern blot analysis, whether any of the three identified *U maydis* chitin synthase genes are differentially expressed in the yeast and filamentous growth forms of the fungus. Differential expression of chitin synthase genes occurs in *Candida albicans*, a fungus displaying dimorphic growth patterns similar to *U maydis*. We are also constructing strains disrupted in each of these chitin synthase genes.

1325 A NECROSIS-INDUCING PEPTIDE FROM THE BARLEY PATHOGEN *RHYNCHOSPORIUM SECALIS* IS A PUTATIVE AVIRULENCE GENE PRODUCT Wolfgang Knogge, Matthias Hahn and Lutz Wevelsiep. Max-Planck-Institut für Zuchtungsforschung, Department of Biochemistry, Carl-von-Linné-Weg 10, D-5000 Köln 30, Federal Republic of Germany

Rhynchosporium secalis is the causal agent of barley leaf scald. Fungal race US238.1 is avirulent on barley line "Atlas 46" carrying resistance gene *Rrs1* and virulent on the near-isogenic line "Atlas" lacking this gene. While F_1 plants displayed an intermediate phenotype, a phenotypic segregation of 1:2:1 was found in the F_2 generation. This demonstrates that resistance is based on a single codominant gene. In this system, host defense does not include a hypersensitive response. However, inoculated primary leaves of the resistant barley line respond with the accumulation of mRNA encoding a thaumatin-like pathogenesis-related protein, PRHv-1, which is expressed to a significantly lower level in leaves of the susceptible line. A necrosis-inducing peptide from *R.secalis*, NIP1, which occurs only in race US238.1, also strongly induced this mRNA accumulation in the resistant line. In contrast, a very weak response was detected in the susceptible line, while F_1 plants displayed an intermediate phenotype. *Rrs1* was originally introgressed into the "Atlas" genetic background from cultivar "Turk". NIP1 was found to induce the accumulation of PRHv-1 mRNA in this cultivar as well, while a barley cultivar lacking any *Rhynchosporium* resistance genes did not respond at all. In F_2 individuals, the NIP1-induced accumulation of PRHv-1 mRNA appears to cosegregate with the resistance phenotype. This implicates NIP1 as the product of the fungal avirulence gene complementary to resistance gene *Rrs1* and a NIP1-binding plasma membrane protein as the primary candidate for the product of *Rrs1*.

1326 CIRCULAR PLASMID DNA OF THE FUNGUS VERTICILLIUM DAHLIAE. Ibragimov A.A., Chodjabaeva S.M. Institute of Microbiology, UzSSR Academy of Sciences, USSR.

In the last years considerable interest has been devoted to studies of extrachromosomal genetic elements, particularly, to plasmid of phytopathogenic fungus of cotton. As an object of investigation it was used the 10-th generation of V.dahliae by multiple inoculations and reextractions of the original fungus form from the resistant to this strain cotton variety Tashkent-1. By means of 0,7% agarose electrophoresis of total fungus DNA preparation, it was found the circular DNA plasmid of V.dahliae. The discovered DNA with molecular weight of 140 kb corresponded to F-plasmid of E.coli 4264(F:Tn-5).In our experiments we have establesned the correlation of V.dahliae DNA plasmid-replication with the terms of appearing of cotton disease. This observations support our hypothesis that isolated circular DNA plasmid of V.dahliae plays a possible role in mutual relations between the pathogen and the cotton as a host plant.

1327 GENOME PLASTICITY IN USTILAGO HORDEI AND KARYOTYPES OF RELATED SMUT FUNGI. Kevin McCluskey, and Dallice Mills Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331-2902

Fungal chromosomes have been shown to be highly variable with respect to both size and number. To estimate genome plasticity among strains of a plant pathogenic fungus, chromosome specific DNA clone libraries were constructed from chromosome-length bands resolved by CHEF PFGE for U. hordei strains 8.1a and 10.1a. Strain 8.1a is derived from a collection of teliospores that has been shown by others to have the greatest number of avirulence genes. Strain 10.1a has the greatest number of chromosomes, and was heat-shocked to generate a mutant, strain 10.1a-1 that has an altered colony morphology. This mutant was subsequently shown to have lost a single chromosome of 960 kilobases, and to be deficient in mating response. The identity of the missing 960 kb chromosome has been investigated by hybridization with DNA probes derived from the chromosome specific library from the 960 kb band in strain 10.1a. DNA probes from several chromosome specific libraries were used to identify homologs in strains representing fourteen different pathogenic races, and demonstrated chromosome-length polymorphisms for many chromosomes. The demonstration that most single copy probes hybridize with chromosomes of similar size suggests that the variability in karyotype is not generated by fragile sites or translocation. Karyotypes for several related Ustilago species are used to define species boundaries. A model that associates fungal karyotype with life cycle is presented.

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1328 A PLANT-INDUCED NUCLEASE FROM *FUSARIUM SOLANI* MAY BE A COMPONENT OF VIRULENCE. Lee A. Hadwiger, David Gerhold and Andrew Pettinger Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430

F. solani f. sp. *phaseoli* a bean pathogen produces and releases a nuclease in the presence of pea endocarp tissue or plant supplemented media. The nuclease causes single strand breaks in both single stranded and double stranded RNA and DNA. 10 mM Ca^{++} concentration greatly enhances ds DNA degradation. Nuclease activity is stable to boiling, trypsin, chymotrypsin but not to SDS or proteinase K. This ~ 22,000 M.W. nuclease has been purified to a single electrophoretic band, and has been partially sequenced. Nuclease production occurs within 30 min after inoculation of pea pods, and fungal nuclease activity is detected in nuclei isolated from pea tissue within 2 hrs. Pure nuclease applied to endocarp tissue prior to the bean pathogen renders susceptible this normally immune tissue. Production of related heat stable nucleases by other *Fusaria* species suggest their nucleases could potentially influence other host/parasite interactions.

1329 CLONING OF GENUS SPECIFIC DNA FRAGMENT FROM FUNGUS FUSARIUM OXYSPORUM B.K.Irisbayev, A.S.Kraev, A.A.Abdukarimov, K.G.Skryabin A.S.Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR,Tashkent,700143;Centre of Bioengineering, Academy of Sciences of the USSR, Moscow 117984

The fungus Fusarium oxysporum is a pathogen of many plant species, including cotton, and causes wilt desease. DNA diagnostics of this fungus would be possible with the availuability of genus specific probes. We report on the cloning of a DNA fragment from the genome of this fungus. By using specific amplification by the polymerase chain reaction with the primers, partially homologous to terminal regions of 18S RNA and 5.8S RNA genes, we generate an intergenic spacer sequence of 209 bp, that is genus specific. The spacer sequence is substantially dissimilar with any such sequences from other related fungi, including another wilt pathogen, Verticillium dahliae.

1330 DOUBLE-STRANDED R N A POPULATIONS IN THE FUNGUS FUSARIUM OXYSROPUM IN CENTRAL ASIA. D.A.Khanazarova, and A.A.Abdukarimov. A.S.Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent 700143

130 isolates of Fusarium oxysporum, a plant pathogenic fungus,have been tested for the presence of double-stranded RNAs. In 10 isolates such RNA species were detected.In all cases the RNAs were of viral origin.Four RNA profiles were found, three of them belonging to the same group of viruses. One RNA profile showed slight differences in all cases, that are likely to result from genome rearrangements. Multiheterogenic dsRNAs were found in one of the isolates.

1331 REGULATION OF CUCUMBER CLASS III CHITINASE GENE EXPRESSION. Kay A. Lawton, Leslie Friedrich, James Beck, David Negrotto and John Ryals. CIBA-GEIGY Biotechnology Research Division, Research Triangle Park, NC 27709

The onset of systemic acquired resistance (SAR) in *Cucumis sativus* (cucumber) is correlated with increased levels of salicylic acid, which is thought to be the endogenous signal molecule for SAR . An acidic, extracellular chitinase purified from tobacco necrosis virus (TNV)-infected cucumber leaves is induced at the RNA level by TNV or salicylate. In cucumber, chitinase accumulation is associated with induction of SAR. To understand the molecular events in this signal transduction pathway we have further examined the accumulation of chitinase mRNA in response to exogenous applications of salicylic acid and other SAR-inducing agents. We have also investigated the effect of the cytoplasmic protein synthesis inhibitor, cycloheximide, on the expression of this gene. Promoter deletion analysis to identify DNA regulatory sequences has been carried out in a heterologous tobacco system and is ongoing in cucumber Results of these experiments will be presented and discussed.

1332 BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF PR PROTEINS OF TOMATO THAT ACCUMULATE AFTER INFECTION BY CLADOSPORIUM FULVUM, CAUSAL AGENT OF TOMATO LEAF MOLD. Matthieu H.A.J. Joosten, Jan A.L. van Kan, Jos P. Wubben and Pierre J.G.M. de Wit, Agricultural University, Department of Phytopathology, Wageningen, The Netherlands.

The interaction between tomato and Cladosporium fulvum, which is based on a gene-for-gene relationship, is used as a model system to study recognition events between plant and fungus. Growth of the biotrophic fungus remains confined to the apoplast, which can be explored by the isolation of intercellular washing fluid (apoplastic fluid). After inoculation of tomato leaves with an avirulent race of C. fulvum there is a rapid accumulation of several pathogenesis-related (PR) proteins in the apoplast. From apoplastic fluid three basic PR proteins of 14 kD in molecular weight were purified and corresponding cDNA clones were isolated from a cDNA library made with poly(A)$^+$-RNA isolated from a compatible C. fulvum-tomato interaction. Two of the proteins (P4 and P6) were found to be isomers of the earlier characterized tomato PR protein P14 and were 60% identical to the tobacco PR-1 protein family. The third protein (P2) was shown to be related to the tobacco PR-4 protein family. Besides the presence of two chitinases (26 and 27 kD) and one 1,3-ß-glucanase (35 kD) in the apoplastic space, two intracellular chitinases (30 and 32 kD) and one intracellular 1,3-ß-glucanase (33 kD) were identified. All chitinases and 1,3-ß-glucanases were purified and corresponding cDNA clones were isolated. The pure enzymes were tested for inhibition of fungal growth of C. fulvum in vitro and in planta

1333 SESQUITERPENOID STRESS METABOLITES: A FACTOR OF SUSCEPTIBILITY IN POTATO-FUSARIUM TUBER ROT. Mostafa H. Mostafa Dept. Plant Pathology, Ain Shams Univ., Cairo, Egypt.

The role of sesquiterpenoid stress metabolites(SSM) which always associated with Fusarium tuber rot infection in disease incidence was studied. Time course of SSM production was determined in potato tuber slices susceptible or resistant inoculated by severe(F.solani)or weak pathogen(F.moniliforme) were determined. SSM greatly produced and accumulated in a very short period in susceptible cv. inoculated by severe pathogen in comparison with other cases. Increasing of SSM production in inoculated tissue by chloramphenicol not only increased disease incidence in susceptible cv. inoculated by a weak pathogen but also changed the type of the interaction between resistant cv. and weak pathogen from state of resistance to susceptibility. Correlation coeffecient between disease severity and SSM production reached +0,83. The term PHYTOPATHOALEXIN was postulated to describe the compound(s) with plant origen which accumulate during infection and play a role in disease susceptibility.

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1334 EVALUATION OF CYANIDE DETOXIFICATION AS A MECHANISM OF FUNGAL PATHOGENICITY. P. Wang and H. D. VanEtten. University of Arizona. Department of Plant Pathology. Tucson. AZ 85721.

Cyanogenic plants such as sorghum (*Sorenum bicolor* L) release HCN when the tissue is damaged during events such as fungal infection. The production of HCN may have evolved as a defense mechanism in the plant against potential pathogens. However, fungal pathogens of sorghum are tolerant of HCN, and this tolerance was thought to be a means by which successful pathogens overcome a resistance mechanism based on HCN production. The high tolerance of HCN in many sorghum pathogens, such as *Gloeocercospora sorghi*, could be explained partially by the production of formamide hydro-lyase (FHL), an enzyme which detoxifies HCN. Previous attempts to evaluate the role of FHL in fungal pathogenicity by creating FHL$^-$ mutants were hampered by the failure to generate such mutants using conventional mutagenesis methods. We decided to create FHL$^-$ mutants in *G. sorghi* by using gene disruption techniques. As a prerequisite, we cloned the gene which encodes FHL. This was done by identifying immunologically positive clones from a cDNA expression library with an FHL antibody. The positive cDNA clone was used to obtain its genomic copy, and verification of the gene was accomplished by obtaining FHL$^+$ transformants in *Aspergillus niaulans*, a fungus that is normally FHL$^-$. The FHL gene was sequenced, and an internal fragment was ligated into a vector which was then used to transform *G. sorghi*. One FHL$^-$ transformant was identified, and Southern analysis indicated that the mutation was achieved by homologous integration. The mutant was highly sensitive to HCN, however, its pathogenicity on sorghum was indistinguishable from that of the wild type. This study indicates that FHL is not required for pathogenicity in *G. sorghi*. In addition, it suggests that cyanogenic compounds serve some functions in plants other than to protect plants from microbial infections.

1335 THE DOUBLE STRANDED RNAS VIRUSES IN COTTON FUNGI FUSARIUM OXISPORUM F.VASINFECTUM. R.S.Mukhamedov*, D.K.Khanazarova, and A.A.Abdukarimov. Institute of Bioorganic Chemistry, 7000143 Tashkent,USSR and *Department of Molecular Biology and Genetics , University of Guelph ,Guelph, Ontario, Canada N1G 2W1.

Viruses and virus-like particles are commonly found in fungi. The presence of isometric virus particles has been detected in 3 of 45 strains of the fungus Fusarium oxisporum f. vasinfectum. Virus particles, have no infectious cycle and there is no evidence of virus-induced lysis. Spores ultrathin sections revealed virus particles (30-32 nm)clustered in the fungi vacuoles. From the mycelium, we isolated virus particles were purified on a sucrose density gradient . Nucleocapsids were precipitated by ultracentrifugation and analysed by electron microscopy after negative staining using 1% freshly prepared uranyl acetate. Icosahedric capsids about 32 nm were evident. We identified RNA polymerase activity associated with virus particles. Double stranded RNA (dsRNA) has interferon activity in mammalian cells. Nucleic acids isolates , separated on 1% agarose gel,contained dsRNAs,measuring(1.6 MD, 2.02 MD), (1.58 MD, 2.18 MD), and (1.45 MD, 1.98 MD) . Ds RNA was resistant to DNAase I , or higher salt (2xSSC) RNAase treatment, but was susceptible to low salt (0.1xSSC) RNAase treatment.

1336 HOST-SPECIFIC ACT-TOXIN FROM ALTERNARIA ALTERNATA AND THEIR BIOSYNTHETIC PATHWAY. S. Nakatsuka, K. Ueda, Y. Itoh* and K. Kohmoto*. Gifu University, Bioorganic Chemistry, Gifu 501-11, and *Tottori University, Plant Pathology, Tottori 680, Japan

Certain pathotypes of the collective species Alternaria alternata produce highly potent host-specific toxins (HST) on spore germination: AK-toxins of the black spot pathogen affecting Japanese pear, AF-toxins of the black spot pathogen attacking strawberry, and ACT-toxins of the brown spot pathogen parasitising mandarins and tangerines. These HSTs have been structurally identified; they contain multiple molecular species. They share in structure the common ω-epoxy-8-hydroxy-9-methyl-decatrienoic acid moiety (1, 2). Substitutions at the 8 position of the HSTs associate with each selective toxicity comparable with the host range for the pathogen. Of these HSTs, ACT-toxin I affects susceptible tangerines and mandarins in orchards, and additionally, Japanese pear cultivars that were experimentally found as a host of the producing pathogen in laboratory tests. Deoxygenation at the 5" position of ACT-toxin I gives a decrease in toxicity to tangerines by a 2,000ths and vice versa an increase in toxicity to Japanese pear by 10 times. When ^{13}C-acetic acid was fed to a growing culture, it was efficiently incorporated into the decatrienoic acid which was detected as a predominant intermediate metabolite in cultures. Meanwhile, 4,6-dimethyl-2-hydroxy-4,6-octadienoic acid and N-(4,6-dimethyl-2,3,4-trihydroxy-6-octenoyl)-valine were isolated from cultures as methyl esters. A possible biosynthetic route for ACT-toxin will be suggested.

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2. Feng, B. et al. Agric. Biol. Chem. 54: 845-848 (1990)

1337 ENHANCEMENT OF THE VIRULENCE OF RHIZOCTONIA SOLANI DURING WHEAT SEEDLING INFECTION BY THE FUNGAL LECTIN. Willy J. Peumans and Jan T.C. Kellens. Catholic University of Leuven, Laboratory for Phytopathology and Plant Protection, Willem de Croylaan 42, 3001 Leuven, Belgium.

Mycelium and sclerotia of Rhizoctonia solani contain an intracellular N-acetylgalactosamine-specific lectin called Rhizoctonia solani agglutinin or RSA. Studies of the developmental control of RSA indicated that it can be considered as a sclerotium-specific protein that accumulates during sclerotium formation. Since the RSA levels drop dramatically during germination of the sclerotia a role as a major fungal storage protein has been proposed.

Recent observations, however, indicate that RSA is somehow involved in the infection process. A first argument in favour of a role in pathogenesis is the fact that the purified lectin restores the virulence of exhausted sclerotia, which are almost avirulent, to the same level as that of fresh sclerotia. The possible involvement of RSA in the process of pathogenesis is further supported by the observation that both plant cell walls and pectin (at low concentrations) induce the accumulation of lectin in the mycelium.

1338 STRUCTURE-FUNCTION OF ELICITINS SIGNAL PROTEINS INVOLVED IN THE PLANT-*PHYTOPHTHORA* INTERACTIONS Jean-Claude Pernollet, Claude Nespoulous and Jean-Claude Huet Laboratoire d'Etude des Proteines INRA 78026 Versailles FRANCE

With the exception of the tobacco pathogen *Phytophthora nicotianæ*, the other fungi *Phytophthora* cause systemic leaf necrosis on their non-host tobacco In culture they secrete proteins, called elicitins which elicit similar necrosis and are responsible for the incompatible reaction These proteins protect tobacco against invasion by the agent of the tobacco black shank *Phytophthora nicotianæ* unable to produce such an elicitor Elicitins form a novel family of 10 kDa holoproteins The large scale purification of parasiticein a 4th elicitin led to the determination of its sequence We compared the necrotic activities and the primary and secondary structures (determined through circular dichroism) of 4 elicitins On tobacco plants, they could be classified in 2 classes α (comprising capsicein and parasiticein, less necrotic) and β (comprising cryptogein and cinnamomin very toxic with a necrosis threshold of 0 1 µg per leaf) We demonstrated that elicitins were able to migrate in the tobacco leaf and to interact directly with the target cells without chemical alterations No difference in migration was observed between cryptogein and capsicein Thus differences in necrotic properties should be due to structural features involved in the interaction of elicitins with the leaf target cells About 75 % identity was observed between the 4 sequences only 2 short terminal regions are heterologous while the central core is mainly conserved The CD spectra showed that the secondary structure of the elicitins was largely conserved All of them exhibited *ca* 50% α-helix and no β structure The comparison of the complete sequences, the aminoacid composition, the calculated isoelectric points the hydropathy index and the secondary structure predictions (optimized with the CD measurements) correlated with the necrotic classification α elicitins corresponded to acidic molecules with a valyl residue at position 13, while β elicitins were basic with a lysine Position 13 appeared to be the best putative active site responsible for necrosis induction involving residues likely bulging at the surface of the molecule

1339 IN VITRO TRANSLATION PRODUCTS FROM COMPATIBLE AND INCOMPATIBLE COMBINATIONS OF BREMIA LACTUCAE AND LETTUCE. Hilde van Peit-Heerschap and Olga Smit-Bakker. Research Institute for Plant Protection, P.O.Box 9060, 6700 GW Wageningen, the Netherlands.

Bremia lactucae Regel, the causal organism of lettuce downy mildew, is a frequent occuring pathogen of field and glasshouse crops. The contact of the fungus with the host plasmalemma or later the formation of the fungal secondary vesicles may probably contribute to differences in compatible and incompatible combinations. Study of the differences in gene activation in both the fungus and the plant for resistant and susceptible interactions may show gene products which determine the specificity. We started to study the gene activation by means of two-dimensional polyacrylamide gel electrophoresis of in vitro translation products in two near isogenic lettuce lines (F8 backcross). One line is susceptible to the physiological race NL12 and resistant to NL15, the other line is susceptible to NL15 and resistant to NL12. At least 5 differences in translation products were found between the mRNAs isolated from the two not inoculated isogenic lines. No differences were found between translation products of mRNAs isolated from water, NL12 and NL15 inoculated leaves of each isogenic line within one day after inoculation. Therefore we conclude that the differences in gene activation may be restricted to the lettuce epidermal cell layers. mRNA will futher be isolated from epidermal cell layers stripped off leaves at different time periods after the inoculation with the fungus.

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1340 MOLECULAR CLONING, INDUCTION AND TAXONOMIC DISTRIBUTION OF CAFFEOYL-COA 3-O-METHYLTRANSFERASE.
Ulrich Matern, Doris Schmitt and Anne-E. Pakusch
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Upon challenge with fungal elicitors, parsley cells reinforce their cell wall with ferulic esters and accumulate furanocoumarin phytoalexins. Reinforcement requires feruloyl-CoA, which is formed from 4-coumaroyl-CoA by the consecutive action of elicitor-responsive hydroxylase and methyltransferase activities. The methyltransferase was purified and cloned using complementary synthetic oligonucleotides. The full size cDNA codes for the 241-amino acids enzyme subunit and contains an extraordinarily long 5'-untranslated region of 370 bp, which suggests a complex mode of translational regulation. Non-induced control cultures contained considerable methyltransferase activity, which nevertheless increased upon elicitation of cells to one transient maximum between 10 and 15 h. Changes in activity correlated to the amounts in specific enzyme protein and were preceded by the transient induction of specific steady-state mRNA levels. The course of induction suggested an operational coordination of the methyltransferase with the general phenylpropanoid pathway, indicating a new meaning, aside from phytoalexin synthesis, for the concomitant induction of 4-coumarate:CoA ligase. Enzyme assays and Northern hybridizations revealed the wide taxonomic distribution of such methyltransferases in plant cells and the common, rapid increase of specific RNA levels in response to elicitation. The results suggest that high methyltransferase activity is generally necessary for the expression of plant disease resistance.

1341 **The effect of the photoactivated fungal toxin cercosporin on the expression of maize antioxidant defense genes.** John D Williamson and John G. Scandalios
North Carolina State University, Department of Genetics, Raleigh, NC 27695

Fungi of the genus *Cercospora* produce a photoactivated polyketide toxin called cercosporin In the presence of light an excited triplet form of this molecule reacts directly with molecular oxygen to produce (depending on the redox potential of the environment) singlet oxygen and/or superoxide radicals. Utilizing both crude and purified cercosporin we have developed a "bench-top" system for the analysis of the response of the maize antioxidant defense genes catalase and superoxide dismutase to these activated oxygen species. Under the assay conditions used, total catalase activity, protein and RNA steady-state levels changed in parallel in response to applied toxin. Responses were not identical for all catalase isozymes, but appeared to vary with tissue and developmental stage. In contrast, neither total superoxide dismutase activity nor individual isozyme protein levels changed in response to toxin treatment However, steady-state RNA levels for several of the superoxide dismutases changed dramatically For example, our analyses show that there are two distinct classes of *Sod3* message, and that each shows a different response to applied toxin. As with the other SODs, corresponding steady-state levels for both SOD-3 proteins remained constant. This suggests that protein turnover might be an important aspect of the SOD's response to activated oxygen species

1342 THE CLONED AVIRULENCE GENE AVR9 OF THE FUNGAL TOMATO PATHOGEN CLADOSPORIUM IS RESPONSIBLE FOR CULTIVAR SPECIFIC RESISTANCE. Guido F.J.M. van den Ackerveken, Jan A.L. van Kan and Pierre J.G.M. de Wit, Agricultural University, Department of Phytopathology, Wageningen, The Netherlands.

Different races of Cladosporium fulvum have arisen as a result of adaptation of the fungus to resistance genes present in cultivated tomato. These new races have overcome their initial avirulence by evading recognition by the plant. In this way several fungal races have overcome resistance gene Cf9 of tomato by a deletion of the avirulence gene avr9 encoding the corresponding race-specific elicitor. Genomic clones carrying the avr9 gene were isolated from a genomic library (λEMBL3) of race 5 of C. fulvum using a cDNA-probe encoding the race-specific elicitor. Sequence analysis revealed a 59 bp intron, a possible TATA-box and several repeats in the promoter- and terminator-region. Stable transformants were obtained after co-transformation of the avr9 gene with pAN7-1 (Hygromycine resistance) to race 2.4.5.9.11, which is virulent on tomato cultivar Cf9. Cultivar specificity of these transformants was converted phenotypically from virulent to avirulent on tomato cultivar Cf9, indicating that the introduced avr9 gene has changed the genotype of race 2.4.5.9.11 into race 2.4.5.11. This is the first report on the cloning of a fungal avirulence gene of which the processed gene product (28 aa.) is the primary inducer of the hypersensitive response (HR in a resistant cultivar. Many avirulence genes of plantpathogenic bacteria have been cloned until now, but for none of them the gene product itself induces HR.

1343 MOLECULAR ANALYSIS OF PATHOGENESIS AND POPULATION EVOLUTION OF BARLEY POWDERY MILDEW IN AUSTRALIA
Dara L. Whisson and Mitra Mazarei. S.A. Department of Agriculture, Waite Agricultural Research Institute, Glen Osmond, S.A. 5064 Australia.

One of the best genetically characterized pathogen/plant interactions is that of powdery mildew, Erysiphe graminis f.sp. hordei with it's host, barley. This obligate biotrophic fungus was probably introduced from Europe into Australia where it has proliferated in virtual isolation and with very little selection pressure from resistant cultivars. In spite of this lack of selection pressure, the pathogen has retained its virulence to resistance genes never used in Australia. In order to obtain a better understanding of the evolution of the fungal population, an RFLP analysis of isolates collected from various parts of the country is being carried out with probes from the U.K. and Denmark. From results so far, all the mildew isolates contain a DNA plasmid and 80% contain different mixtures of double-stranded RNAs. Several contain the same sized dsRNAs as those detected in an isolate collected in Sweden in 1964. As these dsRNAs constitute a large proportion of the total nucleic acid content of the powdery mildew spore, experiments are in progress to resolve the molecular structure and possible function of these elements.

1344 MAPPING STRATEGY FOR RESISTANCE GENES BASED ON RFLPs BETWEEN TOMATO CULTIVARS: CF9 ON CHROMOSOME 1.
Ruud Verkerk[1], Hans van der Beek[2], Pim Zabel[1] and Pim Lindhout[2].
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Recently, the extent and distribution of genetic variation within the genus *Lycopersicon* has been determined on the basis of Restriction Fragment Length Polymorphisms (RFLP). Unlike the extensive variation between wild species, limited variation was found among modern *L esculentum* cultivars, most likely as a result of extensive breeding. Over the past four to five decades, the genetic diversity of tomato cultivars has been broadened by interspecific crosses. Introgression of chromosomal segments from various *L.spp* into *L esculentum* has been realized, mainly for the induction of resistances to pests, diseases and to environmental stress conditions. As 'old' cultivars are devoid of any introgressed resistance genes, in the present study the assumption was made that polymorphisms between 'old' and modern tomato cultivars are mainly due to the introgressed chromosomal segments of wild species, present in the modern cultivars. RFLPs between 'Moneymaker' (old cultivar without disease resistance genes) and 'Sonatine' (cultivar with six disease resistance genes) were analyzed for association with these resistance genes, using near isogenic lines and commercial cultivars, varying for the resistance genes. When such association was found, linkage was confirmed by testing a segregating population of the resistance gene. It is demonstrated that an intelligent choice of tomato genotypes allows resistance genes to be mapped effectively. As an example we present the mapping of the resistance gene Cf9, which confers resistance against *Cladosporium fulvum*, on the short arm of chromosome 1

1345 RELEASE OF ELICITOR-ACTIVE COMPOUNDS BY GERMINATING ZOOSPORES OF *PHYTOPHTHORA MEGASPERMA* F. SP. *GLYCINEA*. T. Waldmüller, E. G. Cosio, H. Grisebach and J. Ebel.
Biologisches Institut II der Universitat, D-7800 Freiburg, FRG.

An important first step in the initiation of plant defense responses is the perception of a pathogen-derived signal. We show that growing germtubes of encysted zoospores of *Phytophthora megasperma* f. sp. *glycinea* release compounds which induce phytoalexin synthesis in soybean (*Glycine max* L.). Fungal cultivation fluids were purified by gel permeation, ion exchange and Concanavalin-A-affinity chromatography. Fractions with elicitor activity in a cotyledon bioassay also showed the ability to displace a labelled hepta-ß-glucoside elicitor from ß-glucan-binding sites which are associated with the soybean plasma membrane. Structural analysis by GC/MS demonstrated that the purified fungal cultivation fluids contain 1,3-1,6-glucans. Furthermore the elicitor response of unwounded root tips of soybean seedlings to fractions from different purification steps was investigated. Short-time kinetics of elicitor release by growing germtubes support the assumption that these glucans are present at very early stages of the infection process. (Supported by the Deutsche Forschungsgemeinschaft)

POSTER ABSTRACTS

1346 ENNIATIN SYNTHETASE FROM FUSARIUM SCIRPI. AN ENZYME MOSTPROBABLY INVOLVED IN PHYTOPATHOGENIC EVENTS.

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Enniatins are a group of cyclic hexadepsipeptides produced by many Fusaria and belong to a variety of low molecular weight phytotoxins from these fungi. The biosynthesis of the enniatins is well understood. A multifunctional enzyme (enniatin synthetase=ESyn), consisting of a single 250 kDa polypeptide chain, synthesizes the enniatins from their primary precursors, i.e. 2-D-hydroxyisovaleric acid, a branched chain L-amino acid (e.g. valine, isoleucine or leucine) and S-adenosyl-L-methionine under consumption of ATP in a socalled "thiotemplate mechanism". We have recently demonstrated, that in submerged cultures of F. scirpi ESyn was present in the mycelium in all phases of the fermentation and even in spores of the organism. Similarly, ESyn-mRNA was present at every stage of the fermentation, leading to the assumption, that ESyn is a constitutive enzyme, the expression of which is not regulated by any specific mechanism. The present poster describes the molecular cloning of the ESyn gene, which has a size of about 10 kb and shares high homology with tyrocidin synthetase from Bacillus brevis. Furthermore data on the expression of the ESyn gene in plant deseases caused by Fusaria will be presented.

1347 HMG-CoA REDUCTASE OF POTATO IS ENCODED BY A MULTIGENE FAMILY

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3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) catalyzes the first committed step in the pathway leading to isoprenoids, e.g. growth regulators, photosynthetic pigments, membrane components, and phytoalexins in solanaceous species. A partial length cDNA obtained through PCR was used as a probe to screen a genomic library of potato cv. Lemhi constructed in a · FIX II vector. From the screening of 5×10^5 pfu 18 positives were isolated and grouped into 7 classes based on their restriction maps. Sequencing around the translation initiation codon establishes the identity of these clones as HMG-CoA reductase. A long untranslated leader sequence (ca 500 bp) was indicated by primer extension analysis. All the clones are nearly identical up to 131 bp upstream from the ATG codon. Thereafter, clone HMGR-22 diverges. Other clones diverge from each other at 340 bp upstream of the ATG codon. The translation initiation codon for one clone, HMGR-7 is GTG. Experiments are currently underway to understand the expression of these genes.

1348 DEFENSE RESPONSES AND THE ROLE OF MEDICARPIN IN RESISTANCE IN ALFALFA TO COLLETOTRICHUM TRIFOLII.

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The role of phytoalexins in resistance to anthracnose in alfalfa was examined by determining the effectiveness of medicarpin in inhibiting growth stages of isolates of races 1 and 2 of Colletotrichum trifolii, and by quantifying the accumulation of medicarpin and phenylalanine ammonia lyase in seedling and cotyledon tissues during compatible and incompatible interactions. Medicarpin was the major phytoalexin among 7 fungitoxic compounds extracted and was inhibitory to spore germination and growth of pre-germinated spores but not to hyphal growth of race 1 and 2 isolates. The activity of defense enzymes PAL and chalcone synthase related to the accumulation of alfalfa phytoalexins in cultivars with specific resistance genes. Hybridizable PAL and CHS mRNA accumulated in total RNA fractions from alfalfa coytledons during race-cultivar specific interactions. A cDNA expression library was constructed from mRNA extracted from resistant and susceptible alfalfa tissues and was used to identify defense related genes.

1349 PHOTOAFFINITY LABELING OF A SOYBEAN MEMBRANE PROTEIN THAT SPECIFICALLY BINDS A HEPTAGLUCOSIDE PHYTOALEXIN ELICITOR.

Eric G. Cosio, Thomas Frey and Jurgen Ebel
Biologisches Institut II der Universität, D-7800 Freiburg, FRG.

A 2-(4-azido-3-^{125}I-phenyl)ethylamino conjugate of a hepta-ß-glucoside phytoalexin elicitor was prepared by coupling 2-(4-aminophenyl)ethylamine to the reducing end of the glucoside followed by radioiodination of the aminophenyl moiety and conversion of the arylamine into an arylazide. The photolabile derivative displayed the same binding characteristics to the glucan-elicitor binding sites of soybean membranes as the underivatized glucoside. Photoaffinity labeling and SDS-PAGE of fractions from a partial purification sequence of the glucan-binding proteins revealed specific labeling of, primarily, a 70 kD band. The labeled protein accounted for over 90% of the specifically incorporated radioactivity in all fractions starting from detergent-solubilized samples onwards. Analysis of the affinity of label incorporation by displacement and ligand saturation experiments gave apparent K_d and B_{max} values of 3.5 nM and 1.5 pmol/mg protein, respectively. These are in full agreement with data obtained from radioligand binding studies. Improved signal to noise ratios and higher intensity of the 70 kD band was observed in all procedures leading to partial enrichment of the glucan-binding activity. Analysis of glucan-elicitor eluted fractions from an affinity chromatography matrix also showed specific labeling of the 70 kD protein along with two minor bands with apparent M_r values of 100 and 170 kD. Silver-stained SDS gels of the affinity-purified fraction also showed a main band with an apparent M_r of 70 kD. This protein appears to be the main form of the heptaglucoside-binding sites in detergent-solubilized soybean membranes. (Supported by the Deutsche Forschungsgemeinschaft)

1350 STRUCTURE AND EXPRESSION OF THREE GENES FROM THE CHITINASE MULTIGENE FAMILY IN POTATO.

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Probes specific for the 3' untranslated regions of three different potato (*Solanum tuberosum*) chitinase genes were used to investigate patterns of expression in response to ethylene. Steady-state message levels for gene CCH4 are higher in leaves than in stems. Treatment with ethylene induces mRNA levels approximately seven-fold in leaves and 3.5-fold in stems. Transcripts for a second gene, CCH12, are more abundant in stems than in leaves. Gene CCH12 is induced approximately five-fold in stems by ethylene and to a lesser degree in leaves. Transcript levels for both genes are extremely low and uninducible in root tissue. A third gene, CCH19, is currently under investigation. Northern hybridization analysis of CCH19 suggests that it is much less abundant than either CCH4 or CCH12.

1351 STNDIES ON THE PRODUCTS OF RICE BLAST RESISTANCE GENES USING RICE NEAR-ISOGENIC PAIRS.

Danian Huang, Wei Yang, Jinxia Wang, Zaifen Fan and Qiu Zou (China National Rice Research Institute; Hangzhou 310006, China)
Zuhua He and Zongtan Shen (Zhejiang Agricultural University, Hangzhou 310029, China)

(1). Construction of near-isogenic pairs resistant/susceptible to rice blast disease
The cultivar Tetep or At-Jiao-bai-mi-zi was chosen as resistant (R) donor to cross with the susceptible (S) variety Zhen-Shan 97B. Then, the acquired F1 progeny was backcrossed for 3 generations to B3F1 using Zhen-Shan 97B as a recurrent parent, followed by a series of Selfing (up to 10 generations) to B3F10.
(2). Water soluble proteins were extracted from leaves of three-leaf stage seedlings without the inoculation of pathogen. The results of 2D-electrophoresis indicated that: a) in a certain near-isogenic pair, there were several extra protein spots in R Line whereas not in S line. b) The generation of rice blast disease may have a close association with the proteins in the area of pI7.5—8.5 and molecular weight of 30 KD to 45 KD. A further study also showed that the protein variance occured mainly in this area when the host plants were inoculated with blast pathogen.
(3). An absorption phenomenon was occured between the conidia of rice blast pathogen and water soluble proteins for some near-isogenic pairs.
The conidia from rice blast pathogen were chosen to mix with a certain amount of water soluble protein solution. The conidia were removed by centrifugation. The supernatant were taken to run on the isoelectric focusing gels(IEF). It can be observed that some kinds of proteins were absorbed by the conidia. This phenomenon means that there is perhaps a direct interaction between some proteins and the conidia.

POSTER ABSTRACTS

1352 FUNGAL INFECTION INDUCES THE EXPRESSION OF A PATHOGENESIS-RELATED PROTEIN IN GERMINATING MAIZE SEEDS. J.M.Casacuberta, P.Puigdoménech and B. San Segundo.
Dept. Genética Molecular, Centro de Investigación y Desarrollo (CSIC). Jordi Girona 18, 08034 Barcelona, SPAIN.
Pathogenesis-related proteins (PRs) are plant proteins produced in leaves in response to infection by pathogens including viruses, viroids, fungi and bacteria. Most of the work on PR proteins has been done on dicotyledoneous plants whereas information on monocotyledoneous species is limited. In addition, very little information is available on seeds producing PR proteins. We have recently cloned a gene coding for a basic PR-protein, named PRms, which is specifically expressed during germination of maize seeds. This PR-protein is encoded by a single copy gene in maize and its expression is induced upon infection with a natural pathogen of maize, the fungus Fusarium moniliforme. Furthermore, treatment with fungal elicitors, or specific chemicals and hormones (gibberellic acid) also induces PRms gene expression. The PRms gene has been found to be expressed in the aleurone layer and embryo of germinating maize seeds. Within the embryo, its expression is confined to the scutelium and, to the scutellar epithelial cells. Despite the fact that the function of the PRms protein, and its role as a part of the defense response against fungi in cereal seeds, remains unknown, our results indicate a correlation of PRms mRNA accumulation with tissues specialized in the production and secretion of hydrolytic enzymes. Finally, the PRms is a attractive model for studies of the molecular mechanisms involved in the regulation of gene expression and disease resistance in plants.

1353 ENHANCMENT OF A PREFORMED ANTIFUNGAL COMPOUND BY CO_2 TREATMENTS IN AVOCADO FRUIT. D. Prusky, R. Plumbley, I. Kobiler and Y. Fishman. Department of Fruit and Vegetable Storage, Volcani Center, Bet Dagan, Israel.
The preformed antifungal compound, 1-acetoxy-2-hydroxy-heneicosa-12,15-diene involved in the resistance of avocado fruits to postharvest disease could be induced by a 24 h treatment of 30% CO_2 at 20°C. The treatment enhanced the activity of phenylalanine ammonia lyase and the level of epicatechin in ripening fruits. The enhanced level of epicatechin inhibits fruit lipoxygenase and enzyme involved in the diene breakdown. We suggest that the mechanism of induction of resistance by the abiotic elicitor is by inhibition of the antifungal diene breakdown.

1354 OLIGOGALACTURONIDES INDUCE H_2O_2 PRODUCTION IN CUCUMBER HYPOCOTYL SEGMENTS. Øystein Svalheim and Børre Robertsen, Department of Plant Physiology and Microbiology, University of Tromsø, Norway
Lignification is an important defence mechanism in many plants. The last step in the lignification process is the peroxidase catalyzed polymerization of cinnamoyl alcohols into lignin. This reaction consumes hydrogen peroxide. We have studied the production of H_2O_2 in cucumber hypocotyl segments in responce to α-1,4 linked oligomers of galacturonic acid. Oligomers with 8 to 14 units of galacturonic acid induce the deposition of lignin in cells and cell walls of cucumber hypocotyl segments with oligomers of 10 to 11 units being the most effective elicitors. Here we present results showing that the same oligomers also induce H_2O_2 production. Addition of catalase to the incubation solution abolished the accumulation of H_2O_2. The increase in H_2O_2 concentration could be detected after 4 hours and reached a maximum of approximately 27 nmol ml^{-1} incubation solution after 10 hours. After 22 hours the H_2O_2 concentration had decreased to the levels seen in intact, untreated plants. Using actinomycin D and cycloheximide to block transcription and translation, respectively, we showed that at least part of the H_2O_2 induction is dependent upon de novo protein synthesis

1355 GENERATION OF A BARLEY cDNA LIBRARY ENRICHED FOR SEQUENCES SPECIFIC FOR RESISTANCE TO ERYSIPHE GRAMINIS. Jennifer Walsh Weller and Shauna Somerville. Michigan State University, MSU-DOE Plant Research Laboratories, E. Lansing MI 48824, USA. The strategy of subtractive cDNA hybridization is being utilized to enrich for barley cDNAs corresponding to the Ml-a gene, which encodes race-specific resistance to the fungal pathogen Erysiphe graminis f.sp. nordei. Two near-isogenic barley lines, which differ phenotypically only in their susceptibility to powdery mildew, were used as the initial mRNA donors. Coleoptile tissue was used as the source of the mRNA to increase the proportion of cells affected by the pathogen (epidermal) and to decrease the background of transcripts from irrelevant sources, such as the photosynthetic apparatus. Our assumption is that the Ml-a resistance gene is constitutively expressed and that its mRNA differs substantively, either in sequence or abundance, between the two isolines. Modification of published protocols for cDNA synthesis and amplification have been necessary to allow us to obtain sufficient material. Some of these modifications include the stringent removal of contaminating carbohydrate and care in reducing secondary structure of the mRNA prior to first-strand cDNA synthesis. It is more efficient to tail the cDNA with oligo(dC) and use oligo (dG) as a PCR amplification primer than to ligate cloning adaptors to the cDNA. During the amplification process great care must be taken to avoid amplification of nucleic acids contaminating Taq polymerase and the cDNA reaction enzymes. An initial assessment of the subtractive library produced will be presented.

1356 A NOVEL GENE STRUCTURE ENCODES A LECTIN FROM STINGING NETTLE (*Urtica dioica*) David R. Lerner and Natasha V. Raikhel. MSU-DOE Plant Research Laboratory, East Lansing, Michigan 48824-1312
Plants respond to pathogenic infection and herbivory with a variety of constitutive and inducible defenses. Chitin binding proteins, found in both monocot and dicot plants, are thought to contribute to these defense mechanisms through interactions with chitin in the cell walls of fungi or in the midgut of insect larvae. These proteins are related by the presence of a conserved cysteine and glycine rich, 43 amino acid, chitin binding domain. This domain has been duplicated in the Gramineae lectins, which have four repeats. The chitin binding domain has also been fused with unrelated domains in the genes for the basic chitinases, the hevein gene isolated from rubber tree latex, and the potato wound inducible genes Win1 and Win2. *Urtica dioica* agglutinin (UDA) is a small chitin binding lectin found in the rhizomes of stinging nettle. This lectin has been shown by *in vitro* experiments to have both antifungal and insecticidal activities. We have produced a synthetic gene for UDA as a probe to isolate and clone the gene for UDA. The clone contains an open reading frame encoding a polypeptide of 373 amino acids, approximately four times the length needed to encode UDA. This deduced amino acid sequence includes a putative signal peptide (23 aa), the two chitin binding domains (43 aa each), followed by unexpected regions encoding a spacer' domain (19 aa) and a carboxyl domain (244 aa) with 45% identity to the basic and acidic (class I and II) chitinases. Thus, UDA represents a new class of chitin binding proteins with both a duplication of the chitin binding domain and a fusion of these domains to the unrelated chitinase-like domain. Overexpression of the entire UDA gene, the chitin binding encoding domain or the chitinase-like carboxyl domain in *E. coli* and transgenic plants will allow us to address questions of the activities of these separate domains and their possible functions *in vivo*

1357 EVIDENCE FOR THE LOCATION OF VICTORIN BINDING PROTEINS IN OAT PLASMA MEMBRANES. D. Loschke, H. Chen, L. Tomaska, Z. Hong, D. Gabriel* and B. Rolfe, Plant-Microbe Interaction Group, Australian National University and *Dept. of Plant Pathology, University of Florida.
Oats containing the dominant *Vb* allele are susceptible to the fungal toxin victorin. The *Vb* allele is either the same as or very closely linked to the *Pc-2* gene, a dominant rust resistant gene. The identification of a specific binding protein for victorin in oat leaf slices has been reported by Wolpert and Macko (*PNAS* vol. 86: pp.4092-4096, 1989). By using radioactively labeled purified victorin, we have found several victorin binding proteins in oat protoplasts and leaf tissues. Further studies have suggested that the proteins are located in the plasma membrane of oats as determined by the binding to proteins associated with plasma membranes purified by aqueous two-phase partitioning. Cellular membranes depleted of plasma membranes exhibited only slight binding perhaps due to the presence of endoplasmic reticulum and Golgi complex membranes containing proteins in transit to the outer plasma membrane.

POSTER ABSTRACTS

1358 ISOLATION AND CHARACTERIZATION OF A TOBACCO GENE ACTIVATED DURING THE HYPERSENSITIVE RESPONSE TO THE PATHOGENIC BACTERIA : PSEUDOMONAS SOLANACEARUM.

Laurence Godiard, Didier Froissard, Dominique Roby and Yves Marco. Laboratoire de Biologie Moleculaire, INRA/CNRS UMR 05, Auzeville, 31326 Castanet-Tolosan, France.

In response to infection by an incompatible pathogen, many plant species have been shown to develop an hypersensitive reaction (HR). A gene whose corresponding mRNAs accumulate to a much higher level in incompatible than in compatible interactions of tobacco with a pathogenic bacteria : *Pseudomonas solanacearum*, has been characterized. Southern blot analysis indicates that this gene is a member of a small multigene family ; at least two members are transcriptionally activated during the incompatible interaction : two different cDNAs clones have been indeed isolated.

Although this gene can be considered as a part of the plant defense potential, no detectable transcript accumulation was observed in response to treatments by various elicitors. The nucleotide sequence of a genomic clone (HSR 203) has been determined : an open reading frame of 1005 bp, composed of a single exon, has been found. Using GUS fusion gene constructs, tested in a tobacco transient gene expression system, it has been found that its activation by the incompatible bacteria is contained within 1.4 kb of the 5' flanking region.

1359 ROOT BORDER CELL SPECIFIC GENE EXPRESSION· IMPLICATIONS FOR PATHOGENESIS

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The root tips of many plant species systematically release masses of living cells into the soil. The long term objective of our laboratory is to determine the function of these root "border" (BRD) cells, particularly with respect to their possible role(s) in regulating populations of microorganisms associated with the root. BRD cells exhibit specificity in their responses to bacterial and fungal pathogens, and certain traits are specifically expressed in BRD cells only after they separate from the root cap. We propose to test the hypothesis that BRD cell specific traits occur as a result of the expression of BRD cell specific genes, and that BRD cell specific gene expression influences susceptibility of plant roots to soilborne fungal pathogens. Preliminary experiments demonstrated that upon differentiation into BRD cells, protein profiles from Pisum sativum BRD cells are distinct from those of cells prior to separation from the root.

1360 ENGINEERING RESISTANCE TO ROOT-KNOT NEMATODES (*Meloidogyne* spp).

Michael G.K. Jones, Plant Sciences, School of Biological and Environmental Sciences, Murdoch University, Perth, Western Australia 6150.

Root-knot nematodes (*Meloidogyne* spp) are serious pests of a wide range of agricultural and horticultural crops particularly in sub-tropical and tropical regions of the world. Root-knot nematodes are endemic to the sandy soils and vegetation of Western Australia and can limit production of a range of important crop plants.

As part of the Western Australian State Centre for Agricultural Biotechnology, a programme has been established to engineer resistance to these nematodes. The aim of the work is to disrupt feeding cell ("giant cell") formation in host plants, in so doing to render these plants resistant to the nematodes.

Juveniles invade roots of host plants and induce the development of about 6 discrete giant cells. These form by repeated mitosis without cytokinesis, fill with cytoplasm and have enlarged nuclei. They develop wall ingrowths typical of transfer cells, specialised for taking up nutrients from the vascular system of the plant. The giant cells provide a nutrient source for the developing nematodes, which enlarge and produce eggs to repeat the cycle. The nematodes are completely dependant on the giant cells for survival, and will die if the giant cells are disrupted. This system will be described, including culture on *Arabidopsis*, together with current status of molecular approaches to the problem.

1361 EVIDENCE FOR RESISTANCE TO *PHYTOPHTHORA PARASITICA* IN TRANSGENIC TOBACCO EXPRESSING CONSTITUTIVELY A NEWLY-DISCOVERED PATHOGENESIS-RELATED cDNA.

Danny Alexander*, Christopher Glascock*, Jeffrey Stinson*, Patricia Ahl-Goy‡, Robert Goodman*†, and John Ryals#. *Calgene, Inc., 1920 Fifth St, Davis, CA 95616. †Univ of Wisconsin, 1630 Linden Dr, Madison, WI 53706. ‡CIBA-GEIGY Ltd., Basle, Switzerland. #CIBA-Geigy Biotechnology, Research Triangle Park, NC 27709.

A new pathogenesis-related (PR) gene family was discovered by differential screening of a cDNA library from tobacco mosaic virus (TMV)-induced Xanthi nc tobacco. mRNAs for at least one of these genes were induced to high levels in leaves following a hypersensitive response to TMV infection or by treatment with salicylic acid. Transgenic plants were engineered to express constitutively a full-length cDNA of one of the family members, using the CaMV double 35S promoter. One line of transgenic plants homozygous for the engineered gene was selected that expressed the gene at a level similar to or higher than that seen in non-engineered plants induced by TMV inoculation. Plants expressing the cDNA in a "sense" orientation with respect to the promoter were highly resistant to infection by *Phytophthora parasitica* in two independent tests. Resistant plants showed much delayed and attenuated symptom development, with slight wilting after 11 days post-inoculation. Non-transgenic plants, and transgenic plants lacking the PR cDNA insert or with the cDNA present in the "anti-sense" orientation showed identical symptoms of severe wilting and death within 8-10 days. Experiments are under way to test the role of this gene in disease resistance using plants from independent transformation events.

1362 A PEROXIDASE PROMOTER RESPONDS TO WOUNDING AND FUNGAL ATTACK IN TRANSGENIC TOBACCO

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Suberin is a biopolymer that is deposited on the cell walls of plants in response to wounding and fungal attack. The gene encoding a highly anionic peroxidase that is involved in the polymerization of the phenolic moieties of suberin was isolated from tomato and sequenced. In order to study the spatial and temporal activation of the suberin-specific tomato anionic peroxidase (TAP-1) gene by wounding and fungal attack, the DNA sequences representing the TAP-1 gene promoter and coding region for the putative leader sequence were fused to the β-glucuronidase (β-GUS) marker gene and introduced into tobacco. Wounding of the transgenic tobacco leaves caused β-GUS gene expression in a manner that correlates with the time course of suberin deposition in wound-healing tomato tissues. When transgenic tobacco leaf tissues were inoculated with *Phytophthora parasitica*, transcriptional activation of the chimeric gene was observed with 10^3- 10^4 zoospores/ml, a concentration range at which resistance to the fungus has been observed in this host-pathogen system. β-GUS gene activation was restricted to the site of wounding or pathogen attack. These observations support the hypothesis that the expression of the suberin-specific peroxidase gene plays an important role in plant defense. Studies are under way to identify the wound and pathogen-responsive DNA elements of the anionic peroxidase gene in an effort to understand the regulation of this defense related gene.

1363 EXPRESSION OF A RIBOSOME INHIBITING PROTEIN (RIP) LEADS TO FUNGAL RESISTANCE IN TRANSGENIC TOBACCO PLANTS.

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A full length cDNA clone encoding a ribosome inhibiting protein (RIP) was isolated from barley seeds. In vitro studies with purified RIP protein demonstrated that low concentrations of RIP protein are cytotoxically active against fungal cells. The RIP-cDNA clone was transcriptionally fused with the wound- and pathogen-inducible promoter wun1 and transformed into tobacco. Transgenic plants expressing RIP-mRNA and RIP-protein were infected with the plant pathogenic fungus Rhizocotonia solani. Whereas the growth of control plants was drastically reduced because of root and stem disease, RIP-transgenic plants showed less infection symptoms and were growing nearly as fast as uninfected tobacco plants. The fungal resistance of wun1-RIP-transgenic tobacco plants is stably inherited.

POSTER ABSTRACTS

1364 TRANSGENIC PLANTS WITH ENHANCED RESISTANCE TO *RHIZOCTONIA SOLANI*. Richard Broglie, Karen Broglie ,Ilan Chet[1], and Nicole Benhamou* E.I. Du Pont de Nemours & Co., Agricultural Products Department, Wilmington, DE. 19880. * Universite Laval, Departement de Phytologie, St Foy, Quebec, Canada.

Chitin is an important component of the cell wall of many pathogenic fungi. One method by which plants protect themselves against potentially pathogenic organisms is by the production of lytic enzymes, such as chitinase, which enable them to partially hydrolyze the cell walls of invading pathogens. In healthy, uninfected plants, chitinase levels are low or undetectable. However, treatment with ethylene, oligosaccharide elicitors, or infection results in an increase in chitinase mRNA levels and an increase in enzyme activity. Transgenic tobacco plants containing a chimeric gene composed of a bean chitinase promoter fused to the coding region of the reporter gene β-glucuronidase (GUS) have been used to study chitinase gene expression in response to fungal infection. Our results indicate that gene activation is greatest in tissues immediately surrounding the site of fungal infection and coincides with the induction of endogenous defense genes. We have also produced transgenic tobacco and canola plants which express a bean chitinase gene modified so that the native inducible promoter is replaced by a high level, constitutive promoter. These transgenic plants exhibit increased chitinase enzyme activity and increased resistance to the pathogenic fungus *Rhizoctonia solani*. The resistant phenotype is characterized by a delay in the development of disease symptoms and an increased ability to survive in infested soil. Ultrastructural analysis of infected transgenic plants show that invading hyphae of *R. solani* suffer from poorly developed cell walls leading to cell disruption and protoplasm leakage.
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1365 DIFFERENTIAL EXPRESSION OF DEFENCE GENES IN BEAN NILs DIFFERING BY A PARTICULAR GENE FOR ANTHRACNOSE RESISTANCE. Aline Mahé, Priscilla Bettini, Jeanine Grisvard and Michel Dron. Dept. Biol. Mol. Vég., URA CNRS 1128, Bat. 430, Univ. Paris Sud, 91405 Orsay (France).

In response to infection by pathogens, plant tissues have been shown to induce both locally at the site of infection, and systemically, a complex biochemical response, generally described as the plant defense response. Several studies on different plant-pathogen interactions have demonstrated that this response involves phytoalexin accumulation, induction of cell wall depolymerases, accumulation of products involved in cell wall thickening, etc. These responses are induced whatever the pathogen, and both in incompatible and compatible interactions; nevertheless, the timing differs, the reaction being earlier in an incompatible interaction than in a compatible one. The aim of the present work is to study the kinetics of the defense response in relation to the genetic background and to the particular resistance gene which is present. Defence gene markers known as induced after Colletotrichum lindemuthianum infection on bean (Phenylalanine Ammonia Lyase, Chalcone Isomerase, Chitinase, Hydroxyproline Rich Glycoproteins), as well as genes involved in the production and detoxyfication of free radicals (Superoxyde dismutase, Peroxidase, Lipoxygenase), have been employed, and their expression compared in different genetic contexts.
Authors are grateful to Hubert Bannerot (INRA-CNRA Versailles, France) for bean NILs.

1366 CHARACTERIZATION OF A BEAN ANTHOCYANIN MARKER GENETICALLY LINKED TO A SPECIFIC ANTHRACNOSE RESISTANCE GENE. Pascal Gantet, Priscilla Bettini, Hubert Bannerot (1), Judy Brangeon (2), Jeanine Grisvard and Michel Dron. Dept. Biol. Mol. Vég. and (2) Struct. Metab. Plant. URA CNRS 1128, Bat. 430, Univ. Paris Sud, 91405 Orsay (France); (1) St. Am. Plantes, INRA-CNRA, Rte de St. Cyr, 78026 Versailles (France).

Anthracnose is a major bean disease caused by a fungal pathogen, Colletotrichum lindemuthianum. This plant-pathogen interaction seems to obey the classical Flor's gene for gene rule, according to which a specific recognition involves a dominant avirulence gene in the pathogen and a dominant resistance gene in the host plant. During breeding, a genetic linkage has been observed between a particular resistance gene, Mex2, and the accumulation of anthocyanins in the pods. A more detailed genetic analysis has shown that the accumulation of anthocyanins in the pod is under the control of a dominant gene (Anp). The phenotypic expression of Anp is itself controlled by another set of dominant genes. Anp presents linkage to the Mex2 resistance gene; the genetic distance between the two genes has been estimated to be 2.3 ± 1.3 cM by F2 segregation analysis of a cross between the bean cv. AFN (Anp, Mex2) and the cv. La Victoire (anp, mex2). TLC and HPLC analysis have shown that malvidin glycosides are accumulated under light and developmentally controlled in the pods of AFN, but not in La Victoire. Both transcriptional and immunocytochemical analysis of several genes coding for enzymes of the phenylpropanoid and flavonoid biosynthetic pathway suggest that the mutation in La Victoire involves one of the latest steps of the flavonoid biosynthesis.
Authors are grateful to Dr. J.P. Roggero (Avignon, France) and Dr. Bourzeix (Narbonne, France) for help in anthocyanin and proanthocyanin analysis. P.G. is an ORSAN-CIFRE pre-graduate fellow; P.B. is a CEE-BAP grantee.

1367 PURIFICATION OF A PR 1-TYPE PROTEIN AND A CHITINASE FROM MILDEW-INFECTED BARLEY. Tomas Bryngelsson. The Swedish Agricult. Univ., Dept. of Plant Breeding, S-268 00 Svalöv, Sweden.

The hypersensitive reaction of barley inoculated with powdery mildew (Erysiphe graminis f. sp.hordei) is accompanied by the de novo synthesis of so-called pathogenesis-related (PR) proteins. We have previously isolated an acidic, thaumatin-like PR protein from barley and we now report the purification and characterization of two basic PR proteins. The Hv-4 protein consists of a single polypeptide (pI 10.5) with an Mr of 16 kD. The protein was cleaved with endoproteinase Lys-C and three individual fragments were sequenced. Alignment of the amino acids demonstrated high homology (60-75%) to the PR 1-type proteins in tobacco and tomato. The Hv-5 protein is a basic protein (pI > 10.5) with an apparent molecular weight of 26 kD. The protein was blocked at the N-terminal but it was possible to obtain sequence information after cleavage. Sequence comparisons revealed homology to several plant chitinases. Hv-5 also exhibits chitinase activity.

1368 ROLE OF CHITINASES IN BARLEY-POWDERY MILDEW INTERACTIONS. Seiji Ouchi, Hideyoshi Toyoda, Yoshinori Matsuda, Takako Yamaga, Masahiro Morita, Seishi Ikeda and Takayuki Tamai.
Faculty of Agriculture, Kinki Univ., Naka-machi, Nara 631, Japan

Chitinases have been characterized as PR proteins in both dicotyledonous and monocotyledonous plants and implicacted in their defenses to fungal pathogens. In view of detection of chitin in haustorial cell wall of Eryshiphe graminis and of chitinases in stressed barley leaves, this study aims at determining the role of the enzymes in barley powdery mildew interactions. When barley coleoptile cells harboring mature haustoria were treated with a chitinase (from Streptomyces griceus) after fixation, haustoria were completely digested by the enzyme, confirming chitin involvement in the cell wall. Chitnase was then introduced by microinjection into haustoria-harboring intact cells to determine its effect on haustorial function. Injection at the stage of primordial formation resulted in complete digestion of haustoria, hence inhibition of secondary hyphae development. Microinjection after haustorial maturation rarely caused observable morphological change, but considerably suppressed the secondary hyphae elongation. These results support the notion that chitinases in fact play a significant role in host defense. One of chitinase genes of Streptomyces sp. with a high chitinolytic activity was cloned into EMBL3 λ phage and subcloned with pUC19 to a 1.2 kb fragment which gave a strong signal in Northern hybridization. Base sequence analysis of this fragment revealed the presence of the ribosome binding sequence, GTG initiation codon common to many Streptomyces spp., and one open reading frame in its entire sequence. This gene ligated to CaMV-35S promotor was found transiently expressed in coleoptile cells when microinjected. Microinjection is an efficient technique for the analysis of function of genes and their products in host-pathogen interactions.

1369 LIPOXYGENASE IN *PHASEOLUS VULGARIS*
Hans-Georg Eiben, Brigit M. Meier and Alan J. Slusarenko Universitat Zurich, Institut fur Pflanzenbiologie, Zollikerstrasse 107, Zurich CH-8008, Switzerland.

Lipoxygenase (linoleate:oxygen oxidoreductase EC 1.13.11.12) is a dioxygenase catalysing the hydroperoxidation of fatty acids containing a *cis,cis*-1,4-pentadiene structure, *e.g.* linoleic, linolenic and arachidonic acids. The enzyme is apparently ubiquitous in eucaryotes. In animals the hydroperoxides produced from arachidonic acid by different lipoxygenases are precursors of important classes of chemical messengers such as leukotrienes and lipoxins. In plants no clearly defined physiological role has been demonstrated for lipoxygenase, but the hydroperoxides formed from linoleic and linolenic acids are further metabolised and produce several substances with pronounced physiological activity. Rôles for lipoxygenase in plant growth and development, senescence, wound responses and resistance against pathogens and pests have all been proposed.

A 2.2kb bean cDNA clone was fished from a lambda NM1149 bean library using a pea LOX cDNA probe. The bean clone showed 76% identity at the nucleotide and amino acid levels with published sequences for a soybean LOX-3 clone. The bean cDNA was used to fish bean genomic clones from a lambda-DASH library and one of these, g8, has been completely sequenced. It shows high homology to the soybean LOX-3 gene, including conserved exon-intron boundaries. The bean cDNA was used to probe Northern blots of RNA from bean and tomato tissue in compatible and incompatible combinations with plant pathogenic bacteria. Data on the pathogen-related changes in LOX transcript accumulation will be presented. The developmental and organ-specific expression of LOX in bean will also be described.

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1370 DOWNY MILDEW OF *ARABIDOPSIS THALIANA* CAUSED BY *PERONOSPORA PARASITICA*: A MODEL SYSTEM FOR THE INVESTIGATION OF THE MOLECULAR BIOLOGY AND PATHOLOGY OF HOST-PATHOGEN INTERACTIONS. Brigitte Mauch-Mani, Kevan P.C. Croft and Alan J. Slusarenko. Universität Zürich, Institut für Pflanzenbiologie, Zollikerstrasse 107, Zürich CH-8008, Switzerland.

We are studying various aspects of the *A. thaliana - P. parasitica* pathosystem using resistant and susceptible ecotypes of *Arabidopsis*. In order to get information on the genetic control of resistance in this system, complete diallele crosses between resistant and susceptible ecotypes as well as backcrosses of F1 plants to the respective homozygous recessive parents are being performed. We plan then to follow the co-segregation of the phenotypic resistance trait with mapped RFLP markers to localize and eventually isolate the resistance genes.

The screening of mutants of resistant ecotypes for changes to increased susceptibility, and the investigation of induction of systemic acquired resistance in susceptible plants by treatment with dichloro-iso-nicotinic acid (INA), will also be described.

Finally we report on our studies concerning the response of *Arabidopsis* at the biochemical level to fungal attack. For example, the induction of phytoalexin synthesis and the behavior of various enzymes (chitinase, peroxidase, superoxide dismutase, lipolytic acyl hydrolase, lipoxygenase, catalase, ascorbate peroxidase, and a lipid hydroperoxide decomposing enzyme).

1371 SUPEROXIDE DISMUTASE, PEROXIDASE AND CHITINASE IN THE HYPERSENSITIVE AND SUSCEPTIBLE RESPONSES OF TOMATO.
Kevan P.C. Croft, Eckhard Koch and Alan J. Slusarenko, Universitat Zurich, Institut für Pflanzenbiologie, Zollikerstrasse 107, Zürich CH-8008, Switzerland.

Pseudomonas syringae pv. *tomato* causes bacterial speck in the susceptible tomato cultivar "Moneymaker", whereas the non-host pathogen *P.s. pv. syringae* gives a typical hypersensitive response (HR). Activities of superoxide dismutase (SOD), peroxidase (POX) and chitinase (CHT) were followed upto 72 hours after inoculation for both the susceptible response (SR) and HR. SOD and POX are involved in the regulation of potentially damaging active oxygen species. By 24 h after inoculation SOD had increased to almost 5 times basal level in the HR but in the SR to only 2 times basal level. Staining for SOD activity in native gels showed that most of the increase was due to higher levels of Cu,Zn-SOD isozymes, although Mn-forms also increased. POX, which may also be involved in lignification as well as H_2O_2 removal, showed a large increase in activity during the HR- at 10 h after inoculation the level of POX was double that in the SR and by 72 h was 5 times greater. In the SR, after an initial increase in POX activity, no significant changes were observed. CHT increased in both the HR and SR, particularly after 24 h. By 72 h after inoculation CHT was at the same level in both the HR and SR, at approximately 10 times basal level.

SOD and POX may increase during the HR as a reaction by the plant to increases in active oxygen species generated during resistance responses. In the SR, SOD did not increase to the same levels as seen in the HR and POX remained close to basal levels. CHT was induced by both the non-host and host pathogens at approximately the same time and to the same levels and thus appears to be unrelated to resistance in this plant/pathogen system.

1372 FUNCTION AND TRANSCRIPTIONAL REGULATION OF DEFENSE-RELATED PLANT GENES. Imre E. Somssich, Petra Kawalleck, Ulla van de Locht, Iris Meier, Giampiero Trezzini and Klaus Hahlbrock. Max-Planck-Institute f. Zuchtungsforschung, Dept. of Biochemistry, 5000 Koln 30, Germany.
Part of the early induced defense response observed in plants following pathogen attack is the transcriptional activation of defense-related genes. Often, expression of the same set of genes can be detected both during host and non-host incompatible interactions. Employing a model system consisting of parsley cells and a fungal elicitor that closely mimics a true non-host interaction, we have isolated numerous defense-related parsley genes and their corresponding cDNAs (designated ELI and PR genes). Interestingly, similar genes appear evolutionarily conserved among different plant species. 15 out of 20 parsley cDNAs were successfully used to isolate their genomic counterparts from Arabidopsis thaliana.
We have focussed our investigations on the elucidation of the biochemical functions encoded by these genes and on the molecular mechanisms regulating their expression. In vivo DNA footprinting and/or elicitor-responsive parsley protoplasts have been used to identify cis-acting sequence elements within the promoters of the parsley PR1 and PR2 genes. Furthermore, cDNA clones encoding putative transcription factors that bind to a functionally important motif within the promoter of the PR1 gene have been isolated by Southwestern screenings of appropriate parsley and Arabidopsis expression libraries.

1373 COMPARISON OF PROTEIN MODIFICATIONS IN TOBACCO ROOTS DURING INTERACTIONS WITH PATHOGENIC OR SYMBIOTIC FUNGI
Abdessamad Tahiri-Alaoui, Silvio Gianinazzi, Vivienne Gianinazzi-Pearson and Eliane Dumas
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We are investigating the molecular basis by which a same host plant distinguishes between infection by a soilborne pathogen (*Chalara elegans*) and symbiotic vesicular-arbuscular endomycorrhizal (VAM) fungi. By comparing the protein profiles of root extracts from tobacco plants infected or not by *C. elegans* we have shown that the accumulation of Pathogenesis-Related (b) proteins (PR-b) is strongly induced upon infection with the pathogenic fungus [1]. In contrast, root infection with three different endomycorrizal fungi is characterized by the production of Endomycorrhiza-Related (ER) proteins, not related to PR-b proteins[2]. Western blotting of soluble proteins extracted from tobacco roots infected with VAM fungi and probed with antibodies against different PR-b proteins does not reveal any PR-b protein induction or cross-reaction with ER-proteins. However, indirect immunogold labelling shows that PR-b_1 protein is present in endomycorrhizal tobacco roots but to a much lesser extent than in pathogen-infected roots. Whilst in the latter subcellular localization of PR-b_1 protein can be observed throughout tissues of infected zones, in endomycorrizal roots it is limited to the intracellular interface of living arbuscules in parenchymal cells. These results suggest that plant defence genes are only weakly activated during development of an endomycorrhizal association and that another set of genes are expressed in the symbiotic interaction.
1. A. Tahiri-Alaoui, E. Dumas, S. Gianinazzi (1990) Plant Molecular Biology 14: 869-871.
2. E. Dumas, A. Tahiri-Alaoui, S. Gianinazzi, V. Gianinazzi-Pearson (1990) Endocytobiology IV, INRA press, Paris, 153-157.

1374 CHAPERONES AS A CANDIDATE CONDUIT FOR GENERAL PLANT PATHOGENESIS RESPONSE. Tamar Lotan and Robert Fluhr, Department of Plant Genetics, Weizmann Institute of Science, P.O. BOX 26, Rehovot, Israel 76100
Pathogenesis-related proteins were originally observed in conjunction with TMV infections in tobacco, but have since been shown to occur in a bewildering variety of fungal, bacterial and environmental responses. While the ubiquity of their response is enigmatic, the lytic enzymatic functions associated with some of their activities support their involvement as antipathogen agents. We have now formulated a universal theory that effectively explains the plants reactivity to general pathogen and environmental stress. It states that environmental or pathogen based-cues are monitored by the plant cell *via* perturbations in the homeostatic machinery of protein biosynthesis. These perturbations are in turn monitored in an autoregulatory manner by the chaperone class of polypeptides in particular the plant analogue to the immunoglobin binding protein, BiP (GRP-78). The principle is based on our novel observations that inhibitors of protein glycosylation such as tunicamycin and a variety of amino acid analogues are very potent inducers of pathogenesis-related proteins accumulation. Both elicitors are widely divergent in their mode of action, but are known to cause the generation of misfolded proteins that can obstruct the equilibrium of polypeptide binding to chaperones. With each elicitor we have monitored a concomitant increase in BiP accumulation that precedes the response of plants to pathogenesis. Protein folding was directly shown to be an intrinsic parameter of the pathogenesis response by the use of protein destabilization agents. Chemical agents such as DTT that can reduce intramolecular disulfide bonds and the amphipathic agent NP40 that can destabilize nonpolar interactions, were all found to be potent inducers of pathogenesis-related proteins and of BiP accumulation as well. Thus the signal transduction pathway for general plant pathogenesis response may be qualified to resolution of the autoregulatory network of BiP induction. The existence of this signal transduction pathway obviates the need for searching for specific receptors for each type of elicitor in general plant pathogenesis response.

1375 CLONING AND CHARACTERIZATION OF cDNA CLONES ENCODING MAIZE β-1,3-GLUCANASE AND CHITINASE. Shenchuan Wu, Alan L Kriz and Jack M Widholm, University of Illinois, Department of Agronomy, Urbana, Illinois 61801
The enzymes β-1,3-glucanase and chitinase in higher plants have been found to accumulate following pathogen attack and environmental stress, which indicates their involvement in plant defense responses. In this study, full length β-1,3-glucanase, and nearly full length chitinase, cDNA clones have been isolated from a library corresponding to maize seedling mRNA and sequenced. We are in the process of characterizing the expression patterns of these genes in various maize tissues subsequent to challenge of the plants with the fungus *Aspergillus flavus*. Because *A. flavus* is capable of producing the highly carcinogenic compound aflatoxin, effective control of *A. flavus* infection of maize grain would have a significant impact on the grain industry. Such control might be achieved through the use of genetic engineering to enhance β-1,3-glucanase and chitinase activities in grain tissue.

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1376 PATHOGEN - INDUCED GENES IN WHEAT.
Felix Mauch, John Bull, Reto Kessler and Robert Dudler.
Universitat Zurich, Institut fur Pflanzenbiologie, Zollikerstrasse 107, Zurich CH-8008, Switzerland.

The resistance of wheat (*Triticum aestivum*) to the fungal pathogen *Erysiphe graminis* f.sp. *tritici* is increased following a previous inoculation with the non-pathogen *E. graminis* f.sp. *hordei*. The onset of this induced resistance correlates with the activation of putative defense genes. Four classes of such genes have been identified and characterized. Two gene classes encode peroxidase (WIR 3) and thaumatin-like protein (WIR 2) respectively, both well known stress proteins. WIR 5 encodes a glutathione-S-transferase (GST), an enzyme function known to be associated with detoxification processes, but so far not with defense reactions. The WIR 1 genes encode small proteins (85-88 aa) with a hydrophobic N-terminal and a hydrophilic C-terminal domain rich in glycine and proline. Based on its structure, the WIR 1 gene product is speculated to be an integral membrane protein with its C-terminus sticking to the cell wall. To determine their possible involvement in resistance reactions, attempts are in progress to localize the gene products in induced wheat and in transgenic tobacco overexpressing the WIR proteins. We will also report on the differential induction of the WIR genes by chemical inducers.

1377 IDENTIFICATION AND CHARACTERIZATION OF SYSTEMICALLY INDUCED RICE GENES CORRELATING WITH ACQUIRED RESISTANCE AGAINST THE RICE BLAST FUNGUS *PYRICULARIA ORYZAE*. Cornelia Reimmann, Felix Mauch and Robert Dudler, Institut für Pflanzenbiologie, Universität Zurich, Zollikerstrasse 107, CH-8008, Zurich, Switzerland.

Induced systemic resistance in rice against *Pyricularia oryzae* has been demonstrated in response to various chemical treatments. Recently it was shown that these defence reactions of the plant could also be activated by a previous infiltration of rice leaves with the non-host phytopathogenic bacterium *Pseudomonas syringae* pv. *syringae*. As a consequence of the bacterial infection, the plant activates a whole set of genes. We hypothesize that such genes might contribute to the expression of systemic resistance against the blast fungus.
Towards the understanding of the molecular basis of induced resistance in rice we have constructed cDNA libraries from rice plants induced with *P. syringae* pv. *syringae*. Data will be presented on the identification of genes which are locally and/or systemically induced in resistant plants.

1378 TOPOISOMERASE INTERACTIONS WITH PEA DEFENSE GENE 49 David Gerhold, David Culley and Lee A. Hadwiger, Department of Plant Pathology Washington State University Pullman, WA 99164-6430

Topoisomerases are enzymes which can relieve transcriptional DNA supercoiling (topoisomerases I and II) and mediate DNA attachment to chromatin scaffolds" (topoisomerase II) in vivo Our interest in topoisomerases stems from the ability of several topoisomerase inhibitor drugs to stimulate transcription of pea defense response *gene 49* This gene is also flanked by putative consensus recognition sites for the *Drosophila* topoisomerase II enzyme Clusters of such sites are indicative of "Scaffold Attachment Regions" (SAR's) which are believed to interact with chromatin proteins DNA is thus reportedly organized into discrete loops which impact gene regulation We are characterizing topoisomerases from tobacco callus with respect to drug sensitivities, and consensus DNA binding/cleavage sites We hope to understand the sites of topoisomerase action in and around *gene 49* and how this gene is induced by topoisomerase inhibitors as well as by fungal attack

1379

Structure and expression of endochitinase genes from sugar beets

Kirsten Bojsen[1], Lars Berglund[1,2], Klaus K. Nielsen[1] and Joern D. Mikkelsen[1]. 1) Biotechnology Research Division, Danisco A/S, 1 Langebrogade, DK-1001 Copenhagen K. 2) Laboratory of Gene Expression, University of Aarhus, Gustav Wieds Vej 10, DK-8000 Aarhus C

Fourteen different chitinase isozymes have been purified to homogeneity from sugar beet leaves infected with Cercospora beticola. Polyclonal antibodies have been raised against six of the proteins. Four different serological chitinase classes have been identified. Partial amino acid sequences were obtained for ten of the chitinase isozymes. Using either specific antibodies or gene probes derived from the amino acid sequence, expression cDNA libraries were screened, and cDNA genes coding for three of the four sugar beet chitinase classes were isolated. In addition genomic genes coding for two of the chitinase classes have been obtained and characterized by sequencing.
The sugar beet chitinase 4 class includes enzymes which constitute a new type of basic chitinase containing much shorter N-terminal hevein and catalytic domains compared to the well-know basic chitinases from tabacco and bean.
The expression level of the three chitinase classes in C. beticola infected sugar beet leaves will be reported.

1380 A NEW TYPE OF ANTIFUNGAL PROTEINS FROM *MIRABILIS JALAPA* L SEEDS. Bruno P.A. Cammue, Miguel F.C. De Bolle, Franky R.G Terras, J Vanderleyden and Willem F. Broekaert. F.A. Janssens Laboratory of Genetics, Catholic University of Leuven, Leuven, Belgium.

Using a combination of ion exchange chromatography, gel filtration and reversed phase chromatography, two antifungal proteins, designed *Mj*-AFP1 and *Mj*-AFP2, were isolated from seeds of *Mirabilis jalapa* L. *Mj*-AFP1 and *Mj*-AFP2 are dimeric proteins each composed of two identical subunits (4 kDa and 3.5 kDa, respectively) held together by disulphide bridges. Isoelectric focusing experiments revealed that both proteins have pI values of about 10.5 . The amino acid sequence was identified for 37 and 35 residues of *Mj*-AFP1 and *Mj*-AFP2, respectively, representing the major part of the primary structure A homologous search on the EMBL bank revealed sequence similarity of the *Mirabilis* proteins to ω-agatoxins, a class of insecticidal neurotoxic peptides isolated from the venom of the spider *Agenelopsis aperta*. The antifungal activity of *Mj*-AFP1 and *Mj*-AFP2 was not affected by heat treatment at up to 100°C (10 min), and was stable over the pH range from 2 to 11. Both proteins exhibit a broad spectrum of antifungal activity since they inhibited growth of 13 tested fungi. Compared with other antifungal plant proteins, it appeared that *Mj*-AFP1 and especially *Mj*-AFP2 exhibit a high antifungal potency. Concentrations required for 50 % inhibition of fungal growth (IC_{50}) varied from 6 to 300 µg/ml for *Mj*-AFP1, and from 0.5 to 20 µg/ml for *Mj*-AFP2, depending on the test organism. In parallel tests, a thionin isolated from wheat endosperm exhibited IC_{50} values ranging from 0.5 to 15 µg/ml, whereas *Urtica dioica* agglutinin inhibited growth of the fungi at IC_{50} values varying from 20 to over 1.000 µg/ml. In contrast to the thionin, however, which is toxic to most types of cells, the toxicity of the *Mirabilis* proteins seemed to be restricted to fungal cells

1381 STRUCTURE OF A NOVEL PROLINE-RICH PROTEIN WHOSE mRNA IS DOWN-REGULATED BY FUNGAL ELICITOR AND WOUNDING. Jinsong Sheng and Mona C. Mehdy, Dept. of Botany, University of Texas at Austin, Austin, TX 78713 We are studying the structure and expression of a cDNA clone (PvPRP1) isolated from a cDNA library prepared from fungal elicitor treated bean (Phaseolus vulgaris) cells. Sequence analysis of the 1.1 kb insert revealed a complete open reading frame which encodes a 32 kD protein. The protein resembles other proline-rich proteins in plants but possesses several unique features: 1) the N-terminal half of the protein is proline rich and contains 3 identical repeats of PPVPVHPPVK and 5 related repeats; 2) the proline rich region contains 2 tracts of 6 histidine residues; and 3) the C-terminal half is low in proline and lacks repeats. Genomic blotting experiments suggest the occurrence of 1-2 PvPRP1 genes within the bean genome. A dramatic decrease in PvPRP1 mRNA levels occurs within 1 hr after elicitor treatment of cell cultures. The PvPRP1 mRNA is present at a moderate level in hypocotyls. Upon wounding, the mRNA level initially decreases over 5 hr and then reaccumulates over a period of 25 hr. Based on the substantial homology with other proline-rich proteins, including the presence of a putative signal peptide, it is likely that the PvPRP1 protein is targeted to the cell wall. The expression of the PvPRP1 gene appears to be integrated with the remodeling of the plant cell wall during the defense response.

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1382 THE STRUCTURE AND ORGANIZATION OF CHALCONE ISOMERASE GENES IN BEAN. Mona C. Mehdy, Marina Zabelshansky and Connie Hinojos. University of Texas at Austin, Department of Botany, Austin, Texas 78713.

We are studying the structure of chalcone isomerase (CHI) genes in bean (Phaseolus vulgaris). CHI catalyzes a step necessary for the biosynthesis of isoflavonoid phytoalexins and flavonoid pigments. We have previously reported that CHI mRNA accumulates in response to fungal elicitor treatment, fungal infection, and wounding. While a single CHI gene within the bean genome was suggested by our previous genomic blot data, we now show that there are 5-6 CHI genes based on genomic reconstruction experiments. The genes appear to be highly homologous in both their coding and flanking regions based on the near absence of restriction site polymorphisms. We have obtained the sequence of the CHI mRNA (1 kb) by isolating overlapping cDNA clones. The bean CHI protein exhibits 67% amino acid homology with the petunia chalcone isomerase CHIA, allowing conservative substitutions. A genomic clone has been identified and characterized. Sequence analysis of the genomic clone in comparison to the cDNA clone sequence demonstrated the locations and sizes of three introns. Present data suggest that the overall transcription unit is slightly larger than 2 kb. In addition, the transcription start site is currently being mapped by primer extension experiments. We will present comparisons of the 5' upstream CHI sequence with the previously reported promoter sequences of the coordinately regulated phenylalanine-ammonia lyase and chalcone synthase genes in bean.

1383 USE OF YEAST ARTIFICIAL CHROMOSOMES FOR THE RECOVERY OF DISEASE RESISTANCE GENES FROM LACTUCA SATIVA. Marc C. Fortin, Wei-Ching Wang and Richard W. Michelmore. Dept. of Vegetable Crops, Univ. of California at Davis, Davis, California, 95616

Our objective is the characterization and recovery of genes and loci responsible for disease resistance in Lactuca. Linked RFLP markers have already been mapped surrounding those genes. We need to determine the recombination frequency in the areas surrounding these loci in order to estimate the physical distance separating the markers from target genes. Yeast artificial chromosome (YAC) clones are being used for that purpose, and for long range chromosome walking from RFLP markers. Large molecular weight DNA (>7 Mbp) has been isolated from lettuce protoplasts and cloned into the pYAC-MG31 vector. This YAC vector was designed to allow easy recovery of end fragments using rare cutting restriction sites flanking the cloning site, or to allow probe synthesis through the use of RNA polymerase promoters. Clones ranging from 50 to 250 kbp have been recovered. End fragments have also been obtained by PCR amplification using primers flanking the cloning site. The frequency of recombination over defined physical distances can be determined using RFLP segregation analysis of the terminal sequences from YAC clones.

1384 MOLECULAR CLONING OF THE Hm1 DISEASE RESISTANCE GENE IN MAIZE. Gurmukh S. Johal and Steven P. Briggs. Pioneer Hi-Bred International, Johnston, IA 50131

The Hm1 locus of maize controls resistance to a severe leaf blight and ear mold disease caused by the fungal pathogen *Helminthosporium (Cochliobolus) carbonum* race 1. To understand the mechanism and evolution of disease resistance, and to manipulate this trait in plants, we have cloned the Hm1 gene by transposon tagging. Several mutations at the Hm1 locus were generated using both the Robertson's Mutator and the Ac/Ds transposable element systems. Cosegregation of the mutant hm1 alleles with transposable element containing restriction fragments indicated that the mutant hm1-656 and hm1-1369 alleles were tagged with Mu-1 and Mu-3, respectively. Appropriate fragments from these mutants were cloned, subcloned, and restriction mapped. It turned out that the element insertions in these mutants were within a few hundred base pairs of each other. In another mutant, hm1-1790, which showed aberrant transmission frequencies, the DNA sequences homologous to hm1-656 and hm1-1369 probes were deleted. It is not known how big the deletion is but hybridization with flanking RFLP markers indicated that the deletion was localized and extended no farther than 5 cM on either side of the Hm1 locus. The hm1-1369 probe also detected what appeared to be an insertion in the hm1-1040 allele. This mutant was derived from the Ac/Ds mutagenesis experiment. However, after cloning this mutant allele we found that the insertion was an Spm/dSpm element. A number of putative revertants of hm1-1040 have been isolated and in all of these revertants the mutant allele has reverted back to the progenitor allele.

1385 A PUTATIVE KINASE C INHIBITOR AND A CHAPERONE ARE PRESENT AMONG RESISTANCE RESPONSE GENES OF BARLEY H. Thordal-Christensen, J. Brandt, P.L. Gregersen, B.H. Cho, H. Walther-Larsen, V. Smedegaard and D.B. Collinge. Dept. of Plant Biology, Sect. f. Plant Pathology, The Roval Veterinary and Agricultural University. DK-1871 Frederiksberg, Denmark.

From barley plants exhibiting papilla resistance to the powdery mildew fungus, five classes of resistance response cDNAs have been isolated. The expression profiles of the corresponding genes have been demonstrated by northern blotting, and show that transcript maxima occur twice, around four hours and around ten hours after inoculation. This result is believed to reflect synchronized conidia germination involving two different germ tubes, in response to which the plant develops papillae. Sequence data reveals that the cDNA clones represent genes encoding 1) a protein homologous to mammalian kinase C inhibitors. Homologues to this kind of kinase inhibitors, not previously cloned from plants, may participate in the regulation of the resistance response. Use of the original clone for further screening of a cDNA library and for production of RACE primers has resulted in the isolation of a further two barley sequences highly homologous to our original clone. 2) A protein homologous to endoplasmin - a resident ER protein - member of the HSP90 family. Proteins of this family are believed to act as chaperones, and such a protein may somehow be involved in protein assembly and export during the resistance response. Endoplasmin sequences have not previously been isolated from plants. 3) A basic peroxidase, 4) a sucrose synthase and 5) a protein of unknown function.

1386 CHARACTERIZATION AND ANALYSIS OF THE PHENYLALANINE AMMONIA-LYASE MULTIGENE FAMILY FROM POTATO. E.L. Virts, J. Collier and L. Fitzmaurice. SIBIA, P.O. Box 85200, San Diego, CA 92186.

L-phenylalanine ammonia-lyase (PAL) is an enzyme which catalyzes the first step in the biosynthesis of one class of plant defense compounds, the phenylpropanoids. Transcription of PAL is activated by a wide variety of elicitors including the fatty acid, arachidonic acid. Southern analysis of total genomic DNA isolated from Solanum tuberosum cv. Desiree showed that the PAL genes of this cultivar were present as a multigene family with possibly as many as 50 members. Two partial cDNA PAL clones were purified from a library constructed using total RNA isolated from Desiree tissue culture cells treated with arachidonic acid. Genomic libraries of total Desiree DNA were screened using these cDNA clones, and 43 genomic clones were isolated. Seven clones were characterized further. From sequence analysis, it appears that two clones are identical, four clones show greater than 95% sequence similarity to each other, and one clone is unique. RNase protection assays were used to determine which of these clones encode an inducible PAL gene(s). The results of this analysis will be presented.

1387 PATHOGEN-INDUCED ACCUMULATION AND DISTRIBUTION OF THE STH-2 PATHOGENESIS-RELATED GENE PRODUCT IN POTATO Peter Constabel, Daniel P Matton, and Normand Brisson. Department of Biochemistry, Université de Montréal, Canada

The pathogenesis-related gene STH-2 is one of several genes rapidly activated following infection or elicitor treatment of potato tubers. The function of the gene is unknown; however, homologous pathogen-induced genes have been isolated from several other plant species. In order to obtain a specific probe which would allow us to study the STH-2 gene product and its distribution in more detail, we have raised antibodies to a β-galactosidase-STH-2 fusion protein expressed in *E. coli*. The antiserum recognizes a protein of the expected 17 kD size in extracts of wounded, elicited, or infected tuber tissues when analysed by immunoblot or immunoprecipitation. In control tuber extracts this band is not detected. However, in both treated and control tubers a second protein of 18 kD is also recognized by the antibody. Infection of tubers with an incompatible race of the pathogen *Phytophthora infestans* results in higher levels of STH-2 protein accumulation than infection with a compatible race. This differential response is also seen in *P. infestans*-infected leaves and suggests that expression of STH-2 is closely associated with resistance to infection.

POSTER ABSTRACTS

1388 CHARACTERIZATION OF CIS-ACTING ELEMENTS INVOLVED IN THE REGULATION OF THE POTATO PATHOGENESIS-RELATED GENE STH-2. Daniel P. Matton and Normand Brisson. Department of Biochemistry, Université de Montréal, Montréal, Canada

The potato pathogenesis-related (PR) gene STH-2 is part of a small gene family. It encodes a small protein of 17 kD of unknown function whose amino acid sequence is highly similar to those of other PR proteins from pea, parsley and french bean. All the genes encoding these proteins are activated by their respective pathogen and by various elicitors. STH-2 also shares sequence identities with the major birch pollen allergen. STH-2 mRNAs accumulate to high levels in wounded and pathogen or elicitor-stressed potato tissues. To get information about the regulatory regions that could be involved in gene activation or repression, we have introduced deletions in the 5'-upstream sequence of the STH-2 gene. Truncated promoters and chimeric constructs containing all the 5'-untranslated leader sequence and part of the STH-2 coding sequence were fused to the *E. coli uidA* (β-glucuronidase) reporter gene. Electroporation of potato leaf mesophyll protoplasts with these constructs led to the identification of several domains which could influence reporter gene activity. The domain comprised between nucleotides -135 and -52 had the characteristics of a true enhancer sequence since it could activate reporter gene expression in both orientations and also when placed 1 kb away from its normal position in the genome. This region could also activate an heterologous minimal promoter (-90 CaMV 35S). Possible sequence identity with domains from other plant genes will be discussed.

1389 DEVELOPMENTALLY REGULATED AND PATHOGEN INDUCIBLE EXPRESSION OF THE *ARABIDOPSIS* ACIDIC CHITINASE GENE IN TRANSGENIC PLANTS. Deborah A. Samac and Dilip M. Shah. Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, MO 63198

Chitinase gene expression is induced in a wide variety of plant species during pathogen attack suggesting that this fungal cell wall hydrolase is involved in plant defense. To study the tissue specific developmental and inducible pattern of expression of the acidic chitinase gene from *Arabidopsis*, a chimeric gene composed of 1.2 kbp of 5' upstream sequence was fused to the β-glucuronidase (GUS) coding region and introduced into *Arabidopsis* and tomato plants. Northern blot analyses of expression of the acidic chitinase gene detected low levels of expression in *Arabidopsis* roots. The GUS reporter gene was a much more sensitive assay of gene expression. Constitutive GUS expression was observed in roots, leaf vascular tissue, hydathodes, guard cells of open stomates and pollen of healthy plants. Expression in these cells is consistent with the role of chitinase as a preformed defensive enzyme. GUS expression was induced in mesophyll cells surrounding lesions after infecting transgenic *Arabidopsis* plants with *Rhizoctonia solani*. In transgenic tomato plants, GUS expression was induced around necrotic lesions caused by *Rhizoctonia solani*, *Septoria lycopersici* and *Phytophthora infestans*-a fungus lacking significant cell wall chitin. Analysis of a series of 5' promoter deletion constructs in *Arabidopsis* indicated that the proximal region of the promoter to -200 was sufficient to establish the constitutive and induced pattern of expression. Elements further upstream are involved in quantitative expression of the gene. A negative regulatory element is located between -400 and -600 and a number of positive regulatory elements are located between -1200 and -600.

1390 GENETIC COMPLEXITY OF THE MAJOR CLUSTER OF DOWNY MILDEW RESISTANCE GENES IN LETTUCE. Ilan Paran, Richard Kesseli and Richard Michelmore. University of California, Department of Vegetable Crops, Davis, California 95616.

The interaction between lettuce and the obligate fungus *Bremia lactucae* that causes downy mildew is characterized by a "gene-for-gene" relationship. Fourteen dominant resistance (*Dm*) genes have been identified in lettuce, each is matched by a dominant avirulence gene in the fungus. The resistance genes are clustered in four linkage groups, the largest one consists of seven genes and spans over 19 cM. Molecular markers, RFLPs and RAPDs that are linked to the *Dm* genes were identified using two methods. Near-isogenic lines that differed for *Dm* genes were screened for polymorphisms which were subsequently mapped using segregating populations. Secondly, two pooled DNA samples were formed, one from F_2 individuals that were homozygous for the resistance allele and one from F_2 individuals that were homozygous for the susceptibility allele. The two pools were screened for polymorphisms in a similar manner to the near-isogenic lines. Several of the identified RAPD markers that were amplified using the same primer, were linked to more than one cluster of *Dm* genes. The relationship between these markers and the sequence complexity in the regions containing the *Dm* genes is being investigated.

1391 THE EVIDENCE OF GENE-FOR-GENE COMPLEMENTARITY. Yishen Zeng, Liang peng Yang, Junqi Huang and Quanzhou Tao. Shanghai Institute of Biochemistry, Academia Sinica, Shanghai 200031, China

Ellingboe and Vanderplank proposed two contrary models to interpret Flor's gene-for-gene relationship respectively. However, the recognizable combination of the models has not been verified at molecular level. A convenient method to identify the race-specific resistance proteins of fusarium-wilt in cotton, bacterial blight in rice, fusarium head blight in wheat and verticillium-wilt in cotton has been developed by our laboratory. The race-sepecificities of these proteins bioassayed in vitro are alway consistent with the phenotypes in the fields. The previous two cases supported Ellingboe's model (incompatible combination model) and the latter two cases supported Vanderplank's model(compatible combination model). A comparison of general features that distinguish incompatible combination model from compatible combination model of plant diseases has been summaried.

1392 ARABILEXIN, A NOVEL PHYTOALEXIN FROM *ARABIDOPSIS THALIANA*. J. Tsuji[1], R. Hammerschmidt[2], and S.C. Somerville[1,2] Michigan State University, [1]MSU-DOE Plant Research Laboratory and [2]Department of Botany & Plant Pathology, East Lansing, Michigan 48824.

Leaves of *Arabidopsis thaliana* inoculated with the non-host pathogen *Pseudomonas syringae* pv. *syringae* were found to accumulate the phytoalexin arabilexin. 2.31 mg of arabilexin was purified from 1.28 kg of elicited leaf tissue by a combination of flash chromatography, TLC, and reverse phase HPLC. The phytoalexin was identified as indole-3-thiazole by UV, IR, MS, ^{1}H NMR, and ^{13}C NMR data. Arabilexin accumulated within 12 hours post inoculation with *P.s. syringae* and reached a maximum level by 36 hours. The accumulation of arabilexin was negatively correlated with the *in planta* growth of *P.s. syringae*. The *in vitro* growth of both the fungus *Cladosporium cucumerinum* and the bacterium *P.s. syringae* was inhibited by arabilexin.

1393 ANALYSIS OF A BEAN CHALCONE SYNTHASE PROMOTER IN TRANSGENIC ALFALFA. Karen Dalkin, Ian Dubery[1], Christopher J. Lamb and Richard A. Dixon. Plant Biology Division, The Samuel Roberts Noble Foundation, P. O. Box 2180, Ardmore, OK 73402. [1]Department of Chemistry and Biochemistry, Randse Afrikaanse University, Auckland Park, Johannesburg, Republic of South Africa. Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037.

A number of plant defense response genes are under complex developmental and environmental regulation. In legumes, such as alfalfa (Medicago sativa L.) and bean (Phaseolus vulgaris L.) chalcone synthase (CHS) genes are involved in the biosynthesis of antimicrobial isoflavonoids during induced defense against pathogenic fungi and bacteria, in the formation of flavonoid compounds involved in flower pigmentation, UV-protection, hormone transport, and as rhizopnere signals for root nodulating bacteria. These multiple functions suggest that the regulation of CHS gene expression is likely to be multi-faceted. Alfalfa cell suspension cultures, and protoplasts derived from them respond to fungal elicitor with rapid increases in the activities of a number of enzymes, including CHS, involved in the synthesis of isoflavonoid phytoalexins. We describe a system for the functional analysis of an elicitor-inducible bean CHS promoter in transgenic alfalfa plants. We compare expression of the transgene to the expression patterns of the endogenous alfalfa CHS genes, and describe the effects of 5'-deletions in the CHS promoter on developmental and environmental control of transgene expression.

POSTER ABSTRACTS

1394 EVALUATION OF INTERGENERIC NAKED DNA TRANSFER TO PRODUCE S. CEREVISIAE AND A.CHROOCOCCUM CAPABLE OF GARILC ANTIBIOTIC PRODUCTION. Kamel A..Ahmed(1) Ahmed N.SharaF(1) and nasan A. Nohammed(1) Department of Genetics, Faculty of Agriculture, Cairo University and (2) Genetic Engineering Research Unti, National Reseaerchg Centre, Egypt.

The specific broad spectrum antifungal antibacterial effect of Egyptian garlic extract was assessed. Garlic antibiotic was transfered via its naked DNA to S. cerevisiae and A.chroococcum. Treansformants of both microbial species capable of producing the garlic antimicrobial substance were isolated. Justification of gene transfer in transformants was carried out by mode of antimicrobial inheritance. paper chromotography for protein analysis and electrophersis for their DNA.

1395 CYANOPHAGE GENOME PARTICIPATES IN CODING NITROGENASE COMPLEX OF CYANOBACTERIA. F.Kamilova, M.Muradov, R.Muchamedov, A.Abdukarimov, A.Khalmuradov. Institute of Microbiology and Institute of Bioorganic Chemistry Uzbek Academy of Sciences Tashkent USSR

The temperate cyanophages of NP-1T series have collected in water reservoirs of Uzbekistan. Clones resistant to phages have been shown to fix atmospheric N_2. Nitrogenase activity estimated by acetylen reduction to ethylene increases from 4-20 to 200-250 nmol C_2H_4/h per 1 mg protein. ^{32}P DNA of pSA30 containing nif H D K genes from Klebsiella was probed with the EcoR32-digested genomic DNA from NP-1T(2) cyanophage and with the total DNA of Nostoc sp., both lysogenic and primary cultures in Southern blot- and dot hybridization experiments. The total DNA from lysogenic culture of Nostoc sp. and 12.1-kb EcoR32 fragment in cyanophage genome were nomologous to DNA of pSA30. Total DNA from Nostoc sp. without propnage wasn't homologous to molecular probe. These data testify to the occurence at least one of the structural genes of nitrogenase complex in NP-1T(2) cyanophage genome

1396 NITRATE REDUCTION AND NITROGEN FIXATION RELATIONSHIPS IN BRADYRHIZOBIUM SP. BACTEROIDS. Manuel A. Chamber. D. G. I. E.A., Apdo. Oficial, Alcalá del Río. 41200-Sevilla. SPAIN.

Nitrate reduction can take place in nodules cytosols and also in bacteroids during N_2 fixation. Strains belonging to two Bradyrhizobium species (japonicum and lupinus) were studied under either the absence or the presence of combined $N-NO_3^-$ (8 mM) along the plants cycle of growth. Several parameters related to the rates of fixed N_2 were measured, namely nitrogenase (ARA) and hydrogenase (Hup) activities, nodulation biomass, nitrogenase relative efficiencies, C/N in nodules, and total N and NO_3^- in shoots. The evolution of these parameters and their relationships were analyzed. We found two types of nitrate-reduction activities in the bacteroids (NRA), the constitutive type seemed to be related to ARA and its occurrence improved yields of shoots, therefore, it could be involved in an assimilatory process. Hup activities and NRA were highly and negatively correlated, otherwise, relative efficiencies and NRA showed up as a quite independent variables. Total N yields increased proportionally to C/N in nodules and NRA in bacteroids.

1397 A MUTANT OF THE CYANOBACTERIUM *ANABAENA* 7120 ALTERED IN HETEROCYST SPACING Jihong Liang and Robert Haselkorn, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

The filamentous cyanobacterium *Anabaena* 7120 grows in continuous chains of homogeneous vegetative cells under conditions where fixed nitrogen is available as nitrogen source. When the filaments are deprived of fixed nitrogen, some cells differentiate into thick-walled heterocysts that become incapable of photosynthesis but can reduce atmospheric nitrogen to ammonia, which is in turn incorporated into glutamine and transported to neighboring vegetative cells. Heterocysts develop both terminally and intercalarily along the filaments at intervals of about 10 to 20 cells. The mechanism controlling this spatial pattern is not yet understood. Using Tn5 mutagenesis, we have isolated a mutant of *Anabaena* 7120 in which the development of heterocyst is delayed by two days compared to wild type cells, and the interval between heterocysts is shortened to about 5-10 cells. The mutant grows poorly in medium containing no combined nitrogen but grows as well as the wild type in medium that contains combined nitrogen. The mutant was complemented by conjugation with a cosmid library of wild type DNA fragments, selecting for rapid growth on medium lacking combined nitrogen. From complemented colonies, cosmids containing a common 4-kb fragment were isolated. Further analysis of this fragment is in progress.

1398 THE ROLE OF SEVEN REGULATORY GENES (nifA, nifL, vnfA, anfA, nfrX, ntrC, and rpoN) IN EXPRESSION OF GENES FOR THE THREE NITROGENASES IN Azotobacter vinelandii. Paul Woodley, Jean Walmsley, Gonzalo Blanco, and Christina Kennedy. AFRC IPSR, Nitrogen Fixation Laboratory, University of Sussex, Brighton, Sussex BN1 9RQ, UK

Azotobacter vinelandii has the potential to synthesize three different nitrogenase enzymes which differ in metal content: molybdenum nitrogenase, also containing iron, has subunits encoded by nifHDK; structural subunits of the vanadium nitrogenase, which also contains iron, are encoded by vnfHDGK in two operons; the subunits of the third nitrogenase, apparently containing only iron, are encoded by anfHDGK. None of these structural gene operons are closely linked to each other. The products of five other nif genes, nifB, nifM, nifV, nifS, and nifU are essential for the function of all three nitrogenases. We have examined the role of seven regulatory genes, rpoN, nifA, nifL, vnfA, anfA, nfrX, and ntrC in expression of the nifHDK, vnfH, vnfDGK, anfHDGK, nifB, and nifM genes. A model will be presented showing the complex interrelationships of the seven regulatory gene products in the expression of the genes necessary for function of the three nitrogenases. Experiments which define the vnf gene promoter sequences necessary for activation by the VNFA protein will be presented along with an analysis of factors controlling vnfA expression.

1399 NOD METABOLITES OF BRADYRHIZOBIUM JAPONICUM THAT INDUCE A PLANT NODULATION RESPONSE. Herman Spaink[1], Russell Carlson[2], Ramadas Bhat[2], Ton van Brussel[1], Mark Barbour[3], Juan Sanjuan[3], Ben Lugtenberg[1], and Gary Stacey[3]. [1]Dept. of Plant Molecular Biology, Leiden University, The Netherlands; [2]Complex Carbohydrate Research Center, University of Georgia; [3]Center for Legume Research, The University of Tennessee.

Present evidence suggests that the nod genes of rhizobia are involved in the production of signal molecules that induce the plant nodulation response. We have examined cultures of B. japonicum for the production of nod factors such as those previously described from R. meliloti. Flavonoid-induced cultures were labeled with ^{14}C-acetate, butanol extracted, and the extract analyzed by thin-layer chromatography. Labeled compounds were detected that were specific to genistein or seed extract-induced cultures. Furthermore, induced cultures from NodA$^-$, NodB$^-$, or NodC$^-$ mutants did not produce these compounds. Mutations in other genes were found to affect the pattern of compounds produced. The extracts containing the ^{14}C-labeled compounds induced a TSR (thick-short-root) phenotype when added to soybean roots. These extracts also induced root hair deformation and curling. The nod factors detected have been purified and their chemistry is under investigation.

POSTER ABSTRACTS

1400 TRANSFORMATION OF FRANKIA BY ELECTROPORATION. Brent L. Nielsen, Barbara Steele, D. Bernie Steele and Joe Shaw. Auburn Univ., Dept. of Botany & Microbiology, Auburn, AL 36849.

Frankia is an actinomycete which plays an important role in biological nitrogen fixation by forming a symbiosis with a wide variety of woody plants (Alnus and Casuarina). This symbiosis is especially important in the tropics and in forest regeneration worldwide. Despite this importance, an understanding of the molecular biology of Frankia has been limited by a lack of a genetic transformation system for this organism. We have cloned into pUC18 various restriction fragments from an endogenous Frankia plasmid. The purified recombinants have been introduced into Frankia strain NPI-0136010 by electroporation under various conditions. Successful Frankia transformants have been selected by ampicillin resistance. The recombinant plasmid was rescued from the transformed Frankia cells into E. coli. The recovered plasmid exhibits the same restriction pattern as the original clone. The presence of the recombinant plasmid in transformed Frankia cells after extended culture was confirmed by Southern blot hybridization.

1401 RHIZOBITOXINE IS DIFFERENTIALLY EXPRESSED IN LEGUME NODULES IN A GENOTYPE AND SPECIES SPECIFIC MANNER. Xiaoan Ruan and N. Kent Peters. The Ohio State University, Columbus, OH 43210.

Rhizobitoxine, a phytotoxin synthesized by some strains of *B. japonicum* and *Bradyrhizobium* spp., causes chlorosis on leaves of soybean plants. In previous studies, its structure and biochemical activities have been determined, but little is known of it's regulatory or biological functions. In order to explore the importance of rhizobitoxine in bacterial-plant interactions, Tn5 induced mutants of *B. japonicum* strain USDA61 were isolated based on their inability to synthesize rhizobitoxine in culture. Southern blot analysis reveals that each of the mutants carries a single copy of Tn5. Growth tests on minimum medium indicate that each mutant is prototrophic. Inoculation of the mutants on to host soybean plants shows that all the mutants are symbiotically effective and have similar kinetics of nodulation on soybean. The mutants can be distinguished by their *in planta* phenotypes of rhizobitoxine production. One group of mutants has completely lost the ability to produce rhizobitoxine in culture or in nodulo A second group produces variable amounts of the toxin in nodules of the susceptible cv. Lee and the resistant cv. Williams. Another set of mutants hyperproduce toxin in the nodules causing chlorosis in the resistant cv Williams This hyperproduction is not evident on other hosts of USDA61 such as cowpea and *Glycine soja*, where little toxin is detected in nodules.

1402 RHIZOBITOXINE IS INVOLVED IN OVERCOMING THE Rj_4 GENOTYPE SPECIFIC HOST RESTRICTION IN SOYBEAN Thomas Stokkermans, Xiaoan Ruan and N. Kent Peters. The Ohio State University, Columbus, OH 43210.

Several genes controlling the nodulation response of soybean with *Bradyrhizobium japonicum* have been described The dominant Rj_4 allele conditions an ineffective nodulation response with bradyrhizobial strain USDA61. Tn5 induced mutations of USDA61 which hyperexpress rhizobitoxine in planta overcome the Rj_4 restriction, forming nodules at similar rates as nonrestricted strains. Rhizobitoxine inhibits ACC synthase, a key enzyme in ethylene biosynthesis. The host restriction, therefore, seems to be mediated by ethylene. The implication of ethylene in the host restriction is further supported by experiments in which addition of an ethylene biosynthesis inhibitor, AVG, resulted in higher numbers of nodules on Rj_4 plants nodulated by USDA61. Preliminary results show differential expression in nodules formed by restricted versus non-restricted symbionts of genes for cell wall proteins and of genes involved in the phenylpropanoid pathway

1403 **Insertional Mutagenesis in *Rhizobium meliloti* utilizing new mini-Tn5 transposon derivatives with rare restriction enzyme cutting sites - mini-Tn5(XSP).** Kwong Kwok Wong, Bruno W.S. Sobral and Michael McClelland. California Institute of Biological Research 11099 N. Torrey Pines Rd. La Jolla, CA92037.

The Tn5 transposon has proven to be of great utility for the insertional mutagenesis of a variety of gram-negative bacteria. In *Rhizobium meliloti*, Tn5 has a high degree of randomness of insertion. We have constructed a set of mini-Tn5 derivatives called mini-Tn5(XSP). The mini-Tn5(XSP) carries several rare restriction cutting sites - M.XbaI/DpnI (TCTAGATCTAGA), NotI (GCGGCCGC), SwaI (ATTTAAAT), PacI (TTAATTAA) and SpeI (ACTAGT). Since cleavage of the *R. meliloti* genome by PacI, SwaI and SpeI produces only 4, 6 and 35 fragments, respectively, the insertion of a mini-Tn5(XSP) in the genome could be visualized as the loss of one fragment and the gain for two smaller fragments when the genomic digest is resolved by pulsed-field electrophoresis. The transposons are located on a R6K-based suicide delivery plasmid that provides the $IS50_R$ transposase mp gene *in cis* but external to the mobile element and whose conjugal transfer to recipients is mediated by RP4 mobilization function in the donor (de Lorenzo et al., J. Bact. 172:6568-6572 1990). We are generating two sets of random mini-Tn5(XSP) insertions. Each set will carry a different selectable marker, one Tc^R and the other Cm^R. These sets of mini-Tn5(XSP) insertions will be useful for the physical and genetic analysis of the *R meliloti* genome and the genomes of many other agronomically important bacteria.

1404 DO ISOFLAVONOID POOLS CHANGE IN SOYBEAN ROOT EXUDATES AND EXTRACTS DURING NODULATION? Renee M. Kosslak[1], J. Dieter[1], H-J. Wang[2], B. Smith[2], P. A. Murphy[2], J. Imsande[2] and R. G. Palmer[1]. Departments of Zoology and Genetics[1], Food Science and Nutrition[2] and Agronomy[2], Iowa State University, Ames, IA 50011.

An axenic system has been developed to study changes in isoflavonoid pools in the rhizosphere and roots of hydroponically grown soybeans. Important features include the use of a dry seed sterilization protocol and a sensitive method to detect slow-growing seed-associated microorganisms. With these precautions, we have been able to characterize isoflavonoid profiles both in root extracts and exudates under conditions which do not elicit stress or pathogen related responses in the soybean host. Currently we are investigating whether internal or external pool sizes of biologically active isoflavonoid compounds change following exposure to Bradyrhizobium japonicum and/or to seed-associated microorganisms.

1405 TISSUE-SPECIFIC EXPRESSION OF CELL SURFACE ANTIGENS DURING PEA NODULE DEVELOPMENT. Nicholas J Brewin, Silvia Perotto, Anne L Rae and Elmar L Kannenberg. John Innes Institute, Norwich NR4 7UH, UK

The *Rhizobium*-legume symbiosis is characterised by close physical interaction between plant and bacterial cell membranes and by progressive physiological adaptation of these membranes to accommodate the symbiotic state. In order to monitor the changing composition of membranes during pea root nodule development, we have isolated many monoclonal antibodies and studied the *in situ* expression of the corresponding epitopes using immunogold staining and silver enhancement. Epitope variation of *Rhizobium* lipopolysaccharide (LPS) in different regions of the nodule appears to reflect the physiological status of the symbiosome compartment: oxygen concentration, pH and ion status all affect the expression of the corresponding epitopes in free-living culture. These observations are discussed in relation to our model for pH balance in symbiosis. The morphology of pea nodules inoculated with LPS-defective mutants suggests that *Rhizobium* LPS is important both for endocytosis and for survival in the symbiosome compartment. Plant membrane glycolipids also vary in expression during nodule development. These glycolipids may function in cell surface interactions with *Rhizobium*; in the targetting of associated proteins to specific cellular compartments; and in physiological adaptation to *Rhizobium* invasion and symbiotic nitrogen fixation.

POSTER ABSTRACTS

1406 ENGINEERING AN INSECT RESISTANCE GENE INTO *RHIZOBIUM* AND LEGUMES. Leif Skøt and K. Judith Webb. AFRC Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, Wales.

The clover, pea and bean weevil *Sitona* sp is an important pest of forage and grain legumes. The adult insects feed on the foliage, but the larvae invade and eat the root nodules formed by N_2 fixing *Rhizobium* bacteria. This can result in serious reduction in N_2 fixation capacity and yield. The *cry*IIIA gene from *Bacillus thuringiensis* subsp *tenebrionis* encoding the δ-endotoxin crystal protein toxic to coleopteran insects was cloned and transferred to various *Rhizobium* strains with a view to using these in the biological control of *Sitona* larvae. Lower levels of the *cry*IIIA gene product were detected in *Rhizobium* bacteroids from pea root nodules than in free living rhizobia. Results of work with two new *cry*IIIA constructs driven by i) the constitutive neomycin resistance or ii) the nodule specific *nif*H promoter from *R. trifolii* designed to increase expression will be presented. Direct transformation of the forage legume *Lotus corniculatus* with the *cry*IIIA gene driven by the plant promoter CaMV 35S is being pursued. Using *Agrobacterium rhizogenes* as the vector transformants were obtained, but little or no gene product was detected. Another construct was made in which the *cry*IIIA gene is fused to the nodule specific soybean leghaemoglobin lbc_3 promoter. Putative kanamycin resistant transformants are being analysed.

1407 MUTATIONAL ANALYSIS OF THE RHIZOBIUM MELILOTI NIFA PROMOTER. Peter G. Agron, Gary S. Ditta and Donald R. Helinski. University of California, San Diego, Department of Biology and Center for Molecular Genetics, La Jolla, CA 92093

The nifA gene of Rhizobium meliloti, the bacterial endosymbiont of alfalfa, is a regulatory nitrogen fixation gene required for the induction of several key nif and fix genes. Transcription of nifA is strongly induced in planta and under microaerobic conditions ex planta. Induction of nifA in turn is positively controlled by the fixL and fixJ genes of R. meliloti, the sensor and regulator, respectively, of a two-component system responsible for oxygen sensing by this bacterium. Chemical and site-directed mutagenesis were conducted to reveal nucleotides essential for induction. Eighteen mutants, including fourteen single point mutants, were analyzed for microaerobic induction in R. meliloti. Critical residues were identified in an upstream region between -54 bp and -39 bp relative to the transcriptional start site. Evidence for direct and specific interaction of the promoter with FixJ was obtained in E. coli by isolating a promoter mutant capable of partially suppressing an inactive FixJ mutant altered in the recognition helix of a putative helix turn helix DNA binding region. Attempts at identifying an upstream activating sequence by separating regions of the promoter will also be discussed.

1408 HEME-BINDING AND KINASE DOMAINS OF THE R. MELILOTI OXYGEN SENSOR, FIXL. Ellen K. Monson, Gary S. Ditta and Donald R. Helinski. University of California, San Diego, Department of Biology and Center for Molecular Genetics, La Jolla, CA 92093

Transcription of nitrogen fixation (nif) genes in R. meliloti is induced in response to a decrease in oxygen concentration. Two regulatory genes that are responsible for mediating the oxygen response of nif genes, fixL and fixJ, have been cloned (Virts, et. al., David, et. al.). The fixL gene product is homologous to the sensor class and the fixJ gene product is homologous to the regulator class of bacterial two-component system proteins. (David, et. al.) FixL, the oxygen sensor, is an oxygen binding hemoprotein and a kinase (Gilles-Gonzalez, et. al.). Deletion derivatives of FixL were constructed, overexpressed in E. coli, and purified. An N-terminal fragment of FixL binds heme, retains the ability to bind oxygen, and has no detectable kinase activity. A C-terminal fragment of FixL is active as a kinase and fails to bind heme. Data will be presented on the domain structure of FixL.

David, et. al., Cell 54:671-683 (1988).
Gilles-Gonzalez, et. al., Nature 350:170-172 (1991).
Virts, et. al., PNAS 85:3062-3065 (1988).

1409 IDENTIFICATION AND CHARACTERIZATION OF A PROMOTER FROM FRANKIA. Joyce V. Bock, Ty Battershell, and Jerry D. Johnson, University of Wyoming, Department of Molecular Biology, Laramie, Wyoming 82071

Frankia are gram-positive, filamentous bacteria which nodulate a wide variety of woody plants and shrubs. They are capable of symbiotic nitrogen-fixation in a process which, in some ways, resembles that seen in Rhizobium-legume symbioses. Random fragments of Frankia chromosomal DNA were cloned into a promoterless LUX cassette, transformed into E. coli, and screened for bioluminescence. From 2-3000 colonies, 12 showed significant stimulation of light production. A cell-free extract which supports in vitro transcription has been derived from Frankia. Plasmid DNA was isolated from each of the luminescent clones and used as a template for in vitro transcription reactions in the cell-free extracts. One of these clones selectively stimulates transcription by the Frankia extract. The insert in this clone is a 1.9 kb Sal I fragment. Sequence analysis of this DNA has identified regions with similarity to ntr promoters from other nitrogen fixing bacteria as well as canonical elements from Streptomyces and E. coli 5'-flanking sequences. Deletion mapping and primer extension analyses have localized the promoter sequence and identified two major transcription start sites, each in a cluster of purines, approximately 50 bp apart. The upstream site is 100 bp downstream from the putative ntr element and 25 bp downstream from a motif, GANA-4GAGTT, found in the 5'-flanking region of a Frankia glutamine synthetase II gene. Supported by NSF RII-8610680.

1410 PHYSICAL MAPPING OF *Rhizobium meliloti* STRAIN 1021 REPLICONS. Rhonda J. Honeycutt, Alan G. Atherly*, Michael McClelland, and Bruno W.S. Sobral. California Institute of Biological Research, La Jolla, CA 92037. *Department of Zoology and Genetics, Iowa State University, Ames, IA 50011.

Rhizobium meliloti, the nitrogen-fixing endosymbiont of alfalfa, has its genome divided into three circular replicons. Genes involved in symbiosis and nitrogen fixation have been identified and localized on low-resolution genetic maps of each replicon. We are constructing physical maps of all three replicons from *R. meliloti* strain 1021 using pulsed-field gel electrophoresis (PFE). The physical maps ultimately will be integrated with the existing genetic maps. We have identified conditions whereby the three replicons can be separated by electrophoresis. We are using Southern hybridization with cloned genes and large DNA fragments to integrate the genetic and physical maps of each replicon. Physical maps of two of the replicons are presented (for the restriction enzymes *Pac*I, *Spe*I, and *Ase*I) along with the PFE conditions for electrophoretic separation of all three replicons.

1411 DICARBOXYLIC ACID TRANSPORT IN Azotobacter vinelandii. Mechthild Filser and Christina Kennedy. AFRC IPSR Nitrogen Fixation Laboratory, University of Sussex, Brighton, BN1 9RQ, UK

A functional dicarboxylic acid transport system is required for effective nitrogen fixation by Rhizobia during symbiosis. Transcription of the gene encoding the dicarboxylic acid transport protein (DCTA) requires RNA polymerase + sigma-54 which is also necessary for expression of nitrogen fixation genes in these organisms. In A. vinelandii sigma-54 is necessary for the expression of genes encoding both dicarboxylic transport and nitrogen fixation. We have identified two regions of the A. vinelandii chromosome which hybridize to the dctA gene of R. leguminosarum. Screening of a lambda GEM12 gene bank of A.vinelandii allowed us to isolate clones carrying these two DNA regions. One has been further subcloned and subjected to transposon mutagenesis. Introduction of these mutations into wild type A. vinelandii gave rise to a mutant which was unable to use succinate, fumarate or malate as carbon source. Data concerning the phenotype of this mutant and the genetic organization of the dicarboxylic acid transport system in A. vinelandii will be presented.

POSTER ABSTRACTS

1412 MAPPING AND ISOLATION OF AN IMPORTANT DEVELOPMENTAL LOCUS OF SOYBEAN (*GLYCINE MAX*) BY MOLECULAR GENETIC MEANS. Peter M. Gresshoff, Deborah Landau-Ellis, Roel P Funke, Randy Shoemaker* and Alexander Kolchinsky
Plant Molecular Genetics and Center for Legume Research, University of Tennessee, Knoxville, Tennessee, 37901-1071, and *USDA/ARS, Ames, Iowa, USA

Nodulation involves a wide range of biological processes fundamental to differential gene expression during development. The supernodulation trait (nts) of soybean was originally detected after chemical mutagenesis of seeds (Carroll et al, 1985, PNAS, USA 82:4162-4166). The mutation is recessive, nuclear, and segregates in a classical mendelian fashion. Twelve different alleles were isolated. The phenotype is characterized by extensive nodulation over large parts of the root system, the ability to form nodules and increased nitrogen fixation in the presence of nitrate. The locus is thought to control the amount of continued cell division permitted in the root cortex (Caetano-Anollés and Gresshoff, 1991, Annu. Rev Microbiol 45 345-382). Using an RFLP map produced from a cross between *Glycine max* and *G. sova*, we positioned the *nts* locus on tentative linkage group E (Landau-Ellis et al, 1991, MGG in press). The map produced at ISU in Ames spans about 2800 cM and contains about 300 markers. Soybean has 20 chromosomes (2n=40) and a reported genome size of 1.07×10^9 bp per haploid genome (as determined by flow cytometry). Analysis of F2 plants from a nts382 x *G. sova* cross revealed that the *nts* locus was less than 1 cM from marker pA-132. We used 4 closely linked RFLP markers in the pA-36 cluster, which is about 10 cM from pA-132 to calculate the approximate conversion ratio of genetic and physical distance in this region of the genome. By pulsed field gel electrophoresis on plant DNA digested by rare cutting enzymes like *Not*I and *Sfi*I we showed that one cM is less than 450 kb in this region (Funke and Gresshoff, 1991, in review). We were able to detect tetraploidization for part of the pA-36 cluster, while the region coding the *nts* locus, pA-36 and pA-132 were present in a diploid condition. We detected DNA fragments of about 200 kb in size by homology to pA-132. It is possible that these, or contiguous fragments carry part of the *nts* locus. We plan to compare such fragments from near-isogenic plant material using single short and arbitrary primers employed in the DAF (DNA amplification fingerprinting, Caetano-Anollés et al, 1990, BioTechnology 9:553-557) approach to determine the molecular nature of the *nts* mutations and to analyze the regulation of the gene.

1413 RICE LECTIN SYNTHESIS AND INDUCTION BY ASSOCIATIVE NITROGEN-FIXING BACTERIA. Sariya Boonlawat, Srimake Chaobongpang, Chetsadaporn Pitaksuthepong, Jiraporn Limpananont and Preeda Chaisiri.
Chulalongkorn University, Faculty of Science, Department of Biochemistry, Bangkok 10330, Thailand.

Rice lectin level in root and shoot of 4-day-old rice seedlings determined by ELISA in 6 indica rice and 2 japonica rice were found to be different. Similar results were obtained when excised roots and shoots were incubated with 5 S-cysteine and lectin biosynthesis were compared among different rice cultivars under the same condition. Immunoblot of denaturing lectin showed the majority of 18-kD subunit in every cultivar, and the 23-kD in some cultivars. Different rice variety respond differenty to inoculation by associative nitrogen-fixing Klebsiella oxytoca strain R15. In general root lectin increased up to 1.5-3 fold after inoculation by R15 for 2 weeks.

1414 IN-VIVO LOCALIZATION OF TRANSCRIPTION OF hetA. A GENE INVOLVED IN THE DIFFERENTIATION OF HETEROCYSTS. D. Holland*, C. Peter Wolk and J. Elhai**. *Dept. of Plant Breeding, ARO, Volcani Center, Bet Dagan 50250 Israel. **MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 U.S.A.

The spatial distribution of transcription of hetA, a gene involved in the process of differentiation of heterocysts of Anabaena sp. PCC 7120 has been followed by the use of hetA transcriptional fusions to the genes (luxAB) encoding luciferase from Vibrio fischeri. Video intensified photon-counting microscopy enabled us to detect light emitted from cells expressing the luciferase. hetA is transcribed in a pattern along the Anabaena filament, before obvious heterocysts are observed. Later, transcription of hetA is observed in well-defined proheterocysts. Transcriptional signals from the hetA promoter were also amplified by a binary system in which the gene (rpoT7) for the RNA polymerase from coliphage T7 was transcriptionally fused to a T7 promoter. The rpoT7-mediated transcriptional system could be a general method for amplification of transcription of genes that are weakly transcribed in organisms other than Escherichia coli.

1415 CHARACTERIZATION OF GENES FOR AN ALTERNATE NITROGENASE IN THE CYANOBACTERIUM *ANABAENA VARIABILIS*. Teresa Thiel, Department of Biology, University of Missouri - St. Louis, St. Louis, MO 63121.

Anabaena variabilis ATCC 29413 is a heterotrophic, nitrogen fixing cyanobacterium that has been reported to fix nitrogen and reduce acetylene to ethane in the absence of molybdenum (Kentemich et al., FEMS Microbiol. Lett. 15: 19-24, 1988). DNA from this strain hybridized well at low stringency to both the alternate nitrogenase gene clusters of *Azotobacter vinelandii*: similar restriction fragments hybridized to both the nitrogenase 2 (*vnf*) genes, and the nitrogenase 3 (*anf*) genes. The gene cluster was cloned from a λ EMBL3 genomic library of *A. variabilis*, mapped and partially sequenced. The nucleotide sequence obtained to date indicates that these genes do not code for a conventional nitrogenase. The deduced amino acid sequence is only about 50% similar to the *nif* genes of *Anabaena* sp. strain PCC 7120, but is about 75-89% similar (depending on the particular gene) to the *vnf* genes of *Azotobacter*. Similarity to the *anf* genes of *Azotobacter* is somewhat lower. Because of the high degree of similarity of the *A. variabilis* genes to the *vnf* genes of *Azotobacter*, we call these genes *vnf*-like. The *vnfD*-like and *vnfK*-like genes are contained within a 3.3 kb *Cla*I fragment, and that fragment may also contain a *vnfG*-like gene. A *vnfH*-like gene is not contiguous with the *vnfD*-like gene, but may be some distance away. By Southern hybridization to chromosomal DNA, using the *A. variabilis vnfDK*-like genes as probe, this gene cluster does not appear to be present in *Anabaena* sp. PCC 7120, or in the heterotrophic strains, *Nostoc* sp. strain Mac, or *Nostoc* sp. ATCC 29150. A hybridizing band of very similar size to the *A. variabilis* band is present in DNA isolated from four independent, cultured isolates of *Anabaena* from the *Azolla* symbiosis.

1417

β-KETOACYL (ACP) REDUCTASE HAS STRONG AMINO ACID SEQUENCE HOMOLOGY TO THE NOD G GENE PRODUCT

Antoni R. Slabas+, Diana S. Slabas+, Philip Sheldon$, Richard Safford*, Roy Kekwick *, Ikuo Nishida α & Robert Mackintosh§

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Plant fatty acid synthetase is a type II dissociable multienzyme system consisting of a central acyl carrier protein (ACP) and at least 6 catalytic proteins. We have purified the NADH-dependent β-ketoacyl(ACP) reductase, which catalyzes the first reduction step, from both Avocado and rape seed. Northern blots have shown that the size of the mRNA is approximately 1.1 kb. cDNA clones have been isolated both from Arabidopsis and rape seed. We have compared the amino acid sequence deduced from the cDNA clones, this shows very high homology to the Nod G gene product.

1418 COMPARISON OF TWO BARLEY NITRATE REDUCTASES Karel J. Angelis, Robert L. Warner, John Miyazaki, Kirk Schnorr, Mila Juricek and Andris Kleinhofs.
Dept. Crop & Soil Sciences, Washington State University, Pullman, WA 99164-6420

The NADH-specific and NAD(P)H-bispecific nitrate reductase (NR) genes from barley have been cloned and sequenced. Sequences for NADH-specific NR genes have been reported for several monocot and dicot species. The NAD(P)H-NR gene encodes 891 amino acids and is shorter than all NADH-NRs. Comparison of amino acid sequences indicate that the NAD(P)H-NR has homology to NADH-NRs from dicots (64%) and monocots (69%). The barley NADH-NR has much greater homology to other monocot NADH-NRs (84%) and similar homology as NAD(P)H-NR to dicot NADH-NRs (68%) . Differences in homology between NAD(P)H NR seqence and the NADH NR sequences are in the N-terminal sequences and the putative hinge regions between functional domains of the NR Both NADH and NAD(P)H NRs are quickly induced in barley by nitrate. Expression of both NRs will be discused

POSTER ABSTRACTS

1419 CHARACTERIZATION OF ALLANTOINASE IN SOYBEAN ROOT NODULES. Xiaoyan Tang, Judy Lindell, and Mary Alice Webb. Purdue University, Department of Botany and Plant Pathology, West Lafayette, Indiana 47907

Allantoinase is important in both the biogenesis and degradation of ureides in soybean. In soybean root nodules the enzyme catalyzes the final step in assimilation of fixed nitrogen into allantoic acid, the primary nitrogenous product exported from nodules to the shoot. We have used polyclonal antibodies produced against allantoinase purified from soybean seeds to study allantoinase in nodules of soybean. In previous studies we determined that antibodies reacted specifically with the 30 kD subunit of the seed enzyme in Western blot analysis of crude seed extracts. In contrast, Western blot analysis of a crude nodule extract probed with the anti-allantoinase antibodies revealed several immunoreactive bands, ranging in apparent molecular weight from about 20 to 80 kD. No reactive bands were observed on blots probed with preimmune serum. When a partially-purified nodule extract (180-fold purification) was probed with anti-allantoinase antibodies, a single band of approximately 45 kD was immunoreactive. This was also the predominant band observed in a silver-stained gel following SDS-PAGE analysis of the partially-purified extract. Antibodies reacting with the 45-kD band were purified by eluting them from nitrocellulose blots, and these affinity purified antibodies were used to reprobe a crude nodule extract. The resulting pattern of immunoreactive bands was similar to that observed with the original antibody preparation. We have not yet determined whether the multiple bands result from immunologically related isozymes or from a non-specific reaction. The sequence for the first 19 amino acids from the amino terminus of the 45-kD polypeptide was determined by Edman degradation. There was no homology between this sequence and the sequence determined for the amino terminus of allantoinase purified from soybean seeds. Continuing studies will be aimed at identifying the gene encoding the 45 kD polypeptide and comparing DNA sequence data for seed and nodule enzymes.

1420 CHARACTERISATION OF cDNA AND GENOMIC CLONES FOR A *LUPINUS ANGUSTIFOLIUS* NODULE-ENHANCED TRANSCRIPT. Kevin J.F. Farnden, Stephen J. Rice, Murray R. Grant# and Paul H.S. Reynolds#
Department of Biochemistry, University of Otago, Dunedin, N.Z. and #Fruit and Trees Division, DSIR, Palmerston North, N.Z.

Nodulins are plant gene products, expressed specifically in the root nodules of leguminous plants and are necessary for the development and maintenance of the nitrogen-fixing legume-*Rhizobium* symbiosis.

Nodulin-45 is encoded by a lupin nodule-enhanced transcript: two cDNA clones have been sequenced, one of 0.7 kb and another of 1.3 kb. The longer cDNA clone was used to isolate a genomic clone (14 kb), which appears to contain parts of two tandem-repeated nodulin-45 genes. The upstream region of one of the nodulin-45 genes was sequenced and the transcription start site was determined by primer extension and S1 nuclease mapping. The nodulin-45 transcript appears to contain a short 5' untranslated region of 13 bases and a 3' untranslated region of at least 280 bases. The open reading frame is sufficient to encode a protein of 37 kDa. The predicted translation start site is the first AUG in the transcript: flanking sequences are consistent with an initiating AUG. There is a TATA box at -27 and two CCAAT boxes at -62 and -74. There are also two copies of each of the CTCTT and AAAGAT motifs in the upstream region. These sequences may confer nodule-specific transcription.

The predicted amino acid sequence of nodulin-45 contains a possible N-terminal signal peptide, and two large glycine-rich repeated regions, of 60-70 amino acids. The function of nodulin-45 is unknown.

1421 STRUCTURE ANALYSIS OF AN EARLY NODULIN GENE FROM VIGNA UNGUICULATA. I. Dehning, A. Trese, S. Pueppke, W.J. Broughton, A. Krause. Universite de Geneve, LBMPS, 1, ch. de l'Imperatrice, CH-1292 Chambesy, Switzerland.

Compatible Rhizobium species induce the expression of nodule-specific genes in the root-hairs of legumes. These genes are classified according to the timing of their expression during nodule development into early and late nodulin genes. pVuB, a 1.3 kb cDNA fragment, was isolated from a cDNA library constructed from cowpea Vigna unguiculata (L.) Walp cv. Purple Hull root epidermis inoculated with Rhizobium fredii USDA 257. The transcription of clone pVuB was induced within 1.5 days after inoculation, indicating a response to some early signals produced by nodulation competent rhizobia.
Hybridizations between the cDNA clone pVuB and total DNA of V. unguiculata cv. Red Caloona showed the presence of a single homologous gene with a similar restriction pattern in this variety. A genomic library of V. unguiculata cv. Red Caloona was constructed in the lambda substitution vector EMBL3. The structure of genomic clones homologous to pVuB have been analysed and will be partially sequenced to determine the promoter region.

1422 Early nodulins in Vigna unguiculata induced by Rhizobium sp. NGR234. Krause A. and Broughton W. J.; L.B.M.P.S., University of Geneva, 1, ch. de l'Imperatrice, 1292 Chambesy/Geneve, Switzerland

Developmental changes in the synthesis of root-hair proteins were followed in the symbiosis between the promiscuous legume Vigna unguiculata and the broad host-range Rhizobium sp. NGR234. Comparison of the two-dimensional electrophoresis patterns of proteins isolated from root-hairs inoculated with wild-type or hair-deformation minus mutants revealed a series of 12 symbiosis-specific proteins, which can be divided into two classes. Synthesis of three proteins were repressed 4 days after inoculation with Rhizobium (class I), one of which was also a root-hair specific protein. Nine proteins were induced by Rhizobium 1, 2 or 4 days after inoculation (class II), of which three proteins (hadulins 15, 31, and 44 kD) were transiently expressed. Five proteins (including hadulins 15 and 31) were first visible 24 h after inoculation, while only one of these (hadulin 15) was not induced by R. fredii USDA257S1 (Nod^+, Fix^- on V. unguiculata). Hadulin 15 may therefore represent a strain-specific and legume-specific protein. After 2 days, three additional proteins became apparent, while another (hadulin 44) appeared on day 4. All 12 proteins seem to be involved in root-hair deformation and nodule development.
To clone the genes which encode symbiosis-specific proteins identified in V. unguiculata, a cDNA-library was constructed from mRNA extracted from root-hairs inoculated with Rhizobium sp. NGR234. Root-hairs were isolated 1 and 4 days after inoculation. Differential screening of the library is in progress.

1423 TOXIGENICS: A NOVEL APPROACH TO THE STUDY OF PLANT DEVELOPMENT. E. B. Corben, M. J. Bennett, K. Moffat, J. V. Cullimore, C. J. O'Kane, J. M. Lord. University of Warwick, Department of Biological Sciences, Coventry CV4 7AL. UK. We are currently investigating the use of toxigenics to study the development of N_2-fixing root nodules in the legume, Lotus corniculatus. Toxigenics provide a simple method for studying the effect of ablating specific cell lineages in plants by the expression of a toxin under the tight control of a cell or tissue-specific promoter. Using recombinant DNA techniques we are transforming Lotus with the toxic A chain of ricin or diphtheria toxin linked to nodule-specific promoters, (e.g. leghaemoglobin, Enod 2 (courtesy of Dr. T. Bisseling and Dr. K. A. Marcker)). We are presently assessing the effect of cell ablation within these transgenic root nodules on the development and physiology of the nodules and on the legume itself. As a further refinement to this approach we have also used conditional mutants of ricin A chain. Hence, by temperature shifts we can choose at which stage of nodule development to synthesize active toxin, and thereby control the timing of cell ablation during development. Using such an approach we can gather information on both the temporal and spatial patterns of gene expression in root nodules.

1424

Induction of early nodulin genes: effect of the R. leguminosarum nod factors on the expression of the ENOD5 and ENOD12 early nodulin genes in pea root hairs.
Nizlós Ladus[1,2], Beatrix Horvath[2] and Ton Bisseling[1]
1. Dept. of Molecular Biology, Agricultural University, Dreijenlaan 3, 6703 HA Wageningen, Netherland
2. Inst. of Molecular Genetics, Agricultural Biotechnology Center, H 2100 Godollő, Szent-Gyorgyi Albert u. 4., Hungary

During the early steps of the interaction between R. leguminosarum and pea (Pisum sativum) the bacteria secretes a mixture of nod factors (substituted oligomers of glucosamine). The bacterial nod factors can induce root hair deformation on Vicia in low concentration range (10^{-11}-10^{-14} mol/l). Based on this observation we studied whether these compounds are able to induce the expression of the infection related early nodulin genes: ENOD12 and ENOD5. Using reverse transcription and PCR amplification we could show that the mixture of factors can induce ENOD12 gene expression when factors are applied in the 10^{-8}-10^{-11} mol/l concentration range. Although there is induction of ENOD5 at 10^{-11} mol/l concentration as well, it is expressed at lower level than the ENOD12 gene.
The effects of purified factors present in the mixture will be discussed on the poster.

POSTER ABSTRACTS

1425 MsENOD12, A PROLINE RICH NODULIN IS ENCODED BY A LARGE GENE FAMILY IN ALFALFA. Manan Löbler and Ann M. Hirsch, University of California Los Angeles, Dept. Biology, 405 Hilgard Ave, Los Angeles, CA 90024

Many bacterial genes involved in the early events of the symbiosis between alfalfa (*Medicago sativa* L.) and *Rhizobium meliloti* are well characterized. We are interested in identifying plant genes which can serve as molecular markers during early nodule development. We have isolated a clone from an alfalfa root-nodule cDNA library using the PsENOD12 cDNA (1) as a probe. Subsequently we have characterized additional cDNA clones that are highly homologous to MsENOD12. Sequence comparison of the cDNAs reveals that at least three different MsENOD12 genes are expressed in alfalfa nodules. Southern analysis shows a high number of DNA fragments hybridizing to the ENOD12 cDNA,suggesting the presence of 10-15 genes homologous to MsENOD12. MsENOD12 codes for a 112 amino acid protein with a proline-rich region that is only 26% homologous to PsENOD12. The signal peptides of both pea and alfalfa ENOD12 show 92% identity. MsENOD12 is expressed only in nodules and not in flowers, leaves, or roots. MsENOD12 is not expressed in "empty" nodules induced by an exopolysaccharide mutant of *R. meliloti*. It is expressed at low levels in pseudonodules induced by NPA. As for PsENOD12, MsENOD12 transcripts are localized by *in situ* hybridization to the invasion zone of the nodule. Although the proline-rich regions of the two ENOD12 differ, the protein seems to be involved in early stages of the infection process in both pea and alfalfa.

1 Scheres, B., Van de Wiel, C., Zalensky, A., Horvath, B., Spaink, H., Van Eck, H., Zwartkruis, F., Wolters, A.-M., Gloudemans, T., Van Kammen, A., and Bisseling, T. (1990) Cell 60, 281-294

1426 REGULATION OF GLUTAMINE SYNTHETASE GENE EXPRESSION IN NODULES OF MEDICAGO TRUNCATULA. Anne Stanford, Knud Larsen, David Barker and Julie Cullimore. Laboratoire de Biologie Moléculaire des Relations Plantes -Microorganismes, CNRS-INRA, BP27, 31326 Castanet-Tolosan Cedex, France.

The plant enzyme glutamine synthetase (GS) plays a key role in the assimilation of fixed nitrogen in legume root nodules. Observations by Knight and Langston-Unkefer (1988) suggest that a change in the activity of plant GS may lead to an increase in the rate of nitrogen fixation, the degree of nodulation and the overall yield of alfalfa plants. Here we describe, firstly, the characterisation of glutamine synthetase genes from the model legume *Medicago truncatula*, and secondly, their application in genetically engineering altered levels of GS activity in transgenic plants in order to investigate the effect on nitrogen fixation and plant productivity. Three distinct genomic clones probably encoding cytosolic GS have been isolated from a library of *Medicago truncatula* and characterised through sequence analysis. These different members of the GS gene family have been shown to exhibit differential organ-specific expression, with one gene corresponding to a mRNA which dramatically accumulates during the development of the nodule, prior to the onset of nitrogen fixation. Fragments of this nodule-enhanced GS gene have been used in antisense constructions aimed at inhibiting the levels of either specific or total GS gene expression in the nodules of transgenic plants, both in the homologous host *Medicago truncatula* and the heterologous host *Medicago sativa*. Progress to date will be reported.

Knight,T.J. & Langston-Unkefer,P.J. (1988) Science, 241, 951-954.

1427 ISOLATION OF A NODULATION FACTOR FROM THE STELE OF THE ROOTS OF *Pisum sativum*. Gerrit Smit, Ben J. J. Lugtenberg and Jan W. Kijne. Leiden University, Department of Plant Molecular Biology, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands.

By releasing low-Mw mitogenic signals, *Rhizobium leguminosarum* biovar viciae bacteria induce formation of nodule meristems in the inner cortex of pea roots. Since ±90% of the nodule meristems are found in the cortex areas opposite protoxylem poles, induction appears to be determined by the position of the xylem in the central vascular tissue (the stele) of the pea root. A similar developmental pattern was found for expression of the early nodulin ENOD12 (1) and for localization of pea lectin on growing pea root hairs (2). Culturing pea root explants on SM medium supplemented with auxins and cytokinin, Libbenga et al. (3) found induction of cell divisions at similar positions in the root cortex. Addition of an extract from vascular tissue of pea roots changed this pattern into induction of cell divisions throughout the whole cortex. Apparently, (a) xylem factor(s) determine which cells respond to *Rhizobium* signals by starting mitogenic activity. Results on purification and identification of this stele factor will be presented.

(1) Scheres et al. 1990. Cell 60, 281.
(2) Díaz et al. 1986. Planta 168, 350.
(3) Libbenga et al. 1973. Planta 114, 29.

1428 A MOLECULAR AND BIOCHEMICAL ANALYSIS OF UREASE IN *ARABIDOPSIS THALIANA*. Laura E. Zonia and Joseph C. Polacco University of Missouri, Dept. of Biochemistry, Columbia, Mo. 65211

Our primary research focus is the molecular genetics of the urease isozymes in soybean. To expand these studies we chose *Arabidopsis* as a model system. An *Arabidopsis* genomic library was screened using heterologous probes derived from the soybean ubiquitous urease gene. Four unique clones were isolated. Restriction mapping analysis revealed that the four clones originated from a single locus and had extensive regions of overlap. The clones represent a full length urease gene which appears to be single copy. An RFLP for the *Arabidopsis* urease gene was identified and allowed the gene to be mapped to Chromosome 1, approximately 1.2 cM from the genetic marker *ap-1*. Urease transcript and enzyme activity were highest in young germinants. The *Arabidopsis* urease had a single pH optimum, similar to the seed-specific isozyme of soybean. The *Arabidopsis* urease crossreacted with antibody to soybean seed urease and by immuneprecipitation criteria the *Arabidopsis* urease showed closer similarity to the seed-specific than the ubiquitous urease of soybean. Gel filtration analysis gave a molecular weight of 165 kD for the holoenzyme. We developed a strategy to select urease mutants using toxic levels of urea. Seeds were mutagenized with 25 kR of γ-irradiation, M_2 seeds were collected and screened, and 51 plants were isolated which all show reduced urease activity. To determine if the lesion is in the urease structural gene, the mutants will be crossed to *ap-1* plants and F_2 segregation of *ap-1* and urease negativity will be determined.

1429 TRANSCRIPTION OF THE PLANT FERRITIN GENE IN *Phasolus vulgaris* SEED TISSUE AND *Glycine max* NODULES. Diane McLaughlin, Michael Spence, Champa Sengupta-Gopalan and Peter Lammers. Dept. of Chemistry and Program in Molecular Biology, New Mexico State University, Las Cruces, N.M. 88003.

A cDNA containing the entire coding region for the iron storage protein ferritin (*pfe*) has been isolated from the French bean plant, *Phaseolus vulgaris* using ferritin-specific antisera. A 1.2 kilobase-pair immunopositive phage DNA insert was isolated and the derived amino acid sequence shows substantial similarity with other ferritin sequences. The 251 bp 5' untranslated region contains two out of frame AUG codons and a region of biased nucleotide composition in which 61 of 66 residues are pyrimidines. In addition, potentially stable secondary structure exists in the region of the predicted translational start site. A 1.4 Kb transcript is present in young (150 mg) *P. vulgaris* seed tissue, increases in abundance at the mid-maturation point of seed development (500 mg) followed by a decline in transcript abundance in mature seeds. The *P. vulgaris pfe* clone also identifies a 1.4 Kb transcript in soybean root and nodule tissue. The steady-state level of *pfe* transcripts in developing soybean nodule tissue declines as nitrogenase activity is acquired, and is nearly undetectable 21 days after infection. However, *pfe* transcript levels do not drop in nodules derived from infections of soybean plants with *Bradyrhizobium japonicum nifA* and *nifDK* mutants. Northern blots reveal higher levels of *pfe* transcripts in the mutant associations than in wild type nodules. Western blots demonstrate that ferritin protein levels decline in wild-type nodule tissue but not in nodule tissue from the mutant associations.

1430 LOCALIZATION OF A GALACTOSE-BINDING LECTIN IN PEANUT ROOT NODULES. K. A. VandenBosch[1], L. C. Rodgers[1], and B. D. Kishinevsky[2]. [1]Texas A&M University, Department of Biology, College Station, Texas, 77843, and [2]Agricultural Research Organization, The Volcani Center, P.O.B. 6, Bet-Dagan 50-250, Israel.

Seed lectins are well known from leguminous plants, where they are sequestered in protein storage bodies derived from vacuoles. Lectins are also secreted to the surface of root hairs in the infectable zone of legume roots. Lectin is thought to mediate early interactions between the root and potential symbionts in the family Rhizobiaceae, and it appears to play a role in determining the specificity of infection thread formation. In peanut (Arachis hypogaea L.), a galactose-specific lectin and a mannose-specific lectin are expressed in root nodules (B.D. Kishinevsky, et al., 1988. Planta 176:10-18). Since peanut is not infected via root hairs, nor does it form infection threads, possible roles for these lectins are obscure. We have used immunogold labeling to map the distribution of the galactose-binding lectin in peanut nodules. The lectin was found in vacuoles in cells infected by Bradyrhizobium sp. and in uninfected cells in the nodule cortex. In infected cells, the lectin was also possibly present within the symbiosome. A symbiosome location is being studied further using cell fractionation. Secreted lectin was detected in extracellular spaces of the cortex. Future work will focus on the distribution of lectin during infection.

POSTER ABSTRACTS

1431 CHARACTERIZATION OF A GLYCOPROTEIN FOUND IN BACTEROIDS OF RHIZOBIUM LEGUMINOSARUM FROM ROOT NODULES OF PISUM SATIVUM. D.A. Dreyer and K.A. VandenBosch. Dept. of Biology, Texas A&M University, College Station, TX, 77843-3258, USA.

In continuing studies of the protein composition of the symbiosome of nodules from Pisum sativum, we have isolated a monoclonal antibody, MAC 254, which reacts with a distinctive group of symbiosome proteins. Numerous proteins in the soluble fraction of the symbiosome lumen bear the MAC 254 epitope, as does an abundant 50 kD protein found in bacteroids (BGP50). These proteins are not found in the free-living Rhizobium leguminosarum. The MAC 254 epitope is sensitive to mild oxidation with periodate, indicating that it is a carbohydrate moiety. BGP50 is bound by concanavalin A, a lectin that specifically binds mannose, giving further evidence that BGP50 is a glycoprotein. Since gram negative bacterial glycoproteins have been reported rarely, we propose that BGP50 is of plant origin. Work is ongoing to test this hypothesis. We are purifying BGP50 for the purposes of N-terminal amino acid sequencing and production of oligonucleotide probes to enable us to determine the location of the BGP50 gene. Purification of BGP50 will be detailed.

1432 FUNCTIONAL ANALYSIS OF THE PEA GLUTAMINE SYNTHETASE 3A GENE PROMOTER IN TRANSGENIC TOBACCO AND ALFALFA Timothy Brears and Gloria M. Coruzzi, Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021

The full-length GS3A promoter and a series of 5' deletions have been fused to ß-glucuronidase (GUS) and introduced into transgenic tobacco and alfalfa. In transgenic tobacco the GS3A promoter directed GUS expression in the phloem cells of the vasculature in leaves, stems, and roots. GUS expression was also detected in the vasculature of cotyledons, and the root tips of germinating T1 seedlings. The promoter conferred a similar expression pattern in transgenic alfalfa, and expression was also observed in root nodules. Nodule expression was located in nodule primordia, and the meristem, the early symbiotic zone, and vasculature of mature nodules. The promoter was found to be active even when deleted to -132 relative to the start of transcription. DNA mobility-shift analysis identified a protein, designated GS3A-F1, which was present in nuclear and whole cell plant extracts and which bound to a DNA element contained within the minimal -132 promoter required for expression. The sequence of the shortest DNA element capable of binding GS3A-F1 was TTGATCTAGTAGATAAA which comprises an imperfect palindrome spaced internally by 3 bp

1433 ANALYSIS OF NODULE-ENHANCED GENE EXPRESSION IN ALNUS GLUTINOSA. Paula Goetting-Minesky and Beth C. Mullin. Botany Department, Plant Physiology and Genetics Program and The Center for Legume Research, University of Tennessee, Knoxville, Tennessee 37996.

Alnus glutinosa is an actinorhizal plant capable of symbiotic interaction with the nitrogen-fixing bacteria Frankia. Initiation and maintenance of the root nodule environment essential for nitrogen fixation and assimilation is believed to be directed by specific differential gene expression of the host plant and the bacteria. Such gene expression has been identified in legume-Rhizobium symbioses, however none has yet been reported for this actinorhizal symbiosis. To identify host nodule-specific sequences, a nodule cDNA library has been constructed in a phagemid and screened with a subtracted probe enriched for nodule-specific sequences. Further screening for nodule-specific cDNA clones will be accomplished by differential (+/-) screening of those hybridizing clones with nodule and root mRNAs. The hybridization pattern of a recovered nodule-specific cDNA to nodule RNA and Southern analysis of the alder genomic DNA will be reported.

1434 TRANSPOSON TAGGING IN LEGUMES OF THE *LOTEAE* Jens Stougaard, Thomas Thykjær and Kurt Handberg Laboratory of Gene Expression, Department of Molecular Biology and Plant Physiology, University of Aarhus, Gustav Wiedsvej 10, DK-8000 C. Denmark.

Successful use of the legume *Lotus corniculatus* as a host for expression of nodulin genes in transgenic plants, has inspired a search for "model" legumes within the genus *Lotus*. Relative small genome sizes of diploid selffertile *Lotus* species has further sharpened this interest. A progress report on screening for a useful model legume among these species and attempts to use the maize transposable element *Ac* for tagging developmental control genes will be presented.

1435 ISOLATION AND CHARACTERIZATION OF cDNA CLONES REPRESENTING PEA ROOT HAIR SPECIFIC GENES. Panagiota Mylona, Marja Moerman, Wei Cai Yang, Ton Bisseling and Henk J. Franssen. Agricultural University, Department of Molecular Biology, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.

The outermost cell layer of the root is a uni-cell layer composed of only 2 cell types that are distinct from each other. The cells with tubular outgrowths are root hairs, and cells that do not produce these outgrowths are epidermal cells. The root hairs form the major surface of the root and are involved in uptake of minerals and water and most likely these hairs also in sensoring the environment. During Rhizobium-legume interaction Rizobium secretes factors like the recently described BF, that interfere with epidermal differentiation into root hairs and Nod Rm-1 that induces root hair deformation. Therefore studies on Rhizobium-legume interaction might provide tools to study root hair development.

Two strategies were applied to isolate marker genes that represent either root hairs specific genes or Rhizobium induced genes in root hairs.

A cDNA library was constructed against polyA+ RNA isolated from root hairs after inoculation of pea plants with Rhizobium leguminosarum. Subsequent differentially screening of this library either with cDNA prepared against polyA+ RNA from total roots and from isolated root hairs or with cDNA against polyA+ RNA isolated from root hairs from non-inoculated and Rhizobium inoculated plants, revealed the isolation of several cDNA clones representing root hair specific genes.

A second approach to isolate root hair specific genes was the isolation and purification of abundant root hair specific proteins. After partial amino acid sequence determination, oligonucleotides were designed and used in a PCR.

Data on the characterization of the various clones representing root hair specific genes will be described.

1436 EARLY GENE EXPRESSION IN ALFALFA NODULE DEVELOPMENT J.B. Cooper, R.C. Wilson, J. Sanchez and A. Belcher. UCSB, Dept of Biological Sciences, Santa Barbara, CA 93106.

We are interested in isolating and characterizing plant genes expressed specifically in the meristem of symbiotic root nodules. Since 3-4 day old alfalfa nodules are enriched in both menstematic and infection activities, we isolated RNA from 3 day spot-inoculated nodules and used this RNA to construct a cDNA library in λZAPII. Two strategies are being utilized to identify early nodulin cDNAs. First, since arabino-galactan proteins (AGPs) are known to be expressed at high levels in mature root nodules, and are thought to be involved in the organization of the root meristem, we screened the cDNA library with a 26 bp oligonucleotide probe degenerate for an AGP consensus peptide sequence. Nine recombinant clones showed strong hybridization to the AGP probe, and insert DNAs from several of these clones show differential hybridization to root and nodule cDNA probes. Preliminary sequence analysis reveals homology between one of the putative AGP clones and PsENOD5, an infection-specific early nodulin. Expression of the putative AGP genes is being studied by RNA blot analysis using RNAs isolated from spot-inoculated nodules at various developmental stages, and nodules induced by mutant strains of *Rhizobium*. To determine the spatial pattern of AGP homolog gene expression in developing nodules, *in situ* hybridizations of nodule sections using anti-sense probes are being performed. The early nodule cDNA library is also being screened differentially with root and nodule cDNA probes. Nodule-specific clones identified in this screen will be characterized in a similar manner to that described above for the AGP clones.

POSTER ABSTRACTS

1437 EARLY NODULIN GENES IN *MEDICAGO TRUNCATULA*
J.B. Cooper, J. Patterson, E.M. Maruoka, J. Sanchez, and A. Belcher
UCSB, Dept of Biological Sciences, Santa Barbara, CA 93106.

Cloned early nodulin genes can provide powerful tools for investigating the pathway(s) leading to morphogenesis and invasion of symbiotic root nodules. To develop such tools, we are characterizing ENOD2 and ENOD12 gene homologs from *M. truncatula*. We constructed an *M. truncatula* genomic library and isolated several ENOD2 homologs by low-stringency screening with an alfalfa ENOD2 cDNA probe. DNA inserts were restriction mapped, and hybridizing fragments were used to probe northern blots. One homolog (Mt8-2) specifically hybridizes to a transcript in nodules, but not roots, of both *M. truncatula* and *M sativa*. An internal 0.7 kb Sal I fragment of 8-2 has been sequenced and shows 65% identity to the A2ENOD2 cDNA sequence. A second homolog (Mt7-1) hybridizes with transcripts in both roots and nodules indicating that ENOD2 may have evolved from a root proline-rich protein gene. Homologs 7-1 and 8-2 are being sequenced; internal subclones are being used to localize transcripts in nodule and root tissues by *in situ* hybridization. We also screened the library for ENOD12 homologs using a pea ENOD12 probe generated by PCR. Two classes of clones were isolated, one of which hybridizes to a nodule-specific transcript. The DNA sequence of this putative ENOD12 homolog is currently being determined. Transcription start sites for the ENOD genes are being mapped by primer extension, and the 5' flanking regions will be fused to the GUS reporter gene in a new *A. rhizogenes* cointegration vector. Transgenic root nodules containing the GUS fusions will be used to analyze the cis-acting regulatory elements responsible for the observed expression patterns and the response of these elements to symbiotic signal molecules.

1438 PROMOTER STUDIES OF ENOD12 EARLY NODULIN GENES OF PEA (*PISUM SATIVUM*)
Irma Vijn, Igor Kardailsky, Francine Govers and Ton Bisseling, Agricultural University, Department of Molecular Biology, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.

During root nodule formation of pea (*Pisum sativum*), which is induced by *Rhizobium* bacteria, the ENOD12 early nodulin genes are expressed at the early stages of the *Rhizobium*-legume interaction, where they are involved in the infection process. The ENOD12 genes can be induced in root hairs of pea plants with purified nod factors (substituted oligomers of N-acetyl glucosamine) of *R leguminosarum*. Therefore the ENOD12 genes can be target genes of the nod factors and this is the reason why we want to study the promoters of the ENOD12 genes.

In pea there are two different ENOD12 genes. By using gene specific sequences it was shown that both genes are expressed in a similar way during the *Rhizobium*-plant interaction. Both ENOD12 genes have been isolated from a genomic library and the promoters will be studied. To unravel the mechanisms involved in the regulation of the two ENOD12 genes a first step will be the identification of the cis-elements. Initially these promoter studies will be carried out in transgenic *Lotus corniculatus* plants.

1439 MAIZE GLUTAMINE SYNTHETASE GENES: REGULATED EXPRESSION DURING DEVELOPMENT AND IN RESPONSE TO METHIONINE SULFOXIMINE.
Min-gang Li and D. Peter Snustad, University of Minnesota, Department of Genetics and Cell Biology, St. Paul, Minnesota 55108. Glutamine synthetase (GS) plays a central role in the assimilation of ammonia in higher plants. Maize contains a single chloroplastic GS (MGS_2) gene and at least five cytosolic GS (MGS_{1-1} through MGS_{1-5}) genes. The MGS_2 transcript is 1.5kb in size, whereas the MGS_1 transcripts are all about 1.4kb in length. Thus, MGS_1 and MGS_2 transcripts are easily distinguished on northern blots. The sequence of the MGS_2 cDNA predicts a precursor protein with a 41-amino-acid chloroplast transit peptide and a mature GS of size 42kD, whereas the MGS_{1-1} cDNA sequence predicts a cytosolic GS of size 39kD. The MGS_{1-1} and MGS_2 sequences predict proteins with 79% sequence identity. Characterization of the other MGS_1 cDNAs is in process. Noncoding 3' gene-specific hybridization probes (GSPs) have been constructed for MGS_{1-1} and MGS_2 and used to investigate the patterns of transcript accumulation for these genes. Northern blot analyses of poly(A+)RNAs from leaves, stem, shoot, root, root tip, embryo, endosperm, immature cob, pollen and young tassel probed with the GSPs showed that the MGS_{1-1} transcript is present almost exclusively in roots, whereas the MGS_2 transcript accumulates preferentially in green tissues. Northern blot analyses of RNAs isolated from control and methionine sulfoximine (MSO)-treated Black Mexican Sweet culture cells demonstrated that MGS_1, but not MGS_2, transcript levels are dramatically up-regulated in response to MSO. Current experiments are focused on characterization of the observed regulation in BMS cells and on extending the analysis to maize seedlings.

1440 GENE EXPRESSION IN INCOMPATIBLE ASSOCIATIONS BETWEEN *RHIZOBIUM* AND PEAS.
S. Ulvang, B Solheim and T. V. Bhuvaneswari, University of Tromsø, Norway

Cytology and host gene expression in incompatible interactions between *Pisum sativum* cv. Afghanistan and the European strains of *Rhizobium leguminosarum* biovar *viceae* was studied. Cytological observations indicate that both the blocking strain and the isogenic non-blocking mutant of this strain infect and also initiate cortical cell divisions. The expression of selected early nodulin (ENOD) genes and disease resistance response genes (DRRG) in the different associations were investigated by *in situ* hybridization with ^{35}S labelled antisense RNA probes. There were no qualitative differences in the expression of these host genes in the compatible and incompatible associations, except for the expression of ENOD2, which was only detected in the nodules.

1441 STRUCTURE AND EXPRESSION OF NODULE-SPECIFIC GLUTAMINE SYNTHETASE GENE FROM YELLOW LUPIN.
L. Boron[1], K. Szczygłowski[1], L. Willmitzer[2], and A. B. Legocki[1]
[1]Institute of Bioorganic Chemistry, Noskowskiego 12, Poznań, Poland.
[2]Institut für Genbiologische Forschung Berlin GmbH, Ihnestraße 63, 1000 Berlin 33, Germany

Two genomic clones coding for nodule-specific form of glutamine synthetase (LINGS) in yellow lupin were identified and characterized.

The LINGS gene did not hybridize with root RNA, and was expressed on the 21st day after the plant had been infected with *b. lupini*. It reveals exon-intron organization similar to alfalfa GS gene. We present the analysis of the promoter region, the transcription start side (position -80 upstream of initiating AUG) and location of regulatory motifs.

To approach the mechanism of its regulation, chimeric gene fusion was made between the GS promoter region and GUS reporter gene, and the construct was assayed in transgenic *N. tabacum* and *L. corniculatus* plants.

Fragment 1.6 kb from 5' flanking region was found to be able to direct the appropriate expression patterns of GUS expression in both plants.

1442 CHARACTERIZATION OF THE SOYBEAN ASPARTATE AMINOTRANSFERASE ISOZYME-GENE SYSTEM.
Gregory J. Wadsworth, Barbara J. Wilson, and Benjamin F. Matthews, USDA/ARS, Plant Molecular Biology Laboratory, 10300 Baltimore Ave, Beltsville, MD. 20705

Aspartate aminotransferase (AAT) plays multiple metabolic roles in plants including nitrogen assimilation, hydrogen shuttles, and amino acid biosynthesis. Plants characteristically have multiple isozymes of aspartate aminotransferase encoded by multiple genes. In soybean we can distinguish five different isoforms electrophoretically, named according to their mobility from slowest to fastest (1-5). Two of the isozymes are spatially and temporally limited in their expression. AAT-1 is detected only in the postgermination cotyledon and AAT-3 is found only in green tissues such as green leaves. Organelle fractionation on sucrose gradients indicates the AAT isozymes are localized in different subcellular compartments. AAT-1 is localized in the glyoxysome, AAT-4 is localized in the mitochondria, and AAT-5 is localized in the plastids. AAT-2 and AAT-3 do not sediment with membrane bound organelles in the sucrose gradients and may be cytosolic isozymes. We have isolated three soybean cDNA clones which can encode proteins with significant homology to carrot and vertebrate AAT. Restriction and sequence analyses indicate that these three clones represent three different soybean AAT genes. These cDNA clones hybridize to soybean genomic blots in simple but unique patterns, indicating there is one or very few copies of each of these AAT genes. Transcripts corresponding to these cDNA clones were detected in a wide variety of soybean tissues with Northern blot analysis. The transcripts levels in each tissue differed for each of the cloned AAT genes. The exact relationship between the cDNA clones and the AAT isozymes has not been determined.

POSTER ABSTRACTS

1443 SOYBEAN GENES ENCODING ASPARTOKINASE AND HOMOSERINE DEHYDROGENASE. Jane M.Weisemann, Barbara J. Wilson and Benjamin F. Matthews. USDA/ARS/Plant Molecular Biology Laboratory, Building 006, BARC-West, Beltsville, MD 20705.

Aspartokinase (AK) is the first enzyme in the pathway for synthesis of the amino acids lysine, threonine, and methionine from aspartate. Homoserine dehydrogenase (HSDH) catalyzes the third step in the branch of the same pathway leading to formation of threonine and methionine. In soybean (Glycine max) two forms of AK and four forms of HSDH have been identified. We have isolated a partial cDNA clone which appears to encode a bifunctional AK-HSDH protein with homology to carrot AK-HSDH and E. coli AK-HSDH. A fairly complex hybridization pattern is observed when this clone is used to probe soybean genomic DNA on Southern blots. The pattern suggests that soybean AK-HSDH may be part of a small family of related genes. When hybridized to RNA from soybean tissues and cell suspension cultures, a transcript of 3 kb and a smaller RNA band are detected.

1444 THE AT BINDING DOMAINS OF SOYBEAN NODULIN AND FRENCH BEAN SEED STORAGE PROTEIN GENES. H. Gamblel and C. Sengupta-Gopalan, Plant Genetic Engineering Laboratory, Mol. Biol. Prog., Agron & Hort. Box 3 GL, New Mexico State University, Las Cruces, New Mexico 88003.

AT-rich DNA sequences present in the 5' flanking region of some plant genes, have been implicated to have a role in the regulation of their expression. Our study has focused on the AT-rich elements of a soybean nodulin (N-20) gene and genes for two seed proteins (β-phaseolin and phytonemagglutenin-L) from french bean. We have shown that these AT elements from the different genes interact with nuclear extracts to form similar patterns of low and high order complexes in vitro. AT binding domains were found in the 3' flanking region (and in at least one intron) of the N-20 and β-phaseolin gene which are capable of forming the same set of complexes as the elements in the 5' flanking region. Though the pattern of complex formation could not be attributed to tissue specific factors, the ability to form higher order complexes did vary with the source of nuclear factors. Partial purification and southwestern analysis indicate that these interactions are due in part to a widely distributed class of high-mobility group-like proteins that appear to specify alternative chromatin structures. Data will be presented to test working models that integrate the potential structural and regulatory roles of these proteins and DNA elements in vitro.

1445 GENETIC MANIPULATION OF GLUTAMINE SYNTHETASE GENE EXPRESSION IN TOBACCO AND ALFALFA. S.J. Temple[1], P.J. Unkefer[2] and C. Sengupta-Gopalan[1]. New Mexico State University[1], Plant Gen Eng Labs, Dept. of Agron. and Hort., Las Cruces, NM 88003. Los Alamos National Labs[2], Los Alamos, NM 87545

Glutamine synthetase (GS) which plays a key role in ammonia assimilation has been the primary focus of attention in our attempt to understand the regulatory mechanisms associated with N_2 fixation, nodulation, and ammonia assimilation in crop plants. In an attempt to understand the regulatory role(s) of GS, we have transformed both tobacco and alfalfa via *Agrobacterium tumefaciens* mediated gene transfer with a full length alfalfa cytoplasm GS cDNA both in sense and antisense orientation behind the CaMV 35S promoter. Leaves from tobacco plants transformed with the sense construct contained greatly elevated levels of GS transcript and a 39 kD immunoreactive polypeptide that comigrates with tobacco GS_1. Activity staining of native PAGE demonstrated that this novel GS polypeptide was correctly assembled into an active GS isoenzyme. Analysis of the tobacco plants transformed with the antisense GS construct, showed a reduction in the levels of endogenous leaf GS polypeptides. Transgenic tobacco plants with sense and antisense GS construct, showed a reduction in the levels of endogenous leaf GS polypeptides. Transgenic tobacco plants with sense and antisense GS constructs are being analyzed to check if altered GS expression results in changes in the level of expression of other relevant genes associated with N_2 and carbon assimilation. Transformed alfalfa plants will be tested for the effect altering GS expression levels has on nodule initiation and N_2 assimilation potential in nodules.

1446 EXPRESSION OF MEMBERS OF THE GLUTAMINE SYNTHETASE GENE FAMILY DURING ROOT NODULE DEVELOPMENT IN SOYBEANS. D. Roche, S.J. Temple and C. Sengupta-Gopalan. New Mexico State University, Dept. of Agron. & Hort., Molecular Biology Program, Las Cruces, NM 88003

Onset of N_2 fixation during root nodule development in soybeans is accompanied by an increase in glutamine synthetase activity. In order to understand if increased GS activity is due to the induction of gene members not normally expressed in roots, we have isolated and characterized several GS cDNA clones from a nodule cDNA library. DNA sequence analysis of these cDNA clones showed high sequence conservation in the coding region while the 3' noncoding region showed heterogeneity. Difference in the 3' noncoding region included changes in the selection of polyadenylation sites and complete divergence in sequence in some cases. The 3' noncoding regions are being used as gene member specific probes to monitor expression of the corresponding genes during the different stages of root nodule development. To check if induction of some of the GS gene members are regulated by N_2 fixation, gene member specific probes will be used to monitor expression in nodules formed by *B. japonicum* strains that are defective in structural nitrogenase genes.

1447 CHARACTERIZATION AND GENOMIC ORGANIZATION OF A HIGHLY EXPRESSED NODULIN GENE SUBFAMILY. Hannes E. Richter and C. Sengupta-Gopalan. New Mexico State Univ, Dept. of Agron & Hort., Mol. Bio. Prog., Las Cruces, NM 88003.

Several plant genes are specifically induced in the soybean root nodule coincident with the release of bacteria from infection threads and the formation of the peribacteroid membrane. Several of these highly expressed genes encoding for nodule specific proteins, have conserved domains that translate into a hydrophobic amino terminal end typical of signal peptide and protein domains resembling metal binding sites (1). Five of these related gene members have been characterized to some extent (1,2). Our study has focused on the identification and characterization of new gene members. Based on the sequence analysis of cDNA and genomic clones, SDS PAGE analysis of their hybrid select translation products and genomic Southerns, we have identified a gene subfamily consisting of three functional genes and a truncated member. Our results suggest linkage of the four genes and show high degree of sequence conservation not only in the coding region but also in the 5' and 3' flanking regions. The gene subfamily is expressed at higher levels than the leghemoglobin gene family and the encoded proteins are membrane associated. Recent duplication, gene conversions and selection pressure are suspected to control the evolution of this gene subfamily. Furthermore, our results demonstrate the existence of extensive RFLP among different soybean cultivars containing this gene cluster suggesting rapid evolutionary changes.

1. Sandal et al., (1987) Nuc Acid Res. 15:1507-1519.
2. Jacobs et al., (1987) Nuc Acid Res 15:1271-1280.

1448 INDUCTION OF PHENYLALANINE AMMONIA LYASE AND CHALCONE SYNTHASE GENES IS RELATED TO CORTICAL CELL PROLIFERATION IN SOYBEAN NODULE DEVELOPMENT. Elizabeth M. Estabrook, Inez Feder , and Champa Sengupta-Gopalan. New Mexico State University, Mol. Bio. Prog., Dept. of Agron. & Hort., Las Cruces, NM 88003.

Flavonoids released by the legume root interact with the Rhizobium/Bradyrhizobium NodD protein to activate transcription of other nod genes to allow nodulation to begin. Phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) play key roles in the phenylpropanoid pathway from which flavonoids are derived. Our studies have focused on the expression of PAL and CHS genes in soybean nodule development to determine other roles phenylpropanoid compounds may have in symbiosis. We have shown the induction of specific PAL and CHS genes two days following infection of soybean roots with *B. japonicum*. The level of induction is correlated to the number of successful infections. While the genes are induced normally in nodules arrested at the cortical cell proliferation stage, induction does not occur in plant-bacterial interactions that do not allow root hair curling or infection thread formation. The results suggest that in root nodule development, flavonoid synthesis is associated with cortical cell proliferation. Since flavonoids have been shown to act as auxin transport inhibitors and also as cytokinins, we can infer that cortical cell proliferation results from hormonal changes at the sites of nodule initiation; hormonal changes being reflected by changes in flavonoid content. We are presently testing the signaling mechanism involved.

POSTER ABSTRACTS

1449 STUDIES ON THE REGULATION AND REGULATORY ROLE OF GLUTAMINE SYNTHETASE IN LEGUMINOUS PLANTS.
Malcolm J. Bennett, Gregory J. Anderson, Andrew P. Morby and Julie V. Cullimore. Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, UK.

Glutamine synthetase (GS) assimilates the ammonium derived from the reduction of the primary nitrogen source, for example from dinitrogen fixation and nitrate reduction, and from deaminating reactions such as those which occur during photorespiration and amino acid catabolism. GS is therefore in a key position to control the acquisition of nitrogen, the major limiting nutrient for plant growth, and its cycling during metabolism. In the legume *Phaseolus vulgaris* there are four functional GS genes which encode three cytosolic GS polypeptides and a precursor to the chloroplast-located GS. These genes are expressed to different extents in various organs and we have shown that changes in expression of these genes lead to alterations in the abundances of the GS polypeptides and the activities of the isoenzymes. Thus transcription appears to be a major factor regulating GS activity. In order to study the possible role of GS in regulating the flux through the nitrogen assimilatory pathway, constructs have been prepared to alter the activity of this enzyme in transgenic plants. In this poster results on the regulation of this enzyme in *P. vulgaris* and in these transgenic plants will be presented.

1450 NODULIN GENE EXPRESSION IN CHICKPEA (Cicer arietinum L.) NODULATION MUTANTS. P. V. Shri and Thomas M. Davis, Depart. of Plant Biology, Univ. of New Hampshire, Durham, NH 03824.

Heterologous nodulin cDNA probes from alfalfa and soybean were used to investigate nodulin gene expression in chickpea through northern analysis of ineffectively nodulating mutants PM405 and PM638 (white, non-fixing nodules), PM796 (poorly fixing, prematurely senescent nodules) and non-nodulating mutant PM233. These plant mutants are isogenic to wild type parent ICC640, differing only with respect to single nodulation gene mutations. Total RNA from roots/nodules was probed with cDNA clones A2ENOD2, leghemoglobin (Lb) and glutamine synthetase (GS) from alfalfa and nodulin-24 (N-24) and Lb from soybean. Lb mRNA was present at wild type levels in PM796 nodules, reduced levels in PM638 nodules, but absent in PM405 nodules and in root tissue of PM233 and uninoculated wild type. ENOD2 mRNA was detected in ICC640, PM796, PM405 and PM638 nodules but not in PM233 and uninoculated wild type root tissue. No differences were found in levels of mRNA hybridizing to N-24 and GS probes in uninoculated root or inoculated wild type root/nodule tissue. Results of northern analysis of chickpea nodulation mutants using other nodulin probes [including cDNA clones sucrose synthase and uricase (from soybean) and ENOD3 (from pea)] will be presented.

1451 MOLECULAR CHARACTERIZATION OF THE NODULIN 30 GENE FAMILY FROM Phaseolus vulgaris ROOT-NODULES. *Francisco Campos, Carolina Carsolio, Mario Rocha-Sosa and Federico Sánchez. Center for Genetic Engineering and Biotechnology, Department of Plant Molecular Biology, Universidad Nacional Autónoma de México, Apdo Postal 510-3 Cuernavaca Morelos, México.

Nitrogen-fixing nodules are novel organs that develop in the stems or roots of leguminous plants elicited by Rhizobium or related genera of bacteria. During the formation of the nodule, a set of nodule-regulated genes or nodulin genes are differential expressed. One of the most abundant nodulin transcripts in P.vulgaris nodules is nodulin-30 (Npv-30). In our group we have cloned and sequenced several cDNAs and a genomic clone. DNA sequence and genomic blot data suggest that this nodulin is encoded by a gene family. Nodulin-30 deduced protein sequence indicates the presence of two "Zn finger" motives, the aminoterminal domain contains a putative signal peptide for membrane translocation and a stretch of 20 proline residues at the carboxy end. This nodulin has some regions of homology in the putative "Zn finger" motives with nodulin-27 from soybean. We have generated a DNA probe from the region that contains the most conserved Zn finger motives between nodulin-27 from soybean and nodulin-30 from common bean, in order to establish the number of members from this Zn finger gene family. Specific antibodies have been raised in rabbits against a fussed protein expressed in E. coli. In situ hybridizations and immunocytological studies with the specific antibodies are in progress in order to attain insite into the possible role of this nodulin in common bean root nodules.
*This provect is partially supported by an EEC. Grant.

1452 CLONING OF A TIMOTHY NITRATE REDUCTASE GENE BY PCR AMPLIFICATION OF LEAF RNA. Serge Laberge[1], Isabelle Ciprès[2], Hank A. Margolis[2], Wilbur H. Campbell[3] and Louis-P. Vézina[1]. Research Station, Agriculture Canada, Ste-Foy, Québec, Canada G1V 2J3[1]; Département des Sciences Forestières, Faculté de Foresterie et Géodésie, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4[2]; Department of Biological Sciences, Michigan Technological University, Houghton, MI 49931 USA[3].

Timothy (*Phleum pratense*), a perennial grass, is the most important forage crop in Eastern Canada. Since its cultivation requires substantial addition of fertilizers after each harvest, much effort has been directed towards minimizing fertilizer inputs by optimizing its use of mineral nitrogen. In this project, we were looking for a rapid method of producing a probe specific for nitrate reductase in timothy in order to use it in time course studies on gene expression. The nucleotide sequences of various plant nitrate reductase genes were compared and two strongly conserved regions (90 bp each) were identified which were about 250 bp apart. Two oligonucleotides were synthesized for each conserved region, giving rise to four possible oligonucleotide combinations. We were unable to obtain any specific PCR amplification of the nitrate reductase gene from the total DNA of timothy. Using ss-cDNA synthesized from mRNA or total RNA, we were able to amplify specific fragments using two oligo combinations. DNA sequencing was done on a 240 bp fragment and the sequence analysis showed that this fragment is highly homologous to various other plant nitrate reductases. The DNA was then used as probe on a Southern blot which contained restriction enzyme digests of total DNA from various other plants. The fragment specifically hybridized with the timothy DNA. We are currently trying to isolate nitrate reductase genes from various plants using the same oligonucleotides in order to determine the extent to which this method can be applied to other plants. We are also isolating the complete timothy nitrate reductase gene which will then be used as a probe in physiological studies.

1453 TARGETING AND ANCHORING OF SOYBEAN NODULIN-24 TO THE PERIBACTEROID MEMBRANE. C.-I. Cheon & D. P. S. Verma, Ohio State Biotechnology Ctr., 1060 Carmack Rd, Columbus, OH 43210

Nodulin-24 (N-24) of soybean, a peribacteroid membrane (PBM) protein, was shown to be co-translationally processed into a 20kD protein. However, antibody against nodulin-24 reacted in western blotting of PBM with a protein of 33kD. In order to follow the PBM targeting and processing mechanisms, nodulin-24 cDNA was cloned into pGEM2 having a bacteriophage T7 promoter and was transcribed *in vitro*. The resulting RNA was translated using rabbit reticulocyte lysate and ^{35}S-Met or ^{3}H-Leu with/without canine microsomal membranes. Signal sequence cleavage was observed. Processed product of N-24 was subjected to Edman degradation and the cleavage site was determined to be between Ala(25) and Arg(26). N-24 cDNA was fused to GUS reporter gene and the construct was transcribed and translated *in vitro* in the presence of microsomal membranes. The fusion product was found to be co-translationally translocated into the membrane and was resistant to the tryptic digestion. In addition, N-24 was found in the supernatant fraction when microsomal membranes were briefly sonicated and centrifuged after *in vitro* translation. These results and sequence analysis data indicated that N-24 has no membrane spanning region and following translation it is present in the lumen of the microsomes. Since N-24 is a PBM protein, it must be post-translationally anchored to the membrane and targeted to the symbiotic compartment. The attachment apparently occurs in the Golgi apparatus before fusion of the vesicles to the PBM. To find out the region responsible for PBM targeting various deletions of N-24 cDNA were fused to GUS gene and introduced into *Lotus corniculatus* under the 35S-CaMV promoter, and the presence of GUS protein was examined using immunocytochemistry. Using the same transgenic plants, it is being investigated where the N-24 is localized in the absence of PBM compartment.

1454 FRUCTOSE 1,6-BISPHOSPHATE ALDOLASE mRNA LEVELS IN ALFALFA ROOTS AND NODULES. Veena Sangwan[1], Ruby Larson[2], Susan Miller[2], Carroll Vance[2,3] and Steve Gantt[1]. University of Minnesota, Departments of Plant Biology[1] and Agronomy and Plant Genetics[2] & USDA-ARS, Plant Science Research[3], St. Paul, Minnesota 55108

Fructose 1,6-bisphosphate aldolase catalyzes the conversion of fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Plants have two types of aldolases, one that functions in Calvin cycle reactions which occur in the chloroplast and the other, located in the cytosol, that functions in glycolysis and gluconeogenesis. We have isolated a cDNA that encodes the cytosolic form of aldolase by antibody screening an alfalfa lambda gt11 library prepared from root nodule poly(A)$^+$ RNA. The encoded protein is 82% identical to maize cytosolic aldolase. Expression of the cytosolic aldolase gene is induced during anaerobiosis in roots. Therefore, in aerobically grown alfalfa plants we expected that this mRNA would be more abundant in nodules than in roots due to the microaerobic environment of the nodule. Unexpectedly, Northern blot analysis of total RNA extracted from roots and nodules shows a greater amount of aldolase mRNA in roots than in nodules. Expression of the aldolase gene at the cellular level will be examined by *in situ* hybridization experiments.

POSTER ABSTRACTS

1455 CLONING AND CHARACTERIZATION OF AN ASPARTATE AMINOTRANSFERASE cDNA FROM ALFALFA ROOT NODULES AND ITS EXPRESSION DURING NODULE DEVELOPMENT. Sudam Pathirana[1], Ruby Larson[2], Mark Farnham[2], Susan Miller[2], Carroll Vance[2,3] and Steve Gantt[1]. University of Minnesota, Departments of Plant Biology[1] and Agronomy and Plant Genetics[2] & USDA-ARS, Plant Science Research[3], St. Paul, Minnesota 55108

Aspartate aminotransferase (AAT) catalyzes the reaction Glutamate + Oxaloacetate ↔ Aspartate + α-ketoglutarate. In plants, AAT plays an essential role in the assimilation of reduced nitrogen into aspartate and asparagine, particularly in legume nodules; the transfer of fixed carbon from mesophyll to bundle sheath cells in C_4 plants; and in the transfer of reducing equivalents to chloroplasts, mitochondria and peroxysomes through the malate aspartate shuttle. To ascertain the molecular genetic characteristics and biochemical regulation of AAT, we have isolated a near full length cDNA clone encoding the nodule enhanced form of AAT, AAT-2, by screening an alfalfa root nodule cDNA library with AAT-2 antibodies. The identity of the clone was verified by its ability to rescue an *Escherichia coli* AAT⁻ mutant. The increase in AAT activity during effective nodule development is accompanied by an 8-fold increase in AAT-2 mRNA and a comparable increase in enzyme protein. AAT-2 mRNA is present in plant-controlled ineffective nodules but at a much lower level than in effective plants. Expression of AAT-2 appears to be regulated by at least two events: the first is independent of nitrogenase activity and the second is associated with nodule effectiveness

1456 EARLY NODULIN GENES FROM THE MODEL LEGUME *MEDICAGO TRUNCATULA*. Magalie Pichon, Etienne-Pascal Journet, Annie Dedieu, Françoise De Billy, Georges Truchet and David G. Barker. *Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganismes, CNRS-INRA, BP 27, 31326 Castanet-Tolosan Cedex, France.*

A number of host genes are specifically expressed during the earliest stages of the *Rhizobium*-legume interaction (root hair infection) and also during nodule organogenesis, processes which lead to the formation of the nitrogen-fixing root nodule. For example, the pea ENOD12 gene is expressed in the nodule primordium, and in the infection zone throughout subsequent nodule development[1]. Furthermore, ENOD12 mRNA can be detected in pea root hairs following the addition of either the intact microsymbiont or extracellular factors produced by the *Rhizobium* after *nod* gene induction. The gene product of ENOD12 is comprised of (hydroxy)proline-rich repeat motifs and is thought to be a cell-wall protein involved in the infection process[2]. We are currently studying the regulation of nodule-specific genes in *Medicago truncatula*, a diploid self-fertilizer which we have adopted as model legume in order to combine both molecular and genetic studies of the host in the symbiotic interaction with *Rhizobium meliloti*. By using the pea ENOD12 cDNA as probe, we have isolated a number of genomic clones from a *M. truncatula* gene library. Analysis of the coding sequences has shown that one of these genes is homologous to the pea ENOD12 gene, whereas a second gene encodes a different (hydroxy)proline-rich polypeptide. Both genes are expressed at high levels in young developing nodules, with a dramatic decrease in mRNA abundance during nodule maturation. So far, transcripts corresponding to the two genes have not been detected in uninoculated roots or other plant organs. The cell-specific expression of these two *M. truncatula* early nodulin genes is being studied by *in-situ* hybridization using nodules at various stages of development. Experiments are also in progress to examine the effects of the addition of *R. meliloti* extracellular symbiotic factors such as NodRm-1 on ENOD12 expression in *M. truncatula* plantlets, root cultures and cell suspension cultures

1) Scheres *et al.* (1990) *Cell* 60 281-294
2) Barker *et al.* (1990) *Plant Molecular Biology Reporter* 8, 40-49

1457 DNA SEQUENCE ANALYSIS OF ASPARTATE AMINOTRANSFERASE GENOMIC AND cDNA CLONES FROM LUPIN. Paul Reynolds, James Dickson, William Jones, Karen Rodger, Stephen Jones, Christopher Winefield[1], Alan Carne[1] and Kevin Karnden[1]. DSIR Fruit and Trees, Palmerston North, New Zealand and [1]Biochemistry Department, Otago University, Dunedin, New Zealand.

Two isoforms of aspartate aminotransferase, AAT-P1 and AAT-P2, have been characterised from lupin nodules. The second of these is implicated in assimilation of ammonia following the onset of nitrogen fixation.
AAT-P2 was immunopurified using a specific monoclonal antibody against this isoform. The purified protein was subjected to tryptic cleavage and the fragments purified using a pepRP column. Seven of these fragments was sequenced. Five of the fragments were aligned against AAT amino acid sequences from animals and E. coli and two showed no homology against the database. Two oligonucleotides were synthesised to encode (a) part of one peptide which aligned near the amino terminus and (b) part of a second peptide which showed no homology with database AAT amino acid sequences. RNA was isolated from lupin nodules and, using reverse transcriptase, a DNA copy was made which served as a template for PCR using the two oligonucleotides as primers. This resulted in a 158bp product which lined up with amino acids 7 to 58 from the database. The same primers yielded a 1100bp product using genomic DNA as template. Lupin nodule cDNA and genomic libraries have been screened and clones are currently being characterised.

1458 CLONING AND CHARACTERIZATION OF TWO DIFFERENT cDNA FAMILIES ENCODING α-AMYLASE IN POTATO. Jette D.Kreiberg* and Kirsten Gausing. University of Aarhus, Department of Molecular Biology and Plant Physiology, DK-8000 Aarhus C, Denmark; Present address:* DANISCO A/S, MARIBO Biotechnology, Langebrogade 1,DK-1001 Copenhagen K.

cDNA clones encoding α-amylase were isolated from a potato sprout cDNA library using a barley α-amylase as probe. The potato α-amylase cDNA clones can be subdivided into two families. The first (called AmyZ1) shows 64-67% amino acid homology with the barley α-amylases and 73-78% homology with a mung bean α-amylase. The second family (called AmyZ3/4) is divergent and is the first reported example of an α-amylase showing only 42-46% homology to either the dicot (potato and mung bean) or the monocot (barley, wheat and rice) α-amylases. The AmyZ3/4 family encode a full length α-amylase polypeptide of 407 amino acid residues including a putative signal peptide of 18 amino acids. Southern analysis of potato DNA with AmyZ1 type sequences indicate that this α-amylase family is encoded by a few genes whereas the AmyZ3/4 α-amylase probably is encoded by a single gene.

1459 NODULIN GENE EXPRESSION AND LOCALIZATION IN COMMON BEAN NODULES FORMED BY BACTERIAL MUTANTS. Jaime E. Padilla, Federico Sanchez. Dept. Plant Mol. Biol., CIIGB-UNAM, A. Postal 510-3, Cuernavaca, Mor. 62271 Mexico.
Nodulin genes are expressed in distinctive temporal and spatial patterns during legume nodule organogenesis and function. Conditions involved in the initial and coordinated regulation are not fully understood. Bacterial mutants in symbiotic genes have aided in finding or discriminating some conditions (1). ENOD2, uricase-II, leghemoglobin and nodulin-30 (Npv-30) expression were evaluated in nodules formed by Rhizobium phaseoli mutants and an Agrobacterium transconjugant. Uricase-II mRNA was detected in 'empty' nodules formed by noninfective mutants, suggesting regulatory conditions prior to bacterial release. Accumulation of some nodulin transcripts was observed in delayed nodulation, and blocked in arrested-developed nodules. Ineffective (Fix-) nodules showed a normal initial development and nodulin expression, but in the absence of nitrogen fixation, terminal mRNA levels decreased (2). Expression of these nodulins is affected differentially by blockages of development and involves several conditions. Results from immunodetection and in situ hybridization of nodulins from wild-type and mutant-induced nodulation will be presented.
1. Sanchez et al. 1991. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42:507-528; 2. Padilla et al. 1991. Molec. Plant-Microbe Interact. In press. (Partial support by DGAPA-UNAM & the EEC)

1460 GENETIC MAP OF PLANT HOST GENES FOR NITROGEN FIXATION IN GARDEN PEAS. Jiang Lu[1], Norman F. Weeden[1] and Thomas A LaRue[2]. Cornell University. [1]Department of Horticultural Sciences, Geneva, NY 14456 and [2]Boyce Thompson Institute, Ithaca, NY 14853
Nitrogen fixation is the result of symbiotic interactions between plants and bacteria which include recognition, infection, nodule formation and efficient symbiotic nitrogen fixation. In legumes, several probably overlapping classes of plant host genes (e.g. sym genes, nodulin genes and lectin genes) are involved in nodule formation. Sym genes alter the nodulation phenotype, usually by partly or completely inhibiting nodulation. Nineteen sym genes have been defined in garden peas so far, and a cluster of 4 (*sym* 2, *sym* 5, *sym* 18 and *sym* 19) are located on chromosome 1. The remaining sym genes are scattered on six chromosomes. Leghemoglobin, the best known nodulins functioning to diffuse O_2 into nodules, is closely linked with the cluster of sym genes in chromosome 1. At least 5 RAPD (Random Amplified Polymorphic DNA) markers have also been found in this region. Lectins have been identified as determinants of host-plant specificity in the rhizobium-legume symbiosis. We have located a lectin gene family on chromosome 7 between isozyme markers Amy-1 and Gal-2. One of the sym genes (*sym* 15) also maps to this region.

POSTER ABSTRACTS

1461 CHARACTERIZATION OF THE HEME BINDING DOMAIN OF *CHLORELLA* NITRATE REDUCTASE. A.C.Cannons, C.J.Kay, M.J.Barber & L.P.Solomonoson. Dept. Biochem. & Mol. Biol., USF Col. Medicine, Tampa, Florida 33612

Assimilatory Nitrate Reductase, (NR), catalyzes the reduction of nitrate to nitrite. This is the first, and rate-limiting step in the overall pathway of nitrate assimilation, the major route of inorganic nitrogen acquisition for higher plants and algae. NR, a member of the superfamily of cytochrome b_5 enzymes, contains a b_5-type cytochrome, Mo-pterin and FAD, in 1:1:1 stoichiometry per subunit. The known E_m' values of the heme centers of algal, fungal and higher plant NRs are lower than those of other cytochrome b_5-containing proteins. To identify structural features contributing to the low heme potential of *Chlorella* NR (E_m' = -164mV) we have constructed a clone that codes for the entire heme-binding domain (92 amino acids). This clone was synthesized by PCR utilizing primers to the 5' and 3' ends of the domain, using a cDNA to *Chlorella* NR as a template. The derived amino acid sequence of the heme domain contains the 13 invariant residues required for the b_5 fold and a 32% homology to calf cytochrome b_5. The amplified fragment was cloned into pET3a, resulting in the expression of a heme-containing 10kDa polypeptide antigenic to anti-*Chlorella* NR. Amino acid sequencing of the N-terminus of this polypeptide revealed 100% identity with the 5' end amino acid sequence of the heme-binding domain of NR. The visible spectrum of the purified protein was identical to that of the b_5-type heme of *Chlorella* NR. Visible potentiometric titration in the presence of dye mediators yielded an E_m' of +16mV, significantly different from native *Chlorella* NR, but close to values reported for other members of the b_5 supertamily. This suggests the very negative value for the *Chlorella* NR heme potential may result from an interaction between the MoCo and/or FAD domains rather than the heme domain itself. Also this expressed protein is synthesized as a 45kDa product, implying a tetramer and suggesting the subunit binding site is located in this domain. Here the heme Em, heme ring exposure, visible and CD spectral parameters of native NR and the heme domain expressed in isolation are compared to assess the role of domain interactions in determining the thermodynamics of the heme prosthetic group. (Supported by NSF and NIH)

1462 LOCALIZATION OF A DIMERIZATION REGION WITHIN THE PHYTOCHROME POLYPEPTIDE Michael D. Edgerton and Alan M. Jones, Dept of Biology, University of North Carolina, Chapel Hill, NC 27599

Phytochrome is a plant photoreceptor involved in photomorphogenic events ranging from seed germination and de-etiolation to the induction of flowering. In solution, phytochrome exists as a homodimer of two independently phototransformable subunits. Kinetic descriptions of phytochrome responses have been used to develop a dimeric model for phytochrome action, thus dimerization is thought to be essential for phytochrome action. To better understand the mechanism of phytochrome action, we have begun a search for structures responsible for phytochrome dimerization. Toward this end, we have developed and used a novel assay based on protein fusions to lambda repressor to localize a small region in the carboxy-terminal domain of phytochrome which is capable of mediating dimerization. Using both in vivo and in vitro assays, fusions between the DNA-binding domain of lambda repressor and segments of an oat phytochrome gene have been assayed for increased repressor activity, an indication of dimerization. Using these assay systems, a 50 amino acid segment of oat phytochrome (phyA gene ap3) has been shown to increase repressor activity, indicating that this portion of the polypeptide may be involved in dimerization of phytochrome. The corresponding part of the gene is highly conserved between all members of the phyA gene family and contains 3 potential amphipathic alpha helices.

1463 REGULATORY ELEMENTS AND TRANSCRIPTIONAL FACTORS INVOLVED IN PHYTOCHROME REGULATION OF A LEMNA GIBBA cab GENE. Kehoe, D.M. and Tobin, E.M. Department of Biology, UCLA, Los Angeles, Ca. 90024

The cab gene family, encoding the chlorophyll a/b- proteins of photosystem II in Lemna gibba, have been shown to be transcriptionally regulated by phytochrome (Silverthorne and Tobin, 1984, PNAS 81:1112-1116). One member of this gene family, AB19, has been sequenced (Karlin-Neumann et al.1985. J. Mol. Appl. Genet 3:45-61) and has been shown to be transcriptionally regulated in vivo (Tobin et al. 1991. In: Phytochrome Properties and Biological Action. ASI Cell Biology Series, Thomas, B., ed., in press). Using a transient assay system previously described (Rolfe and Tobin, 1991. PNAS 88:2683-2686), various deletions of the AB19 promoter fused to a luciferase gene have been assayed for their ability to respond to phytochrome action. These studies have shown that a 70 base pair fragment directly upstream of the CAAT box is necessary and sufficient to confer phytochrome regulation to this promoter. Identification of the element(s) responsible within this region is being pursued via further analysis, and the factor(s) which interact(s) with this element(s) are being characterized by use of gel shift analysis and footprinting.

1464 RICE AND *ARABIDOPSIS phyB* GENES ENCODE FUNCTIONAL PHYTOCHROMES AS SHOWN BY OVEREXPRESSION IN *ARABIDOPSIS THALIANA*. Doris Wagner, James Tepperman and Peter H. Quail, Department of Plant Biology, University of California, Berkeley CA 94720 and Plant Gene Expression Center, Albany CA 94710.

Phytochrome has recently been found to be encoded by a multigene family in *Arabidopsis* and rice (1,2). *phyA* has been shown to encode the well characterized etiolated-tissue or Type1 phytochrome whereas the *phyB* product has yet to be characterized. The sequences derived from the rice and *Arabidopsis phyB* genes are 73% identical to each other but only 50% identical to available *phyA* sequences. Thus phytochrome B contains conserved regions not present in the *phyA* product. To better understand phytochrome responses it has to be determined initially whether *phyB* encodes a functional photoreceptor. If so, what is it's biological function? The comparatively low degree of sequence homology between *phyA* and *phyB* may indicate distinct functions. Alternatively, the two photoreceptors could be functionally redundant, but act in different tissues or at different stages of development. As one approach to these questions we have overexpressed both the rice and *Arabidopsis phyB* proteins in *Arabidopsis* using the 35S promoter. We have obtained a phenotype that indicates that *phyB* encodes a functional phytochrome. Homozygous lines containing single insertions have been generated to further analyze the phenotype. Monoclonal antibodies raised against the rice *phyB* product have been used to characterize the behavior of the overexpressed protein and spectral analysis of phytochrome B has been performed. The results of these experiments will be discussed.

1. Sharrock, R.A. and Quail, P. H., 1989. Genes and Development 3: 1745-1757.
2. Dehesh ,K., Tepperman, J., Christensen, A. H. and Quail ,P.H. 1991. Mol Gen 225: 305-313.

1465 CLONING AND CHARACTERIZATION OF RICE AND OAT cDNA CLONES THAT BIND TO THE PE-1 ELEMENT OF THE OAT PHYTOCROME A3 GENE (*phyA3*) PROMOTER. Jorge Nieto-Sotelo and Peter H. Quail. University of California, Berkeley/U.S. Department of Agriculture, Plant Gene Expression Center, 800 Buchanan Street, Albany, CA 94710.

Phytochrome down-regulation of the expression of its own *phyA* genes is the fastest light-induced effect at the gene expression level reported to date in plants. Functional analysis of the promoter has revealed 3 elements upstream of the TATA box region involved in positive regulation of oat *phyA3* gene expression: PE1, PE-2 and PE-3 (Bruce and Quail, 1990, Plant Cell 2, 1081). Oat and rice cDNA clones encoding PE-1 binding activity were detected by screening oat and rice λgt11 expression libraries, respectively. The libraries were screened by using a 29 bp catenated oligonucleotide containing the PE-1 element of the oat *phyA3* gene promoter as a probe. The rice library yielded two independent recombinant phage: R2 and R4. The oat library yielded two independent recombinant phage, O1 and O2. Binding to the PE-1 element seems to be sequence specific. Sequence substitution mutations in the 3' one-third of the PE-1 element decrease binding by 3 orders of magnitude for all clones indicating that nucleotides in this region are the most crucial for binding. The deduced amino acid sequence of the protein encoded by O2 reveals proline-rich regions but does not demonstrate a known DNA-binding domain. mRNA hybridization analyses show single homologous transcripts expressed in oat and rice etiolated aerial tissues and indicate that these cDNA inserts are not full length. Currently we are performing sequence analyses of the R2, R4 and O1 cDNA inserts and further characterizing these clones.

1466 DNA BINDING FACTOR GT3 FROM *ARABIDOPSIS THALIANA*. Robert M. Kuhn, Timothy Caspar, Katyoon Dehesh and Peter H. Quail. Univ. of California, Berkeley/USDA Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710

cDNA clones of a gene encoding a DNA-binding factor have been obtained from *Arabidopsis* by DNA hybridization with a GT2 factor cDNA clone from rice (Dehesh et al., 1990, *Science* 250:1397). The GT3 gene is present in a single copy in the *Arabidopsis* genome, is transcribed as a 2.1 kb mRNA which is not strongly light-regulated, and encodes a protein of about 55 kD. There is limited amino acid homology to the rice factor, including three regions of approximately 100 amino acids each of greater than 60% identity. Other than these conserved regions, GT3 has no similarity to other sequences in the Genbank database. GT3 does, however, have acidic, basic, proline/ glutamine-rich, and helix-loop-helix motifs which are found in several other well-characterized transcription factors. Studies with *lacZ* fusion constructs show that GT3 has binding characteristics similar to the rice GT2 factor. GT3 binds to the sequence GGTAAT, which regulates the expression of the rice phytochrome gene, but not to the closely-related Box II sequence, GGTTAA, which has been characterized as being important in the light regulation of the pea rbcS3a gene (Lam and Chua, 1990, *Science* 248:471). Mutation of the GG to CC in the binding sequence eliminates binding by the GT3 factor.

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1467 MULTIPLE G-BOX BINDING FACTORS FROM *ARABIDOPSIS THALIANA*. Anne E. Menkens, Ulrike M. Schindler and Anthony R. Cashmore. Plant Science Institute, Department of Biology University of Pennsylvania, Philadelphia, PA 19104

We have isolated and characterized a family of bZIP transcription factors (GBF-1, 2 and 3) from *Arabidopsis thaliana* that bind to the G-box sequence, an element that we and others have characterized for a variety of plant promoters. Each GBF contains a DNA-binding domain consisting of a leucine zipper and a basic region, as well as a proline rich domain. The DNA binding domain is strongly conserved between the three factors. however the proline-rich regions are divergent. We are interested in elucidating the expression characteristics of each of the three G-box binding factors and the role that they play in the regulated initiation of transcription. Experiments are in progress to explore the protein/protein interactions among these three transcription factors and the DNA/protein interactions between the GBF's and different G-box-like sequences.

1468 MUTATIONAL ANALYSIS OF OAT PHYTOCHROME IN TRANSGENIC ARABIDOPSIS. Margaret Boylan and Peter Quail, Plant Gene Expression Center, Albany, CA 94710.

An oat cDNA, encoding phyA, the major type of phytochrome found in etiolated plants, has been overexpressed in transgenic Arabidopsis. At the seedling stage, the hypocotyls of the phytochrome overexpressors are distinctly shorter than those of their wild type siblings. We are currently using the short hypocotyl phenotype as a means of identifying regions of the oat phytochrome molecule that are important for its function as a photoreceptor in Arabidopsis. Our initial attention has focused on three parts of the protein: first, regions that are involved with chromophore interaction; second, domains that are implicated in dimerization; third, amino acid residues that have been conserved during phytochrome evolution. We have created deletions and point mutations in these parts of the molecule and have overexpressed the mutant phytochrome clones in transgenic Arabidopsis. The effects of these mutations on oat phyA function will be discussed.

1469 PHOTOREGULATION OF PHENYLALANINE AMMONIA LYASE IN PHOTOMORPHOGENETIC MUTANTS OF TOMATO (*Lycopersicon esculentum*). R. Sharma[1,2], K.V.Gaud[2], R.E.Kendrick[3], M.Furuya[1].

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Photoregulation of phenylalanine ammonia lyase (PAL) (E.C. 4.3.1.5) was analyzed in wild (wt) and photomorphogenetic mutants viz: phytochrome deficient (*au*), high pigment producer (*hp*) and double mutant (*au/hp*) of tomato. Red light acting via phytochrome stimulates the PAL activity in the cotyledons and hypocotyls of tomato seedlings. The kinetics of photoinduction of PAL in cotyledons of mutants (*au/hp*) and wild type seedlings are similar in the profiles except in hp seedlings which maintains a high level of PAL after attaining a peak induction. In hypocotyls also the induction of PAL follows the similar profiles in mutants (*au, au/hp*) seedlings, however in *hp* seedlings induction of PAL is greater in the magnitude. The induction of PAL is dependent on *de novo* synthesis of protein and nucleic acids. The use of PAL specific inhibitor α-amino β-phenylpropionic acid indicated that PAL is an essential part in anthocyanin biosynthetic pathway of tomato seedlings. However, the comparison of anthocyanin biosynthesis and PAL induction data revealed that phytochrome mediated induction of PAL and anthocyanin in tomato seedlings are not directly correlated. While *au* and *au/hp* mutant seedlings shows similar induction of PAL level as in wild type, there is a little induction of anthocyanin in these seedlings. These results would be discussed in relation with photoregulation of anthocyanin and PAL by different molecular species of phytochrome viz. *PhyA* and *PhyB* respectively.

1470 PHYTOCHROME RESPONSES IN TRANSGENIC TOBACCO PLANTS WHICH OVER-EXPRESS RICE PHYTOCHROME I. Akira Nagatani[1], Naoko Nishizawa[2] Steve A. Kay[3], Nam-Hai Chua[3] and Masaki Furuya[1] [1]Frontier Research Program, RIKEN Institute, Wako, Saitama, Japan; [2]Department of Agricultural Chemistry, The University of Tokyo, Bunkyou-ku, Tokyo, Japan; and [3]The Rockefeller University, New York, NY 10021-6399

The photoreceptor phytochrome mediates a wide variety of photomorphogenetic responses in plants. To elucidate the molecular mechanism of its action, we have generated transgenic tobacco lines overexpressing rice phytochrome I (PI) (Kay et al., 1989). Analysis of hypocotyl elongation in young seedlings of these transgenic plants suggested that the Pfr form of the introduced rice PI inhibited stem elongation through an endogenous signal transduction pathway (Nagatani et al., 1991) In the present study, we have investigated this phenomenon further. Light-intensity dependence analysis of the response to continuous irradiation with red light indicated that the transgenic plants were about 10^3 times more sensitive than the wild type to the light. Expansion of cotyledons was also affected in a similar way. On the contrary, the amount of chlorophyll per seedling under the same light conditions was not affected by the presence of exogenous rice PI. Electron microscopic observation by liquid helium rapid-freezing method could not reveal any significant difference in ultrastructure of the chloroplasts of these plants. Potentiation of chlorophyll accumulation by a red light pulse was also tested but the exogenous PI caused no observable effects. To correlate the amount of exogenous rice PI to these responses, immunoblotting analysis of the plants grown under similar light conditions is in progress

1471 STUDIES ON THE PRODUCTION OF Pisum sativum *PhyA* MULTIPLE TRANSCRIPTS UNDER DIFFERENT PHOTOPERIODIC CONDITIONS Martin J. G. Hughes , Ken-Ichi Tomizawa and Masaki Furuya. Frontier Research Program, RIKEN, Wako-Shi, Saitama Pref. 351-01, JAPAN

It has been reported that the Pisum sativum phytochrome gene (which resembles the *PhyA* gene of Arabidopsis) produces three transcripts of different sizes (Sato 1988) which are differentially regulated by light (Sato 1988, Tomizawa *et al.* 1989) The function of these multiple transcripts has not as yet been characterized Initial characterization has been carried out on the location of production the two smaller transcripts (RNA1 and RNA2) using S1 nuclease mapping (Tomizawa 1991) Difficulties have been experienced when this method has been used to study RNA3 levels, due to low levels of production, and putative multiple start sites of the transcript. To overcome this problem, we have synthesised a probe specific to the RNA3 segment of the transcript, and have used this to investigate RNA3 levels in different areas of tissue. Additionally, probes for RNA2 and RNA1 as well as that for RNA3 have been used to study the levels of the transcripts under different photoperiodic conditions

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1472 DETECTION OF *phyA* AND *phyB*-LIKE GENE PRODUCTS IN TWO COMPLEMENTARY PHYTOCHROME MUTANTS. Enrique López, Akira Nagatani, Richard Kendrick[1] and Masaki Furuya. Riken Inst., Wako, Saitama 351-01, Japan; [1]Agricultural University, Wageningen, The Netherlands.

The phytochrome photoreceptor is encoded by a gene family with at least phy*A*, *B* and *C* subfamilies. Different phytochrome forms, at the physiological and biochemical levels, specifically light-labile and light-stable pools, have also been recognised. We have raised a monoclonal antibody, mAT1, against a partial product of a tobacco *phyB* gene (courtesy of Prof. N.-H. Chua and coworkers, Rockefeller University, New York) expressed in *E. coli*. mAT1 recognises a light-stable polypeptide of phytochrome size in tobacco extracts, whereas an antibody (mAP5) against the well characterised phytochrome from dark-grown tissue reacts against a light-labile polypeptide in the same samples. We have characterised two phytochrome-related mutants with complementary phenotypes: cucumber *lh*, which shows greatly reduced shade-avoidance reaction, and tomato *au*, defficient in a *phyA* gene product and in phytochrome responses related to greening Cucumber *lh* seedlings specifically lack a polypeptide recognised by mAT1 antibody, while the protein recognised by mAP5 is indistinguishable from the wild type one in terms of amount, apparent molecular size and kinetics of degradation in the light. In tomato *au* plants, mAT1 stains a polypeptide apparently present at wild-type levels . These data are discussed in relation to the phenotype of the mutants and the possible roles of the different phytochrome forms.

POSTER ABSTRACTS

1473 RHYTHMIC EXPRESSION OF CAB GENES IN ETIOLATED WHEAT AND TRANSGENIC TOBACCO SEEDLINGS. Barbara Wehrmeyer, Ferenc Nagy and Eberhard Schäfer. Universität Freiburg, Institut für Biologie II, Schänzlestr. 1, 7800 Freiburg, FRG.

In etiolated wheat and (transgenic) tobacco seedlings the expression of cab genes is under phytochrome control. The rhythmic cab mRNA accumulation is induced by either a 5 min red light, 5 min far-red light or 5 min RG9 pulse. Even small amounts of active phytochrome (ca. 2% P_{fr} after a 5 min far-red light pulse or less than 0.01% P_{fr} after a RG9 pulse)) induce the rhythmic expression of cab genes (low and very low fluence response). Kinetics of the cab gene expression up to 60 h in darkness after the inducing pulse show three maxima: in etiolated wheat seedlings around 10, 36 and 52 h, in etiolated tobacco seedlings maxima of the endogenous cab gene expression occur at 4, 24 and 44 h, and the wheat cab gene in tobacco seedlings shows maximal expression 8, 24 and 44 h. This indicates that the transgene follows the endogenous (tobacco) cab gene expression.

To investigate the interactions between phytochrome and the endogenous rhythm, second light pulses were given 6 and 12 h after the inducing 5 min far-red light pulse. The second pulses induce an additional maximum of cab gene expression, irrespective whether a 5 min red light pulse or a 5 min long-wavelength far-red light , RG9, pulse are used. This shows that wheat and tobacco seedlings are very sensitive even to small amounts of P_{fr} (ca. 0.01% after a RG9 pulse). In none of the cases a phase shift of the endogenous rhythm was observed, the peak positon of the subsequent maxima remained constant. Some conclusions may be drawn from these results: P_{fr} induces and/or synchronizes a preexisting rhythm of the expression of cab genes in etiolated seedlings; once the rhythm is set, P_{fr} can transiently modulate the cab gene expression, but no longer interfere with the endogenous clock.

1474 CHROMOPHORE SUBSTRATE SPECIFICITY FOR HOLOPHYTOCHROME ASSEMBLY*. Liming Li and J.Clark Lagarias, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

A full length *Avena* phytochrome cDNA has been cloned into the yeast-*E.coli* shuttle vector, pMAC-105. Under induction conditions, transformed yeast cells express full length, 124 KDa apophytochrome. Zinc-dependent bilin-peptide fluorescence and spectroscopic studies show that this recombinant apophytochrome covalently binds to phytochromobilin (PΦB), the natural linear tetrapyrrole (bilin) chromophore of *Avena* phytochrome, and to the chromophore analog phycocyanobilin (PCB), to form photoactive holophytochrome adducts *in vitro*. In this study, we present experiments to identify potential bilin substrates/inhibitors of holophytochrome using apophytochrome produced with this yeast expression system. A number of bilins containing the ethylidene substituent in the A-ring but varying in the B-, C-, D-rings have been tested. Zinc-dependent fluorescence studies have revealed that phycoerythrobilin (PEB), a bilin which differs from the natural *Avena* phytochrome chromophore (phytochromobilin) by missing the double bond at C15-C16, covalently attaches to apophytochrome. Difference spectral assay shows that this PEB-holophytochrome adduct is not photoreversible. A kinetic analysis method has also been developed for the study of the bilin substrate specificity for the holophytochrome assembly reaction. The implications of such studies with regard to the structure of the chromophore binding site of apophytochrome will be discussed.

* Funded by USDA (GAM 89-001162)

1475 CHARACTERIZATION OF REQUIREMENTS FOR CHROMOPHORE ATTACHMENT TO *ESCHERICHIA COLI* EXPRESSED APOPHYTOCHROME.* Jill A. Wahlethner and J. Clark Lagarias. University of California, Davis, Department of Biochemistry and Biophysics, Davis CA 98616. The phytochrome polypeptide functions as an enzyme that directs the covalent attachment of its linear tetrapyrrole (bilin). To identify structural features of the apoprotein involved in this prosthetic group assembly process, we have developed an *in vitro* assembly assay. Full-length, soluble apophytochrome has been expressed in *E. coli*. One gram of *E. coli* cells produces 9.51 mg of apophytochrome; this can be enriched to .058% of the soluble protein by ammonium sulphate precipitation. When the soluble protein fraction of an *E. coli* lysate is incubated with phycocyanobilin, a phytochrome chromophore analog, 62% of the apoprotein covalently attaches to the chromophore. The resulting holoprotein is photoreversible. Using this sytem, we have examined the effects of deletions and other mutations in the phytochrome coding region on the ability of the expressed apoprotein to functionally assemble with phycocyanobilin. The implications of these results with regard to "active site regions" within the apoprotein will be discussed.

*Funded by USDA (GAM89-001162).

1476 PROGRESS TOWARD CLONING THE *DE-ETIOLATED-1* (DET-1) LOCUS IN *ARABIDOPSIS THALIANA*. Terrence P. Delaney, Alan E. Pepper and Joanne Chory. Plant Molecular Biology Laboratory, The Salk Institute for Biological Studies, La Jolla CA 92186-5800

Our laboratory has taken a molecular genetic approach to elucidate features of light-signal transduction pathways regulating light-dependent seedling development. Using mutant screening procedures of dark grown *Arabidopsis thaliana* seedlings, we have identified a number of mutations that cause plants to develop many features of light-grown plants while grown in total darkness. When grown in the dark, *det1* plants have short hypocotyls, expanded cotyledons and leaves, form chloroplasts and express both nuclear and chloroplast photogenes at near light-growth levels. Because *DET1* mutations are both recessive and pleiotropic, we have proposed that the *DET1* gene product acts as a negative regulator of many light-dependent developmental processes. To understand the molecular nature of this regulation, we have undertaken chromosome walking experiments aimed at isolating the *DET1* gene. Through genetic analysis, we have localized *DET1* to a short region on chromosome 4, between the RFLP markers cosmid 2616 and λ518 (H. Goodman and E. Meyerowitz collections, respectively). Using these as probes, we have identified yeast artificial chromosome (YAC) clones from the region around *DET1*. The ends of each YAC have been isolated and used to identify new RFLPs, establish genetic linkage to *DET1*, and rescreen the YAC libraries. At this writing we have determined orientation to and taken several steps in the direction of *DET1*. Estimates of physical distance based on genetic distance suggest that we should link up the two walks in the next few steps. We are currently examining cosegregation of *det1* with the end probe RFLPs to delineate a smaller region that contains this gene.

1477 GENETIC SUPPRESSORS OF THE *det1-1* MUTATION OF *ARABIDOPSIS THALIANA* Alan E. Pepper and Joanne Chory. Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037. The *DET1* locus of *Arabidopsis thaliana* is defined by recessive single gene mutations that result in plants that display the characteristics of light grown plants when grown in complete darkness. It is our hypothesis that the *DET1* gene product exerts negative regulatory control over the process of seedling photomorphogenesis. We further propose that the *DET1* gene product may be an intermediary in a signal transduction pathway that is initiated by red (phytochrome) and blue light photoreceptors and by tissue related factors (possibly including phytohormones), and which modulates such downstream responses as leaf development, chloroplast differentiation and photoregulated gene induction.

In order to define the genetic loci that encode other molecules essential to this signal transduction process, including molecules which might interact directly with the *DET1* gene product, we have isolated second-site genetic suppressors of the *det1-1* mutation. A homozygous *det1-1* strain was mutagenized with EMS and M_2 seed pools were screened in the dark for plants which exhibited some or all of the characteristics of the etiolated wild-type plant. Recessive and dominant mutations, unlinked to the *det1* locus, were obtained. A subset of these mutations fully restore the wild-type pattern of photoregulated seedling development, while in the homozygous *det1-1* genetic background. One class of dominant mutants exhibited increased lethality (due to a failure to de-etiolate) after exposure to light) when in the homozygous state. Genetic mapping and complementation studies will further define the loci that have been revealed by these mutations.

1478 PHYTOCHROME CONTROL OF HRGP GENE EXPRESSION IN TOMATO SEEDLINGS. G. Dechamp-Guillaume, D. Rumeau and M.T. Esquerre-Tugaye. Centre de Physiologie Vegeta'e, C.N.R.S. URA 1457, Université Paul Sabatier, 118 route de Narbonne, F-31062 Toulouse cedex, France.

Expression of hydroxyproline-rich glycoprotein (HRGP) gene is affected by number of different conditions such as infection, ethylene, elicitor treatment and wounding... We have investigated the effects of light on HRGP gene expression in tomato seedlings.

Northern analysis demonstrated that :

1) HRGP mRNA level is highly increased in etiolated tomato seedlings as compared to HRGP mRNA level in light-grown seedlings.

2) Red light irradiation induces a rapid and transient decrease of HRGP mRNA.

3) When followed by Far Red irradiation, the Red light effect is suppressed.

Taken together the data suggest that phytochrome is involved in HRGP genes regulation.

Results will be discussed with regard to the possible involvement of phytochrome in transduction pathways leading to defense responses.

POSTER ABSTRACTS

1479 THE ISOLATION OF ARABIDOPSIS THALIANA MUTANTS DEFECTIVE IN PHYTOCHROME SIGNAL TRANSDUCTION. Judy Brusslan and Elaine Tobin. Dept of Biology, UCLA, Los Angeles, CA 90024

In an attempt to isolate phytochrome signal transduction mutants, we are using two different selection schemes. The first is based on the observation that germination is dependent on phytochrome being in the active Pfr form (Shropshire, W. et al. 1961. Plant and Cell Phys. 2 63-69). Thus we are screening for mutants that do not germinate in response to red light, but can be rescued with gibberellic acid which bypasses the red light requirement. Among the mutant lines isolated are non-germinating alleles of hy1 and hy2 (Koornneef, M. et al. 1980. Z. Pflan. Bd. 100.147-160). hy1 and hy2 are thought to be defective in phytochrome chromophore biosynthesis (Parks and Quail, personal communication), but previously isolated alleles have not been defective in germination (Cone and Kendrick 1985. Planta 163:43-59). We are continuing to use this screen to look for mutants defective in the signal transduction pathway, but containing normal levels of phytochrome.

Our second selection scheme utilizes the ims2 (iaaH) gene under the control of a phytochrome-regulated cab gene as a suicide marker that allows putative mutants to survive on an exogenously added substrate for the ims2-encoded enzyme (Karlin-Neuman, G.A., Brusslan, J.A., and E. Tobin. The Plant Cell, in press). We are doing this selection in the presence of GA so that non-germinating signal transduction mutants can be isolated.

1480 A DNA BINDING PROTEIN TO THE CAB140 PROMOTER REGION IMPORTANT FOR PHYTOCHROME-REGULATED GENE EXPRESSION IN ARABIDOPSIS THALIANA. Lin Sun, Ruth Doxsee and Elaine Tobin. UCLA, Department of Biology, Los Angeles, CA 90024

In *Arabidopsis thaliana* the *cab* genes that encode light-harvesting chlorophyll a/b-proteins are regulated by phytochrome at the transcriptional level, and *cab*140 is the most responsive member of the gene family with respect to phytochrome action (Karlin-Neumann, G.A. *et. al.* (1989) Plant Physiol. 88, 1323-1331 and Karlin-Neumann, G.A. *et al.* (1991) Plant Cell, in press). We have partially purified a DNA binding activity from *Arabidopsis thaliana* which binds to an 86 bp *cab*140 promoter fragment in gel shift assays. This region has been shown to be necessary for phytochrome-regulated reporter (CAT) gene expression in transgenic tobacco lines that were kindly provided by G. An (Ha, S-B. and An, G. (1988). Proc. Natl. Acad. Sci. USA, 85, 8017-8021). The protein-DNA interaction is specific for this DNA fragment, because other DNA fragments (poly dIdC, two regions from the cab140 promoter flanking the 86 bp fragment, or a region downstream of a transcribed *Lemna gibba cab* gene) failed to compete for the binding activity while the 86 bp fragment itself or a longer DNA fragment consisting of the 86 bp fragment and the two flanking regions competed very well in *in vitro* binding competition experiments. The protein protected a CA-rich region in DNase I footprinting analyses, and therefore has been designated CA-1.

1481 CHARACTERIZATION OF A LIGHT-REGULATED DNA-BINDING FACTOR (LRF-1) IN L. GIBBA AND A. THALIANA. Sharlene Weatherwax and Elaine Tobin, UCLA, Dept. of Biology, L.A., CA 90024

Phytochrome has been shown to mediate changes in expression of the *rbcS* genes (encoding the small subunit of Rubisco) in some species [Tobin, E. and Silverthorne, J. (1985) *Ann. Rev. Plant Physiol.* 36, 569-593]. We have previously identified a nuclear DNA-binding protein (designated LRF-1) whose activity increases in response to light and which interacts in a specific manner with the upstream portion of the *rbcS* SSU5B gene in *Lemna gibba* [Buzby, J.S., Yamada, T., and Tobin, E.M. (1990) *Plant Cell* 2, 805-814]. In addition, the LRF-1 footprinted sequence corresponds to a region implicated in transient expression studies of *cis*-acting phytochrome-responsive regulatory elements in the SSU5B promoter [Rolfe, S.A. and Tobin, E.M. (1991) *Proc. Natl. Acad. Sci USA* 88, 2683-2686]. We have identified an LRF-1 cognate activity in whole cell extracts from *Arabidopsis thaliana* and have initiated biochemical characterization of the factor and molecular characterization of its activity. The behavior of the *A. thaliana* LRF-1 with respect to heterologous competitors in electrophoretic mobility shift assays has been examined and is consistent with that of the *L. gibba* LRF-1. Quantitation of the amount of LRF-1 activity in extracts from seedlings grown under various light regimes is being examined to define the response of LRF-1 activity to phytochrome control. Finally, studies are in progress to identify the genes which encode LRF-1 in *L. gibba* and *A. thaliana*.

1482 PHYTOCHROME OVER-EXPRESSED IN *E. coli* SELF-ASSEMBLES INTO A PHOTOCHEMICALLY FUNCTIONAL HOLOPROTEIN *IN VITRO*. Brian M. Parks, David E. Somers, Margaret Boylan, and Peter H. Quail. UCB/USDA Plant Gene Expression Center, Albany, California 94710

We have engineered *Avena* and *Arabidopsis* phytochrome cDNAs into a bacteriophage T7 vector for over-expression in *E. coli*. Using this system, we have generated full-length polypeptides encoded by the *phyA* genes from *Avena* and *Arabidopsis*. The majority of over-expressed phytochrome polypeptide is found in the insoluble fraction from disrupted *E. coli* cells, but a small portion is soluble. When phycocyanobilin (PCB), the chromophore for c-phycocyanin and a close analog to phytochromobilin, is added to the soluble over-expressed phytochrome polypeptides, a stable adduct is formed *in vitro*, and each of these phytochromes self-assembles into a photochemically functional holoprotein. These adducts show complete photoreversibility with red and far-red light. Difference spectra for the *Avena phyA* polypeptide-PCB adduct are blue-shifted approximately 15 nm when compared to purified phytochrome from etiolated *Avena*. We have also produced two carboxy-terminal deletion constructs for the *Avena phyA* cDNA. Soluble over-expressed gene product from either of these modified cDNA constructs (75 kDa and 68 kDa polypeptides) also self-assemble into a photochemically active chromoprotein *in vitro*, thereby demonstrating that the amino-terminal half of the phytochrome polypeptide is sufficient for functional chromophore attachment. We have also produced over-expression constructs which contain the cDNAs for the *phyB* and *phyC* genes of *Arabidopsis*. Similar to our results using the phyA over-expression vectors, small portions of the over-expressed full-length *phyB* and *phyC* polypeptides are found in the soluble fraction of disrupted *E. coli* cells. When PCB is added to these soluble polypeptides, they also self-assemble into photochemically active holoproteins. This provides direct evidence that the *phyB* and *phyC* genes encode proteins which are capable of assembling into photochemically functional chromoproteins in plant cells.

1483 THE *hy3* MUTANT OF *ARABIDOPSIS* IS DEFICIENT IN *phyB* TRANSCRIPT AND PROTEIN. David E. Somers[1], Robert A. Sharrock[2], James Tepperman[1] and Peter H. Quail[1]. [1]Department of Plant Biology, University of California, Berkeley, CA 94720; [1]Plant Gene Expression Center, Albany CA 94710; [2]Department of Biology, Montana State University, Bozeman, MT 59717.

The six non-allelic *hy* mutants of *Arabidopsis thaliana* share the common feature of an elongated hypocotyl when grown in white light. The varied responses of these mutants to irradiations of differing wavelengths have suggested that some of the lines may lack elements of the phytochrome or blue light signal transduction pathways. Such speculation has been confirmed in recent studies which have demonstrated that *hy1*, *hy2* and *hy6* lack spectrophotometrically detectable phytochrome in etiolated tissue, although immunochemically detectable phytochrome levels in the three mutants are normal (1, 2).

The cloning of *phyA* and *phyB* cDNAs from *Arabidopsis* has allowed us to obtain large amounts of relatively pure *phyA* and *phyB* apoprotein through a T7-based overexpression system in *E. coli*. Using the gel-purified protein, we have developed and identified monoclonal antibodies which are specific for the *phyA* and *phyB* products and which have enabled us to further probe the nature of the *hy* lesions. The Bo64 allele of *hy3* was compared to the wild type for the presence of *phyA* and *phyB* transcripts and proteins. This line exhibits a reduced *phyB* transcript level and a severely reduced *phyB* protein level in etiolated tissue. *phyA* transcript and protein appear normal. Further characterization of the expression of these gene products in *hy3* under various light conditions and in comparison to the wild type and the other *hy* mutants will be presented.

(1) Chory, J., Peto, C. D., Ashbaugh, M., Saganich, R., Pratt, L., and Ausubel, F. 1989. The Plant Cell 1, 867-880.

(2) Parks, B. M., Shanklin, J., Koornneef, M., Kendrick, R. E., and Quail, P. H. 1989. Plant Mol. Biol. 12, 425-437.

1484 PRESENCE OF ACTIVE PHYTOCHROME AND A WELL FUNCTIONING RESPIRATORY CHAIN IN A BARLEY MUTANT HAVING NO CAPABILITY OF SYNTHESIS OF δ-AMINOLEVULINIC ACID FROM CHLOROPLAST $tRNA^{GLU}$. Wolfgang R. Hess, Thomas Börner, Anke Koch and Wolfhard Rüdiger*, Humboldt-Universität Berlin, O-1040, Germany, *Ludwig Maximilians Universität, W-8000 München 19, Germany.

The barley albino mutant *albostrians* contains no plastid ribosomes and no chloroplast-encoded $tRNA^{glu}$. In accordance with this result the mutant synthesizes only traces of chlorophylls or chlorophyll precursors. In contrast to the widely established fact that synthesis of tetrapyrroles in higher plants requires chloroplast $tRNA^{glu}$ as universal precursor for δ-aminolevulinic acid we show that in this mutant only chlorophylls are missing meanwhile other tetrapyrrole-dependent functions are nearly normal. Consequently a reaction circumventing $tRNA^{glu}$-dependent formation of δ-ALA has to be drawn into account. This might either be a reaction synthezising δ-ALA in the chloroplast independent from $tRNA^{glu}$ or, more probably, a second pathway of δ-ALA synthesis depending in no way from functioning chloroplasts.

POSTER ABSTRACTS

1485 PROGRESS TOWARD CLONING THE *ARABIDOPSIS* DET2 GENE. Punita Nagpal and Joanne Chory. Plant Biology Laboratory, The Salk Institute, San Diego, CA 92186-5800.

DET2 is a genetic locus involved in light-mediated early seedling development in *Arabidopsis thaliana*. Mutations in the DET2 gene are recessive and pleiotropic. When grown in the dark, *det2* mutants display many characteristics (in morphology and gene expression) that are light-mediated in the wild-type. However, *det2* mutants do not initiate the chloroplast developmental program in the dark and thus allow separation of two downstream light-regulated responses (gene expression and morphology) from a third, the development of chloroplasts. In addition to its role in de-etiolation, DET2 also appears to be involved in later stages of vegetative growth as *det2* mutants have a prolonged vegetative phase, are late-flowering and show lack of leaf senescence. In our efforts to study the molecular mechanisms of signal-transduction, specifically the role of the important regulatory gene DET2, we have mapped it to chromosome 2 and have started chromosome-walking experiments from the two closest flanking RFLP markers. We have estimated the physical distance between the RFLP marker proximal to DET2 and the end of one of the YACs picked up with the distal RFLP marker, to be about 475 Kb. Using Columbia-Landsberg recombinants, we have oriented our walk from both the markers and have travelled about 300 kb from the proximal marker and 150 kb from the distal marker. Our most recent results will be presented in the poster

1486 EXPRESSION AND PHYSIOLOGICAL ROLES OF phyA IN RICE: AN APPROACH USING TRANSGENIC RICE PLANTS. Junko Kyozuka, Steve A. Kay•, Nam-Hai Chua• and Ko Shimamoto. Plantech Research Institute, 1000 Kamoshida, Midori-ku, Yokohama 227 Japan. •The Rockefeller University, Department of Plant Molecular Biology, New York, NY10021.

Phytochrome is the best characterized photoreceptor in plant. In rice, two distinct phytochrome genes, phyA and phyB, have been isolated and sequenced. Their physiological roles, however, remain to be elucidated.

As a first step toward understanding the role of the phyA whose expression is strongly downregulated by light we introduced 35S-phyA cDNA into rice. Progeny seeds (R1 generation) were obtained from 12 independent primary transgenic plants. A preliminary analysis showed that a high level of phyA mRNA was detected in progeny seedlings grown under light. They are now being examined for possible alterations in a number of morphological and physiological traits.

As a second approach, phyA promoter-gus constructs were introduced into rice and progeny seeds were obtained from 15 independent primary transgenic rice plants. Tissue localization and downregulation of its expression in R1 plants are being analyzed.

The results of these experiments will be discussed.

1487 CHARACTERIZATION OF THE PHYTOCHROME SYSTEM IN POTATO Arnd Heyer and Christiane Gatz, Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, 1Berlin 33, Germany

The photoreceptor phytochrome mediates many aspects of light regulated growth and development in higher plants. This photoreceptor is interconvertible between its inactive Pr form and its active Pfr form by red and far red light. The presence of at least three different genes and gene products for phytochrome has been first shown in Arabidopsis thaliana. We are interested in characterizing the phytochrome system in potato, because potato shows a number of unique phytochrome controlled developmental processes like tuberization, sprouting of the tuber and photomorphogenesis of the sprouts. We want to assign defined functions to the different components of the phytochrome system by specifically inhibiting the expression of one type of phytochrome. We have started the project by isolating and sequencing a full length cDNA clone for type A phytochrome. On the mRNA level, we observe light regulated expression of the gene in all tissues. Protein levels however accumulate only in the dark. We will present data on the physiological effects of expression of anti-sense RNA, as well as sense RNA, under the contol of different promoters.

1488 PHYTOCHROME MEDIATED CHLOROPHYLL LOSS AND PROLINE ACCUMULATION IN RICE DURING LEAF SENESCENCE. H.K. Patra, N.K. Sen and D. Mishra. University of Utkal, Department of Botany, Bhubaneswar 751 004, India.

The involvement of phytochrome system in the regulation of rice leaf senescence was assessed by estimating proline accumulation, chlorophyll and protein loss under in vivo and in vitro conditions. This experiment indicated the possible existence of a unique photoreversible photoreceptor in senescing rice leaves which can be converted to active form by red light(R) exposure and reverted to inactive form if leaves were subsequently exposed to far red light(FR). A brief irradiation with red light is as effective as natural light or kinetin in arresting the loss of chlorophyll and protein in detached leaf segments and attached whole leaves of rice. Proline accumulated steadily during senescence of both intact and excised leaves. There was a slower accumulation of proline in R or R-FR-R light treated leaves which is similar to those leaves grown under natural light or by kinetin treatment. Comparatively a much higher level of accumulation of proline was noted for F or R-FR treated leaves as in the darkness. The effect of red light was completely reversed by a consecutive brief irradiation with far red light. The results suggest the involvement of phytochrome system in regulating leaf senescence in rice during plant growth and development.

1489 NEGATIVE TRANSCRIPTIONAL REGULATION OF THREE GENES IN *LEMNA GIBBA* BY PHYTOCHROME ACTION. Patricia Okubara and Elaine Tobin, UCLA, Biology Department, Los Angeles, CA 90024.

The developmental photoreceptor phytochrome can act to either increase or decrease transcription of specific genes. We have isolated three distinct genes, designated NPR (Negatively Phytochrome Regulated) 1, 2 and 3, from the aquatic monocot *Lemna gibba*. The NPR mRNAs show reduced levels in plants grown in continuous white light or intermittent red light, and increase in abundance within 6 hours of darkness. In both green and etiolated plants, NPR transcription increases within 6 to 12 hours of darkness. Far-red light given to etiolated plants prior to darkness results in more rapid accumulation of the mRNAs and greater rates of transcription in the dark, indicating that P_{fr} exerts an inhibitory effect on NPR gene expression. Furthermore, a single red light treatment causes a reduction of NPR gene transcription, and this reduction is reversed by far-red light given immediately after the red light. The effects of red and far-red light are observed in etiolated plants but not in green plants, suggestive that a switch from phytochrome to another light receptor for negative regulation of the NPR genes occurs during greening. 900 base pairs of the NPR2 promoter confer negative phytochrome regulation on a luciferase reporter gene when introduced into *Lemna* by microprojectile bombardment. We will perform promoter deletion analyses on the two most highly transcribed NPR genes, to identify *cis* elements involved in negative phytochrome regulation.

Ref. Okubara PA and Tobin EM (1991) *Plant Physiol* 96. (In press).

1490 GT-2: A TRANS-ACTING FACTOR THAT BINDS IN A HIGHLY SEQUENCE SPECIFIC MANNER TO A GT MOTIF IN THE RICE *PHYA* GENE PROMOTER. Katayoon Dehesh, and Peter H. Quail. University of California, Berkeley / U.S. Department of Agriculture, Plant Gene Expression center, Albany, CA 94710.

Phytochrome regulates the expression of its own *phyA* genes in monocots at the transcriptional level. To elucidate the molecular mechanism underlying this regulation we are studying the 5' flanking cis-elements and the trans-acting factors involved in the expression of this gene. Utilization of microprojectile mediated gene transfer has enabled us to identify sequences required to drive a high level of transcription of the *phyA* gene. Among these sequences is a GT motif. Previously we reported the isolation of a partial cDNA clone, designated GT-2 that binds in a highly sequence specific manner to this functional GT motif in the rice *phyA* gene promoter (1). Recently we have obtained the full length GT-2 protein sequence by isolation of overlapping cDNA and genomic clones. Sequence analysis data shows that GT-2 is a polypeptide with a predicted size of 81 kDa. However the polpeptide produced by *in vitro* transcription-translation runs at 110 kDa on SDS gel electrophoresis. A combination of western blot analysis with antibodies raised against the GT-2 polypeptide, and southwestern analysis using the GT-2 binding site as a probe shows that the *in vitro* transcription-translation product comigrates with a reactive band in nuclear extracts. This result verifies that the new GT-2 clone encodes the full length protein, which runs aberrantly on SDS gel electrophoresis. Sequence analysis has revealed that this protein is very hydrophilic, perhaps accounting for the aberrant mobility. Although GT-2 does not have any striking homology with any sequences in the data bases, it does have certain features defined for other transcription factors such as acidic and basic, proline-glutamine rich, and helix-loop-helix domains. Genomic Southern blot analysis suggests that GT-2 is present as a single copy gene. Northern blot analysis shows that GT-2 transcript level decreases in white light.

(1) Dehesh, K. Bruce, W.B. and Quail, H.P. Science 250, 1397-1399 (1990)

POSTER ABSTRACTS

1491 LIGHT-INDUCED CHANGES OF TUBULIN AMOUNT AND BEHAVIOUR IN MAIZE COLEOPTILES. Peter Nick, Masaki Furuya, Riken-Institute,Wako, 35101 Saitama, Japan.

α and ß-tubulin can be purified from maize coleoptiles using ethyl-N-phenylcarbamate (EPC) sepharose affinity-chromatography. Irradiation of the plants with red light produces two effects on tubulin: (1) The amount of tubulin is dramatically decreased, (2) the strength of tubulin binding to EPC-sepharose is increased. Both red-light effects can be mimicked by blue light. Together with R/FR reversibility experiments suggests a very low fluence (VLF) response. Other cytoskeletal proteins (actin, calmodulin, an unknown 60 kDa protein recognised by a polyclonal antiserum raised against chicken gizzard muscle actin) are copurified with tubulin. The spatiotemporal pattern and degradation experiments are presented. The data are discussed with respect to light-induced differential expression of different tubulin isotypes.

1492 REGULATION OF GENES CODING FOR AUXIN-BINDING PROTEINS
Etienne Schwob, Soon-Yong Choi, Klaus Palme* and Dieter Söll
Yale University, Dept. of Molecular Biophysics and Biochemistry, New Haven, CT 06511, USA and *MPI for Breeding Research, Cologne, FRG.

Plant hormones are the major determinants of growth and developmental responses in plants. Auxins, a major class of plant hormones, are involved in cellular differentiation, cell elongation and the initiation of the gravitropic growth response. Auxin's mode of action is mainly accounted for by the acid-growth and gene expression theories that imply plasma membrane or intracellular receptors, respectively. The characterization of these receptors and the elucidation of the signal transduction cascade leading to the hormone-specific biological response are a major challenge in plant molecular biology. The axr1 gene from corn codes for such a putative receptor, but the recent characterization of several other auxin-binding proteins (ABPs) from corn (24, 60 kD), zucchini (42 kD) and *Hyoscyamus* (31 kD) having different cellular localization (plasma membrane, cytosol, endoplasmic reticulum) raises the question of how complex the molecular mechanism of auxin action really is. In order to try to understand the biological role of these various ABPs, we have decided to study how their genes are regulated.

The axr1 gene encodes the major 22 kD auxin-binding protein from corn coleoptile membranes whereas the axr4 cDNA codes for a protein which is 65% identical to the mature axr1 protein. Both proteins contain the C-terminal KDEL sequence which specifies their retention in the lumen of the endoplasmic reticulum; they differ significantly only in their signal peptide. We have now isolated and sequenced the genomic axr1 and axr4 genes from maize, as well as the corresponding AXR1 gene from *Arabidopsis*. We will describe their molecular structure and discuss their evolutionary origin. The tissue-specific and developmentally regulated expression of these genes will be discussed.

1493 BLUE LIGHT STIMULATES PHOSPHORYLATION OF A 114 kDa PROTEIN IN MAIZE COLEOPTILES. Julie M. Palmer, Timothy W.Short, Winslow R. Briggs. Carnegie Institution of Washington, 290 Panama Street, Stanford, California 94305.

Phosphorylation of a 114 kDa protein in plasma membranes from maize coleoptiles is induced by exposure to blue light within a range of fluence levels sufficient to stimulate phototropism. The phosphorylation response is detected at total fluences of 10^1 umoles m^{-2} either upon *in vitro* irradiation of extracted plasma membranes or upon irradiation of tissue *in vivo* following perfusion with radioactive orthophosphate. Similarly, exposure of intact coleoptiles to a series of blue light fluences in the absence of exogenous phosphate results in decreasing phosphorylation of the 114 kDa band during subsequent *in vitro* irradiations with gamma-labeled ATP. Presumably, *in vivo* irradiation causes sites on the protein to be phosphorylated by endogenous non-radioactive phosphate, which remains stably bound during extraction of the membranes and through at least one freeze-thaw cycle. The *in vivo* irradiation fluence series indicates that 10^2 umoles m^{-2} is sufficient to saturate phosphorylation of the 114 kDa protein, which correlates with the level of blue light required to saturate the phototropic response in maize coleoptiles. Maximal phosphorylation on a per unit protein basis occurs in the extreme tip of the coleoptiles and no blue light-dependent phosphorylation is detected in tissues from the node or mesocotyl. These results suggest that phosphorylation of the 114 kDa protein may be an early step in the signal transduction chain of blue light-mediated phototropism in maize coleoptiles.

1494 FLAVIN AS A POSSIBLE BLUE-LIGHT PHOTORECEPTOR CHROMOPHORE IN PEA. Katherine M.E. Warpeha, Lon S. Kaufman* and Winslow R. Briggs. Carnegie Institution of Washington, Stanford, California 94305, and *University of Illinois, Department of Biological Sciences, Chicago, Illinois 60680.

Blue light regulates many photomorphogenic processes in higher plants, but the photoreceptor(s) have not yet been isolated. Blue light significantly enhances the binding of GTP to proteins associated with the plasma membrane from apical buds of dark-grown pea seedlings (Warpeha et al., *Supp to Plant Physiol* 93: 30, 1990). Blue-light induced binding of GTP is inhibited when flavin antagonists phenyl acetic acid or potassium iodide are included in the reaction mixture. The low level of binding of GTP to non-irradiated plasma membranes is unaffected. Fluorescence spectrophotometry indicates that a flavin is present in the plasma membranes of dark-grown pea seedlings. The flavin fluorescence is only visible after the plasma membranes have been boiled with 0.1% SDS, indicating that the putative flavin may be associated in a protein complex in the plasma membrane. The flavin fluorescence is quenched when the inhibitors phenyl acetic acid or potassium iodide are used in concentrations similar to those effective in the GTP-binding assay. These data indicate that a flavin may be the chromophore for a blue-light photoreceptor in the apical bud tissue of dark-grown pea seedlings.

1495 MORPHOLOGICAL AND MOLECULAR CHANGES IN LEAVES OF *BRASSICA NAPUS* DURING ADAPTION TO UV-B STRESS. Michael I. Wilson and Bruce M. Greenberg. Department of Biology,University of Waterloo,Waterloo,Ontario,Canada N2L 3G1.

Plants can acclimate to environmental stresses by altering morphology, anatomy and biochemical composition (1). UV-B radiation is interesting because it is of current environmental concern (depletion of the ozone layer) and because plants photomorphogenetically respond to UV-B. For example, if plants are unable to adapt, basic processes such as photosynthesis are damaged. However, they can be protected from damaging radiation by accumulating UV-absorbing pigments (e.g. flavonoids), synthesizing radical scavenger systems and adjusting leaf thickness. These adaptation processes are regulated mainly at the gene level, and genetic elements and factors required for UV-B-induced transcription have been identified. As part of an ongoing investigation towards understanding UV-B effects and identification of photoreceptors, we are studying UV-B responses of *Brassica napus* at the anatomical, molecular and biochemical levels. After germination, plants were grown for two weeks in visible light combined with low levels of UV-B. Compared to controls, there was some growth retardation and leaf curling. However, net photosynthetic activity was not affected. The UV-B treatment also caused an increase in leaf thickness and flavonoid content, which may protect photosynthetic proteins by screening UV-B. Four varieties of *B. napus* have also been compared for adaptability to UV-B radiation. They exhibit a range of morphological responses and susceptibilities to UV-B. Two varieties with contrasting tolerances for UV-B were selected to study molecular biological changes in more detail.

1496 CHARACTERIZATION OF THE 40 AND 42 kDa AUXIN-BINDING PROTEINS IN PLASMA MEMBRANES OF ZUCCHINI. Glenn R. Hicks, Margaret S. Rice and Terri L. Lomax, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902

We have identified auxin-binding proteins (ABPs) of 40 and 42 kD in plant plasma membranes using photoaffinity labeling with azido-IAA (^{3}H-5N$_3$-IAA). These ABPs are present in lower quantities or display greatly reduced photoaffinity labeling in hypocotyl membranes of an auxin-insensitive, agravitropic mutant of tomato (*diageotropica, dgt*) compared to the isogenic parent (VFN8). We are currently characterizing these putative auxin receptors in purified plasma membranes from zucchini and developing purification protocols. Enrichment via a temperature-induced phase separation in the nonionic detergent Triton X-114 results in partitioning of the 40-42 kD ABPs into the aqueous (hydrophilic) rather than the detergent (hydrophobic) phase. This behavior is similar to that of certain integral proteins that aggregate to form water-filled channels through membranes. Gel filtration suggests that the ABPs may associate to form a dimer of ca. 80 kD under native conditions. Two-dimensional electrophoresis of the ABPs revealed radiolabeled proteins having isoelectric points of ca. 7.2 and 8.2 , and the results of enzymatic and cyanogen bromide peptide mapping suggest that the ABPs are closely-related polypeptides. These studies will be useful for polypeptide sequencing and designing molecular probes to examine the role of the 40-42 kD ABPs in auxin perception.

POSTER ABSTRACTS

1497 PHYSIOLOGICAL CHARACTERIZATION OF THE lazy-2 GRAVITROPIC MUTANT OF TOMATO J.C. Gaiser and T.L. Lomax. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA, 97331-2902.

The *lazy-2* (*lz-2*) mutant of tomato exhibits normal negatively gravitropic (away from gravity) shoot curvature in the dark, but upon exposure to light *lz-2* shoots become positively gravitropic (curvature in the same direction as gravity). The roots of this mutant are wild-type under either condition. The shift from negative to positive gravicurvature observed in the shoots of this mutant appears to be under the control of phytochome due to its dependence upon red light. When light-grown *lz-2* plants are oriented horizontally, the shoots initially (~1-2 hrs. after reorientation) exhibit a wild-type response, that is, the hypocotyl begins to curve upward. But after ~2 hrs. this upward curvature ceases and no further curvature is observed until >12 hrs. after reorientation when one can observe *lz-2* hypocotyls exhibiting downward curvature. Wild-type hypocotyls normally attain full vertical reorientation within 8 hrs. The phenotype may indicate an altered auxin physiology in *lz-2*. Our initial investigations of polar transport reveal an greater flux of IAA through *lz-2* stems than in wild-type. We are currently testing the nature of this difference in transport as well as the ability of *lz-2* shoots to respond to exogenous IAA. We are especially interested in the degree and direction of auxin asymmetry in gravistimulated *lz-2* hypocotyls relative to wild-type. Also, a transmission electron microscope (TEM) analysis has been undertaken to compare the subcellular anatomy of the mutant with wild type; specifically with regard to the nature of the amyloplasts, which have been postulated as gravisensing organelles. We feel a thorough physiological analysis of this mutant will lay the groundwork for future experiments aimed at understanding the molecular mechanism of transduction of a signal (gravity perception) to a physiological response (stem curvature).

1498 UV-B INDUCED CHANGES IN ARABIDOPSIS GENE EXPRESSION AND METABOLISM. Tsai-Mei Ou-Lee, Suzanne Fellows, Richard M. Raba, Robert G. Amundson and Robert L. Last. Boyce Thompson Institute for Plant Research, Ithaca, NY 14853-1801

We are pursuing a combined molecular biological, biochemical and genetic approach to understand how plants respond to ultraviolet-B (UV-B: 280-320 nm) radiation and to determine what costs are associated with this response. Changes in the composition of the stratosphere are projected to cause significant increases in the amount of UV-B radiation reaching the biosphere. Plants are thought to synthesize light absorbing pigments, including flavonoids, as an adaptive mechanism to protect against UV-B damage to macromolecules. We are using the plant *Arabidopsis thaliana* to test the efficacy of flavonoid pathway pigments in protecting plants from UV-B damage and to understand the genetic regulation of the biosynthesis of these pigments in response to UV-B radiation. Preliminary results show that mRNA levels for phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) increase several-fold within hours following shifting wild-type plants from -UV-B to +UV-B growth conditions. The growth rate and leaf morphology of plants are dramatically altered when grown under these UV-B conditions. Data will be presented documenting physiological changes and pigment accumulation in Arabidopsis plants grown under UV-B light. We will also compare the UV-B response of wild-type and flavonoid deficient *tt4* mutant plants.

1499 GRAVITY-INDUCED CHANGES IN CARBOHYDRATE METABOLISM IN SOYBEAN COTYLEDONS.

William C. Piastuch and Christopher S. Brown. The Bionetics Corporation, J. F. Kennedy Space Center, FL 32899 USA.

Evidence obtained during space flight indicates morphological and physiological changes may occur in plants growing in an altered gravity environment. To examine the relationship between plant carbohydrate metabolism and gravity conditions, a series of ground-based experiments involving clinorotation and centrifugation have been performed. Six-day-old soybean cotyledons showed decreased levels of starch when grown on a horizontal clinostat (4.2% of the total dry weight) when compared to vertically rotated control plants (5.4%) or seedlings exposed to hypergravity conditions via centrifugation (7.1%) It is interesting to note that other investigators have shown that plants exposed to true microgravity consistently had reduced starch concentrations relative to ground-based controls. Sucrose concentrations were greater in the cotyledons of the horizontally rotated plants (3.5%) than in either the centrifuged plants (1.0%) or the vertically rotated plants (1.2%) Plant length and dry weight measurements also exhibited differences between gravity treatments. To determine the mechanism(s) responsible for these gravity-induced changes, studies of the activities of key enzymes and expression of genes encoding for these enzymes (alpha-amylase, ADP-glucose pyrophosphorylase, and invertase) are underway.

1500 ALANINE AMINOTRANSFERASE PURIFICATION FROM BARLEY
A. G. Good and D. G. Muench, Dept. of Genetics, University of Alberta, Edmonton, Alberta.

Alanine aminotransferase (AlaAT) activity in barley root tissue increases four-fold under anaerobic conditions. In order to understand the regulation of AlaAT activity under anaerobic conditions we have purified AlaAT for the purpose of producing antibodies and amino acid sequencing. Three isoforms of AlaAT are evident in root tissue, only one being anaerobically induced This isoform was purified to homogeneity by ammonium sulphate precipitation, ion exchange chromatography, gel filtration and chromatofocusing AlaAT is a multimeric enzyme with the single subunit having a molecular weight of approximately 52 kD The kinetics of this enzyme will also be presented

1501 ANALYSIS OF ABA RESPONSIVE GENES IN WHEAT DURING DROUGHT STRESS. S. W. King, C.P. Joshi and H.T. Nguyen. Plant Genetics Laboratory, Texas Tech Univ., Lubbock TX 79409-2122

The overall objective of this project is to determine the molecular basis of drought tolerance in winter wheat. Our current objective was to isolate cDNAs corresponding to the *rab* family of drought inducible genes in winter wheat. Preliminary data indicated that considerable changes occur in translatable mRNA and *rab* gene expression during drought stress in winter wheat. In order to further charactenze the *rab* genes in wheat a cDNA library was constructed using mRNA from the drought tolerant wheat line TAM W-101 in the lambda Uni-Zap XR vector system (Stratagene). The cDNA library was screened and cDNAs representing the *rab* gene family were isolated. Sequencing of the cDNAs revealed considerable homology with previously reported *rab* genes at the carboxy terminal of the protein coding region and stretches of diversity at the amino terminal of the coding region. Nucleic acid sequence comparisons, patterns of gene expression and genomic orgnaization data will be presented.

1502 ANALYSIS OF *Cis*-ACTING ELEMENTS AND *trans*-ACTING FACTORS OF OSMOTIN PROMOTER. K.G. Raghothama, D.E. Nelson, N.K. Singh, P.M. Hasegawa and R.A. Bressan. Purdue University, Department of Horticulture, W. Lafayette, IN 47907

Several environmental, developmental and hormonal signals have been shown to regulate the expression of the osmotin gene. Thus, the osmotin promoter provides an excellent system to study *cis* and *trans*-acting elements responding to multiple signals. 5' deletions of the osmotin promoter were cloned into a promoter-less GUS-NOS plasmid (pTac), and used in transient expression studies with a Biolistic gun. The transient expression studies demonstrated that a minimum promoter sequence to -105bp is required for basal expression. A distinct enhancement of GUS activity was observed with increasing size of the promoter up to -1046bp. However, a further increase in size (-1377), resulted in decreased activity. We have thus far found that ethylene enhanced expression is associated with the larger promoter fragment and are determining the least sequence required for responsiveness to ethylene and other inducers. *Agrobacterium* (LBA-4404) containing different promoter deletion-fusions were also used to transform tobacco leaf segments. These transgenic plants are at various stages of analysis. Gel retardation studies indicated that the osmotin promoter contains sequences that bind to nuclear proteins from tobacco cell suspensions and leaves. Nuclear protein binding regions were found proximal to the TATA box (-106 to +45), and considerably upstream from the TATA box (-639 to -759 bp). Both DNA binding factors increase in abundance during salt adaptation or after ABA treatment.

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POSTER ABSTRACTS

1503 BOTH CYTOKININ AND SALT TREATMENT ELICIT THE EXPRESSION OF PHOSPHO*ENOL*PYRUVATE CARBOXYLASE IN *M. CRYSTALLINUM*. John C. Thomas, Elizabeth F. McElwain and Hans J. Bohnert, Department of Biochemistry, The University of Arizona, Tucson, AZ 85721

The facultative halophyte *Mesembryanthemum crystallinum* switches carbon assimilation from the C_3 pathway to Crassulacean acid metabolism (CAM) in response to high salinity and drought stress. The increase in the expression of phospho*enol*pyruvate carboxylase (PEPCase) is indicative of the salt induced CAM shift. We have determined that exogenously applied cytokinin, 6-benzylaminopurine (6-BAP), completely substitutes for the salt induced rise in PEPCase in this species. When applied to plants grown in hydroponics, 6-BAP concentrations between 10^{-7} to 10^{-5}M stimulate PEPCase mRNA and protein accumulation. The extent and the response time of 6-BAP-mediated PEPCase induction is equivalent to that of control plants that are treated with 400mM NaCl. At the level of steady state mRNA, PEPCase levels increase in excess of 30 fold in both salt treated and 6-BAP treated plants. No further increase in the amount of PEPCase mRNA is observed when NaCl is added after 6-BAP treatment. 6-BAP treatments of *M. crystallinum* for as little as one day result in detectable changes in PEPCase mRNA at the level of RNA blot analysis. After treatment with 6-BAP, there is no increase in endogenous abscisic acid (ABA) levels as is observed in salt treated plants. Further studies including exogenously supplied ABA indicate that the actual *in planta* response to an exogenous NaCl challenge involves the interaction of both plant growth regulators, ABA and 6-BAP. We interpret these results as further evidence for the interrelation of plant development and the capacity of this plant to modulate its environmental stress response. To our knowledge this is the first account of a salt-responsive gene being regulated by cytokinin. Supported by the USDA.

1504 CHARACTERIZATION OF GENES RESPONSIVE TO DESICCATION (rd) IN ARABIDOPSIS THALIANA. 1. rd GENES ENCODING THIOL PROTEASE ARE INSENSITIVE TO EXOGENOUS ABA. Kazuo Shinozaki, Masahiro Koizumi, Kazuko Yamaguchi-Shinozaki and Satomi Urao. The Institute of Physical and Chemical Research (RIKEN), Gene Structure Laboratory, Tsukuba, 305 Japan.

Under water-deficit condition various proteins are induced, which are thought to protect plant cells from desiccation. Several proteins are induced by plant hormone abscisic acid (ABA), whose synthesis is triggered by osmotic stress. We have started to clone and characterize genes induced by desiccation to understand the molecular process of signal transduction from water stress to gene expression using Arabidopsis thaliana.

We have constructed a cDNA library from poly $(A)^+$ RNA prepared from desiccated Arabidopsis plants, and cloned 9 independent cDNAs by using the differential hybridization method. All the 9 cDNA clones, named RD(Responsive to Desiccation), are induced by desiccation on transcriptional level. In this paper we report gene structure and expression of two RD clones, RD19 and RD21, encoding different thiol proteases, which are not responsive to exogenous ABA. RD19 and RD21 are induced by temperature change from 22°C to 4°C and vise versa. We have cloned genomic DNA of RD19 and RD21, and finished sequencing one of each clone (rd19A and rd21A).

1505 CHARACTERIZATION OF GENES RESPONSIVE TO DESICCATION (rd) IN ARABIDOPSIS THALIANA. 2. rd GENES RESPONSIVE TO EXOGENOUS ABA. Kazuko Yamaguchi-Shinozaki, Masahiro Koizumi, Satomi Urao and Kazuo Shinozaki. The Institute of Physical and Chemical Research (RIKEN), Gene Structure Laboratory, Tsukuba 305 Japan.

We have cloned 9 cDNAs (named RD), which are responsive to desiccation in Arabidopsis thaliana using the differential hybridization method as reported in the preceding paper. In this paper, we report gene structure and expression of two RD clones, RD22 and RD29, which are induced by exogenous ABA. RD22 has local homology with an unidentified seed protein (USP) of Vicia fava. Concerning RD29 we could not find any sequence homology with reported proteins.

RD22 is induced by cold stress as well as osmotic stress. RD22 is responsive to exogenous ABA, which indicates that endogenous ABA synthesized by desiccation or cold stress induces RD22 expression. We have cloned and sequenced its gene, rd22, and analyzed the promoter region. Two step induction of RD29 by water deficit was observed. The first quick induction occurred in 30 min after starting desiccation and the second slow induction was observed in 3 h. ABA spray induced RD29 expression in 3 h, which suggest the slow induction is achieved by endogenous ABA synthesized under water deficit condition. We are analyzing two genes of RD29, rd29A and rd29B.

1506 CIS-ACTING DOMAINS OF A PEPC PROMOTER FROM THE COMMON ICE PLANT (*MESEMBRYANTHEMUM CRYSTALLINUM*) THAT CONFER SALT-STRESS INDUCIBLE GENE EXPRESSION. John C. Cushman, Michael S. Meiners, Hans J. Bohnert. Department of Biochemistry, The University of Arizona, Tucson, AZ 85721. Crassulacean Acid Metabolism (CAM) is a metabolic adaptation to arid environments that allows plants to conduct nocturnal CO_2 fixation. Water loss is reduced by avoiding high transpiration rates during the day. In our model, the facultative halophyte, *Mesembryanthemum crystallinum*, salt stress induces a shift from C_3 photosynthesis to CAM. The key enzyme involved in nocturnal CO_2 fixation, phosphoenolpyruvate carboxylase (PEPC; E.C. 4.1.1.31), is encoded by a small gene family. Only one member of this family (designated *Ppc1*) responds to salt stress *via* transcriptional activation (Cushman et al., 1989) that is modulated over the course of development (Cushman et al., 1990). Transcript levels are also induced to some extent by abscisic acid (ABA). To understand the role of each of these stimuli in the process of transcriptional activation, we have used microprojectile bombardment of intact leaves as an *in vivo* transient assay to define functional regions of the *Ppc1* promoter. A region of only -135 bp upstream of the transcriptional start site is responsible for conferring salt-stress responsiveness. Regions further upstream modulate *Ppc*-1 gene expression in both a positive and negative manner. To complement these studies, we have also characterized *in vitro* DNA-binding protein interactions within the 5'-upstream region of *Ppc1* in an attempt to define the contribution of specific, isolated binding activities to salt and/ or ABA inducibility. A complex set of regulatory mechanisms is emerging for this gene that plays such an important role in basic plant metabolism and Crassulacean acid metabolism. Supported by USDA and Arizona Agricultural Experiment Station.

Cushman, J.C., G. Meyer, C.B. Michalowski, J.M. Schmitt, H.J. Bohnert (1989) Plant Cell 1: 715-725
Cushman, J.C., C.B. Michalowski, H.J. Bohnert (1990) Plant Physiol. 94: 1137-1142.

1507 CLONING BY COMPLEMENTATION OF THE γ-GLUTAMYL KINASE GENE FROM A TOMATO EXPRESSION LIBRARY. M. G. Garcia-Rios[1], P. C. LaRosa[2], R. A. Bressan[2], L. N. Csonka[1] and J. M. Hanquier[1]. Purdue University, Departments of [1]Biological Sciences and [2]Horticulture, West Lafayette, Indiana 47907

Many plants accumulate proline within the cells after exposure to high salinity or drought. The complete biosynthetic pathway of proline and its regulation remain to be elucidated in plants. In bacteria and fungi, the pathway proceeds via the enzymes γ-glutamyl kinase, γ-glutamyl phosphate reductase, and Δ^1-pyrroline-5-carboxylate reductase. We identified four recombinant phage from a λ *gt11* cDNA library from tomato that could complement a γ-glutamyl kinase (*proB*) mutation in *Escherichia coli*. The inserts in these four phage had overlapping restriction maps. A 3.0 kb fragment from one of the phage was used as a probe in Southern analysis, and it hybridized to a single copy tomato chromosomal locus. Assays of γ-glutamyl kinase in crude extracts confirmed the presence of increased activity of this enzyme in the complemented *E. coli proB* mutants. The γ-glutamyl kinase activity of these strains was sensitive to inhibition by proline. The cloned fragments were introduced into an *E. coli* Δ*proBA* strain which lacks both γ-glutamyl kinase and γ-glutamyl phosphate reductase, and they complemented both enzymatic defects. Enzyme assays indicated that this strain had increased activities of both γ-glutamyl kinase and γ-glutamyl phosphate reductase. Our results suggest that the product of the cloned gene might be a bi-funcuonal enzyme with both γ-glutamyl kinase and γ-glutamyl phosphate reductase acuvities.

1508 CLONING THE PEP CARBOXYLASE GENES OF THE INDUCIBLE CAM PLANT *SEDUM TELEPHIUM*. Paul Barnwell, John A. Bryant, Nick Smirnoff and A. Fred Weir*. University of Exeter, Dept. of Biological Sciences, Exeter, England EX4 4QG. * Advanced Technologies (Cambridge) Ltd, Cambridge, England CB4 4WA.

Exposure of *Mesembryanthemum crystallinum* to salt stress leads to the induction of crassulacean acid metabolism (CAM). This is accompanied by a large increase in the activity of phosphoenolpyruvate carboxylase (PEPC). It has been shown that there are two isoforms of PEPC which are encoded by seperate genes. The large increase in total activity is due to an increase in only one of the isoforms (Cushman *et al.*, 1990). *Sedum telephium* can also be induced to enter CAM by exposure to water stress. Biochemical and physiological evidence suggests that there may also be PEPC isogenes in *S.telephium* whose expression governs the response observed under control and drought conditions. In order to clarify this, the genes need to be cloned. However, *S.telephium* has proved to be highly recalcitrant to conventional methods of nucleic acid extraction, although high molecular weight DNA can now be extracted. A strategy has been developed whereby the polymerase chain reaction (PCR) is being used to amplify PEPC-specific sequences. This has been achieved by identifying conserved regions from the PEPC sequences of several plants. PCR primers matching these regions have been made and gene fragments amplified and cloned. Once PEPC gene fragments have been identified, Inverse PCR can be used to isolate the 5′ promoter region. This will permit studies of the upstream regulatory elements involved in the control of PEPC gene expression under control and drought conditions.

Ref: Cushman, J.C., Michalowski, C.B., & Bohnert, H.J. (1990) *Plant Physiol.* 94, p1137-1142.

POSTER ABSTRACTS

1509 DEHYDRINS: A SUBSET OF LEA PROTEINS WITH CHARACTERISTIC PROPERTIES AND BROAD DISTRIBUTION. Timothy J. Close and Raymond D. Fenton, University of California, Department of Botany and Plant Sciences, Riverside, CA, 92521

Dehydrins are a group of proteins characterized by high glycine content, the absence of tryptophan and cysteine residues, pronounced hydrophilicity, and several conserved and repeated amino acid sequence motifs, including the eight amino acid consensus KIKEKLPG. Dehydrins occur widely in plants as a consequence of any of several physiologically related stresses including desiccation, chilling, osmotic shock and exposure to the plant hormone abscisic acid (ABA). The KIKEKLPG consensus sequence occurs at the carboxy terminus of all dehydrins, and elsewhere as few as one time and as many as eight times in barley proteins. A synthetic peptide covering the carboxy terminus of cereal dehydrins was used to raise antibodies and to affinity purify anti-peptide antibodies. Affinity purified antibodies were used to detect ten dehydrin proteins in barley seedlings, ranging in size from 15 to 80 kDa. Some dehydrins are present in hydrated as well as dehydrated barley seedling tissues, and there are obvious organ specificities. Different cultivars of barley have differences both in the number and size of dehydrins, suggesting that antibodies can be used as simple markers of dehydrin alleles. Various numbers and sizes of dehydrins were also observed in a broad range of additional plants within and outside of the Gramineae. Affinity purified anti-dehydrin antibodies were used in sub-cellular localization studies, and to develop an immunoaffinity adsorbent useful for the purification and subsequent biochemical characterization of this set of stress-related proteins.

1510 DESICCATION AND THE CONTROL OF PROTEIN SYNTHESIS IN THE REPAIR OF CELLULAR DAMAGE IN *TORTULA RURALIS*. Melvin J. Oliver and Harlan B. Scott II. USDA-ARS, Lubbock, Texas 79401.

Desiccation-tolerance of the moss *Tortula ruralis* is characterized by a desiccation induced change in gene expression that is evident upon rehydration. Several properties of this system lead to the hypothesis that the desiccation-tolerance mechanism employed by *T. ruralis* is based upon the rapid repair of cellular damage upon rehydration. This report demonstrates that the alteration in gene expression is characterized by the loss or decrease (>5 fold) in the synthesis of 25 hydration (h) proteins and initiation or increase (>5 fold) in the synthesis of 74 rehydration (r) proteins. Experimentally varying levels of water loss allowed the separation of translational controls affecting the synthesis of r and h proteins. The r and h protein synthesis responses are internally co-ordinate, however. Similarly the return to normal levels of h protein synthesis differs from that of the r proteins. Run-off translation of polysomes isolated from gametophytes during drying demonstrates that there are no novel or specific mRNAs recruited for translation during desiccation. Polysomal RNA has been used to produce cDNA probes to screen a moss cDNA library. We have isolated 40 putative r protein and 12 putative h protein cDNA clones. The isolation and characterization of these clones will be presented. The data will be discussed in relation to the desiccation/water loss translational control of gene expression in this plant and to the hypothesis that repair is the basis of this desiccation-tolerance mechanism.

1511 DIFFERENTIAL EXPRESSION AND TISSUE SPECIFICITY OF SALT INDUCED NOVEL ALFALFA GENES. Ilga Winicov. University of Nevada, Reno, Departments of Biochemistry and Microbiology, Reno, Nevada 89557.

Acquired salt tolerance in plant cells and whole plants is accompanied by numerous changes in gene expression. In order to identify those changes that may contribute to the salt tolerance phenotype in crop plants, I have isolated several cDNA clones that represent mRNA species showing enhanced accumulation in the salt tolerant alfalfa cells in culture, when they are grown in the presence of salt. Three of these clones, pA50, pA18 and pA9 have been partially characterized and have some interesting features. The sequence analysis of pA50 and pA18 show them to be unrelated to other sequences in the data banks. However, the pA50 protein may be a metal/nucleic acid binding protein, as indicated by the putative Zn^{++} finger consensus domain. I have regenerated fertile plants from the salt tolerant callus culture that show improved salt tolerance to parent plants under greenhouse conditions. The three clones detect mRNA species expressed in these plants indicating that these genes may represent cellular factors that operate also at the whole plant level While pA50 and pA18 detect mRNA species in both roots and leaves, pA9 shows a significant root specificity of mRNA accumulation. The effect of salt is being investigated on the tissue specific gene expression in these salt tolerant alfalfa plants regenerated from the salt tolerant cell lines.

1512 DROUGHT-INDUCED GENE EXPRESSION IN *ATRIPLEX CANESCENS* (SALT BUSH): TRANSCRIPTIONAL AND POST-TRAN-SCRIPTIONAL RESPONSE. John Cairney[1], Dirk Hays[2], Jing Chen[2], James D. Stockand[2], Edward A. Funkhouser[2], Ronald J. Newton[1]. [1]Dept. of Forest Science and [2]Dept. of Biochemistry and Biophysics, TAES, Texas A&M University, College Station, TX 77843 The rangeland shrub *Atriplex canescens* (saltbush) is extremely drought-tolerant and is capable of growing at water potentials below -40 bar. To discover the molecular basis of this tolerance, we have isolated a number of cDNA clones of drought-stress induced genes. Analysis of the nucleotide sequence and expression of these genes in different tissues and in response to different stresses reveals the diversity of the stress response. Members of a drought-induced, multi-gene family, have been sequenced. Although 95% homologous, non-conservative substitutions result in proteins of different ternary structure. Additionally, the genes are expressed through a number of mature forms of mRNA which may arise by alternative RNA processing. Homologous clones have been isolated from other plants by PCR.

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1513 EFFECT OF DROUGHT STRESS ON PEROXIDASE LEVELS IN LEAF TISSUE FROM TWO COTTON RACESTOCKS. Darryl Ritter, Alesia Reinisch and Norma Trolinder. USDA-ARS Lubbock, Texas 79401

Cotton racestocks T-25 and T-169 have been shown to differ in their response to drought stress, with T-25 being wilt resistant and T-169 being wilt sensitive. These two racestocks were examined to determine the effect drought stress has on peroxidase activity and peroxidase mRNA levels in both expanding and mature leaf tissue. In well watered leaf tissue both racestocks showed a gradual increase in extractable peroxidase activity as the tissue matures. In water stressed leaves there was a dramatic increase in extractable peroxidase activity (as high as a three fold increase) in leaves that were still expanding while fully expanded leaves showed no stress induced increase in peroxidase activity. The level of stress induced peroxidase activity was about two fold greater for the wilting racestock (T-169) versus the wilting resistant racestock (T-25). Three major peroxidase isozymes were extracted from cotton leaf tissue. One of these isozymes was found to disappear during water stress in the expanding leaf tissue of both racestocks. Using a cotton peroxidase cDNA as probe, peroxidase mRNA levels were determined. In well watered plants there were two different size peroxidase messages. One appeared in tissue that is still undergoing cell division while the other one was present in tissue undergoing cell expansion. The peroxidase mRNA was abundant in young expanding leaf tissue but dropped to very low levels in mature leaf tissue. It appears there is a direct correlation between water stress and induced peroxidase activity in expanding leaf tissue. This induced activity could be one of the causes of the slower cell expansion rate seen during water stress.

1514 EFFECTS OF ABSCISIC ACID (ABA) ON GENE EXPRESSION AND PROTEIN SYNTHESIS IN BARLEY PLANTLETS. M. Gulli, M. Marmiroli, Institute of Genetics, Parma; G. Rhao Laboratory of Genetics, Lecce; H.Hartings, EICC Bergamo; S.A. Quarrie, Cambridge Laboratory, Norwich. Several cereal species (e.g. wheat, barley and maize) have been shown to differ in ABA accumulation and lines segregating for the character have been developed. Stresses stimulate ABA accumulation, and the expression of specific genes is known to be induced by ABA-treatment of non-stressed tissues. Use of genotypes differing in ABA accumulation will help determine the value of these genes to a stressed plant such as for water and temperature stress. Our work aims to correlate changes in protein synthesis following ABA treatment of barley seedlings with endogenous ABA content. Modification of protein synthesis in response to ABA treatment was analyzed both in vivo and in vitro to help understand the role of ABA in regulating gene expression and to provide a source of mRNAs to construct a cDNA library from which to isolate ABA induced genes by differential hybridization. Differential screening of these cDNA library allowed us to isolate several ABA positive clones, three of which were analyzed further. There is evidence that the three clones belong to different genes families in as much as they do not cross hybridize and they show dissimilar restriction maps. We are examining the three cDNAs further to see whether they correspond with other previously- reported RAB cDNAs.

POSTER ABSTRACTS

1515 EFFECT OF WATER STRESS ON GENE EXPRESSION IN THE CULTIVATED AND WILD TOMATO.

Ri-Dong Chen and Zohreh Tabaeizadeh. Dept of Biological Sciences, University of Quebec in Montreal, Montreal, Quebec, Canada H3C 3P8

Tomato (*Lycopersicon esculentum*) and its drought tolerant relative (*L. chilense*) were used to study the mechanism of drought tolerance. The induced changes in steady-state polypeptide levels and fluorography of radiolabeled *in vitro* translation products were monitored using one- and two-dimensional polyacrylamide gel electrophoresis. Under water deficit conditions, both species similarly synthesized a new set of proteins. However, some species-specific proteins were also observed. A cDNA library was constructed from leaves of drought treated *L. chilense* plants using Lambda ZAP II as vector. Several clones which seems to be induced by drought and ABA have been obtained by differential screening. Further characterization of these clones is in progress.

1516 *ELECTROPHORETIC CHARACTERIZATION OF STRESS PROTEINS IN PIGEONPEA CALLUS CULTURES ADAPTED TO NaCl. Amrit Sagar, K.Vaidyanath, Department of Genetics, Osmania University, Hyderabad-500007, India.*

Cotyledon derived calli of three known salt tolerant (ICPL-227, ICPL-239 and C-11) and one susceptible genotype (PI-397039) of pigeon pea (Cajanus cajan (L) Mills P) were adapted to NaCl (0.3, 0.5, 0.75, 1.0 and 1.5%) in L6 meidum and induced stress for 12 passages. At the end of 8th, 10th and 12th passages, electrophoretic variation of soluble protein under stress were evaluated employing polyacrylamide disc-gel electrophoresis. The callus protein profiles under stress revealed a wide variation as compared to control. The change in intensity also showed the quantitative differences. The amount of variation was relatively high in the genotypes PI-397039 and ICPL-239. Further, as a result of destress in the 9th and 10th passages, the protein levels decreased and reverted to that of control. Similarly, when the calli destressed and is subjected to reimposition of salt stress in the 12th passage, showed the absence or presence of certain specific bands. The differential banding pattern of soluble proteins observed in the present study under stress, destress and reimposed stress suggests that salt stress is transient in nature and modulates the gene expression.

1517 EVALUATION OF THE CONCEPT OF OSMOPROTECTION: THE NOVEL PRODUCTION OF MANNITOL IN TRANSGENIC TOBACCO.

Mitchell C. Tarczynski, Richard G. Jensen & Hans J. Bohnert. University of Arizona, Department of Biochemistry, Tucson, AZ 85721

Various osmolytes, including the polyol, mannitol, have been proposed to have roles in the mitigation of the effects of water stress on plants. A direct approach has been developed to test the concept that osmoprotectants confer resistance to water stress in higher plants. We have modified for plant-cell expression a bacterial gene that codes for a key enzyme involved in mannitol catabolism. In plants, contrary to the situation in bacteria, the enzyme should lead to mannitol biosynthesis. Several plasmid constructions, varying the promoter element and the mRNA stability domain, were used for transformation of tobacco, which normally does not contain mannitol. With some constructions, extracts of leaves from transgenic plants contained greater than 25% of their total soluble carbohydrate content as mannitol (>200% that of sucrose), as determined by HPLC. Analyses of root tissue from plants transformed with light-responsive promoters showed that mannitol accumulated in this tissue and, in some cases, comprised up to 40% of the total sugar content there. There appear to be no deleterious effects of mannitol production on the growth and the development of the transgenic tobacco. Using selfed progeny from these plants, we are currently investigating the physiological responses of the transgenic plants to various stresses; these responses can then be directly compared to those of wild-type (control) tobacco.

1518 EXCESS CHLOROPLASTIC Cu/Zn SUPEROXIDE DISMUTASE DOES NOT IMPART OZONE TOLERANCE.

L. Pitcher, E. Brennan, A Hurley, P. Dunsmuir, and B. Zilinskas. Dept. of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ 08903 and DNA Plant Technology Corporation, Oakland, CA 94608

Nicotiana tabacum cv 'Wisconsin 38' plants were grown from seeds produced by selfing two original transformants overproducing petunia chloroplastic Cu/Zn-SOD Both transgenic types showed about 15 times overproduction as compared to the non-transformed control. Vegetative plants, aged 3-4 months with 9-12 leaves, were subjected to 0.3 ppm ozone for 6 hours. Forty-eight hours after exposure, leaves 1-8 were rated for visible injury. The results of three experiments showed that, compared to the control plants, there was no protection conferred upon the transgenic plants by the extra Cu/Zn SOD. Additional ozonation experiments and analyses are continuing using the measurement of electrolyte leakage as the means of determining damage. To date, results do not differ from those obtained with visible injury ratings. We are also using these transgenic plants to address the question of the interaction of anti-oxidative enzymes. Specifically, we are studying the responses of ascorbate peroxidase and glutathione reductase to the increase in the chloroplastic Cu/Zn SOD.

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1519 EXPRESSION AND CHROMOSOMAL LOCATION OF A LEA 3 GENE IN DEHYDRATED WHEAT.

Jeffrey L. Ried and M.K. Walker-Simmons, USDA-ARS and Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420 Changing weather patterns and the increased cultivation of marginal lands require drought tolerance in many crops. We are examining responses at the biochemical and molecular level in water-stressed wheat (Triticum aestivum) to identify markers for drought tolerance. In severely dehydrated seedlings, an ABA-reponsive LEA 3 transcript is produced in roots, shoots, and scutellar regions. Accumulation of the corresponding protein, as measured by Western blotting using antibody produced to a bacterial fusion protein, occurred only in the shoots and scutellar regions. In those two tissues, multiple polypeptides were detected. Upon rehydration of the dehydrated seedlings, the shoots and scutellar regions resumed growth while the roots did not. The chromosomal location of the cross-reacting bands was established by Western blotting using nullisomic lines of the wheat cultivar Chinese Spring. The major cross-reacting polypeptide is located on chromosome 1A, while several minor polypeptides are located on chromosomes 1B and 1D. This type of information should prove useful in breeding programs designed to increase drought tolerance in wheat.

1520 EXPRESSION OF BARLEY LACTATE DEHYDROGENASE IN TRANSGENIC TOMATO ROOT CULTURES.

Jean Rivoal and Andrew D. Hanson. MSU-DOE Plant Research Laboratory, East Lansing MI 48824-1312.

The current theories on the role of lactate dehydrogenase (LDH) in plants suggest that LDH activity is only necessary at the onset of anoxia to activate ethanolic fermentation by a pH-stat mechanism, and that uncontrolled lactic fermentation leading to cytoplasmic acidosis is the cause of cell death under anoxia. These hypotheses are not readily reconciled with the finding that LDH is strongly induced in barley root tissues during prolonged anoxia. In order to study the function of LDH in anaerobic metabolism, we have used an *Agrobacterium rhizogenes* based method to introduce cDNAs containing the entire coding sequence of barley LDH into tomato. These constructs are under the control of the CaMV 35S promoter. Several root clones expressing up to approximately 50 times the level of LDH activity of control roots have been isolated. We are characterizing the effects of LDH overexpression on the fermentative metabolism of the transformed roots.

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1521 EXPRESSION OF DROUGHT-INDUCED GENES IN TRANSGENIC TOBACCO. Gabriel Iturriaga, Francesco Salamini and Dorothea Bartels. Max-Planck Institut für Zuchtungsforschung, Department of Plant Breeding and Yield Physiology, Carl von Linne Weg 10, D-5000 Köln 30, FRG.

Barley embryos and the resurrection plant Craterostigma plantagineum are being studied in order to understand the mechanisms by which plants resist to desiccation. In contrast to the majority of higher plants where tolerance to dehydration is restricted to mature embryos in seeds, the foliage of Craterostigma can withstand complete dryness and after rehydration it resumes full physiological activity within hours. Several proteins are induced during water stress in barley embryos and Craterostigma. After the isolation and sequence of their corresponding cDNA clones, here we show the independent expression of four of these clones in transgenic tobacco. The effect of desiccation-related proteins on the physiological performance of tobacco plants will be discussed.

1522 EXPRESSION OF LUCIFERASE IN TRANSGENIC TOBACCO MEDIATED BY A PROMOTER ELEMENT FROM DESICCATION TOLERANT *CRATEROSTIGMA PLANTAGINEUM* M. Bâga, P.L. Dale & A.A. Szalay, Plant Molecular Genetics, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7 and D. Piatkowski, F. Salamini & D. Bartels Max-Planck-Institut fur Zuchtungsforschung, Carl-von-Linne-Weg 10, D-5000 Koln 30, Germany

The phytohormone abscisic acid (ABA) is known to play a key role in drought tolerance of plants. In the desiccation-tolerant *Craterostigma plantagineum*, specific genes are transcriptionally activated in response to desiccation or application of ABA. Five of these genes have been identified and isolated (Piatkowski, D *et al* Plant Physiol 94:1682-1988, 1990). Two kb DNA from the upstream sequences of one of these ABA-induced genes and its truncated derivatives have been fused to the bacterial luciferase reporter fusion gene (*luxF*) resulting in a set of ABA-promoter *luxF* transcriptional fusions. These chimeric genes were used in separate experiments to transform leaf disks of SR1 tobacco plants. In regenerated transgenic tobacco plants, the luciferase activity was monitored *in vivo* during plant development. Light emission was found only in the maturing seeds and not in the vegetative portions of the analyzed plants regardless of the constructs used. In contrast, leaf mesophyll protoplasts from transgenic plants exhibited light emission only in the presence of high osmoticum (13% mannitol) or upon treatment with 10^{-4}M ABA. 5' truncations revealed the location of essential sequences required for seed specific activation as well as for promoter activation in the presence of osmoticum and ABA. The detailed functional analysis of the upstream sequences will help in elucidation of gene activation during dehydration of plants.

1523 FERRITIN GENE TRANSCRIPTION IS REGULATED BY IRON IN SOYBEAN CELL CULTURES. J.F. Briat[1], D. Proudhon[1], M. Ragland[2], E.C. Theil[2] and A.M. Lescure[1]. [1]Laboratoire de Biologie Moléculaire Végétale, Centre National de la Recherche Scientifique and Université Joseph Fourier, BP53X, F-38041 Grenoble cedex, France. [2]Biochemistry Department, NCSU-Raleigh, NC27695-7622, USA.

In higher plants, ferritin protein is specifically localized in plastids, as shown by microscopy observations. It is synthesized as a large precursor that gives the mature chloroplastic subunit by the cleavage of a transit peptide. This transit peptide has been found in the sequence of a partial Soybean hypocotyl ferritin cDNA. Moreover, this cDNA shares strong homologies with animal ferritins but not with *E. coli* ferritin, suggesting a common eukaryotic progenitor. Ferritin has been shown to be inducible by iron overload. In animals, a widely studied translational control is mainly involved, although a low transcriptional induction is also observed. By contrast, regulation of iron response in a Soybean cell culture system is entirely accounted for by transcription. A 60 fold increase of ferritin protein coincides with a 45 fold increase in ferritin mRNA synthesis and a 50 fold increase in ferritin mRNA accumulation.

The iron-induced ferritin has been purified. It is built up from a 28 kDa subunit and contains an average of 1800 atoms of iron per molecule. The major ferritin mRNA from Fe-induced Soybean cells is 100% homologous to normal Soybean hypocotyl cDNA and confirms the identity with animal ferritin genes.

The apparent absence of translational control of iron-induced ferritin synthesis in cultured Soybean cells contrasts with the dominance of translational control of ferritin synthesis in animal cells and may reflect variations in how cells detoxify iron : ferritin in animal cells versus vacuoles in plant cells.

1524 FROST, ABA AND DESICCATION HASTEN CANOLA EMBRYO DEVELOPMENT. A.M. Johnson-Flanagan, X.M. Geng, Z. Huiwen, C. Nykiforuk, D.C.W. Brown and J. Sing, Department of Plant Science, University of Alberta and Plant Research Centre, Agriculture Canada, Ottawa (DCWB, JS)

Seed development in Brassica napus following a mild nonlethal freeze was examined with respect to ABA levels, desiccation and expression of LEA and ICL (isocitrate lyase) transcripts. Plants with seeds of 70 and 55% moisture contents were frozen to -5°C for 3 h, and seed development followed after thawing. In addition, similar processes in Brassica microspore-derived embryos after induction of extreme desiccation tolerance by application of ABA in culture were compared.

A mild freeze/thaw caused a premature switch in seed developmental direction from predesiccation to desiccation as indicated by an immediate and accelerated loss of seed moisture to levels similar to the mature seed in 7 instead of 35 days, elevated ABA levels and induction of LEA76 and ICL transcripts. Similarly, addition of ABA to microspore-derived embryos in culture resulted in the induction of desiccation tolerance and low levels of LEA but not ICL transcripts. In contrast, normal seed development and desiccation of ABA treated (desiccation tolerant) and control (desiccation sensitive) embryos all resulted in the induction of very high levels of LEA76 transcripts. The results suggest that the development of desiccation tolerance whether by a hastening of the maturation process in the developing seed, or by the exogenous application of ABA in haploid embryos in culture, was not accompanied by the induction of high levels of LEA76 transcripts

1525 GENE EXPRESSION IN NaCl-ADAPTED RICE SUSPENSION CELLS Li-Fei Liu (1). Chia-Hui Lee (1) and Su-May Yu (2). (1) Dept. of Agronomy, National Taiwan University, Taipei, Taiwan, 10764 ROC (2) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan 11529, ROC.

Fast growing rice (*Oryza sativa* L. Tainan 5) suspension cells have been initiated from young embryo derived callus. Cell lines adapted to 1, 1.5 and 2% NaCl were selected by step wise method. Cell growth rate was decreased following the increasing of NaCl concentration. In salt adapted cells, the water content was decreased about 10-15%, the sugar, protein, and polyamine contents were increased about 2-3 folds, and proline content was increased about 50 folds. The protein patterns of NaCl-adapted cells were compared to that of non-adapted cells on one-dimentional and two-dimentional polyacrylamide gel electrophoresis. In salt adapted cells, new spots appeared in the 34KD, 23KD and 21KD regions, and spots dissappeared in 54KD, 40KD and 34KD regions. Among them, the decrease of 54KD polypeptide was most prominent, and correlated with NaCl concentration. Whether those polypeptides are associated with salt adaptation is further studied

1526 GENOTYPIC DIFFERENCES IN GROWTH AND ^{14}C SUGAR UPTAKE UNDER PEG STRESS AMONG RAINFED RICE GENOTYPES. Vajranabhaiah, S.N., Reddy, P.C. and Prakash, A.H.University of Agricultural Sciences, Department of Crop Physiology, GKVK, Bangalore-560 065.

Seven rice genotypes-CTH-1, Doddi, Halubbulu, IR, Prabhavathi, Rasi and Valya-cultivated under rainfed situation in south eastern dry plains of India (Karnataka) were employed to determine tolerance limits invitro prior to development of drought tolerant somaclones. Calli derived from seeds were grown in media containing PEG equivalent to 0.8 MPa of stress. Growth, cell number, reducing sugars and ^{14}C (sucrose) sugar uptake were determined. The results obtained indicated that cultivars Rasi, Doddi and CTH recorded higher fresh and dry weights. The cell number under stress were high in C.V. Doddi, Halubbulu, Prabhavathi and CTH. The reducing sugar content under stress were high in Halubbulu, Rasi, Doddi and CTH. Pattern of the uptake of ^{14}C and incorporation into insoluble fraction revealed that stress reduced uptake and also incorporation into insoluble fraction. However, soluble ^{14}C fraction in Rasi, Halubbulu, Doddi and CTH were high under stress. Insoluble fraction of ^{14}C was high in case of Rasi, CTH Prabhavathi and Halubbulu. Ratio between the two (soluble/insoluble) fractions was very low in Rasi and Halubbulu and little high in CTH and Doddi. This suggests that more efficient uptake and incorporation of the ^{14}C to form the dry matter. Results are discussed in the light of the protein fractionation profiles and water relation parameters.

POSTER ABSTRACTS

1527 GLUTAMATE DEHYDROGENASE AS A TARGET FOR IMPROVED AMMONIUM TOLERANCE IN PLANTS. Kerrie M. Jones[1,2], Michael J. McPherson[1], Andrew C. Cuming[2] and John C. Wootton[2]. [1]Department of Biochemistry and Molecular Biology and [2]Department of Genetics, University of Leeds, Leeds, LS2 9JT, UK.

Ammonium is a crucial metabolic intermediate but excessive amounts are toxic to most plants leading to uncoupling of phosphorylation from photosynthetic electron transport, interference of pH regulation and inhibition of some enzymes. Symptoms include stunted growth, distorted morphology, increased susceptibility to other stress and chlorosis by chloroplast degradation. Plants usually assimilate ammonia by the glutamine synthetase/glutamate synthase pathway. Disposal of elevated levels of ammonia may be achieved by accelerating this pathway or by supplementing it with additional ammonium utilising reactions. The detoxification mechanisms which plants use are of interest for a number of reasons including the problem of 'excess nitrogen deposition' and the possibility of reducing nitrate accumulation in crop plants. Arabidopsis thaliana is being used as a model to examine ammonium toxicity and tolerance in plants with a view to engineering improved tolerance. Various evidence suggest that glutamate dehydrogenase (GDH) may be of importance in detoxification including work at Leeds which has demonstrated increased ammonium tolerance in a cyanobacterium carrying a foreign gdh gene. We have been using a nested PCR strategy to clone plant gdh genes using 'universal primers' designed from a multiple sequence alignment. gdh gene fragments have been amplified from Arabidopsis and a variety of other organisms. Progress towards the isolation and characterisation of an Arabidopsis gdh gene will be reported.

1528 **A HISTONE-LIKE GENE THAT IS INDUCED BY ENDOGENOUS ABA DURING DROUGHT STRESS.** Elizabeth A. Bray, Ryozo Imai, Meena S. Moses, Amybeth Cohen, and Aine L. Plant. Department of Botany and Plant Sciences, University of California, Riverside, CA 92521

The plant hormone abscisic acid (ABA) plays a role in the regulation of changes in gene expression during drought stress. The cDNA, pLE20, was isolated from a cDNA library constructed from poly(A)+ RNA isolated from drought-stressed tomato leaves. pLE20 expression requires elevated levels of endogenous ABA. pLE20 mRNA was not expressed in drought-stressed leaves of the ABA-deficient mutant, *flacca*, as it was in the wild type. In addition, ABA application to the mutant resulted in pLE20 mRNA accumulation. The nucleotide sequence of the cDNA and corresponding genomic DNA fragment revealed that the deduced polypeptide sequence is similar to histone A1 and a regulatory protein, AlgR, found in *Pseudomonas*. Sequence similarities, and expression characteristics will be discussed.

1529 HYDROLYSIS OF POLYPHOSPHATES WITHIN ACIDIC VACUOLES COUNTER BALANCES AMINE-INDUCED ALKALINE STRESS. Uri Pick* and Meira Weiss, The Weizmann Institute of Science, Rehovot 76100, ISRAEL.

Amines at alkaline pH induce a transient internal alkalinization and a drop in ATP in cells of the halotolerant alga *Dunaliella*. Within 30 to 60 min the cells recover their motility, ATP, photosynthetic capacity and cytoplasmic pH. The recovery is accompanied by massive swelling of acidic vacuoles and by release of a fluorescent amine indicator from the vacuoles, indicating that the amine is compartmentalized into the vacuoles. The accumulation of amine within the vacuoles is associated with a parallel hydrolysis of long-chain polyphosphates to tripolyphosphates. The results indicate that: (1) Acidic vacuoles serve as the major reservoir for accumulation of amines; (2) Recovery of cytoplasmic pH from alkaline stress is brought about by accumulation and protanation of amines within the vacuoles; (3) The hydrolysis of polyphosphates provides a high-capacity buffering system to counterbalance internal alkalization; (4) Acidic vacuoles possess a phosphatase which is activated at neutral pH, and cleaves long chain to tripolyphosphates.

1530 IDENTIFIATION OF A RICE SALT TOLERANT LINE IN MOLECULAR RESPECTS Shouyi Chen, Lihuang Zhu, Hongjian Zhang, Shaolin Chen. Institute of Genetics, Academia Sinica, Beijing 100101, China

The anther cultures of rice "77-170", a Japanese strain of Oryza sativa were treated with EMS or nontreated, then the calli were selected for NaCl-tolerance on N6 medium containing 0.5, 0.8, and 1% NaCl respectively Three rice salt tolerant lines were obtained from EMS treated anther culture and two from nontreated.

The salt tolerance trait of all five lines have been stably inherited through nine generations. In order to characterize these mutant lines in molecular respects, 130 RFLP genetic markers along 12 pairs of chromorones based on McCouch's rice RFLP linkage map were used to assay polymophysm between the mutant lines and its original variety. It was notable that allelic differences were found at two linked loci RG711 and RG4 on chromosome 7 of three mutant lines which were treated with EMS, only one allelic difference at RG711 was found between the original variety and the mutant line nontreated with EMS. It seems that the difference at RG711 may related with salt-tolerant in mutants. Besides, proteins of roots and leaves between the mutant and the control plants were compared by two dimensional polyacrylamide gel electrophoresis. The Comparison showed that some new proteins appeared in the salt tolerant plant under salinity condition, which might be produced by salt inducible genes.

1531 IDENTIFICATION AND ISOLATION OF SALT-STRESS PROTEINS FROM MICROSOMAL FRACTION OF INDICA RICE CULT. 'SONALI'. Rajat K. Chaudhuri[1], Anup Das[1], Dilip Roy[2], Sudip Bandopadhyay[2] Calcutta University, [1]Botany Department, [2]Biochemistry Department, 35 B C Road, Calcutta 700 019, India.

Exact mechanism of salt tolerance is not yet clear. It is thought to be a quantitative trait. Genomes of several Indian rice cultivars are found to contain this character. CSR4 is one of them. When seeds of such tolerant variety are exposed to 0.1 molar sodium chloride concentration new proteins are found to be synthesised at the microsomal fraction of the cells, with ^{35}S-methionine incorporation, within few hours of salt treatment. On the other hand, sensitive variety like cultivar 'sonali' shows an inhibition of total protein synthesis, after such treatment. Salinity-related proteins are identified in 2 D gels. Works are in progress to sequence the proteins, and also to find their homology with other stress-related proteins. Regulatory functions, if any, of these proteins for stress-related gene expressions shall be looked into. Results will be discussed. Financial assistance of RF Project (#48) is acknowledged.

1532 IN VITRO SELECTION FOR SALT TOLERANCE IN MAIZE. Franca Locatelli[1], Maria Clotilde Lusardi[1], Joan Stadler[2] and Elisabetta Lupotto[1]. 1) Istituto Sperimentale per la Cerealicoltura, 24100 Bergamo, Italy; 2) Zoology/Genetics Dept., Iowa State University, Ames, Iowa 50011

The objective of this study was to characterize the salt tolerant phenotype of NaCl-selected maize embryogenic cell cultures and to test whether in vitro salt tolerance would be stably inherited and expressed in regenerated maize plants. Selection for salt tolerance has been performed in vitro with cell lines of other crop species, but these selected characteristics were not always retained in plants obtained from the resistant cultures. Salt resistant maize cell lines were obtained in embryogenic cultures derived from immature F2 embryos of the cross A188 x W64A by selection on 86 mM NaCl. The salt selected maize somaclonal variants were compared with parental salt sensitive cultures and with cultures obtained from the maize synthetic population Arizona 8601 which is salt tolerant at the plant level. Somaclonal variant and Arizona 8601 cell lines were capable of growth on 129 to 344 mM NaCl, concentrations which strongly inhibit the growth of parental cultures. Plants were regenerated from the in vitro salt selected maize cell lines, and the fresh weight of R2 seedlings grown in saline conditions (12% seawater) was greater than that of parental controls. These results indicate that cellular mechanisms underlying the salt resistance selected in vitro also provide some protection against salt stress at the seedling level.

This is the first report which suggests that it may be feasible to increase salt tolerance in maize by means of in vitro selection.

POSTER ABSTRACTS

1533 IRON INDUCTION OF FERRITIN SYNTHESIS IN ROOTS AND LEAVES FROM MAIZE. J.F. Briat, O. Massenet and S. Lobréaux. Laboratoire de Biologie Moléculaire Végétale, Centre National de la Recherche Scientifique and Université Joseph Fourier, BP53X, F-38041 Grenoble cedex, France.

Plant ferritin is an iron storage protein located in plastids. It has an eukaryotic origin and its synthesis is induced by iron, at the transcriptional level. In order to understand the molecular mechanisms of this control, we have initiated a study of the iron induction of ferritin synthesis in maize.
Ferritin from maize seeds has been purified to homogeneity allowing to raise antibodies against its subunit and to determine its NH_2- terminal amino acid sequence. Antibodies have been used to determine the iron nutrition conditions necessary to induce ferritin protein accumulation in roots and leaves from maize plantlets in hydroponic cultures. From an iron-induced cDNA library from maize roots, three different ferritin cDNAs have been characterized. Two of them are identical, except in their 3' non coding region within which an alternative polyadenylation signal can be used, leading to a difference in length of the UTR. A third clone has a different sequence but still shares a strong homology within its coding region with the other cDNAs. Both clones contain a transit peptide for plastid targetting and a plant specific extension peptide (absent in animal ferritin sequences) in the N terminal part of the protein. Northern experiments have allowed to show that ferritin protein accumulation in response to iron in roots and leaves is preceeded by a transient increase in ferritin mRNA accumulation. This transient increase being reminiscent of stress responses, we have tested the effect of the iron treatment on the accumulation of known maize RAB transcripts. Indeed, a transient accumulation of two different maize RAB mRNAs is observed after the iron treatment. This observation raises the question of an eventual involvement of abscissic acid in the iron induction of maize ferritin synthesis. This hypothesis is actually tested in our laboratory.

1534 ISOLATION AND CHARACTERIZATION OF 1-PYRROLINE-5-CARBOXYLATE REDUCTASE GENES FROM *ARABIDOPSIS THALIANA* Nathalie Verbruggen, Raimundo Villarroel and Marc Van Montagu. Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

In higher plants, proline is believed to play a role in resistance to stress such as salt, drought, and frost. Free proline accumulation is frequently observed in plants grown under such conditions. The mechanisms involved in proline accumulation are still unclear but an enhancement of the proline biosynthesis occurs. We are interested in studying the proline biosynthesis pathway which until now is very poorly understood in plants. For the last enzyme in the pathway, 1-pyrroline-5-carboxylate reductase (P5CR), two different copies have been isolated from a genomic library of *Arabidopsis thaliana* and sequenced. These two copies are more than 90% homologous. Cloning of the corresponding cDNA is in progress. Expression analysis of the P5CR genes upon salt stress will be reported.

1535 ISOLATION, CHARACTERIZATION AND EXPRESSION OF EM-LIKE GENES FROM *ARABIDOPSIS*. G. Hull, P. Gaubier, G. Huestis, M. Raynal, F. Grellet and M. Delseny. Université de Perpignan, Laboratoire de Physiologie et Biologie Moleculaire Vegetale, U.A. 565 du CNRS, 66860 Perpignan, France.

During the late maturation stage of the plant seed, at the onset of desiccation, a particular set of mRNA's accumulate in the embryo. The pattern of expression of the corresponding genes suggests that the proteins which they encode, called Late Embryogenesis Abundant (LEA) proteins, play a role in seed maturation and desiccation, and/or germination. The early methionine (Em) protein is a major LEA protein of wheat which has been shown to be regulated by abscisic acid (ABA). It has been suggested that Em may play a role in maintaining seed viability during desiccation. Using a heterologous cDNA probe from radish, two clones encoding Em-like proteins have been isolated from a genomic library of *Arabidopsis*. Nucleotide sequencing shows that one of the genomic clones (pGEA1) differs from wheat Em in that a 60bp sequence is repeated four times in the coding region. Southern blot analysis shows that the genes belong to a small family of 2-3 members, and preliminary Northern blots indicate that pGEA1 is expressed only in mature seed. Putative seed specific and ABA responsive elements have been identified in the 5' flanking regions of the genes by comparison with published consensus sequences. Chimeric gene fusions of pGEA1 5' flanking DNA linked to the GUS reporter gene have been constructed, and are being used in transformation experiments to determine the role of these elements *in vivo*.

1536 INHERITANCE OF AND MOLECULAR MARKERS FOR OSMOTIC ADJUSTMENT TO WATER STRESS IN GRAIN SORGHUM.

M.M. Ludlow, J. Basnayake, M. Cooper, R. G. Henzell, J. M. Manners and Y. Tao. CSIRO Division of Tropical Crops and Pastures, Dept of Agriculture, University of Queensland and Queensland Department of Primary Industries, St Lucia, Queensland 4067, AUSTRALIA.

Osmotic adjustment to water stress has been shown to enhance the yield of grain sorghum by up to 33% in water-limited environments. Currently studies are underway to determine the inheritance of this trait using classical genetics and to develop RFLP and RAPD markers so that genes for high levels of osmotic adjustment can be introgressed into otherwise well-adapted and high yielding accessions. Twenty parents have been screened for osmotic adjustment and polymorphisms. Ten parents were used to make a half diallel cross of F_1 progeny, and then progeny of three of these parents used to produce F_2 and backcross generations. Based on data from the F_1 and F_2, osmotic adjustment is highly-heritable, and there appears to be 3-4 genes segregating for the trait; one expressing partial dominance, one recessive, and 1 or 2 modifying genes. Linkage studies to develop RFLP and RAPD markers are being conducted on a F_2 population and will subsequently be done on backcross and recombinant inbred F_5 populations.

1537 IN VITRO SELECTION FOR SALT TOLERANCE IN MAIZE. Franca Locatelli[1], Maria Clotilde Lusardi[1], Joan Stadler[2] and Elisabetta Lupotto[1]. 1) Istituto Sperimentale per la Cerealicoltura, 24100 Bergamo, Italy; 2) Zoology/Genetics Dept., Iowa State University, Ames, IA 50011

The objective of this study was to characterize the salt tolerant phenotype of NaCl-selected maize embryogenic cell cultures and to test whether in vitro salt tolerance would be stably inherited and expressed in regenerated maize plants. Selection for salt tolerance has been performed in vitro with cell lines of other crop species, but these selected characteristics were not always retained in plants regenerated from the resistant cultures. Salt resistant maize cell lines were obtained in embryogenic cultures derived from immature F2 embryos of the cross A188 x W64A by selection on 86 mM NaCl. The salt selected maize somaclonal variants were compared with parental salt sensitive cultures and with cultures obtained from the maize synthetic population Arizona 8601 which is salt tolerant at the plant level. Somaclonal variant and Arizona 8601 cell lines were capable of growth on 129 to 344 mM NaCl, concentrations which strongly inhibit parental cultures. SDS-PAGE analyses showed that 26-28 kDa proteins were constitutively overexpressed in the salt tolerant and Arizona 8601 cell lines. Plants were regenerated from the in vitro salt selected maize cell lines, and the fresh weight of R2 seedlings grown in saline conditions (12% seawater) was greater than that of parental controls. These results indicate that cellular mechanisms underlying the salt resistance selected in vitro also provide some protection against salt stress at the seedling level.

This is the first report which suggests that it may be feasible to increase salt tolerance in maize by means of in vitro selection.

1538 A MAJOR PLASMA MEMBRANE 150 kDa PROTEIN IS INDUCED BY SALT AND BY INTERNAL pH CHANGE IN THE HALOTOLERANT ALGA *Dunaliella*. Avi Sadka, Adriana Katz, Mordhay Avron and Ada Zamir, Biochemistry Dept. Weizmann Institute Rehovot 76100, Israel

The genus *Dunaliella* includes extremely salt-tolerant, unicellular green algae that achieve osmotic balance by adjusting their intracellular glycerol concentration in a process not requiring *de novo* protein synthesis. Searching for additional functions involved in the salt tolerance of *Dunaliella salina*, we have detected, purified and characterized a plasma membrane 150 kDa glycoprotein (p150) whose level corresponded to the external salinity and whose synthesis was salt induced. Correlations between p150 induction and cell proliferation strongly suggested p150 was required for sustained growth in saline media (Sadka *et al.*, Plant Physiol. 95, 822 (1991)). Recent evidence indicates that p150 is also induced when cells are adapted to growth in the presence of 18 mM NH_4Cl, a treatment affecting the intracellular pH and previously shown to induce the activity of a Na^+/H^+ antiporter (Katz and Avron, unpublished). Immunochemical analyses suggested p150 belonged to a family of widely conserved proteins. Putative cDNA clones for p150 were isolated with the aid of anti-p150 antibodies and by differential screening of cDNA libraries with mRNA from salt-induced or non-induced cells. In Northern analyses these cDNA clones recognized a single large transcript whose level paralleled the induction of p150 thus providing evidence for transcriptional regulation of the protein.

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1539 A METHYL TRANSFERASE INDUCED BY SALT STRESS AND ABA, BUT NOT DROUGHT, IN THE FACULTATIVE HALOPHYTE *M. CRYSTALLINUM*. Daniel M. Vernon and Hans J. Bohnert, Dept of Molecular and Cellular Biology, U. of Arizona, Tucson, AZ, 85721

The facultative halophyte *Mesembryanthemum crystallinum* conserves water during prolonged osmotic stress by changing from C3 to CAM photosynthesis. The switch to CAM is not immediate. It is preceded by a period of temporary wilting followed by recovery, and is considered separate from the plants' ability to tolerate high salinity in the short term. To investigate the molecular basis of salt tolerance, we studied changes in gene expression distinct from those associated with CAM. Screening of a subtracted cDNA library enriched for stress-induced sequences yielded a dramatically up-regulated mRNA, B4-OMT. This RNA is transiently induced in roots prior to its maximum induction in leaves and shoots. It is encoded by a single-copy gene and is up-regulated transcriptionally. Low temperature and ABA, both of which cause temporary wilting and recovery, trigger increases similar to those observed with salt stress. However, B4-OMT transcript levels are not increased by drought, which causes long-term wilting and does not allow for osmotic adjustment and recovery of turgor. Drought does induce the accumulation of mRNAs encoding CAM enzymes. B4-OMT sequence analysis and activity of the protein expressed in *E. coli* indicate that the transcript encodes a lignin-specific hydroxymethyl transferase. We suggest that there is more to salt tolerance than osmotic adjustment and accumulation of compatible solutes, and that this methyl transferase plays a role in the maintenance of structural integrity during adaptation to saline conditions in this facultative halophyte. Supported by the USDA

1540 MOLECULAR ANALYSIS OF THE PCR-CLONED ALCOHOL DEHYDROGENASE GENE FAMILY FROM SOYBEAN. Tara VanToai and Kurt Newman. USDA-ARS, Soil Drainage Research and Agron Dept, Ohio State Univ, Columbus, OH 43210.

A single ADH isozyme was resolved by native polyacrylamide gel electrophoresis of protein extracts from control and stressed soybean leaves, hypocotyls and roots. The cotyledons of unstressed soybeans contained three ADH isozymes, two were distinct, one was faintly visible. Following anoxic stress, all three isozymes increased in intensity. A multi gene family model was proposed for soybean ADH with organ specific expression of family members. Soybean ADH cDNA's were amplified in-vitro from total RNA by the polymerase chain reaction (PCR). The amplification strategy involved first strand cDNA synthesis from stressed cotyledon total RNA using an oligonucleotide composed of 18 thymine residues as primer. The sense strand primer for PCR was an oligonucleotide homologous to a conserved region near the 5' end of the plant ADH transcripts. The PCR products were directionally cloned into Bluescript (Stratagene). Unique clones were isolated on the basis of size and restriction pattern. Sequence analysis revealed three distinct soybean ADH cDNA. RNA blot hybridizations showed differential expression patterns for the genes. All three genes were induced by anoxia. One gene expressed in all stressed organs, one gene expressed in control tissues and the third gene was predominantly root-specific. The expected cotyledon specific transcript was not cloned.

1541 MOLECULAR ASPECTS OF SALT-ASSOCIATED PROTEIN IN *CITRUS*. Gozal Ben-Hayyim, Zehava Faltin, Doron Holland and Yuval Eshdat. Agricultural Research Organization, Volcani Center, 50250 Bet Dagan, Israel.

Most members of the *Citrus* genera are very sensitive to salt. Studies aimed to understand the molecular mechanisms involved in salt tolerance in *Citrus* revealed a 25 kD (pI 6.1) low-turnover protein which was isolated by two-dimensional PAGE from Shamouti orange (*C. sinensis* L. Osbeck) salt-tolerant cultured cells (Ben-Hayyim, Faltin, Camoin, Strosberg and Eshdat, in preparation). The level of the 25 kD protein was significantly increased by growing the cells in the presence of NaCl, ABA or PEG. Western blot analyses using polyclonal antibodies raised against the purified 25 kD protein indicate that the level of the protein increased in roots, leaves and stems of Etrog citron (*C. medica*) and Cleopatra mandarine (*C. reticulata*) seedlings irrigated with saline water. Partial amino acid sequence of peptides isolated by tryptic digest of the 25 kD protein did not reveal any significant homology with other sequenced proteins, including Osmotin. In addition, no immunological cross reactivity was observed neither for the anti-25 kD specific antibodies with Osmotin nor for anti-Osmotin specific antibodies with *Citrus* cell extracts. In an attempt to further characterize the 25 kD protein and to isolate the gene encoding the protein, a directional cDNA gene expression library in bacteriophage vector λZAPII was constructed from mRNA derived from salt-grown Shamouti orange cultured cells. Screening of this library with antibodies against the 25 kD protein is currently being done.

1542 MOLECULAR CHARATERIZATION OF SALT TOLERANT WHEAT GERM-PLASM DEVELOPED THROUGH WIDE HYBRIDIZATION. S. Farooq, T.M. Shah and N. Iqbal, NIAB, Faisalabad, Pakistan.

Salt tolerant germplasm of hexaploid wheat has been produced by crossing salt tolerant accession of Ae. cylindrica with cultivated wheat used as female parent. The F1 hybrids were backcrossed & BC1 seeds were selfed to get self fertile derivatives. All the derivatives were tested in hydroponics and saline field at conductivity levels ranging between EC 15-20 dS/m; all of them produced grain yield of 19-23% of control. Both the wheat parents were unable to set seed under these conditions. In order to identify the genes of Ae. cylindrica conferring salt tolerance to this germplasm, we are planning to isolate mRNA from Ae. cylindrica growing under saline and non saline conditions for the production of cDNA library and formation of Probes respectively. These probes will be hybridized with cDNA. The unhybridizable colonies will be selected and pocessed for isoaltion of plasmid and cDNA fragment cloned into it. This fregment will be labelled with 32P and hybridized as probe with DNA isolated from salt tolerant wheat lines mentioned above to identify the gene(s) of Ae. cylindrica conferring salt tolerance.

1543 MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION OF PEA SUPEROXIDE DISMUTASES. D. White, L. Pitcher and B. Zilinskas, Rutgers University, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903.

Superoxide dismutases (SODs) are metalloenzymes responsible for the dismutation of superoxide radicals to H_2O_2 and O_2 and are thus central in defense of plants against oxidative stress. We previously reported the cloning and sequencing of a cDNA encoding the chloroplastic Cu/Zn-SOD of pea (Scioli and Zilinskas, 1988). We now describe the isolation and characterization of cDNAs encoding pea cytosolic Cu/Zn-SOD and Mn-SOD. The availability of these cDNAs, as well as antibodies specific for each of the SODs, has made possible the examination of the tissue- and developmental-specificity of the expression of each of the genes encoding these SODs. Activity of each of the SOD isozymes was measured in parallel experiments using nondenaturing gels. These studies revealed that there is a differential regulation of the expression of the Cu/Zn-and Mn-SODs with regard to temporal, spatial and oxidative stress conditions, specifically ozone exposure. This regulation appears to be at least in part at the transcriptional level, as changes in transcript abundance parallel changes in SOD protein level and activity.

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1544 MOLECULAR CLONING OF THE BETAINE ALDEHYDE DEHYDROGENASE cDNA OF A HALOPHYTE. L. Adam[1], R.K. Jain[1,2], S. Jana[2] and G. Selvaraj[1]. [1]Plant Biotechnology Inst., Saskatoon, SK, Canada; [2]Dept. of Crop Science & Plant Ecology, Univ. of Saskatchewan, Saskatoon, SK, Canada.

Kochia scoparia is a halophyte of European origin that has colonized parts of North America. Its ability of withstand osmotic stress has incited an interest in its cultivation for rationed use in animal feed. We have been studying this plant with regard to characterizing its adaptive strategy against osmotic stress. Kochia can synthesize glycinebetaine (GB), a major osmolyte in various organisms including some plants subjected to osmotic stress (1,2). Glycinebetaine synthesis is, therefore, a potential target for manipulating osmotic stress tolerance in plants. Betaine aldehyde dehydrogenase (BADH) is the second of the two enzymes involved in the synthesis of GB from choline (3,4). Recently a spinach cDNA and an *Escherichia coli* gene coding for BADH have been isolated and characterized (4,5). The primary structure of *E. coli* and spinach BADH are similar and show a remarkable resemblance to mammalian aldehyde dehydrogenases. Using a combination of polymerase chain reactions and DNA hybridizations a cDNA clone of Kochia coding for BADH was isolated. This clone contained a 2.1 Kb insert, which included the entire open reading frame coding for the BADH. The structural similarity of this BADH to its counterparts and the results pertaining to the expression of this gene in Kochia will be presented.

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POSTER ABSTRACTS

1545 MOLECULAR CLONING OF GLUTATHIONE REDUCTASE FROM PEA. Gary Creissen[1,*], Anne Edwards[1], Corine Enard[1], Phil Mullineaux[1] and Alan Wellburn[2]. 1 John Innes Institute, Norwich, U.K.; 2. Biological Sciences, Lancaster University, U.K..

Glutathione reductase [GR; EC.1.6.4.2] is a key enzyme of the glutathione-ascorbate (GA) cycle; one of the mechanisms thought to be important in the protection of plant membranes from free radical damage induced by oxidative stress. Under certain oxidative stress conditions (e.g. herbicide treatment, drought, exposure to atmospheric pollutants such as NO_2 and O_3), both synthesis and activity of GR have been shown to increase. In addition, treatment of leaves with glutathione or ascorbate has been found to reduce some types of oxidative damage. Thus components of the GA cycle appear to be important in the plant's response to stress and may be amenable to manipulation.

GR in pea was found to be represented by a single polypeptide of 55kDa on SDS-PAGE, and could be resolved into up to 8 isoforms by 2-D electrophoresis. Cell fractionation studies revealed that GR activity was distributed between chloroplasts (77%), cytosol (20%) and mitochondria (3%). Activity staining of GR isoforms on non-denaturing gels revealed differences in the types and abundance of the different isoforms in crude protein extracts from plants exposed to various forms of oxidative stress.

In order to study the molecular basis of the response of GR isoforms to oxidative stress, we have used an antiserum raised against pea GR to isolate cDNAs from gt11 libraries made from poly(A)+ RNA from control and stressed plants. Two cDNA clones have been sequenced and their derived amino acid sequences determined. The pea enzyme shows a high degree of homology to the human, E.coli and Pseudomonas aeroginosa enzymes. RFLP analysis indicates that the GR genes are nuclear encoded and are located at a single locus within the pea genome. We intend to use these cDNAs to manipulate the levels of GR in transgenic tobacco plants by overexpression in different cell compartments and by antisense RNA technology. This will enable us to address the question of the true role of GR in the oxidative stress response.

1546 PEA ASCORBATE PEROXIDASE: PURIFICATION, CHARACTERIZATION AND CLONING OF ITS cDNA. R. Mittler and B. Zilinskas. Rutgers University, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903.

Ascorbate peroxidase is a key enzyme in the defense mechanisms of plants to oxidative stress in that it rids the cell of H_2O_2. We have purified to homogeneity the cytosolic isozyme from shoots of two-week old pea shoots. The enzyme is a homodimer with a Mr of 57,000, composed of two subunits of 29,500d. Its spectroscopic properties in oxidized, reduced and KCN-inhibited form provide convincing evidence for the heme content of the enzyme. When compared with ascorbate peroxidase activity obtained upon rupture of isolated chloroplasts, the cytosolic enzyme was demonstrated to have higher stability, a broader pH optimum for activity, and the capacity to utilize alternate electron donors. Polyclonal antibodies raised against the cytosolic ascorbate peroxidase of pea did not cross-react with pea chloroplastic ascorbate peroxidase or with horseradish peroxidase. The amino acid sequence of the N-terminal region of the purified isozyme was determined. While little homology was found between the pea cytosolic ascorbate peroxidase, the chloroplastic isozyme of tea, and horseradish peroxidase, high homology was however found with the N-terminal sequence of spinach chloroplastic ascorbate peroxidase. Antibodies were used to screen a λgt11 expression library constructed from pea leaf mRNA. A 1.1 kb cDNA insert was subcloned into a plasmid vector and found to encode an immunoreactive polypeptide of 29,500 Mr. Nucleotide sequence analysis is in progress

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1547 PHOSPHATE STARVATION INDUCED SYNTHESIS OF TOMATO RIBONUCLEASES: PROPERTIES, INDUCTION PROCESS, POSSIBLE FUNCTION. K. Glund, B. Blume, M. Köck, K. Theierl, A. Löffler, T. Nürnberger and S. Abel. Universität Halle, Institut für Biochemie, 4050 Halle, Germany.

We have identified five ribonucleases in cultured tomato cells which are induced upon phosphate (Pi) starvation. One enzyme is secreted into the culture medium and three of the four cellular enzymes studied are located in the vacuoles. These RNases exhibit very similar physical and enzymatic properties. The protein sequence of the extracellular enzyme (RNase LE) displays similarity to fungal RNases T2, Rh, M, as well as to the products of self-incompatibility genes of tobacco and carrot. Using activity and antibody staining on native gels, we showed that these enzymes are rapidly induced by the absence of extracellular Pi or, in the presence of extracellular Pi, by addition of phosphorylatable metabolites to the cell cultures. By immunoscreening of an expression library prepared from RNA of Pi-starved tomato cells, we isolated a cDNA clone encoding sequences of RNase LE. RNase LE cDNA was used to study the induction process in more detail. The induction of multiple RNases upon Pi deprivation coincides with an enhanced rRNA degradation in these tomato cells. Attempts were undertaken to show participation of vacuoles during that process. We propose that these RNases are intra- and extracellular components of a hypothetical Pi starvation inducible rescue system in higher plants.

1548

POLYETHYLENE GLYCOL INDUCED CHANGES IN WATER STRESS ASSOCIATED GENE EXPRESSION IN RICE SEEDLINGS. A H K Rao, B. Karunasree and A R Reddy, School of Life Sciences, University of Hyderabad, Hyderabad 500 134, India.

Response of rice seedlings to water deficit caused by polyethylene glycol (PEG) was investigated in growing seedlings of different indica rice lines. Response was analysed at psysiological, biochemical and molecular level. Single as well as two dimensional PAGE of PEG treated seedling extracts revealed significant changes in polypeptide profiles. Three distinct polypeptides with apparent molecular weights of 15, 23 and 50 KD were found to be prominently induced by PEG. Of these the 15 and 23 KD polypeptides were purified to near homogeneity. The 23 KD polypeptide may be the same as "dehydrin" described previously. RNA dot-blot hybridizations with a heterologous cDNA probe, pcC 27 - 04 from an African resurrection plant Craterostigma plantagineum detected desiccation specific transcripts in PEG treated rice seedlings. These were detected in both water stress sensitive as well as tolerant seedlings under stress. These results are also in agreement with growth response profiles and proline levels in seedlings. The implications of the presence of disiccation resistance associated cDNA "homolog" of pcC 27 - 04 clone in rice genome will be discussed.

1549 PROTEINS FROM *ARABIDOPSIS* SUSPENSION CULTURES AND LEAVES BIND TO THE *Adh* ARE. Alice J. DeLisle. University of Nevada, Dept. of Biochemistry, Reno, NV 89557

***Alcohol dehydrogenase (Adh)* is developmentally regulated and induced by hypoxic conditions in both *Arabidopsis* and maize. In *Arabidopsis*, *Adh* is constitutively expressed in suspension culture cells but *Adh* mRNA is not detectable in leaves. In an attempt to find transcription factors that are responsible for the pattern of *Adh* transcription, I have analyzed extracts from cell suspensions and leaves for proteins that bind to the putative Anaerobic Response Element (ARE) of the *Arabidopsis Adh* promoter. Protein extracts were analyzed by gel mobility shift assays. Crude extracts from cell suspensions and leaves both contain ARE-binding activity. Comparison of the crude extracts shows different gel mobility shift patterns and different relative abundance of proteins that bind to the ARE. Following fractionation of crude extracts on FPLC MonoQ, however, there are few if any differences in the gel mobility shift patterns between fractions from suspensions and leaves. There are multiple proteins from both sources that bind to the ARE, but the ARE-binding proteins are still less abundant relative to total protein in the leaf extracts. Sequence binding specificity of the different bands in the MonoQ fractions are being analyzed using various competitors in gel mobility shift assays.**

1550 PYRUVATE-P_i-DIKINASE IN MESEMBRYANTHEMUM CRYSTALLINUM: INDUCTION BY SALT-STRESS AND NUCLEOTIDE SEQUENCE OF A FULL-LENGTH cDNA CLONE. B. Fißlthaler, M. Piepenbrock, F. Wronski, A. Sheriff, H.J. Bohnert[1], G. Meyer[1] & J.M. Schmitt. Institut für Pflanzenphysiologie, Freie Universität, 1000 Berlin 33, FRG; [1]Biochemistry, University of Arizona, Tucson AZ 85721, USA

The desert plant *Mesembryanthemum crystallinum* is a facultative CAM-plant (Crassulacean-Acid-Metabolism). Salt or drought stress shifts the mode of CO_2 fixation from C_3- to water conserving CAM-type photosynthesis. We have compared the induction kinetics of the stress-induced enzymes phosphoenolpyruvate carboxylase (PEPCase) and pyruvate-P_i-dikinase (PPDK) by determination of mRNA and protein concentrations. In most experiments, mRNA and protein abundance of the two enzymes increase in parallel when plants are stressed and decrease in parallel upon rehydration. However, in detached leaves, wilted for 8 hours, PEPCase transcripts increase with a much higher rate than PPDK transcripts. This indicates a loose regulatory coupling between the two enzymes. As a first step to characterize the promoter sequences involved in the change of abundance of transcript levels we cloned and sequenced the cDNA for PPDK from *M. crystallinum*. The cDNA is 3173 bp in length and corresponds to a polypeptide with a molecular weight of 103.2. The homology of the mature protein to the PPDK from maize and *Flaveria trinerva* is 78% and 80 %, respectively. We also cloned and sequenced the gene for PPDK from *M. crystallinum*. The gene has a minimal length of 12000 nucleotides and consists of at least 18 introns and 19 exons. At the 5' end there is a 1700 bp long intron in the nontranslated region. So far we have sequenced the promoter region up to 400 bp upstream of the putative transcription start site.

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1551 RESPONSES OF MAIZE TO PESTICIDE TREATMENT. Vinnichenko A.N., Shvemenko N.I., Mashara S.V., Martynova V.V. Biology Institute of Dnepropetrovsk State University

Maize of several types, differ on resistance and productivity were grown on control and treated with large dose of atrazine (experimental) fields. Stability of plant and quantity of pesticide in grain had reverse correlation connections. Composition of protein amino acids in grain changed under pesticide stress simultaneously with stability of plant too. Amino acid composition of hybrid, that was taken as a very stable form, didn't have any deviation in comparison with control species on the last stages of maturity and in rape grain. Only samples, picked on first days after pollination, had significantly increased quantity of bound glutamate and proline. Maize grains of line A 204 with average resistance had changed amino acid composition on all investigated stages after pollination due to considerable increase of glutamate content (on 5%). Amino acid composition of grain of line A 204 02/02 - the most unresistant, had the most deviations on all stages of ripening. Significant increase of glutamate (on 8%) and aspartate (on 5%) was found in these samples. Our results have shown, that process of atrasine detoxication took place not only in vegetative organs of maize, but in reproductive too. Process of inactivation of the xenobiotic required participation of protein amino acids and has genetic determination, i.e. depends on genotype of plant.

1552 SEQUENCE OF TWO WHEAT ABA-INDUCIBLE cDNAs AND THEIR EVALUATION AS MARKERS FOR DROUGHT TOLERANCE. Jeanne Curry and M.K. Walker-Simmons, USDA-ARS, Washington State University, Pullman, WA 99164-6420. Two ABA-responsive, dehydration stress-inducible cDNA clones pMA2005 and pMA1949 have been sequenced from wheat. The clone pMA2005 encodes a 224 amino acid, basic, hydrophilic protein with 10 tandem repeats of an 11 amino acid motif and is homologous to other Group 3 LEA (late embryogenesis abundant) proteins. Clone pMA1949 is a partial cDNA and has been deduced to encode a unique hydrophilic protein of at least 317 amino acids. Dehydration stress of young wheat seedlings results in the induction of pMA2005 and pMA1949 transcripts which correlates with a 25-fold increase of endogenous ABA levels. We are currently evaluating these clones as markers for drought tolerance among cultivars. Northern results with shoots from dehydration-stressed seedlings show differences in transcript levels. Additional studies measuring responses to and recovery from dehydration stress for these cultivars are in progress.

1553 SPINACH LEAF PROTEINS ACCUMULATED DURING COLD ACCLIMATION. ARE ALSO ACCUMULATED IN RESPONSE TO SMALL CHANGES IN WATER POTENTIAL. Dale Haskell, Charles Guy, Lisa Neven, and Chris Smelser. University of Florida, Department of Environmental Horticulture, Gainesville, Florida 32611.

The accumulation of 85 and 160 kDa proteins in spinach during cold acclimation is known to be correlated with increased freezing tolerance. Purification and protein sequence analyses of these proteins revealed that the 85 kDa protein was related to members of the LEA/Dehydrin/ABA family of proteins, and suggested that it might also be accumulated in response to desiccation. Western blot analyses of extracts from plants subjected to desiccation confirmed that both the 85 and 160 kDa proteins accumulated during desiccation. Surprisingly, the accumulation of these proteins was far more sensitive to changes in water potential than expected. They began to accumulate when the water potential decreased by 2.0 bars or more from a fully hydrated predawn level of approximately -1.0 bar. Seedlings grown in 100% relative humidity had much lower levels of the two proteins than nonstressed seedlings grown at ambient relative humidity. Desiccated and desiccated/rehydrated plants grown at nonacclimating temperatures contained elevated levels of the 85 and 160 kDa proteins and were more freezing tolerant than cold acclimated plants. This evidence suggests that cold acclimation and desiccation responses involve a common mechanism, and further establishes the linkage of these two proteins in freezing tolerance mechanisms.

1554 SUBCELLULAR LOCALIZATION OF Δ^1-PYRROLINE-5-CARBOXYLATE REDUCTASE. G.-H. Maio, A. Szoke, B. Stein and D. P. S. Verma, Biotechnology Center, The Ohio State University, 1060 Carmack Rd, Columbus, OH 43210.

A soybean nodule cDNA clone expressing Δ^1-pyrroline-5-carboxylate reductase (P5CR) was isolated by functional complementation of an *E. coli pro* C mutant. Northern blot analysis of soybean tissue treated with different salt concentration indicated that P5CR gene is osmoregulated. The *E. coli* expressed P5CR was purified to homogenity. The enzymetic properties of P5CR were determined including substrate specificity *km* and salt-dependent pH optima. Antibodies were raised against the purified enzyme from *E coli* expressing soybean P5CR. They were used to determine subcellular location of P5CR in soybean plants and transgenic tobacco expressing constitutive levels of P5CR. The latter was achieved by introducing the soybean P5CR cDNA under the control of CaMV-35S promoter. A high level of cytosolic P5CR was expressed in transgenic plants.

1555 SYNTHESIS OF A LOW MOLECULAR WEIGHT PROTEIN DURING SEVERE WATER STRESS IN A DESICCATION TOLERANT FERN Tracey L Reynolds and J D Bewley. University of Guelph, Department of Botany, Guelph, Ontario, Canada.

The desiccation tolerant fern, Polypodium virginianum, is able to lose more than 60% of its water content during periods of desiccation and then fully hydrate without any obvious physical damage. Rehydration from the air-dry state occurs rapidly (within 12 hours) with a concurrent initiation of protein synthesis. A low molecular weight doublet (mw 19-20 kDa) is synthesized during water stress and is stable for >48 hours after rehydration. These proteins are expressed when the fully hydrated fronds are subjected to 10^{-3}M ABA for 24 hours and they are not related to the "dehydrin" proteins found in corn. They may have a role in protection from or repair to damage incurred during the drying process. Also, during drying, there is the synthesis of the large subunit of Rubisco, but despite temporary accumulation, the de novo synthesis component is broken down again. The function of this rapid synthesis and turnover is unknown. During rehydration from the air-dry state, unique rehydration polypeptides are produced. These polypeptides are specific for the water content and time of rehydration and are only transiently expressed. This transient expression indicates that they are only necessary during the early stages of rehydration when the rapid initiation of physiological and repair processes is essential.

1556 TOBACCO OSMOTIN GENE EXPRESSION PATTERNS SUGGESTING POST-TRANSCRIPTIONAL CONTROL. P. C. LaRosa, D. E. Nelson, N. K. Singh, P. M. Hasegawa, Z. Chen and R. A. Bressan. Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

The protein osmotin is abundant in cells adapted to low osmotic potentials in the allotetraploid *Nicotiana tabacum* (cv. Wisconsin 38), and is encoded by two genes. Accumulation of both mRNA and protein in intact plants is developmentally regulated. Osmotin protein was most abundant in tobacco roots and peels of the outer stem consisting mainly of epidermis. It was less abundant in calyx and corolla, and was a minor protein in other tissues including developing and mature ovules. The mRNA levels paralleled those of the protein except in some tissues where high levels of mRNA but not protein accrued. Accumulation of osmotin mRNA but not protein was induced in some plant tissues by exogenous ABA, wounding and TMV infection. Ethylene induced the accumulation of osmotin mRNA and to a small extent protein in seedlings, but was ineffective in cultured cells. Exposure of cultured cells and plants to NaCl caused high levels of both mRNA and the protein to accrue. Thus, the accumulation of osmotin mRNA is controlled developmentally and by at least 5 hormonal or environmental signals, some acting independently. However, the accumulation of the final gene product, osmotin protein, occurs only in certain tissues in response mainly to osmotic stress and to some extent ethylene.

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POSTER ABSTRACTS

1557 TOWARD CLONING THE CARBAMYLPHOSPHATE SYNTHETASE GENE FROM ALFALFA. J.A. Malev[1], B.C. Hyman[2] and C.J. Lovatt[1]. [1]Botany and Plant Sciences and [2]Biology, University of California, Riverside, California 92521.

A parallel exists between drought tolerance in alfalfa and de novo arginine and polyamine biosynthesis. Drought sensitive strains under drought stress exhibit reduced arginine and polyamine production and accumulate ammonia, a substrate of the first and key regulatory enzyme in the pathway, carbamyl-phosphate synthetase (CPSase). To study regulation of CPSase in various tissues and under various conditions, we are cloning its gene. We used highly conserved regions of CPSase genes from a wide range of organisms (E. coli, yeast, Drosophila and hamster) to design degenerate oligonucleotide primers for use in the polymerase chain reaction (PCR). One PCR product from alfalfa cDNA template hybridizes to CPSase coding regions from the homologous genes. This product, isolated from the mixture of PCR products by hybridization, is used to probe cDNA libraries from plants to isolate CPSase sequences. In addition to the study of its role in drought tolerance, possession of the CPSase gene will allow evolutionary comparison to CPSases from other organisms.

1558 TRANSFER OF FOREIGN DNA IN RICE (Oryza sativa L.) THROUGH POLLEN-TUBE PATHWAY.

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Porteresia coarctata, a naturally tertraploid wild rice of Sundarban region (South West Bengal, India) is well known for its salt tolerance capability. It is very difficult to incorporate the salt tolerance capability of this wild rice into a cultivar through usual sexual hybridization. An attempt has been made in the present investigation to standardize the steps involved and its effects, if any in incorporation of wild DNA from Porteresia coarctata into a rice cultivar through pollen-tube pathway. An expected transgenic plant showed morphological variations particularly in regard to anthocyanin pigmentation.

1559 UNIQUE ENZYME ACTIVITIES FOR POLYAMINE BIOSYNTHESIS IN DROUGHT-TOLERANT ALFALFA. Suman Bagga, Gregory C. Phillips and Glenn D. Kuehn, New Mexico State University, Molecular Biology Program, Las Cruces, NM 88003 USA

Shifts in polyamine metabolism occur in response to abiotic stresses in plants. Polyamines are postulated to stabilize membranes and nucleic acids under extreme environments. We have reported the occurrence of uncommon long-chain polyamines in drought-tolerant alfalfa, induced by water-deficit stress. A paper electrophoresis technique was used to follow the conversion of radiolabeled substrates into the uncommon polyamines, as a means to identify the associated biosynthetic enzyme activities in partially purified extracts prepared from drought-tolerant alfalfa. In vitro assays identified a single aminopropyltransferase activity that was specific for putrescine as the substrate, yielded spermidine, thermospermine, homocaldopentamine and homocaldohexamine as products, and utilized decarboxylated S-adenosyl-methionine as the propylamine donor. A polyamine oxidase activity that accepted spermidine or spermine as substrates and yielded 1,3-diaminopropane, was distinguished from diamine oxidase activity. 1,3-Diaminopropane was elongated to caldine, thermine, caldopentamine and caldohexamine by repetitious reactions involving Schiff-base formation with L-aspartyl-ß-semialdehyde (serving as the propylamine donor), followed by reduction and decarboxylation in each elongation reaction. This Schiff-base reductase/decarboxylase activity is unprecedented, but has some similarity to the alternative route to spermidine biosynthesis described in a few bacteria and one other drought-tolerant plant. We are seeking the genes corresponding to these enzymatic activities as potential mediators of abiotic stress tolerances in plants.

1560 UNIQUE SPATIAL EXPRESSION PATTERN OF TWO DROUGHT- AND ABA-INDUCED GENES OF TOMATO. Amybeth Cohen, Aine L. Plant, Meena S. Moses and Elizabeth A. Bray. Dept. of Botany and Plant Sciences, University of California, Riverside, CA 92521

Expression of *le16* and *le25* occurs in response to water deficit and elevated levels of endogenous abscisic acid (ABA). Both genes are expressed in drought-stressed leaves, stems, and petioles, however, each gene has unique spatial expression characteristics. *le16* gene expression is confined primarily to aerial, vegetative tissues, while *le25* is predominately expressed in dehydrating seeds. In addition, both genes are differentially expressed in response to salinity, cold stress, heat stress, and ABA treatments, with *le16* expressed only in leaves and *le25* in leaves and roots. *le16* and *le25* encode deduced polypeptides of 12.7 kD and 9.3 kD respectively. The LE16 polypeptide, rich in leucine, glycine, and alanine, contains both hydrophobic and hydrophilic regions. The hydrophobic amino-terminus is characteristic of signal sequences which target polypeptides for export from the cytoplasm. The amino-terminus of the mature LE16 polypeptide has 47.2% identity with the amino-terminus of the maize phospholipid transfer protein. The LE25 polypeptide, rich in alanine, threonine, lysine, and glycine, is overwhelmingly hydrophilic. A 50% identity exists between LE25 and the cotton late embryogenesis abundant protein, D113. The 5' flanking sequences of the *le25* gene contain a consensus ABA response element (ABRE) common to the promoters of many ABA-responsive genes, including *d113*. Such an ABRE is absent in *le16* despite expression in response to elevated levels of ABA during periods of water deficit.

1561 REGULATION OF OSMOTIN PROMOTER ACTIVITY IN OSM/GUS TRANSGENIC TOBACCO PLANTS. D.E.Nelson, A.K.Kononowicz, N.K. Singh, P.M.Hasegawa and R.A.Bressan. Purdue University, Dept of Horticulture, West Lafayette, Indiana 47907.

An osmotin promoter ligated to the ß-glucuronidase (GUS) gene coding region was introduced into *Nicotiana tabacum* L., cv W38 by *Agrobacterium*-mediated transformation. Vegetatively propagated R_0 and R_1 generations of these transformants were studied by means of biochemical and/or histochemical methods for regulation of the osmotin promoter activity by ABA, ethylene, wounding, and adaptation to NaCl and PEG. Seedlings of the primary transformants were treated in bulk with 100 uM ABA or 20 ppm ethylene before MUG assays. In agreement with previous data from nuclear run-on transcription assays, five- and four-fold increases of promoter activity were observed after ABA and ethylene treatment, respectively. Vegetative clones of the primary transformants were adapted in hydroponics to 171 mM NaCl. The tissue specificity of the promoter activity was examined by X-gluc assay and light microscopy of paraffin sections. Adaptation to NaCl stimulates GUS gene expression in rootcap cells (but not in root apex meristems) and in the root elongation zone (epidermis and root cortex parenchyma). Increased GUS activity was observed also in stem (epidermis and xylem parenchyma) and fully developed leaves (trichomes, epidermis, mesophyll, and xylem parenchyma). In both NaCl-adapted and unadapted plants high expression of GUS gene was found in mature pollen grains at the stage of anther dehiscence. However, osmotin promoter activity decreased dramatically during *in vitro* germination of pollen grains. In conclusion: organ and tissue specific activity of osmotin promoter is regulated by growth regulators and environmental stresses. Furthermore, response of osmotin promoter to these factors is (at least in certain cases) related to stage of plant development.

Supported by USDA grant 86-CRCR-1-2064 and NSF grant DCB-9005216.

1562 CLONING OF GENES RELATED TO SALT ADAPTATION. Yi Xu, P.-F. Chang, M. Reuvani, R. Rietveld, N. K. Singh, P. M. Hasegawa, R. A. Bressan. Horticulture Dept., Purdue U., W. Lafayette, IN, 47907.

Plants regenerated from salt-adapted tobacco cells (S-25) have mitochondria with salt tolerance that is partially cytoplasmically inherited. Mitochondrial DNA from S-25 plants that has been specifically rearranged around the F_0-ATPase subunit 9 gene has been cloned and sequenced. The F_0-9 gene coding sequence has not been altered, however preliminary experiments indicate that altered expression of this gene occurs in S-25 plants. Using 2-D polyacrylamide gel analysis, an apparently nuclear encoded protein appears in normal (W38) and S-25 x W38, but not in S-25 mitochondria. The N-terminal peptide sequence (14 mers) of this protein was determined. A 17 mer oligonucleotide was synthesized based on the peptide sequence and used to screen a cDNA library. We have also found a 55 kD protein that is associated with the plasmamembrane and is dramatically induced in salt-adapted tobacco cells. By immunoscreening using anti-55 kD, we have isolated a 1.8 kB clone from a cDNA expression library. About 60 % of this clone has been sequenced and it has a 100 bp region with 58 % sequence identity to the gene of the maize ATP/ADP translocator which is located in mitochondria.

Supported by USDA grant 88-37264-4067 and the McKNIGHT foundation.

POSTER ABSTRACTS

1563 PSYCHROMORBIDIN: A COLD INDUCIBLE, LOW MOLECULAR MASS PEPTIDE FROM THE SNOW MOLD *Coprinus psychromorbidus*. Fawzy Georges, W. Jay Newsted, Mohammed Saleem, Adrian J. Cutler and Sandra Polvi, Plant Bitechnology Institute, National Research Council Canada, Saskatoon, Saskatchewan S7N 0W9. The snow mold *Coprinus psychromorbidus* is a low temperature fungal pathogen which causes extensive damage to winter cereals, grasses and forage legumes in western Canada. Analysis of the protein profile from this organism by Western blotting revealed the presence of a low molecular mass peptide (psychromorbidin, ~3200 Da) exerting a strong cross reactivity with antibodies raised against the winter flounder antifreeze protein (AFP, 3700 Da). Biosynthesis of psychromorbidin appears to be regulated in response to temperature variation. Highest peptide levels were observed at 4°C in both bound and soluble protein fractions. Preliminary data suggest that psychromorbidin may be a glycoprotein. Temperature regulation data, IEF and other properties of psychromorbidin will be presented.

1564 EXPRESSION OF HEAT SHOCK PROTEIN GENES IN RESPONSE TO 2,4-DINITROPHENOL. Kailash C. Upadhyaya, and Ram Kishore, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067 India.

Seedlings of pigeon pea (Cajanus cajan), a tropical legume plant, have been shown to synthesize at least 35 polypeptides ranging in molecular weight from 15 to 91 kD at heat shock temperature (40°C) as resolved by two-dimensional non-equilibrium pH gel electrophoresis. The effect of a known uncoupler of oxidative phosphorylation, 2,4-dinitrophenol which generates endogenous heat signals during the oxidation of NADH has been investigated in activating the heat shock (hsp) genes. Approximately 84 polypeptides, 12 over 60 kD, 41 between 30 to 60 kD and 31 with a molecular weight below 30 kD, are synthesized in the presence of DNP at normal growth temperature (30°C). The synthesis of 15-20 hsps is not initiated by the DNP treatment whereas an equal number of polypeptides appear to be specific for DNP and they are not synthesized following a heat shock. However, 5-6 hsps appear to be synthesized in the presence of DNP. Thus, the DNP treatment induces the synthesis of two different sets of polypeptides, one set unique to the DNP treatment and the other whose synthesis could be initiated both by heat shock as well as by the DNP treatment.

1565 *EVALUATION OF DRYING INJURY TO SUNFLOWER AND PEANUT SEEDS.* *Latha Saratny, G.Gopala Krishna, Adeel Ahmad and P.Sudhakar, Biophysics Laboratory, Physics Dept. Nizam College, Hyderabad, India.*

Rapid imbibition of seeds results in the displacement of the protein and phospholipid components of the membrane giving rise to leakage of electrolytes into the surrounding medium. This leakage will occur until such displacements are reversed either by spontaneous reformation or molecular repair mechanisms. The longer it takes for reconstitution to be completed, the greater the amount of leakage. A typical symptom of drying injury in seeds is an increase in the quantity of electrolytes that are leached from the tissue during imbibition, suggesting loss of membrane integrity and increased membrane permeability. Sunflower and peanut seeds are subjected to drying injury by the accelerated ageing method. Ion efflux of treated as well as viable seeds is mathematically analysed to give an insight into the changes in permeability of seed membranes due to drying injury. The rate of ion efflux $d\sigma^-/dt$ is proportional to the difference between the efflux capacity of the seeds f(t) and the ion efflux σ^- at any time t, plus a constant rate of efflux b during the linear phase. $d\sigma^-/dt = k(f(t)-\sigma^-) + b$ where k is interpreted as a measure of the permeability of the seed. This study indicates that heat injured sunflower and peanut seeds were more permeable than viable ones. Also the energy of activation of heat injured seeds was less suggesting that the permeability barrier has been reduced by the drying injury.

1566 DEVELOPMENTAL REGULATION AND FUNCTION OF HSP80 Luca Comai, Anna Chinn, Elizabeth Haswell, Kai Coshow, and David Parks. Dept. of Botany KB-15, University of Washington, Seattle, WA 98195

When subjected to heat shock, all organisms produce a set of proteins called Heat Shock Proteins. Since HSPs are also produced during development, they may be involved with normal cell functions. We have characterized a tomato HSP80 gene and determined that its mRNA is abundant in all meristems but low in mature leaves. Leaves mRNA levels can be induced three fold by wounding and heat shock. We are studying the regulation and function of this gene. A two kb region upstream of HSP80 translation initiation was spliced to the gus gene and introduced into tobacco. This chimeric gene was under developmental and wound-induced regulation. We are studying the components of the HSP80 promoter by deletion analysis and plant transformation. We are also investigating putative scaffold attachment regions flanking the HSP80 gene. We are studying the effect of these DNA elements on the expression pattern of reporter genes in transformed plants. The mode of expression of HSP80 is consistent with the developing picture of HSP80 as a factor needed in high concentration during normal and stress-induced developmental events of all eukaryotes. It has been shown that HSP80 interacts specifically with the animal steroid receptor family of regulatory proteins. Binding of the receptors by HSP80 occurs in the absence of the respective hormone ligand and temporarily inactivates the DNA-binding and gene regulation activity of the receptor. HSP80's function in plants is unknown. We are trying to elucidate HSP80 function by antisense RNA expression and biochemical studies. We are transforming plants with sense and antisense HSP80 genes driven by the Mac promoter, a mannopine synthase-CaMV 35S hybrid.

1567 **HOMOLOGIES BETWEEN COLD HARDENING AND OTHER STRESSES IN WINTER OATS (*AVENA SATIVA* L.). Andrea S. Quigley, Helen J. Ougham, Colin F. Eagles, Petra H.D. Schünmann. AFRC-IGER Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed, SY23 3EB, U.K.**

We wish to elucidate the physiological and molecular mechanisms underlying the hardening process in winter oats, which are less frost tolerant than most temperate cereals. Exposure to low positive temperatures leads to the development of hardiness, such that plants become able to withstand temperatures that would otherwise be lethal. It has been proposed that cold-hardening and plant responses to drought stress may, in part, be mediated by related processes. We have chosen two varieties which show contrasting levels of hardiness. The American line Kentucky 77-177 is relatively freezing tolerant whereas the Welsh cultivar Bulwark is freezing susceptible. The translation products of mRNAs synthesised under cold hardening conditions have been examined, and a comparison made with gene products induced by a range of other stress treatments, including heat shock, cold shock and drought.

1568 ISOLATION, CHARACTERIZATION AND SEQUENCE ANALYSIS OF cDNA ENCODING 16 - 20 KD LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS OF RICE. C. Y. Lin, T. S. Tseng, K. W. Yen, C. H. Yeh, and Y. M. Chen. National Taiwan University, Department of Botany, Taipei, Taiwan

mRNA obtained from heat shocked rice seedlings was used to construct a cDNA library in phage λgt11. The cDNA library was screened with pCE53, a cDNA clone encoding soybean 17.5 kD heat shock protein (HSP), (kindly provided by Dr. J. L. Key, The University of Georgia, Athens, GA. 30602 U.S.A.). Four clones, pTS1, pTS2, pTS3, and pTS4, were isolated. They were heat shock specific and hybrid-selected mRNAs were translated into the 6 HSPs of 16 to 20 kD. Nucleotide sequence analysis indicates that pTS1 is a full length clone and the apparent mass is 16.9 kD with isoelectric point of 6.15 by the deduced amino acids. When comparison was made to the deduced amino acid sequence we found 69.5% identity to soybean GmHSP 18.5 - C and 80.4% identity to wheat C 5-8 respectively.

POSTER ABSTRACTS

1569 ISOLATION AND CHARACTERIZATION OF cDNA CLONES OF A NEW HEAT SHOCK COGNATE PROTEIN FROM *CATHARANTHUS ROSEUS*. B. Laevens[1], N. Brisson[2] and V. De Luca[1] University of Montreal, Dept. of Biol. Sciences[1] and Biochem[2], C.P. 6128 Station A, Montreal, PQ, Canada, H3C 3J7

The importance of heat shock proteins (HSPs) in cell survival is well known, but their specific biochemical functions are not well-understood. Several studies have shown that HSPs can be implicated in thermotolerance, protein translocation, protein folding and protein degradation. The only enzymatic functions assigned to HSPs involve the ATPase activity discovered in the HSP 70 class of heat shock proteins.Recent studies in our laboratory have resulted in the isolation and characterization of 2 cDNA clones which possess extensive homology to petunia and maize HSP 70. Both clones appear to encode full-length proteins whith apparent Mr 45,000 (Heat Shock Cognates [HSC 45]). Deduced amino acid sequence analysis of HSC 45 revealed that the similarity to HSP 70 did not extend to the ATP binding domain but was restricted to the putative protein binding domain. Heat shock treatment of cell suspension cultures or of intact plants resulted in slight induction of HSC 45 mRNA accumulation, whereas seeds contained HSC 45 which disappeared during germination and seedling development. The role of HSC 45 in seedling development is not known at present but it may be representative of a class of HSPS involved in developmental processes during seed germination which have nothing to do with stress tolerance.

1570 MOLECULAR AND FUNCTIONAL ANALYSIS OF A PLASTID-LOCALIZED HEAT SHOCK PROTEIN. Kathenne W. Osteryoung, Holly Sundberg & Elizabeth Vierling. University of Arizona, Department of Biochemistry, Tucson, AZ 85721.

Plants respond to heat stress with the induction of multiple low molecular weight heat shock proteins (LMW HSPs) whose functions are at present unknown. We are studying the chloroplast-localized LMW HSP from *Arabidopsis thaliana*, HSP21, with the goal of defining the role of this protein in the acquisition of thermotolerance. HSP21 is encoded by a single nuclear gene in *Arabidopsis*. As in other plants, the protein is not detected in the absence of stress, but is highly induced in response to elevated temperatures. Analysis of an HSP21 genomic clone indicates that the promoter region contains consensus heat shock elements common to all heat shock genes, and that the gene contains a small intron (164 bp), an unusual occurrence in an HSP gene. RFLPs have been identified for HSP21 and are being used to map the chromosomal location of the gene. To gain insight into the function of this protein, we are conducting antisense RNA experiments in *Arabidopsis* to test the consequences of reduced HSP21 expression in response to heat stress. Supported by NIH RO1 GM 42762-01.

1571 ANALYSIS OF TWO LIGHT-INDUCIBLE HEAT SHOCK (hsp70) GENES OF CHLAMYDOMONAS REINHARDTII Frank W Muller Dagmar Riegel Erika D v Gromoff, and Christoph F Beck University of Freiburg, Institut fur Biologie III D-7800 Freiburg FRG

Three light-inducible hsp70-homologous heat shock genes (hsp68, hsp70, hsp80) were identified previously in Chlamydomonas reinhardtii The sequence of the hsp70 gene, including its 5 and 3 flanking sequences was determined The deduced amino acid sequence revealed 82% 70% and 49% homology to maize human and E coli hsp70 genes respectively The hsp70 gene contains 5 introns Three heat schock elements were observed: one between the TATA box and CAATT box the second upstream of the CAATT box, and the third within intron 3 In order to define regulatory elements involved in the expression of hsp70 during heat stress and dark-light-shift deletions are introduced in the upstream region These deletion constructs can be tested for altered expression in C reinhardtii after transformation

The primary structure of the hsp80 gene including its 5' and 3' flanking sequences was also determined The deduced amino acid sequence showed similar homology to the E coli dnaK gene product and the C reinhardtii hsp70 gene product This gene, belonging to the hsp70 family in vitro encodes a protein of 80kD

The accumulation of the hsp mRNAs can be induced either by subjecting Chlamydomonas cells to heat shock or by transferring dark grown cells to light During heat treatment the kinetics of mRNA accumulation were unaffected by an inhibitor of cytoplasmic protein synthesis However cycloheximide inhibited the accumulation of hsp68 and hsp70 mRNAs in dark-light-shift experiments Chloramphenicol and DCMU (an inhibitor of PSII) affected the accumulation of hsp68 mRNA These results suggest that the two extrins c signals - elevated temperature and light - regulate expression of two of the genes through different signal chains

v Gromoff E D Treier U Beck C F (1989) Mol Cell Biol 9 3911-3918

1572 MOLECULAR STUDY OF OXIDATIVE STRESS IN PLANTS. Dirk Inzé, Chris Bowler, Wim Van Camp, Luit Slooten[1], Didier Hérouart, Ed Tsang, Chris Sybesma[1] and Marc Van Montagu. Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium); [1] Laboratorium voor Biofysica, Vrije Universiteit Brussel, B-1050 Brussel (Belgium)

Numerous environmental stress conditions are known to mediate damage to cells by enhancing production of reduced oxygen species such as superoxide radicals ($O_2^{\bullet}$), H_2O_2, and hydroxyl radical ($OH^{\bullet}$). These oxy-radicals react instantaneously with virtually every macromolecule in the cell, leading ultimately to cell death. Plants have evolved a wide range of enzymatic and non-enzymatic mechanisms to contend with this problem. Key enzymes in this protection are superoxide dismutases (SODs), which convert superoxide radicals into hydrogen peroxide. *Nicotiana plumbaginifolia* contains a mitochondrial manganese SOD, a cytosolic copper/zinc SOD, a chloroplastic copper/zinc SOD, and a chloroplastic iron SOD, all nuclear encoded. Using different approaches we have been able to clone full-length cDNAs encoding all these isoforms. The expression of the corresponding genes is highly induced, although not co-regulated, in response to exposure to various stress conditions such as high light intensities in combination with high and low temperatures, ozone, and the superoxide-generating herbicide paraquat. The data suggest that the expression of the SODs is governed by the extent of generated oxidative stress within the particular subcellular location of each enzyme. Furthermore, it has been found that high manganese SOD overproduction in either chloroplasts or mitochondria of transgenic plants significantly reduces the amount of cellular damage mediated by oxy-radicals. Most interestingly, the plants have been shown to have a higher tolerance to ozone treatments (in collaboration with the GSF, München, F.R.G.). These data have suggested further experimental strategies for engineering stress tolerance in plants.

1573 EXPRESSION OF ANTIFREEZE PROTEINS IN TRANSGENIC PLANTS. Pamela Dunsmuir, Robin C. Hightower, Catherine S. Badaen, Eva Penzes and Peter Lund. DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608. The quality of frozen plant products can be compromised by the damaging effects of ice crystal growth within the frozen tissue. Several antifreeze proteins have been identified that inhibit ice crystal growth when present in low concentrations. We have introduced the gene for a winter flounder-type antifreeze protein into plants, and have studied its expression and activity in transformed plant tissue. Furthermore, the gene was modified so that the protein it codes for is larger and more easily detected (by fusion to protein A from Staphylococcus aureus). This chimeric gene was introduced into plants and its expression and activity measured. RNase protection analysis demonstrated that the gene is expressed in plant tissue and antifreeze activity is detectable in transformed plants. We are now using simple assays on transgenic fruit to assess the ability of the antifreeze protein to protect against freeze/thaw damage. Ultimately, this approach may improve the quality of frozen fruits and vegetable as well as expanding the range of fruits and vegetables that can be successfully frozen.

1574 EXPRESSION OF CHIMERIC METALLOTHIONEIN GENES IN TOBACCO. Taline Elmayan and Mark Tepfer. Laboratoire de Biologie Cellulaire, INRA de Versailles, 78026 Versailles Cedex, FRANCE.

As an extension of previous studies of expression of a mouse metallothinein gene (mMT-I) in plant cells (V. Pautot and M. Tepfer, Gene, 1989), we have introduced into tobacco a series of genes constructed with a human metallothionein (hMT-II) cDNA, which we have in some cases fused with the GUS reporter gene. These new genes will allow us to quantify the protein in different transgenic tissues, and to observe where the protein is produced. Genes controlled by constitutive promoters (35S promoter of CaMV and Arabidopsis elongation factor EF-1a promoter) express the fusion protein in all tissues of transformants. Finer control of heavy metal partitioning should be obtained with organ specific promoters, such as domain A (-90 to +8) of the 35S promoter, and the promoter of ORF15 of the pRi TL-DNA. Expression and function of the hMT/GUS fusion gene in plants, as well as the possibility of trapping heavy metals in the root system, and thus to lower cadmium content in tobacco leaves, and in food crops in general, will be discussed.

POSTER ABSTRACTS

1575 LOW TEMPERATURE-INDUCED (lti) GENES IN THE CYANOBACTERIUM ANABAENA VARIABILIS M3. Naoki Sato, Department of Biology, University of Tokyo, Hongo, Tokyo, Japan

Our previous studies showed that Anabaena variabilis M3 adapts itself to a low temperature by desaturating the fatty acids of the existing membrane lipids. In an attempt to obtain the genes that are involved in the desaturation and adaptation to low temperature, I screened a λEMBL4 genomic library by differential screening using a pair of single stranded labelled cDNA probes that are complementary to RNAs from the cells grown at 38°C or the cells shifted to 22°C for 2.5 h. Among many isolated clones, two genes, lti12 and lti46, were sequenced. Lti12 encoded an ORF composed of 552 amino acid residues, which was homologous to α-amylases. Lti46 was a polycistronic gene comprising three ORFs, one of which exhibited a significant homology to the surface antigen of Treponema. The levels of transcripts of lti12 and lti46 were both rapidly increased within 1 h after the temperature shift from 38°C to 22°C, and gradually decreased to reach the steady state levels at 22°C, which were as low as the levels at 38°C.

1576 CHARACTERIZATION AND CLONING OF COLD-INDUCED GENES IN POTATO TUBERS. J. van Berkel, F. Salamini, and C. Gebhardt. Max-Planck-Institut fur Zuchtungsforschung, 5000 Koln 30, Germany

The effect of cold treatment on gene expression in potato (Solanum tuberosum L.) tubers has been studied. Polyadenylated RNA was isolated from tubers which had been stored at low temperatures for different time periods and from unstressed control tubers. The RNA was in-vitro translated, and the translation products were analyzed by 2-dimensional electrophoresis. The examination of the patterns revealed changes in the relative amount of at least 26 polypeptides as a response to cold treatment. As the cold-induced mRNA species coding for these proteins accumulated with different kinetics, the translation products could be grouped into several categories. In the second part of the study, cDNA clones which are homologous to cold-induced transcripts have been isolated by differential screening of a cDNA library constructed from polyA$^+$RNA of cold-treated potato tubers. About 25,000 plaques were plated and differentially screened by in-situ plaque hybridization. The polyA$^+$RNA preparation from which the library was derived, and polyA$^+$RNA of unstressed tubers were used as differential probes. 29 differentially hybridizing clones were obtained which could be divided into several groups according to their crosshybridization. Two clones, which did not crosshybridize with each other, were further analyzed by Northern hybridization experiments. Both clones hybridized to mRNA species of 1000 to 1100 bp length, but they differed in their cold induction kinetics.

1577 PHOTOSYNTHESIS RATE AND ASSIMILATE TRANSLOCATION IN RELATION TO FREEZE INJURY AND HARDENING IN BRASSICA.

Ashok K. Dhawan, Haryana Agricultural University, Regional Research Station, Uchani, Karnal-132001, India.

Freezing injury reduces rate of photosynthesis, but causes a slight enhancement in respiration rates in B. juncea Coss & Czern plants. Hill activity of chloroplasts, isolated 24 h after freezing, was comparable to unfrozen control. Chlorophyll content also decreased on freezing. Low temperature hardened plants however, showed no decrease in rates of photosynthesis or hill activity or chlorophyll content. The partioning of photosynthates from leaves to reproductive parts was marginally affected by freezing. Sucrose synthetase and sucrose phosphate synthetase activities show a large decrease on freezing the unhardened plants, but are not affected or show slight increase on freezing the hardened plants. Photosynthetic apparatus of hardened plants thus appears to be better adapted to tolerate freezing stress.

1578 THE HEAT SHOCK RESPONSE OF SENSITIVE AND TOLERANT STRAINS OF FUSARIUM OXYSPORUM. Chen Ginzburg[1], Stanley Freeman[2] and Jaacov Katan[2]. [1]Department of Ornamental Horticulture, ARO, The Volcani Center, Bet Dagan 50250, Israel. [2]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100.

Two pairs of closely related strains of Fusarium oxysporum, which differ in their ability to survive elevated temperatures, were selected. The more thermotolerant strain within each pair had a higher capacity to synthesize proteins, both under regular and under heat-stress conditions. Changes in the patterns of protein synthesis in the different strains were followed during exposure to heat stress at the range of 36-45°C, and during recovery from stress. The more thermotolerant strain within each pair responded more rapidly to heat-shock by attaining earlier a level of heat shock proteins (hsps) commensurate with the stress, by an earlier suppression of hsp synthesis and by an earlier resumption of the regular pattern of protein synthesis. The differences in rates of protein synthesis between the strains are sufficient to account for the ability of the tolerant strain to complete the heat shock response earlier. The results suggest a direct relationship between the intensity of the heat shock response and thermotolerance.

1579 CHILLING - SENSITIVE MUTANTS OF ARABIDOPSIS. Carrie Schneider, Suzanne Hugly, Chris Somerville. MSU - DOE Plant Research Lab, Michigan State University, East Lansing, MI 48824

Chilling-sensitive species show one or more symptoms of injury when subjected to low, though non-freezing, temperatures; for example, the plant may wilt, show ion leakage, become chlorotic, or develop necrotic spots. We are employing a genetic approach to study chilling-sensitivity, using Arabidopsis thaliana, a chilling-resistant plant. Chilling-sensitive mutants were identified in an EMS-mutagenized population by growing the plants at 22°C for two weeks, then shifting to 10°-15°. Of 10,000 M2 plants examined, 20 mutants that began to develop necrosis or chlorosis in the cold were found. In addition, ten could not be rescued. These, and two mutants from a previous screen (PM2 and PM11), fall into four phenotypic groups: Class 1a, the entire plant becomes chlorotic and eventually dies; Class 1b, only the older leaves become chlorotic and die; Class 2, mutants develop random chlorotic patches; and Class 3, the center of the plant becomes chlorotic. The chlorosis is not fatal in classes 2 and 3 - plants continue to develop and eventually flower. Genetic complementation tests indicate that class 1 contains 8 mutants which fall into 4 complementation groups, class 2 has 4 mutants which fall into 2 groups, and class 3 has 11 which fall into at least 3 groups. Since several alleles have been isolated in two of the loci in class 1 and 2 from independently mutagenized seed lots, it appears that mutations in a relatively small number of genes can result in these phenotypes. In order to characterize these mutants, we are examining chlorophyll accumulation, polar and nonpolar lipids and protein synthesis. For example, class 1 mutants accumulate new nonpolar lipids, identified as sterol esters, when transferred to low temperature (Hugly, et al., Plant Physiol. 93: 1053-1062, 1990). At the same time, synthesis of chloroplast proteins ceases, but transcription does not. This evidence suggests that the defect in this group affects the structure or function of the chloroplast ribosome. Further studies will determine whether the defect in protein synthesis is a primary or secondary effect of the mutation.

1580 PROTEIN SYNTHESIS AND mRNA ABUNDANCE DURING RECOVERY FROM HEAT SHOCK IN HEAT TOLERANT AND NON-TOLERANT VARIANTS OF *AGROSTIS PALUSTRIS*

Renuka Shivaji and Dawn S. Luthe, Mississippi State University, Department of Biochemistry and Molecular Biology, Miss. State, MS 39762

The heat shock response was studied in two variants of creeping bentgrass (*Agrostis palustris*). A heat tolerant variant (SB) was derived from the non-tolerant variant (NSB) by *in vitro* cell culture using heat as a selection pressure. *In vivo* protein synthesis measurements indicated that SB recovered from heat shock about 2 hr faster than NSB, and that SB had a longer heat shock response than NSB. Uptake studies suggested that amino acid availability did not account for these differences. SDS-PAGE of the proteins synthesized *in vivo* during recovery confirmed the labeling data and indicated that normal proteins appeared in extracts of SB before they appeared in NSB. SDS-PAGE also indicated that the heat shock proteins began to diminish in NSB sooner than in SB during prolonged heat shock. The abundance of mRNA for hsp 70, hsp 17 and the chlorophyll a/b binding protein was measured during the recovery period for SB and NSB. Northern analysis indicated that the abundance of hsp 17 mRNA decreased faster during recovery in SB than in NSB, but the decrease in hsp 70 abundance was similar in NSB and SB. The abundance of the chlorophyll a/b binding protein mRNA decreased following heat shock and recovered to normal levels after 8 hr of recovery in both SB and NSB. These preliminary data suggest that the more rapid recovery of protein synthesis in SB may be due to some type of translational regulation.

POSTER ABSTRACTS

1581 VECTORS FOR ANTIFREEZE PROTEIN GENE EXPRESSION IN PLANTS. M. Saleem, F. Georges, H. Wang and A.Cutler. Plant Biotech. Inst., SK. Canada S7N 0W9

The winter flounder antifreeze protein (AFP) has shown promise for enhancing the ability of certain plants to avoid freezing (Cutler et al. 1989, J Plant Physiol 135: 351-354). A DNA sequence coding for the AFP was chemically synthesized and inserted 3' to the cauliflower mosaic virus 35S promoter in a series of pGCS vectors. Initially, The AFP sequence was inserted as an N-terminal translational fusion with chloramphenicol acetyl transferase (pGCS1) and was expressed in corn protoplasts following electroporation (Georges et al. 1990, Gene 91: 159-165). Subsequently, an AFP containing vector (pGCS2) was constructed that was suitable for stable transformation with *Agrobacterium tumefaciens*. In this construct, the AFP sequence was inserted as a translational fusion with β-glucuronidase (GUS). This vector was used to transform tobacco and *Arabidopsis thaliana* but was prone to deletions when maintained in *E. coli*. This problem was alleviated in a third series of pGCS vectors in which various leader sequences were incorporated 5' to the AFP coding sequence to facilitate secretion. Initially the 35S promoter was used to drive expression but the effectiveness of other promoters is being investigated. Progress has been made toward characterizing a stress-inducible promoter for use in this system. The effect of removing the GUS sequence on AFP gene expression is being examined.

1582 ACCLIMATION AND VERNALIZATION RESPONSES OF ARABIDOPSIS SEEDLINGS. Leyva. A., J.A. Jarillo and J.M. Martínez Zapater. CIT-INIA. Ctra. de La Coruña Km 7. 28040 Madrid. Spain.

Etiolated seedlings of Arabidopsis are able to develop freezing resistance and to vernaliz when exposed to low non-freezing temperatures. Using a combination of genetic an molecular methods we are analyzing the molecular mechanisms responsible for thos responses to low temperatures and the possible relationships between them Germinating seeds of Arabidopsis are highly resistant to freezing temperatures durin the initial stages of germination This resistance falls down rapidly upon root elongation Etiolated seedlings exposed to 4ºC during different periods of time develop freezin resistance at the same time that they become vernalized. Although etiolated seedlings ca become fully vernalized in the absence of a carbon source, freezing resistance is no complete without an energy supply, indicating that both processes have different energ requirements. Trying to identify if both cold responses, acclimation and vernalization are induced through common signal transduction pathways we are currently analyzin the acclimation responses of Arabidopsis mutants unable to vernalize and we plan t characterize the vernalization responses of acclimation deficient mutants. Finally, w have identified a 10 fold increase in the levels of PAL and CHS mRNAs upon acclimatio of rosette plants to 4ºC during 24 hours. We will present the characterization of thi induction in the wild type plants and mutants unable to acclimate. The relationshi between the increase in the levels of these transcripts and the mechanisms controllin cold acclimation and vernalization will be discussed.

1583 CHARACTERIZATION OF THE POLYPEPTIDE ENCODED BY COR15, A COLD-REGULATED GENE OF ARABIDOPSIS. Chentao Lin[2], Michael F. Thomashow[1,2]. Department of Crop and Soil Sciences[1], Program in Genetics[2], Michigan State University, East Lansing, MI 48824, USA.

Exposure of Arabidopsis to low nonfreezing temperatures results in enhanced freezing tolerance and changes in gene expression. One of our goals is to determine whether Arabidopsis cor (cold-regulated) genes have a direct role in freezing tolerance or some other aspect of low temperature survival. Toward this end, we have isolated cDNAs representing 4 cor genes. One of these genes, designated cor15, encodes a 15 kD polypeptide. This polypeptide, like a number of other cold-regulated polypeptides in Arabidopsis and wheat, has the unusual property of remaining soluble upon boiling. DNA sequence analysis of cor15 cDNAs indicates that the deduced COR15 polypeptide is hydrophilic. Further, the first 50 amino acids bear a striking similarity to the chloroplast stroma-targeting consensus transit peptide. Immunological studies have shown that the COR15 polypeptide is indeed processed in vivo and that it is present in the chloroplasts. Additional biochemical studies have indicated that COR15 has potent cryoprotective activity in a standard in vitro assay; COR15 is very effective in protecting lactate dehydrogenase against inactivation by a freeze-thaw cycle. Current efforts are directed at determining whether COR15 has an important role in cryoprotection in planta.

1584 AN HSP70 (dnaK) HOMOLOG IS ENCODED ON THE PLASTID GENOME OF PORPHYRA UMBILICALIS. Michael Reith and Janet Munholland. National Research Council, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada.

In an attempt to isolate hsp70 gene homologs from the red alga, Porphyra umbilicalis, PCR experiments were carried out using primers based on conserved regions of the protein and total DNA. Interestingly, the purified PCR product hybridized strongly to the plastid rather than the nuclear DNA fraction. An EcoRI library of plastid DNA fragments was constructed in λZAP II and two contiguous clones containing the dnaK gene were isolated. DNA sequence analysis revealed an open reading frame of 630 amino acids with a high degree of similarity to prokaryotic dnaK genes (73% identical to Synechocystis 6803 dnaK, 54% identical to E. coli dnaK). Northern analysis detects a single 2.2 kb transcript that is present in both normal (15°C) and heat stressed (75 minutes at 30°C) cultures, but that accumulates to higher levels under heat stress. Primer extension experiments indicate that both normal and heat-induced dnaK mRNAs begin at the same position. This suggests that the same promoter is used in both the normal and heat-induced expression of this gene. The putative promoter region shows some similarity to the E. coli consensus promoter, but no similarity to E. coli heat shock promoters. Further analysis of the regulation of the P. umbilicalis dnaK gene is in progress.

1585 TRANSFORMATION WITH A FATTY ACID DESATURASE GENE ALTERS THE LOW-TEMPERATURE SENSITIVITY OF *Anacystis nidulans* R2. Hajime Wada, Zoltan Gombos and Norio Murata. National Institute for Basic Biology, Myodaiji, Okazaki 444, Japan

Anacystis nidulans R2 can introduce a single unsaturation bond at the Δ9 position of fatty acids, and is sensitive to low temperature. In this study, we transformed *A. nidulans* with a gene for fatty acid desaturation at the Δ12 position from *Synechocystis* PCC6803. The transformant acquired the ability to introduce the second unsaturation bond at the Δ12 position of fatty acids, and was more tolerant to low temperature than the wild type. Moreover, the second desaturation depended on growth temperature, which was the same characteristic as that in *Synechocystis* PCC6803.

1586 CLONING OF THE cDNA ENCODING THE 200 kD PROTEIN INDUCED DURING COLD ACCLIMATION OF WHEAT. François Ouellet, Mano Houde and Fathey Sarhan. Univ. du Québec à Montréal, Dépt. de Biologie, Montréal, Québec, Canada H3C 3P8.

We have previously shown that a 200 kD protein was specifically induced during cold acclimation of winter wheat (*Triticum aestivum* L. cv Fredrick). The induction and accumulation of this protein were correlated with the degree of freezing tolerance. In order to understand its function during cold acclimation, we have purified this protein and determined the amino-terminal sequences of several tryptic peptides. Two oligonucleotide probes were synthesized and used to screen the cDNA library prepared from cold acclimated wheat. The corresponding clone was identified and partially characterized. Northern analysis demonstrated that the expression of the mRNA was correlated with the induction of freezing tolerance. The sequence comparison indicates that this protein is related to the family of proteins induced by low temperature in cereals.

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1587 CLONING OF A WHEAT GENE ENCODING A MAJOR PROTEIN ASSOCIATED WITH THE DEVELOPMENT OF FREEZING TOLERANCE. Jean Danyluk, Mario Houde, Jean-François Laliberté*, Eric Rassart and Fathey Sarhan. Univ. du Québec à Montréal, Dépt. de Biologie, Montréal, H3C 3P8; *Institut Armand-Frappier, Laval, H7N 4Z3; Québec, Canada. In vivo and in vitro analyses indicate the induction of a 50 kD protein and its mRNA during the development of freezing tolerance in wheat. This mRNA was rapidly induced during cold acclimation, reached a peak after 24 hours, and its level of expression maintained up to the end of the acclimation period. This mRNA decreased rapidly upon deacclimation and was not induced by heat shock, drought or ABA. We have cloned the gene encoding this protein and have completed the nucleotide sequence analysis, which revealed that the gene encodes a basic, hydrophilic protein. The expression of the gene in E. coli using T7 RNA polymerase promoter produced a protein of similar MW and pI. The expressed protein comigrates with that synthesized in vivo and in vitro during cold acclimation. From these results, we conclude that the cloned cDNA has the complete coding region of the gene responsible for the synthesis of this protein. The sequence analysis and the homology to other known proteins will be presented.

1588 IDENTIFICATION OF A GENE FAMILY ASSOCIATED WITH FREEZING TOLERANCE IN WHEAT. Mario Houde, Jean Danyluk, Rajinder Dhindsa*, and Fathey Sarhan. Univ. du Québec à Montréal, Dépt. de Biologie, H3C 3P8; *McGill Univ., Dept. of Biology, H3A 2T5; Montréal, Québec, Canada.

We have identified and cloned several cDNA's from wheat which were specifically induced during cold acclimation. These clones recognize mRNA's with sizes ranging from 0.8 to 7 kb. The expression of these clones was correlated with the induction of freezing tolerance and were not induced by water stress or heat shock. On the other hand, the antibody prepared against one of the major protein (clone pCWS120 encoding a 50 kd protein) recognized most of the proteins induced during cold acclimation and water stress in several cereal species. This similar antigenicity suggests that these proteins may share a common feature and a related physiological role. However, the molecular analysis and sequence information indicate that the cold acclimation and water stress proteins are encoded by different genes. The isolation of promoter regions will help us to understand how this gene family is regulated by different environmental factors. Immunolocalization of these proteins may give us some insight as to their role in cellular adaptation to environmental stresses.

1589 COLD REGULATED GENE EXPRESSION IN BRASSICA NAPUS
J. Singh, W. Orr, E. Weretilnyk, T. White, L. Robert and B. Iu. Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6.

A clone encoding a cold-regulated transcript was isolated from a winter B. napus leaf c-DNA library. Northern blot hybridization of mRNA isolated from B. napus shows that the cDNA clone hybridizes to a 0.8 kb transcript which is present in leaves of cold acclimated plants but cannot be detected among the leaf mRNA of plants grown at 22°C. This transcript can be detected within 1 day exposure of the plants to 2°C, its levels appear to reach an apparent maximum by 2 days and is still present, albeit at reduced levels, following 10 weeks of exposure of the plants to 2°C. When cold acclimated plants are returned to 22°C, the transcript disappeared totally within 24 hours. Labeling of nascent mRNA followed by separation and hybridization of the derivitized RNA suggests that this message is transcriptionally regulated, at least on initial exposure of the plants to 2°C. Exposure of 22°C plants to ABA either by foliar application or vacuum infiltration of leaf-discs does not result in appearance of the 0.8 kb transcript. The clone also cross-hybridizes to transcripts of similar size in several cold acclimated Crucifers. Sequence and restriction analyses of the cDNA and genomic clones respectively is presented.

1590 ISOLATION OF *ARABIDOPSIS* GENES WITH HOMOLOGY TO *E. COLI* DNAK.
Lisa M. Lauzon and Elizabeth Vierling, Department of Biochemistry, University of Arizona, Tucson, AZ 85721.

We are studying nuclear-encoded heat shock proteins (HSPs) which are organelle-localized. To obtain *Arabidopsis* nuclear genes which potentially corresponded to mitochondrial or chloroplast HSP70 proteins, PCR primers were designed based on regions of sequence homology between *E. coli* dnaK (the prokaryotic HSP70 homolog) and yeast mitochondrial HSP70 (the SSC1 gene). The 5' primer chosen was derived from the sequence N G D A W V (amino acids 99 to 103 of dnaK) and the 3' primer was derived from E A A E K A (amino acids 264 to 269 of dnaK). Two distinct DNA fragments of approximately 650 nucleotides were amplified (designated AtdnaK1 and AtdnaK2) when these primers were used in the polymerase chain reaction with *Arabidopsis* genomic DNA. AtdnaK1 and AtdnaK2 were cloned and partially sequenced. Comparison of a 255 nucleotide fragment showed that Atdnak1 and 2 are approximately 85% identical at the amino acid level and 80% indentical at the nucleotide level. When this same region was compared to HSP70 proteins, both AtdnaK1 and 2 were approximately 75% identical (82% similar) to *E. coli* dnaK, whereas they were only 60% identical (75% similar) to pea eukaryotic HSP70. From Southern and Northern data, we concluded that AtdnaK1 and 2 are encoded by distinct single copy genes, and that AtdnaK2 is strongly heat regulated. Genomic clones have been isolated for both AtdnaK1 and 2 and a full length cDNA has been isolated for AtdnaK2. Initial sequencing indicates AtdnaK2 has an amino-terminal transit peptide. Additional sequence data from these two dnaK-like clones compared to pea chloroplast and mitochondrial HSP70s suggest that they have higher homology to the mitochondrial HSP70s.

1591 PERTURBATION OF GENE EXPRESSION IN RIPENING TOMATO FRUIT BY CHILLING AND HIGH TEMPERATURES. Steve Picton*, Chris B Watkins# and Don Grierson*. *University Nottingham, Dept. Physiology & Environmental Science, LE12 5RD, U.K., #D.S.I.R., Horticulture and Processing, Auckland, New Zealand.

Results will be presented and discussed to show that the failure to ripen at elevated or reduced temperature is due, in part, to disruption of the normal pattern of gene expression. Previously identified ripening-relating cDNAs (including those for phytoene synthase, pTOM5, polygalacturonase, pTOM6 and ethylene forming enzyme, pTOM13) have been used to examine the level of expression of ripening related genes during the process of ripening in detached fruit incubated at normal (24-25°C) chilling (4°C) and "heat-shock" (35°C) temperatures. Incubation of fruit at 4°C and 35°C leads to an abnormal pattern of ripening. Climateric ethylene biosynthesis falls, lycopene accumulation is inhibited and softening is retarded. In fruit held at 35°C the level of 12 ripening-related mRNAs show a marked decline, PG mRNA being undetected after 72 hours and EFE levels falling to 8% of control values, in parellel with the reduction in ethylene biosynthesis. Traces of heat-shock proteins are detected by *in vivo* labelling. Application of exogenous ethylene to the fruit does not overcome the inhibition of ripening. At chilling temperatures several mRNAs examined also fell to a low level with the exception of EFE mRNA that continued to accumulate for 10 days before declining. Following a return of pre chilled fruit to 24°C most mRNAs show an increase in level, ethylene synthesis shows a rapid rise and lycopene shows increased accumulation.

1592 IMMUNOLOGICAL CHARACTERIZATION OF THE TISSUE AND CELL TYPE SPECIFICITY OF TWO ZINC-BINDING PROTEINS FROM CITRUS. Kathryn C Taylor[1], L. Gene Albrigo[2], and Christine D Chase[3]. [1]University of Arizona, Dept. of Plant Science, Tucson, AZ 85721. University of Florida, [2]Citrus Research and Education Center, Lake Alfred, FL 33850; and [3]Vegetable Crops Dept., Gainesville, FL 32611 Two zinc-binding proteins (ZBP) were isolated from the phloem tissue extract of Citrus sinensis on Citrus paradisi rootstock. The ZBPs had molecular weights of 5 and 19 Kda, when separated by gradient SDS-PAGE. Preliminary evidence suggested that one of these proteins (5 Kda ZBP) was more abundant in the zinc-accumulating, phloem tissue of blight-affected citrus when compared to healthy citrus. Immunological approaches are underway to determine the tissue and cell-type specificity of the ZBPs in citrus as well as in other species. Both proteins had isoelectric points near pH 7 Both molecules were cysteine-rich and were anionic. The characteristics of these ZBPs will be useful for the determination of the involvement of these molecules in the symptom development and expression of citrus blight.

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1593 GENE EXPRESSION IN BLEACHING PLASTIDS OF *Euglena gracilis* Z. William Ortiz, Lee Newman, and Carl A. Price. University of Oklahoma, Department of Botany and Microbiology, Norman, Oklahoma 73019, and Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759, USA.

Reversible loss of chlorophyll (= bleaching) occurs in growing photoheterotrophic cultures of *Euglena* incubated at 33°C. Prolonged incubations at the elevated temperature bring about an irreversible loss of chloroplast function. We have begun an investigation of the molecular events underlying the bleaching response. After 41 hours at 33°C, protein synthesis by chloroplasts *in vivo* vanishes, whereas cytoplasmic synthesis of proteins targeted to the chloroplasts remains. Is the loss of chloroplast translation due to a disappearance of templates? To answer this question, we probed northern blots of total cell RNA with internal fragments of *psbA*, *psaA*, and *psbE*. Each of these genes contains at least one intron. To complicate matters, *psaA* and *psbE* are transcribed in a hexacistronic transcript. The amount of fully processed transcripts of *psbA* decreases progressively during incubation at 33° until about one-third is left after 41 hours, although translation of *psbA* has stopped altogether. Amounts of *psaA* transcripts decline more rapidly to nearly zero. The behavior of *psbE* is most remarkable: incompletely processed forms of a transcript containing *psbE*, *psbF*, *psbL*, and ORF42 appear to be converted progressively to a fully processed end-product whose concentration remains high, but novel transcripts accumulate transiently during the bleaching process. These data are consistent with a shut-down of chloroplast transcription following transfer to elevated temperatures together with possible disturbances in transcript processing and translation.

1594 EXPRESSION OF PHOSPHOENOLPYRUVATE CARBOXYLASE IN SOYBEAN EXPOSED TO ELEVATED TEMPERATURES. Frank J. Turano and Michael B. McMahon. USDA/ARS, Climate Stress Laboratory, BARC-West. Beltsville, Maryland 20705

A portion of cDNA encoding phosphoenolpyruvate carboxylase (PEPCase, E. C. 4.1.1.31) has been identified in soybean. Degenerate oligo primers were synthesized to conserve regions of known PEPCase genes from *Zea mays*, *Anacystis nidulans* and *Escherichia coli*. The primers were utilized in a polymerase chain reaction (PCR) to amplify a portion of the PEPCase gene from *Glycine max* cv.Williams. In the PCR reaction, first strand cDNA was synthesized from poly $(A)^+$ RNA isolated from 21 day-old plants. The resulting 2.4 kbp fragment, which is in close agreement with the estimated fragment size of 2.393 bp, was subcloned and sequenced. Sequence comparison of the cDNA fragment from soybean with the maize PEPCase gene shows a 65% nucleotide identity. Northern blot analysis was utilized to determine the effect of increased temperature on PEPCase gene expression. Fourteen day-old soybean plants were incubated at 42°C for 0, 15, 30, 45, 60, 90, 120, 150 and 180 minutes. Hybridization of northern blots of total RNA isolated from leaves of heat treated plants show a decrease in PEPCase at 15 minutes and no detectable amounts of PEPCase after 60 minutes.

1595 MOLECULAR CHARACTERIZATION OF STRESS- AND ABA-INDUCIBLE GENES OF ALFALFA MEDICAGO FALCATA CV ANIK. Luo, M., Mohapatra, S.S. and Hill, R.D.
Department of Plant Science, University of Manitoba, Winnipeg, MB Canada, R3T 2N2

ABA has been proposed as a common mediator for plant responses to stresses. We previously reported the role of ABA in acclimatization of plants to low, non-freezing temperatures which resulted in freezing tolerance in alfalfa seedlings. A cDNA library was constructed from the mRNA of cold-acclimatized alfalfa seedlings. Expression of the gene corresponding to one of the cDNA clones, PSM1409, was inducible in response to ABA treatment. In this study, three more cDNA clones were isolated from this cDNA library. They are homologous to PSM1409. The nucleotide sequences of these cDNAs have been analysed. The expression of the corresponding genes at different developmental stages of alfalfa seedlings, and in response to ABA, ABA analog and Gibberellic acid treatment, as well as various stress conditions was examined.

1596 REGULATION OF SPECIFIC CARBON STARVATION INDUCED PROTEINS (STP). Francesca Tassi, Elena Maestri, Francesco M. Restivo and Nelson Marmiroli. University of Parma, Institute of Genetics, Parma, Italy.

Carbon starvation affects cell metabolism and specific protein synthesis in plants. This has been shown in Gerbera jamesonii callus cultures subjected to carbon source deprivation by analyzing in vivo and in vitro the induction of some STP. Two-dimensional analysis of protein synthesis at different times of starvation shows that some STP are induced after only one day of sugar deprivation, whereas other STP appear at longer periods of stress. Indeed the in vitro translation experiments did not show induction of STP mRNA at the earlier stages of starvation, suggesting that the early and the late responses are differently regulated. Induction of most of the STP can be transiently inhibited by an heat shock treatment (40°C) with no effect on cell function and recovery from starvation. This fact suggests that heat-shock proteins and STP may have some common physiological role. This communality is also suggested by a possible identity between hsp70 and one of the STP (STP70). To confirm this we have undertaken immunological characterization of STP70 and Northern analysis with a probe for the cloned HSP70 gene.

1597 MOLECULAR CLONING AND CHARACTERIZATION OF HEAT SHOCK PROTEIN GENES IN WHEAT. Jian Weng, Zi-Fen Wang, and Henry T. Nguyen, Plant Genetics Laboratory, Texas Tech University, Lubbock, TX 79409-2122

The overall objective of this project is to isolate heat shock protein (HSP) genes and investigate their role in the genetic control of heat tolerance in wheat. We have constructed a cDNA library of the heat-tolerant wheat line 'Mustang' in a Lambda ZAP II vector system. After screening, more than 80 positive clones corresponding to different HSP genes were isolated by using plaque-lift and Southern-blot hybridization with available HSP cDNA probes including ubiquitin, low molecular weight (LMW) and high molecular weight (HMW) HSPs. Several cDNA clones were identified and classified into different HSP groups by *in vitro* hybrid selection following *in vitro* translation and 2-D gel analysis. Several LMW HSP clones were sequenced and compared with known HSP genes from other species. Sequencing data show that wheat chloroplast-localized HSP gene Tahsp26.6 encodes a 238 amino acid, 26.6kD protein which is 81.7%, 60.8%, 50.0%, and 38.2% identical to the chloroplast-localized HSPs of maize, soybean, pea, and *Chlamydomonas*, respectively, and wheat LMW HSP gene Tahsp17.3 encodes a 157 amino acid, 17.3kD protein which is 84.1%, 67.5%, and 69.4% identical to class VI HSPs of maize, soybean, and pea, respectively. Similar to previous reports for LMW HSPs including chloroplast-localized HSPs, monocots and dicots have more conserved sequences at the carboxyl-terminal which may reflect their evolutionary conservation of function and more divergent sequences at the amino-terminal which may reflect their species-specificity. The use of these clones in our investigation of gene expression in wheat under heat stress and the identification of potentially important genes for heat tolerance will be discussed.

1598 EXPRESSION OF A MAMMALIAN PCB-METABOLIZING CYTOCHROME P-450 IN *N. TABACUM*. V.D. Wall*, D. W. Galbraith*, J. R. Halpert*, and D. P. Bourque+, Departments of *Pharmacology and Toxicology, and +Biochemistry, University of Arizona, Tucson AZ (USA) 85721.

Polychlorinated biphenyls (PCBs), are resistant to metabolism in most animal species. The dog has the unique ability to metabolize and eliminate certain PCB congeners, as a result of the activity of the cytochrome P-450 isozyme PBD-2. An expressible cDNA coding for PBD-2 has been introduced into the genome of tobacco plants. PBD-2 cDNA and a screenable marker gene coding for neomycin phosphotransferase were introduced into tobacco leaf disks using a binary *Agrobacterium tumefaciens* vector system. Southern and Western blot analyses have confirmed chromosomal integration of the cDNA and expression of the PBD-2 polypeptide. Differential centrifugation and Western blot analyses have shown the PBD-2 protein to be associated with a membrane fraction in transgenic tobacco leaf homogenates. Our goal is to develop transgenic plants in which the PBD-2 protein metabolizes PCBs, thus providing a novel method for bioremediation of PCB-contaminated soils.

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1599 NATIVE STRUCTURE OF A LOW MOLECULAR WEIGHT CHLOROPLAST HEAT SHOCK PROTEIN. Qiang Chen and Elizabeth Vierling, University of Arizona, Depart of Biochem. Tucson, AZ 85721

Nuclear-encoded low molecular weight (LMW) chloroplast HSPs have been found in many plant species. These HSPs have a conserved carboxyl-terminal "heat shock" domain which is also present in all eukaryotic LMW HSPs, and an amino-terminal "methionine bristle" domain which is unique to LMW chloroplast HSPs. To understand the function of chloroplast-localized LMW HSPs, we are studying the native structure of pea HSP21. In its native state, HSP21 is found in a particle of approximately 200 kD (~ 11S) in both leaves and roots, as determined by sucrose gradient centrifugation and native gel electrophoresis. To study the assembly of this particle, *in vitro* translated HSP21 was imported into isolated chloroplasts for 3 min and during post-import incubation, the aggregation state of HSP21 was then examined. When HSP21 was imported into chloroplasts isolated from heat stressed plants, within 20 min of post-import incubation approximately 60% of the protein could be recovered in the 200 kD particle form. These particles were resistant to degradation by the protease thermolysin. However, under the same conditions no HSP21 could be found in the protease resistant, 200 kD particle form when HSP21 was imported into control chloroplasts. Instead, it was recovered in a ~ 4S form which was thermolysin sensitive. The same results were obtained whether the post-import incubation was carried out at ambient temperature or at 4°C. When a truncated HSP21 missing the "heat shock" domain was imported into chloroplasts, no 200 kD form of HSP21 was recovered in either heat stressed or control chloroplasts. These data suggest that other heat stress induced factors or preexisting HSP21 are required for the assembly of the HSP21 particle, and that the presence of the "heat shock" domain is also necessary for assembly. The HSP21 particle is currently being purified to determine the possible association of HSP21 with other chloroplast components. We propose that the 200 kD particle is the functional form of HSP21 and that it may perform a role in plastids similar to that of LMW HSPs in the cytoplasm.

1600 COLD REGULATION OF A LEA PROTEIN IN WINTER CEREALS. Fedora Sutton, Xiang Ding and Don Kenefick. South Dakota State University, Departments of Plant Science and Biology, Brookings, South Dakota 57007

RNA blot analysis of total RNA isolated from both non-acclimated and cold-acclimated barley shoot tissue and that of wheat seedlings were performed with radio-labelled pHVA1 cDNA insert. The pHVA1 cDNA is derived from mRNA that is readily induced by ABA in barley aleurone tissue (Hong, B., Uhnes, S.J., & Ho, T.H.D. Plant Molecular Biology 11:495-506). Results of cold-acclimation studies in this laboratory reveal that this gene is also regulated by cold. Deacclimation studies have also been performed. After two hours of incubation at 25C after removal from 2C no detectable amount of mRNA was found. Similar studies on cultivars differing in potential for freeze resistance without cold-acclimation have revealed no difference in the expression of this gene. Experiments with Fluridone treated and untreated barley shoots reveal that the cold regulation of this LEA gene is mainly via ABA.

1601 GENOTYPIC DIFFERENCES IN GENE EXPRESSION AT LOW NIGHT TEMPERATURES IN ALTITUDINAL ECOTYPES OF THE WILD TOMATO L. hirsutum. C. Eduardo Vallejos. University of Florida, Department of Vegetable Crops, Gainesville, FL 32611. Previous photosynthesis and growth measurements have shown that a high altitude ecotype of the wild tomato species can acclimate to low growth temperatures. Low altitude ecotypes and the cultivate tomato are low temperature (chilling) sensitive and do not acclimate. An analysis of this process at the nucleic acid level has shown that the major difference between the altitudinal ecotypes of L. hirsutum is that transcripts normally found at elevated levels during the dark period under non-stressful temperatures are only marginally affected by low night temperatures in the high altitude ecotype, whereas a drastic reduction in the level of these transcripts is observed in both a low altitudinal ecotype and the cultivated tomato. Low night temperatures also induce the accumulation of specific transcripts (7-9) in all three genotypes. However, an additional 15 transcripts accumulate after a week at low night temperatures in the cultivated tomato. The possibility that these transcripts are the product of a stress response is being investigated.

1602 ENDOMEMBRANE-LOCALIZED SMALL HEAT SHOCK PROTEINS IN PEA AND *ARABIDOPSIS* Kenneth W. Helm, James Schmeits, and Elizabeth Vierling. University of Arizona, Dept. of Biochemistry. Tucson, AZ 85721. Higher plants synthesize multiple low molecular weight heat shock proteins (LMW HSPs) in response to high temperature stress. The functions of LMW HSPs are unknown Plant LMW HSP genes can by classified into four related ancient gene families which are maintained across divergent genera. Of the four families, two are known to encode proteins localized in the cytoplasm, and a third encodes proteins imported into chloroplasts. We demonstrate that proteins from the fourth gene family are localized in the endomembrane system. Two LMW HSP cDNAs from *Pisum sativum* and *Arabidopsis thaliana*, designated PsHSP22.7 and AtHSP22.0, respectively, were isolated and characterized. Comparisons of the derived amino acid sequences showed that PsHSP22.7 and AtHSP22.0 are 64% identical They are most closely related to one family of cytoplasmic LMW HSPs (>41% identical) The amino terminal 25-30 residues of PsHSP22.7 and AtHSP22.0 have characteristics typical of signal peptides which direct cotranslational import into the endoplasmic reticulum (ER) The existence of a signal peptide on PsHSP22.7 was confirmed by in vitro translation of PsHSP22.7 mRNA in the presence and absence of ER-derived canine microsomes. Addition of the microsomes resulted in processing of PsHSP22.7 from a 23 kDa precursor form to a 20 kDa processed polypeptide. Preliminary results suggest that AtHSP22.0 is also processed by canine microsomes in vitro. Antiserum against PsHSP22.7 recognized two LMW HSPs of 20 and 21 kDa localized in ER fractions prepared from heat stressed pea seedlings. The 20 kDa protein comigrated with the microsome processed mRNA translation product of PSHSP22.7 on 2D gels. Similarities in the amino acid sequences of endomembrane-localized and cytoplasmic LMW HSPs argue that these HSPs have analogous functions in their respective compartments Thus, the study of endomembrane-localized LMW HSPs provides a simplified model system for understanding the functions of LMW HSPs in eukaryotes

1603 Cd-INDUCED PROTEINS IN Cd-RESISTANT AND -SENSITIVE CELL SUSPENSION CULTURES OF *DATURA INNOXIA*. P.E.Urwin, Ronald R.D.Croy and Nigel J. Robinson, Department of Biological Sciences, University of Durham, Durham DH1 3LE, U.K.

The effect of Cd on gene expression in suspension cultures of two cell lines (Cd-300 and WDI) of *Datura innoxia* has been investigated. The Cd-resistant cell line, Cd-300, was derived by step wise selection from a Cd-sensitive cell line, WDI (Jackson et al. *Pl. Physiol.* 75, 914, 1984). Resistance in these cells has been correlated with rapid assembly of complexes between Cd and class III metallothionein (phytochelatin; cadystin; poly(γglutamylcysteinyl)glycine) rather than modification of apopolypeptide biosynthesis (Delhaize et al. *Pl. Physiol.* 89, 700, 1989). Cd-induced synthesis of poly(γglutamylcysteinyl)glycine in these cells is largely insensitive to cycloheximide implying that the enzymes involved are constitutive (Robinson et al. *Plant Sci.* 56, 197, 1988). However, [H^3]leucine-*in vivo* labelling studies also show increased synthesis of several proteins in response to Cd. Changes in the levels of specific translatable mRNA species in response to Cd were also observed following 2-D PAGE of *in vitro* translation products (Delhaize et al. *Pl. Mol. Bio.* 12, 487, 1989). We report here the further analysis (by 2-D PAGE) of proteins in extracts from these cell lines following growth in either the presence or absence of Cd or heat shock. Poly(γglutamylcysteinyl)glycine, was removed from cell extracts prior to 2-D PAGE. This was necessary because samples containing these thiol-rich polypeptides inhibited the formation of polyacrylamide when the sample was added to the isoelectric focussing (IEF) gel mixture prior to polymerisation. Samples were incorporated in IEF gels in order to facilitate increased sample loading while avoiding protein precipitation at the gel surface. Two Cd-induced proteins of M_r c.a.20,000 were identified in extracts from Cd-300 cells and designated Cd-1 and Cd-2. These two proteins have not yet been detected in WDI. A third, constitutive, protein of similar pI and M_r has also been selected for further analysis and designated Pr-3. While both of the Cd-induced proteins were refractory to Edman degradation, the N-terminal sequence (30 amino acids) of Pr-3 has been determined by direct microsequencing of the "blotted" protein. This shows sequence similarity to the N-terminal region of pathogenesis related proteins from parsley. Following treatment of Cd-1 and Cd-2 with cyanogen bromide (CNBr) amino acids were detected in the microsequencer eluant necessitating the purification of the cleavage products prior to sequencing.

1604 CHARACTERIZATION OF THE CHALCONE SYNTHASE MULTIGENE FAMILY FROM BLACK SPRUCE

Bob Rutledge, Jack Pitel and Glen Sunohara. Petawawa National Forestry Institute, Forestry Canada, Chalk River, Ontario, Canada, K0J 1J0.

Chalcone synthase (CHS) is a key regulatory enzyme in the phenylpropanoid biosynthetic pathway, central to the production of various classes of flavonoids involved in, among others, floral pigmentation, phytoalexins, root nodulation signals and UV protectants. Although CHS multigene families have been extensively characterized in many angiosperm species, CHS genes in gymnosperm have yet to be characterized. We have utilized PCR (polymerase chain reaction) for the rapid and detailed analysis of CHS genes from the gymnosperm, black spruce (*Picea mariana*). Construction of two opposing universal primers targeted to highly conserved regions within CHS, allowed the simultaneous *in vitro* amplification of the selected region from all members of the CHS multigene family. Combined with a routine subcloning into M13 and sequence analysis of individual subclones, this approach has provided extensive amounts of DNA sequence data, which has indicated that black spruce contains a large and complex CHS multigene family. To date we have identified over 30 CHS genes, each of which can be classified into one of three major families. This approach has thus provided a rapid and powerful method for the characterization of multigene families and clearly has potential for applications in other gene characterization projects.

POSTER ABSTRACTS

1605 PHYTOCHELATIN SYNTHESIS IN CADMIUM TOLERANT TOMATO CELLS.
Peter B. Goldsbrough, Subhash C. Gupta and Jian-Jun Chen. Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

A family of Cd-tolerant tomato cell lines have been obtained by step-wise selection. These cells grow in the presence of up to .6 mM $CdCl_2$, where the ID_{50} for unselected cells is ~0.2 mM. The concentration of Cd within the tolerant cells ranged from 2.3 to 3.4 times higher than in the media, indicating that exclusion of Cd was not the primary mechanism of tolerance. Growth of tolerant cells in Cd-containing media was accompanied by proportional increases in cellular concentrations of phytochelatins (PCs). There was also a trend towards production of higher mol wt PCs in cells grown in higher concentrations of Cd. PC levels increased during the lag phase of the cell culture period and gradually declined thereafter. When grown in the absence of selection, Cd-tolerant cells maintained a degree of increased Cd tolerance over that exhibited by unselected cells, although they did not grow when transferred directly to medium containing the millimolar concentrations of $CdCl_2$ at which they were originally selected. When grown in Cd-free medium, cells selected at 6 mM $CdCl_2$ contained higher concentrations of GSH and PC_2 than unselected cells. The rate of PC synthesis in response to Cd exposure was also higher in selected cells. These results indicate that the capacity to synthesize PCs has been altered in Cd-tolerant cells. Preliminary experiments point to a variable but increased specific activity for PC synthase in selected Cd-tolerant cells in comparison with unselected cells.

1606 STUDIES ON ACID PHOSPHATASE IN RICE PLANTS.
W. S. Chen, S. C. Tso, Y. F. Huang and Y. R. Chen. National Taiwan Univ., Botany Dept., Taipei, Taiwan

Regenerating calli and seedlings of Oryza sativa were examined. Cytochemical study of leaves showed that acid phosphatase mainly distributed in cell wall and ectoplasmic surface of plasmalemma, and the enzyme activity preferentially located in the side of middle lamella; enzyme activity also occassionally detected in other organelles, such as mitochondrion, chloroplast, endoplasmic reticulum, Golgi, vacuole and nucleus, varied from cell to cell. Most cells in green calli had plastids containing aggregated starch grains, and activity of acid phosphatase occured in cell wall, plasmalemma, vacuole and amyloplast. The functions of acid phosphatase related to sugar translocation, autolysis and wall formation had been suggested. Fractionational studies indicated that soluble fraction was the major, ionically bound wall fraction the intermediate and covalently bound wall fraction the minor. Different fractions occupied the variability in responsing to mineral effect. Copper, mercury, floride and molybdate had differential inhibitory effects on the activity of acid phosphatase. However, magnesium, manganese, iron and cobalt had the enhanced effect. Soluble fraction was purified through salting out with ammonium sulfate, gel filtration and ion-exchange columns. A distinct band with molecular weight about 40,000 in SDS-PAGE was collected and dried for antibody induction.

1607 EXPRESSION OF NOVEL NON-HEATSHOCK PROTEINS ASSOCIATED WITH LONG TERM THERMOADAPTATION IN CULTURED COWPEA CELLS.
P Maheswara Reddy, Kelly A. Drake, Joe H. Cherry, and Narendra K. Singh, Department of Botany and Microbiology, Auburn University, Auburn, AL 36849

Suspension cells of cowpea (Vigna unguiculata var. California Blackeye 5) normally cultured at 26°C (unadapted cells) were slowly adapted to grow at 38-40°C (thermoadapted cells), a temperature range normally lethal for unadapted cells. The thermoadapted cells produce several novel proteins not produced by unadapted cells or unadapted cells heat-shocked at 40°C for 4 hours. Thermoadapted cells produce altered levels of some proteins present in unadapted cells and only four heat shock polypeptides (two 22 kDa, 70 kDa, and 80 kDa). Several new polypeptides were present among in-vitro translation products, most notably was a major 37 kDa polypeptide in thermoadapted cells. Thermoadapted cells also exhibit dramatic changes in the ultrastructure. A cDNA library was constructed using poly A$^+$RNA produced by the thermoadapted cells. This library was probed with ^{32}P labeled subtracted probes. Such probes were obtained by hybridization of single stranded cDNA corresponding to A$^+$RNA from thermoadapted cells with 10-20 fold molar excess of biotinylated A$^+$ RNA from unadapted cells and unadapted cells heat shocked at 40°C for 4 hours. Unhybridized cDNA was separated with the help of streptavidin. Six putative cDNA clones have been isolated and a preliminary analysis of the expression of corresponding genes by RNA slot blot shows either unique or highly elevated expression of the corresponding genes in thermoadapted cells. Characterization of these cDNA clones are in progress.

1608 TRANSIENT DNA POLYMORPHISMS IN CEREAL SEEDLINGS EXPOSED TO COLD-HARDENING AND NON-HARDENING TEMPERATURES.
Andre Laroche, Agriculture Canada Research Station, Lethbridge, Alberta, Canada T1J 4B1.

The objective was to determine whether differential DNA methylation would be observed following exposure of seedlings to low temperature. DNA was isolated from young seedlings of winter wheat (cv. Norstar), spring wheat (cv. Capelle), spring triticale (cv. Wapiti) and fall rye (cv. Musketer) grown at 20°C for 20 days or at 5°C for 14 and 46 days. DNA was restricted with enzymes sensitive to methylation and hybridized with the ribosomal DNA probe p71BE covering the IGS region and flanking sequences of the 26S and 18S RNA. Southern blot analyses of DNA restricted with TaqI, HpaII, MspI and MvaI revealed several polymorphisms while none was observed with BamHI, EcoRI or EcoRII. These polymorphisms may result from alterations in the methylation of specific DNA sequences and suggest that temperature would affect the activity of specific methylases and that DNA modifications at low temperature are transient. Furthermore, an important decrease in the signal intensity of rDNA genes was observed in the Capelle cultivar following exposure of seedlings to low temperature while an increase in signal intensity occurred for Norstar. Finally, work is under progress to determine the effect of deacclimation of seedlings on banding pattern and copy number of rDNA genes.

1609 MOLECULAR CLONING AND SEQUENCING OF WHEAT UBIQUITIN GENE
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Heat shock is known to induce a rapid synthesis of a special class of proteins in a variety of organisms ranging from bacteria to plants and animals. It is almost certain that these proteins protect the organism from the deleterious effects of heat shock. A large number of abnormal and denatured proteins also accumulate during this heat stress and cell clean-up and recovery is essential for the survival of the organism. Eukaryotic polyubiquitins perform this vital function by timely and efficient degradation of denatured proteins. A multigene family encodes these polyubiquitins which consist of highly conserved(~100%) identical repeats of 76 amino acids fused together in head-to-tail fashion. Further post-translational processing of these polyproteins results in their monomeric form. Abnormal proteins destined to degradation are tagged by the fusion with these monomer forms of ubiquitin. We have constructed a cDNA library using mRNA from heat shocked wheat leaves. Screening of this library using chicken ubiquitin as a probe resulted in several putative positive clones containing inserts of various sizes. Sequencing of the smallest insert(~400 bp) yielded information regarding last repeat of polyubiquitin and 3' non-coding region. At the protein level, wheat ubiquitin shows 100% identity to other plant ubiquitins. Nucleotide sequence homology however, ranges from 88-91% in maize and barley to 76-81% in other dicot species. The 3' non-coding region exhibits only 23-55% homology among these plant species. We are currently attempting to overexpress the wheat ubiquitin monomer gene in transgenic tobacco plants to understand its role in heat shock.

1610 CLONING OF A COLD-INDUCED cDNA FROM *BRASSICA NAPUS*
Jacqueline A. Pallas, Nigel A.R. Urwin, James Jardine and Gareth I Jenkins. Departments of Biochemistry and Botany, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

Plants acclimate to low temperatures. Exposure to a period of low, non-freezing temperatures increases a plant's ability to survive subsequent cold treatments. Various biochemical and physiological changes occur during the acclimation period and changes in gene expression in response to cold have been identified in several species. We have isolated a cDNA clone (pLF5) encoding a cold-induced gene from oil-seed rape (*Brassica napus*). Transcripts hybridising to the cDNA accumulate dramatically following the transfer of *B. napus* seedlings to cold, reaching a maximum level within 12 h of transfer to 5°C. Upon returning the plants to 20°C the transcripts decrease to a basal level in 4 to 8 h. The response is observed in leaf tissue and also in other tissues. Expression studies in non-cold-treated plants suggest that the gene is expressed more in shoot apices than in other organs. We have found that expression of the gene encoded by the cDNA is not stimulated by wounding or heat shock. It is known that in certain species abscisic acid can induce cold tolerance and expression of cold-induced genes. We are therefore currently investigating whether expression of pLF5 is induced by abscisic acid.

POSTER ABSTRACTS

1611 THERMAL EFFECT ON THE CELL WALL PROTEIN ACCUMULATION BETWEEN SUSPENSION CELLS AND FIBER COMPETENT CELLS.
T. C. Hsieh, W. C. Wang, and N. Trolinder. Texas Tech University and U.S.D.A.-A.R.S., Lubbock, TX.
The purpose of this study was to understand how thermal stress affect on the accumulation of cell wall proteins (CWP) at different growing stages of suspension cells, and how thermal stress affect the expression of genes encoding the CWP during fiber developmental process. For two cotton racestocks (T25-heat tolerant and T169-heat susceptible), cell suspension cultures derived from hypocotyl callus and fiber competent cell suspension cultures derived from ovary-induced callus were established to evaluate the thermal response to CWP deposition. Three temperatures (18°C, 28°C, and 34°C) have been investigated for both culture systems. $CaCl_2$ extractable CWP and proteins from cultured medium were collected to examine the protein changes under different temperature conditions.
Growth pattern for 6 different temperatures in suspension cells are followed the similar trend for both racestocks. T25 has higher growth rate than T169. The optimal growth temperature range is between 28°C to 34°C. Genotypic and temperature differences have been found in the CWP profiles for both suspension cultured cells during the courses of growth. During the growth period, we also found two groups of major proteins (42kd and 24kd) changed sequentially. And almost all of the protein bands from media are present on the gel of CWP, and the protein changes caused by the thermal effect and different growing stages of cells are the same. These suggest the CWP distribution is genotypic, growing stage, and temperature dependent and the proteins secreted into media by suspension cells are the same as CWP of suspension cells.
The role of CWP plays in fiber competent cells under thermal conditions will be reported. The differences and relationship between both culture systems will also be investigated.

1612 ISOLATION AND CHARACTERIZATION OF THE LOW TEMPERATURE-REGULATED RICE *ALCOHOL DEHYDROGENASE 1* GENE. Andrew W. Liu, Rajinder S. Dhindsa* and Virginia Walbot. Stanford University. Department of Biological Sciences, Stanford, California 94305-5020
Increased levels of mRNA and protein for *Alcohol dehydrogenase-1* have been observed in rice seedlings subjected to low temperature stress (Hahn and Walbot, 1989, Plant Physiol. 91:930-938; Christie *et al.* 1991, Plant Physiol. 95:699-706). We have screened a rice IR36 genomic library in Lambda replacement vector EMBL3A and have isolated clones which hybridize to rice *Adh1* cDNA (kindly provided by Dr. Ray Wu, Cornell University). Using restriction analysis, we have been able to ascertain that the clones we have are *Adh1*, not *Adh2*. We are currently sequencing the entire genomic *Adh1* including the 5' flanking regulatory sequences and hope to clone the promoter sequences into expression vectors driving GUS or Luciferase expression. Constructs containing deletions of the upstream region will be used in transient expression studies in rice or BMS maize protoplasts in order to identify the cold-regulated promoter sequences found in *Adh1* of rice. The rice and maize *Adh1* promoter elements will be compared.

1613 Low-Temperature Induction of Maize Genes Associated with Respiration, Oxidative Stress, and the Phenylpropanoid Pathway. Peter J. Christie and Virginia Walbot. Stanford University, Biological Sciences Dept., Stanford, CA 94305-5020 We previously demonstrated that *Alcohol dehydrogenase-1* transcript and protein activity accumulate in maize and rice seedlings to high levels within 24-h of shifting 7-d seedlings from 27°C to 10°C, and decline when cold-stressed seedlings are returned to 27°C (PJ Christie, M Hahn, & V Walbot, 1991, *Plant Physiol.* 95:699-706). Here, we show that cold stress causes a rapid, tissue-specific increase in transcript levels for numerous genes in maize seedlings. Within 24 h of shifting ambiently-grown seedlings to 10°C, transcript levels increase several-fold for specific genes in root and/or shoot tissues:
Roots: Respiration: aldolase, glyceraldehyde-3-phosphate dehydrogenase (*Gpc2, Gpc3*), pyruvate decarboxylase (*Pdc*), alcohol dehydrogenase (*Adh1, Adh2*), alternative oxidase; Oxidative stress: Mn and Cu/Zn superoxide dismutases (SODs), catalase.
No transcript accumulation: sucrose synthase (*Sh1*); anionic peroxidase; β-tubulin, actin.
Shoots: Phenylpropanoid pathway: *C2* (chalcone synthase), phenylalanine ammonia lyase, *R* and *C1* homologs, *A1, A2, Bz1, Bz2*.
No transcript accumulation: alternative oxidase; chalcone isomerase, 4-coumaryl:CoA lyase; Mn and Cu/Zn SODs, catalase, anionic peroxidase; β-tubulin, actin.
Enzyme assays demonstrate a corresponding increase in activity of several gene products, suggesting that cold stress enhances transcription and translation of several genes and enzymatic function of their products. Despite an increase in mRNA abundance during the period of cold stress for the anthocyanin pathway genes, anthocyanin levels remain constant until cold-stressed seedlings are returned to ambient temperature. These findings suggest that cold transcriptionally activates the anthocyanin pathway genes, but posttranscriptional events and/or enzyme function require the removal of cold stress. Ongoing experiments are aimed at identifying *cis*-sequences required for cold-enhanced gene expression in maize

1614 EFFECTS OF TEMPERATURE ON COTTON FIBER PROTEIN SYNTHESIS. W. Xie, N.R. Rao, T. P. Owen, Jr., and C.H. Haigler. Dept. of Biological Sciences, Texas Tech University, Lubbock, TX 79409
Growth of cultured cotton fibers is known to be delayed by cycling cool temperatures both at the stages of fiber initiation and fiber maturation. To understand the metabolic basis of these responses, we examined changes in protein profiles in cotton ovules at different stages grown *in vitro* under different temperature regimes. To study fiber initiation, one day preanthesis ovules that were cultured at constant 34°C or 15°C, or 34°/15°C 12h cycling conditions were labeled with ^{35}S-methionine at temperatures from 7° to 40°C. Very little methionine was incorporated in ovules grown at 7°-15°C whereas the cultures grown between 22°-40°C had many labelled proteins. A heat shock response was prominent at 40°C as indicated by the appearance of specific protein bands. Ovules labelled at 34°C and 15°C showed specific temperature sensitive bands but some of these continued to be synthesized in the opposite part of the 34°/15°C cycle. To study fiber maturation, temperature experiments on proteins from cultured cotton fibers 9 to 35 day old were performed. Analysis of silver-stained gels indicated that cycling at low temperatures delayed physiological development of the fiber. Effects of cold on protein synthesis in 21d fibers was examined by labeling the proteins at different points in the temperature cycle. Protein synthesis recovered in 15°C cultures within 6h after shifting to 34°C. These changes will be further characterized in 2D gels.
Research supported by grants from Cotton Inc. and Texas Advanced Technology Program.

1615 ASSOCIATION OF 70 kDa HEAT SHOCK COGNATE PROTEINS WITH ACCLIMATION TO COLD. Lisa Neven[1], Dale Haskell[1], Charles Guy[1], Nancy Denslow[2], Paul Klein[3], Linda G. Green[3], and Allison Silverman[1] University of Florida, Departments of Environmental Horticulture[1], Biochemistry and Molecular Biology[2], and Pathology[3], Gainesville, Florida 32611.
Exposure of young spinach seedlings to 5°C leads to an increase in the synthesis of several 79 kDa proteins (Cold Acclimation Protein; CAP79) present in leaf tissue grown at 20°C. Protein sequence analyses and immunological cross-reactivity indicate that this group of proteins belongs to the 70 kDa heat shock family. While steady-state transcript levels and protein synthesis are increased, immuno-blot analyses suggest that the concentration of this protein group in leaf tissue is increased by less than two fold at low temperature. It is proposed that this response of several members of the 70 kDa heat shock family could result from an influence of low temperature on protein folding and/or assembly processes.

1616 EFFECTS OF AMINO ACID ANALOG TREATMENT ON SOYBEAN HEAT SHOCK RESPONSE. Lee, Julie Y.-R., Ronald T. Nagao, Chu-Yung Lin[1] and Joe L. Key. University of Georgia, Department of Botany, Athens, Ga 30602. [1]National Taiwan University, Department of Botany, Taipei, Taiwan.
The effect of amino acid analog treatment on the heat shock (HS) response of soybean seedlings was analyzed at both normal growth and HS temperatures. Northern blot analyses revealed that HS genes, except two families represented by cDNA clones, pEV3 and pFS2033, were activated at the normal growth temperature, 28°C, in the presence of azetidine-2-carboxylic acid, a proline analog. The decline of HS mRNA steady state levels after 2 hr continuous 40°C HS (self-regulation) was not observed until 6 or more hr following analog treatment at 28°C. The incorporation of radioactive amino acids into high molecular weight HS proteins (hsps) was detected during analog treatment at 28°C; however, the incorporation into low molecular weight hsps was barely detectable although these hsps were detected by immunoblot analyses. Thermotolerance was not observed for analog treated seedlings even though hsps were synthesized. A 40°C HS in the presence of analog resulted in a full set of HS mRNA accumulation and hsp synthesis. During analog treatment HS mRNA accumulation and hsp synthesis continued for a longer time period than during HS in the absence of analog. Thermotolerance of combined analog and HS treated seedlings was reduced. This suggests that normal hsp synthesis is required for the regulation of HS gene expression, for the acquisition of thermotolerance, and for the onset of self-regulation.

POSTER ABSTRACTS

1617 CHARACTERIZATION OF cDNA CLONES FOR SOYBEAN LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS. Peter R. LaFayette, Ronald T Nagao, Julie Y-R Lee, Joe L. Key. Department of Botany, University of Georgia, Athens, Georgia 30602

Organisms respond to high temperatures and many other stresses by synthesizing a distinct set of proteins termed heat shock (HS) proteins. A unique feature of the plant HS response is the greater number and family complexity of low molecular weight heat shock proteins (hsps) synthesized. cDNAs encoding four families of low molecular weight hsps from soybean were characterized. Sequence and hydropathy analyses of three cDNA clones (pFS2033, pEV2, pEV6 representing hsp in the 21 to 24 kD range) indicates they contain a signal sequence and may be targeted to the endomembrane system or secreted from the cell. Northern analysis of RNA isolated from both free and membrane-bound polysomes substantiates the assignment of pFS2033, pEV2, and pEV6 to the endomembrane system. A fourth clone (pEV1), encoding proteins of similar molecular weight but more different in sequence, is not localized to membrane-bound polysomes. Two of the clones studied (pFS2033 and pEV6) are strikingly similar: at the amino acid level they are 73% identical and 92% conserved and both possess the C-terminal tetrapeptide KQEL, yet can be clearly resolved from one another by two-dimensional gel analysis of hybrid arrest translations. In addition, pFS2033 and pEV6 transcripts may be differentially regulated as evidenced by their response to HS and amino acid analog treatments

1618 INDUCTION OF GENE EXPRESSION BY LOW TEMPERATURE AND WATER STRESS IN *ARABIDOPSIS THALIANA*. Pekka Heino, Kerstin Nordin, Viola Lång, Bjorn Welin and E. Tapio Palva. Department of Molecular Genetics, Swedish University of Agricultural Sciences, Box 7003, S-750 07, Uppsala, Sweden

cDNA clones corresponding to genes induced by low temperature were isolated from an *Arabidopsis thaliana* cDNA library. The cDNA clones were used to study the induction of the expression of the corresponding genes during low temperature exposure, water stress and ABA treatment. All the genes that were studied were found to be induced with these treatments. However, studies with *A. thaliana* plants harboring different mutations in ABA synthesis/responsiveness revealed that the genes can be divided in two categories depending on the involvement of ABA in the induction pathway.

1619 MOLECULAR CELLULAR BIOLOGY OF HEAVY METAL TOLERANCE IN *Schizosaccharomyces pombe*. Daniel Ortiz, David Speiser, Lisa Kreppel and David Ow. Plant Gene Expression Center, Albany, CA 94710

Plant and fission yeast cells share a common response to heavy metal toxicity, the induction of metal-chelating peptides known as phytochelatins (PCs). PCs are derived from glutathione and have the structure of $(\gamma\text{-GluCys})_n\text{Gly}$, where n=2-11. PCs bind Cd to form two different complexes: the more stable of the two contains acid-labile sulfide. In this laboratory, the fission yeast *S. pombe* has been used for characterizing the genetic components of this cellular response. Here, we describe the molecular basis of two Cd-hypersensitive mutants that fail to accumulate wild type levels of the PC-Cd-S^{-2} complex. The first mutant has a defect in a gene encoding a protein with structural and sequence similarities to a large family of integral membrane transporters. In comparison to wild type strains, *S. pombe* with multiple copies of this cloned gene, which we named *hmt1* (heavy metal tolerance), are more resistant to Cd but also accumulates more of the metal. Subcellular fractionation analysis indicates that a HMT1-LacZ fusion protein is directed to the vacuolar membrane and that PCs accumulate in the vacuole. Since Cd and PCs are localized to the vacuoles of tobacco, we postulate that HMT1 is the transporter for vacuolar sequestration of metal-chelates. The deficient accumulation of the PC-Cd-S^{-2} complex in this mutant also suggests that its formation occurs within the vacuole. In the other Cd-hypersensitive mutant, lesions in two genes of the purine biosynthesis pathway were found. The phenotypes of various *ade* mutants suggest that blocking both an early and a late branch of the purine biosynthesis pathway is necessary to produce the Cd-hypersensitive phenotype. Since cysteine sulfinate is an analog of aspartate, we propose that enzymes of the purine biosynthesis pathway can incorporate cysteine sulfinate in place of aspartate and that the sulfide in the PC-Cd-S^{-2} complex is derived from this alternate substrate. A model of the cellular biology of metal tolerance mediated by *hmt1* and *ade* genes will be presented.

1620 PHYTOCHELATIN-Cd-SULFIDE COMPLEXES IN *BRASSICA JUNCEA* AND A GEL ASSAY FOR PCs. David M. Speiser, Susan L. Abrahamson, and David W. Ow. USDA/ARS Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710. Phytochelatins (PCs) are metal-binding peptides produced by higher plants and some fungi in response to heavy metal exposure. PCs are synthesized by transpeptidation of glutathione by phytochelatin synthase, yielding a structure of $(\gamma\text{-glu-cys})_n\text{-gly}$, with n= 2 to 11. PC synthesis is induced by a variety of metals, including Cd, Cu, Zn, Pb, and Hg, but the relative affinity of PCs for different metals has not been well characterized with the exception of Cd and Cu. When cell-free extracts from Cd-induced *Schizosaccharomyces pombe* are analysed by gel filtration chromatography, two distinct PC- and Cd-containing complexes are seen. The first, with a lower apparent MW (LMW PC) is composed of PCs and Cd^{+2} ions. The second, with a higher apparent MW (HMW PC), also contains acid-labile sulfide as part of chelation complex; this complex has a higher affinity for Cd and Cu, and has been demonstrated to be essential for Cd tolerance. Sulfide and sulfite have been described in PC-containing extracts from plants, but no distinct HMW complex has been reported. We have developed a gel electrophoresis assay for PCs which enables examination of a large number of samples simultaneously and have used it to measure the relative affinity of PCs for a variety of metals. By measuring the displacement of bound Cd, we found that Hg and Cu have the highest affinity for PCs and that Se, one of two semimetal inducers of PC synthesis, is able to eliminate binding of Cd to PCs. Zn, however, binds poorly and As, another semimetal inducer, does not appear to compete for binding to PCs We have also used the gel assay to screen a number of different plant species for PC production. *Brasssica juncea*, a wild mustard, produces a more vigorous PC response to Cd exposure than do a number of other species. PCs produced by *B. juncea* were examined by gel filtration and found to contain a distinct HMW complex. This complex was purified and its amino acid composition and Cd/sulfide ratio were found to conform to the HMW complex described in *S. pombe*.

1621 NUCLEAR GENES FOR MITOCHONDRIAL CHAPERONIN CPN60 IN MAIZE. P.S.Close [a,b], S.P. Briggs [b], B.A. Bowen [a,b] T.K. Prasad [a] and R.G. Hallberg [a,c]. [a] Dept. of Genetics/Zoology, Iowa State Univ. [b]Pioneer Hi-Bred International Inc. [c] Dept. of Biological Sciences, Syracuse Univ.

Cpn60 (hsp60) is an abundant mitochondrial protein belonging to a family of proteins homologous to the *E. coli* protein groEL and the chloroplast Rubisco-binding protein. These proteins are designated 'chaperonins' because they are implicated in the folding of peptides and the assembly of oligomeric protein complexes. The identification and sequence of a cDNA clone for maize cpn60 is presented. A transcript of approximately 2.4 kilobases in length was detected in two week old maize seedlings. Expression is elevated after heat shock and in developing maize seedlings. Genomic Southerns reveal two copies of cpn60 in all maize inbreds screened. One copy is present in the *Arabidopsis* genome. Segregation patterns indicate that both maize genes are encoded in the nuclear genome. A maize B73 genomic library has been constructed and screened for cpn60 clones. Two major families of overlapping clones have been identified corresponding to the two gene copies identified on genomic Southern blots. Four minor families of genomic clones which show varying degrees of homology to the cpn60 cDNA have also been identified. In addition, one family of *Arabidopsis* cpn60 genomic clones has been identified.

1622 SOYBEAN HEAT SHOCK GENE *Gmhsp17.5E*: MUTATIONAL DEFINITION OF THE HEAT SHOCK MODULE. D. Barros, E. Czarnecka and W. B. Gurley. Microbiology & Cell Science, Univ. of Florida, Gainesville, FL 32611. The promoter of soybean heat shock gene *Gmhsp17.5E* has two overlapping heat shock elements within the TATA-proximal site 1 (-72 to -49). Five core domains comprised of nGAAn or nTTCn are present in this region and are numbered 1 to 5 according to their proximity to TATA. A detailed site-directed mutational analysis was carried out by changing one to three bases at a time in order to define the optimal configuration of this regulatory region. A total of 32 mutants were tested in sunflower tumors by S1 nuclease hybrid protection analysis using a T-DNA-based vector. In general, mutations within the region spanning core domains 3 and 4 have the most pronounced effect on transcription with potential for both positive and negative modulation of activity. The configuration of site 1 in *Gmhsp17.5-E*, and in other heat shock genes, can be represented by two perfect core domains flanked by two imperfect cores. This configuration is designated as the "heat shock module". Mutations away from the highly conserved GAA or TTC of the core had a negative influence on transcriptional activity. Surprisingly, mutations in the nonconserved bases flanking the cores also had a great impact on the thermoinducibility of the promoter. Determination of optimal activity by mutating bases flanking the cores indicated that the core domain can be more precisely represented as 5'-aGAAg-3' or 5'-cTTCt-3'.

POSTER ABSTRACTS

1623 PLANT GENES WITH SIMILARITY TO METALLOTHIONEIN GENES Andrew M Tommey, Shi Jianguo, William P. Lindsay Julia Bryden and Nigel J. Robinson Department of Biological Sciences, University of Durham, Durham DH1 3LE, U K.

Metallothionein genes have been isolated from vertebrates, invertebrates, some fungi and most recently from prokaryotic cyanobacteria (Robinson et al. *Proc R Soc. Lond. B.* 242. 241, 1990). Functions proposed for metallothioneins include the detoxification of non-essential metals, such as Cd, and roles in essential trace metal ion homoeostasis. Essential trace metals, such as Zn, are required for the activity of enzymes involved in many aspects of metabolism including DNA synthesis, RNA synthesis, protein synthesis and degradation. Some DNA-binding transcription factors require Zn to form an appropriate conformation to interact with DNA and activate transcription and it has recently been reported that thionein (apometallothionein) can modulate DNA binding and transcriptional activation by Zn finger containing factor Sp1 (Zeng et al. *FEBS* 279. 310 1991). The Zn-binding E_C protein from wheat germ (Lane et al. *Biochem. Cell Biol* 65. 1001. 1987) has been designated class II metallothionein and we have reported the isolation of a gene from *Pisum sativum*, $PsMT_A$, which has similarity to metallothionein genes (Evans et al. *FEBS* 262. 29. 1990). Southern blots reveal that $PsMT_A$ is a member of a small multigene family and two further genes, $PsMT_B$ and $PsMT_C$, have now been isolated and sequenced. This small gene family encodes putative proteins with two metallothionein-like domains containing Cys-Xaa-Cys motifs, where Xaa is an amino acid other than cysteine. Related genes have been isolated from several other species including *Mimulus guttatus* (deMiranda et al *FEBS* 260, 277, 1990), soybean (Chino et al. in press) maize (de Framond et al in press) alfalfa, *Phaseolus vulgaris* and *Brassica napus*. The amino acid sequences of the predicted translational products of all of these genes are similar within the two metallothionein-like domains, but less similar in the intervening region. *PsMT* transcripts are much less abundant in leaves than roots 14 days after germination. However, these transcripts were not detected in the embryonic radicle 13 days after flowering but were detected in the embryonic cotyledon 15 days after flowering. The translational product of $PsMT_A$ has been expressed in *E coli* as a C-terminal extension of glutathione-S-transferase The fusion protein has been purified from extracts of cells grown in trace metal supplemented media by glutathione-Sepharose affinity chromatography The fusion protein binds trace metal ions such as Zn The metal-binding characteristics of this protein have been examined and the results of these investigations will be presented

1624 COLD-REGULATED GENES OF WHEAT AND ARABIDOPSIS. WW Guo, R Ward, MF Thomashow. Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, USA.

Many plants increase in freezing tolerance in response to low nonfreezing temperatures, a process known as cold acclimation. It has been established that cold acclimation is associated with changes in gene expression. Two questions that we are addressing are: 1) Do cor (cold-regulated) genes from different plants encode polypeptides with related functions?; and 2) Are cor genes regulated by similar mechanisms in different plants? Work is currently focused on comparing the polypeptides encoded by cor genes of wheat and Arabidopsis and examining their expression in response to temperature and other conditions. The data indicate that a number of cor genes from these plants encode polypeptides that share the unusual property of remaining soluble upon boiling. Further, one of the cor genes of wheat, tentatively designated cor50, encodes a "boiling-stable" polypeptide that is related to the cor47 gene of Arabidopsis, a gene that encodes a 47 kD boiling-stable polypeptide. Transcripts for cor50, like those for cor47, accumulate within 4 hours of placing plants at low temperature. Both cor50 and cor47 are induced in response to ABA and drought. DNA sequence analysis indicates that cor50, like cor47, is related to the Group II family of LEA (late-embryogenesis-abundant) proteins. These data indicate that cold acclimation in wheat and Arabidopsis includes the induction of at least one related gene, the accumulation of transcripts encoding boiling-stable polypeptides, and similarities in cor gene regulation. Future studies will further delineate the similarities and differences in the molecular genetics of cold acclimation in these two plant species.

1625 GENETIC STABILITY OF THE HERBICIDE RESISTANCE GENE PAT IN TRANSGENIC PLANTS AND SUSPENSION CULTURES C. Walter, I. Broer, A. Pühler, University of Bielefeld, Department of Genetics, D-4800 Bielefeld, Germany

The meiotic stability of the phosphinotricin-N-acetyl transferase (pat) gene from Streptomyces viridochromogenes has been analysed in transgenic Medicago sativa plants and suspension-cultures. All cultures were derived from one primary transformant (T304) which carries a single copy of the gene. Single cell suspension cultures could be established, and after inbedding into agarose, regenerated to monoclonal calli. Up to 12% of the cells grown under non-selective conditions and constant temperature (23° C) lost the herbicide resistant phenotype. A ten days' heatshock (37° C) during a 20 weeks' culture period led to 95% phosphinothricin (Pt) sensitive cells. It could be demonstrated that the pat gene is still present but not expressed. The resistance level of the Pt-sensitive transgenic cells is equivalent to that of wildtype cells. Additionally, leaves of Pt-resistant T304 descendants grown for months under non-selective conditions were protoplasted and analysed for their reaction to the herbicide. In all leaves, independant of the culture time, 0.7% to 2% of the cells turned out to be Pt-sensitive. The answer of total plants to the heat shock is under investigation.

1626 TRANSCRIPTIONAL CHARACTERISTICS OF THE DIVERGENT MANNOPINE PROMOTERS (Pmas1'Pmas2') FROM THE Ti PLASMID OF *AGROBACTERIUM TUMEFACIENS*. Cindy Peach and Jeff Velten, Graduate Program in Molecular Biology, New Mexico State University, Las Cruces, New Mexico 88003 USA

Pmas1'Pmas2' are adjacent, transcriptionally divergent promoters originally isolated as a 479 bp fragment located within the TR-DNA. Although the functional characteristics of Pmas1', and to a lesser extent Pmas2', have been independently investigated, this is the first simultaneous and quantitative examination of both dual promoters using separate and independent reporter genes (GUS<-Pmas2'Pmas1'->CAT). We have found (1) basal (uninduced) activities of both *mas* promoters show a polarity within leaf tissue (greater at tips and forward edges), (2) upon wounding, the levels of CAT(Pmas1') and GUS(Pmas2') activities increase with time and (3) the level of induction is proportional to the degree of wounding. However, the ratio of CAT/GUS activities throughout wound induction is about 100-200, suggesting both wild-type promoters are equally induced. All these data are consistent with the hypothesis that both promoters share regulatory elements. Deletion mutations within the promoters have been made and will be used to define regulatory regions important to the functional characteristics of both promoters. The effects of wounding, hormone treatment, methyl jasmonate and phytopathogens on both the wild-type and deleted *mas* promoters will be discussed and compared.

1627 CHROMATIN STRUCTURE OF PROTEINASE INHIBITOR I GENE IN TOMATO LEAVES. Antonio Conconi and Clarence A. Ryan. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Although signal transduction pathways for the wound induction of proteinase inhibitors are becoming better understood, events within the nucleus in response to wounding are still largely unknown. The nuclear DNA of eukaryotic cells is densely packaged by histone proteins in a linear array of nucleosomes. These structures reduce the accessibility of DNA sequences for regulatory factors and processive enzymes such as RNA polymerases. We have therefore investigated the chromatin structure in tomato leaves, in the coding region and in the surrounding flanking regions of the proteinase inhibitor I gene, before and after wound induction. Analysis of the chromatin structure by nuclease digestions (micrococcal nuclease and DNase 1) indicate an open conformation in the coding region of the inhibitor I gene, before and after wounding. Additionally, no structural difference were found between the proximal flanking regions of the inhibitor I chromatin structure from unwounded versus wounded plants. A loose structure was also found in the proximal 800 bp upstream of the transcription initiation site. In contrast, the 3'-end of the gene (~ 1.2 kb), was in the canonical nucleosomal structure, similar to the bulk inactive chromatin. Our results support a model with the inhibitor I gene in a poised preactive chromatin structure, which may be a prerequisite for a functional wound-inducible gene. (Supported in part by the Washington State University College of Agriculture and Home Economics, Project # 1791 and grants from NSF and the McKnight Foundation.)

1628 CHROMATIN STRUCTURE OF TOMATO LEAF rRNA GENES. Antonio Conconi, Josè M. Sogo and Clarence A. Ryan. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

The transcribing ribosomal genes of several animal organisms are devoid of canonical nucleosomes, whereas the non-transcribing gene copies appear to be folded into inactive chromatin. In plant ribosomal DNA (rDNA) clusters, the inactive ribosomal genes are the most predominant population. Using the classical micrococcal nuclease approach for studying plant chromatin, we found that the bulk of ribosomal genes in tomato are folded into inactive chromatin. The structure of a minor active ribosomal gene population was investigated using a newly developed psoralen technique. Isolated nuclei were photoreacted with psoralen and the extent of crosslinking was analysed using gel retardation assays. About 10% of the ribosomal genes were found to be present in an active (open) chromatin structure. We suggest that ribosomal gene activity in plants, as in animals, is correlated with their chromatin structure, which may determine the maximal RNA polymerase I loading. (Supported in part by the Washington State University College of Agriculture and Home Economics, Project # 1791 and grants from NSF and Mc Knight Foundation.)

POSTER ABSTRACTS

1629 ORGANIZATION AND EXPRESSION OF A WOUND-INDUCIBLE (*win*) MULTIGENE FAMILY IN POPLAR TREES. Howard Clarke, John Davis. Harvey Bradshaw, Jr. and Milton Gordon. University of Washington, Departments of Microbiology and Biochemistry, Seattle, Washington 98195. Wounding juvenile poplar trees induces the expression of genes in leaves remote from the site of damage. Several wound-inducible (*win*) transcripts have been cloned by differential cDNA hybridization. One cDNA, *win6*, has significant nucleotide sequence similarity to basic endochitinase genes from tobacco, potato, bean, *Arabidopsis* and barley. Using the *win6* cDNA as a hybridization probe nine *win6* genes have been cloned from the genome of hybrid poplar H11-11 (*Populus trichocarpa* x *P. deltoides*) indicating that *win6* belongs to a multigene family. Restriction mapping and sequence analysis of two *win6* genomic clones, *win6.2* and *win6.39*, demonstrates that multiple *win6* genes are clustered together in the genome in the same transcriptional orientation. The *win6* cluster segregates as a single genetic locus in a three generation pedigree. Genomic clone *win6.2* is derived from the *P. trichocarpa* parent and *win6.39* is derived from the *P. deltoides* parent. PCR analysis of reverse-transcribed RNA from wounded poplar trees shows that at least one *win6* gene from genomic clone *win6.2* is expressed in *P. trichocarpa* and similarly, at least one *win6* gene from genomic clone *win6.39* is expressed in *P deltoides P. trichocarpa* x *P. deltoides* hybrids express both of the parental *win6* genes.

1630 CHARACTERIZATION OF A POTATO PROTEINASE INHIBITOR II GENE THAT IS NOT WOUND-INDUCIBLE. by Sanggyu Park and Robert Thornburg Department of Biochemistry, Iowa State University, Ames, Iowa 50011

A potato Proteinase Inhibitor II gene, pin2T, was isolated from a potato genomic DNA library and was characterized both molecularly and functionally. The 5' flanking region of the pin2T is highly homologous (>91% identity) with that of the wound-inducible gene, pin2K. The 5' flanking region was linked to both CAT and GUS reporter genes in constructions that contained the terminator from the wound-inducible pin2K gene. The chimeric genes were transferred to tobacco plants using *Agrobacterium tumefaciens*. The presence of the constructions in the transgenic tobacco plants was confirmed by polymerase chain reaction (PCR) Expression of CAT or GUS activities driven by pin2T promoter indicated that pin2T is not a wound-inducible gene. GUS activities driven by promoter deletion mutants ruled out the presence of the silencer sequences in upstream portion of the pin2T promoter. Comparison of pin2T promoter sequence with that of pin2K indicates that there are three small deletions in the pin2T promoter which map to those regions required for wound-inducibility of pin2K. When these deleted sequences are searched through the Genebank, we identified a putative wound-inducible sequence, 5'-AGTAAA-3' that is found in a wide variety of other wound-inducible genes but is not easily found in the published promoter sequence of the other plant genes. Nuclear proteins, from unwounded and wounded potato leaves bound to the pin2T proximal promoter downstream of this sequence. Several biochemical compounds known to induce the wound-inducible pin2K-CAT constructions in transgenic plants were also tested for their effects on the pin2T-CAT constructions. The pin2T constructions were not enhanced by sucrose, by abscisic acid (ABA), nor was the gene derepressed in the absence of auxin.

1631 ANALYSIS OF A BEAN PR PROTEIN GENE FAMILY Michael H. Walter, Jian-Wei Liu, Joachim Wünn and Dieter Hess. Universität Hohenheim, Institut für Pflanzenphysiologie -260-, P. O. Box 70 05 62, D - 7000 Stuttgart 70, FRG

We are characterizing a large family of pathogenesis-related (PR) protein genes of unknown biochemical function in bean (*Phaseolus vulgaris* L.), which encodes small acidic proteins without signal peptides. These PR proteins have known homologues in pea, potato, parsley and birch. Their mRNAs accumulate in response to fungal elicitor as in bean, to fungal or bacterial infection, to abscisic acid or in untreated pollen tissue. We have isolated two genomic clones containing complete bean genes and have determined the DNA sequence of transcribed and promoter regions. The two genes are very much alike in coding, intron and promoter regions proximal to the TATA-box, but differ in promoter sequences further upstream and in 3'-untranslated regions. The protein encoded in both genes is not identical to any of the cDNAs characterized so far, but closely related to a PvPR2 protein gene transcript strongly induced by fungal elicitor in bean cells. In the promoters we have identified a 11 bp putative regulatory cis-element proximal to the TATA-box, which is found in the two bean genes and in homologous genes from pea (91% similarity), in potato (73% similarity) and in two parsley genes (55% similarity). Promoter-GUS fusions are being introduced into tobacco to screen various stimuli for their ability to activate these genes. A similar survey is underway to analyze bean plants exposed to such stimuli in Northern-type experiments.

1632 STRUCTURE OF A BEAN MALIC ENZYME GENE AND DEFENSE-RELATED ACTIVATION OF PROMOTER-GUS FUSIONS IN TRANSGENIC TOBACCO. Michael H. Walter, Jörg Schaaf and Dieter Hess. Universität Hohenheim, Institut für Pflanzenphysiologie -260-, P. O. Box 70 05 62, D - 7000 Stuttgart 70, FRG

NADP-dependent malic enzyme (ME, malate dehydrogenase decarboxylating, E.C. 1.1.1.40) is best known for the involvement of a chloroplast form in photosynthetic carbon fixation of C4 plants. Additional MEs are found in the cytosol and in mitochondria. The cDNA λCAD4, which was earlier thought to encode a cinnamyl-alcohol dehydrogenase (PNAS 85, 5546-5550) has now been redesignated as a bean malic enzyme clone (cME1) after finding a very strong homology of the protein encoded to malic enzymes from maize, *Flaveria trinervia*, mouse, rat and man. The protein deduced from cME1 is likely to be a cytosolic ME, since some 50 N-terminal amino acids corresponding to signal peptides in chloroplast MEs are lacking. We have isolated a genomic clone from a bean library and have sequenced a total of 7 kb comprising about 2.6 kb of promoter sequence.The coding region is interrupted by 18 introns, one additional intron is found in the leader region. The 2.6 kb promoter fragment and deletions thereof have been transcriptionally fused to the GUS reporter and introduced into tobacco. Preliminary analysis of several transformants indicates a preferential expression in vascular tissue. Stimuli already shown to activate the full promoter-GUS construct include wounding, elicitor treatment and glutathione (known to cause defense responses in bean). Additional potential inducers are being tested. A putative role of cytosolic malic enzyme as a supplier of reducing equivalents in plant defense and development is discussed.

1633 CINNAMYL ALCOHOL DEHYDROGENASE FROM SUBTERRANEAN CLOVER. Ernest H. Creaser, Kathleen A. Britt, Troy Delbridge and Barry G. Rolfe. Plant-Microbial Interaction Group. Australian National University. Canberra. Australia.

We are studying the molecular defence mechanisms of subterranean clover against environmental conditions such as wounding and the attacks of pathogens and other microorganisms. It has been shown in other plants that lignin formation is a common defence mechanism and that the penultimate enzyme in the lignin biosynthetic pathway, cinnamyl alcohol dehydrogenase [CAD], can be used as a marker for this synthesis. Gel electrophoresis of clover extracts shows that there are at least ten distinct enzymes in leaves and roots which catalyse the CAD reaction. Initial experiments indicate that an NADP requiring CAD of Mr 80000 is the species whose synthesis responds to stimulation by wounding or elicitation. This enzyme has been purified by standard affinity chromatography procedures. It will be sequenced, a DNA probe will be made, the CAD gene isolated from our clover gene bank and then cloned. We will then study the control mechanisms for this gene in relation to environmental changes, fungal and bacterial invasion as well as *Rhizobium* infection of the roots.

1634 REGULATION OF A PHENYLALANINE AMMONIA LYASE GENE FROM TOMATO. Leonard N. Bloksberg and C. I. Kado. University of California, Davis, Davis Crown Gall Group, Davis, CA 95616

Phenylalanine ammonia lyase (PAL) (EC 4.3.1.5) is a key branchpoint enzyme leading to the phenylpropanoid metabolic pathways in plants. In tomato (Lycopersicon esculentum, Mill.), pal is encoded by a seven to ten gene family. One of these genes was cloned from a genomic library and characterized. Its sequence showed strong homology (ca. 77% identity) with the structural regions of other plant pal genes, including similar placement of the intron as observed in the pal genes of bean. Approximately 35% identity was observed with pal clones from yeast. The promoter of the tomato pal gene was subcloned in front of either a GUS or a LUX reporter and proved to be biologically active in tobacco plants by the production of GUS or light. The pal gene was also cloned from a tomato cDNA library. The sequence of the promoter region contains several putative regulatory elements which were shown to modulate expression in response to various environmental challenges during plant development. Such modulation of the pal promoter was measured by the production of light in living transgenic plants.

POSTER ABSTRACTS

1635 ISOLATION AND CHARACTERIZATION OF AN AUXIN DEPENDENT TOBACCO GENE ACTIVATED UPON INFECTION WITH PSEUDOMONAS SOLANACEARUM. Didier Froissart, Philippe Brethenou, Christophe Lacomme, Laurence Godiard, Michèle Axelos, Yves Marco and Dominique Roby. Laboratoire de Biologie Moleculaire, INRA/CNRS, Auzeville, 31326 Castanet-Tolosan, France.

A tobacco gene whose transcripts accumulate during incompatible and compatible interactions with the pathogenic bacteria, *Pseudomonas solanacearum*, has been characterized. This gene, STR 246, is a member of a small multigene family and is regulated positively by auxin, wounding and elicitors from various sources. Its nucleotide sequence has been determined and shows the presence of a 660 bp open reading frame interrupted by a 190 bp intron. A chimeric gene consisting of 2.1 kb of 5' flanking sequences derived from STR 246, has been fused to the coding sequence of B-glucuronidase. Analysis of expression of this construct indicates that this region confers a high level of expression in freshly prepared tobacco protoplasts ; in addition, this expression level is increased by auxin and elicitors. Deletions in the promoter region are being tested in a transient gene expression system. Identification of the cis elements involved in the response to these different effectors will be presented.

1636 REGULATION OF FATTY ACID PEROXIDATION. David F. Hildebrand, Hong Zhuang, Tom Hamilton-Kemp and Roger Andersen. Univ. of Kentucky, Department of Agronomy, Lexington, KY 40546

Fatty acid peroxidation products are thought to be important in plant defense responses and some of these compounds increase massively upon damage to many plant tissues. These compounds may also play a role in plant growth and development. The formation of fatty acid peroxidation products was found to be under tight developmental regulation and treatments which altered development of plant tissues also changed levels of some of the key enzymes. The inaugural event in the formation of fatty acid peroxidation products is thought to be release of fatty acids from glycerolipids, but evidence is accumulating which does not support this. In order to gain a greater understanding of the control of the formation of fatty acid peroxidation products, we have utilized different (but matching) plant tissues with contrasting fatty acid compositions and investigated the formation of lipoxygenase/hydroperoxide lyase products. Of all major lipid classes, glycolipid composition was most closely reflected in patterns of these products formed. This suggests that glycolipids may be the major source of fatty acid substrates for these reactions. Lipoxygenase appears to be the most important enzymatic step in the production of fatty acid peroxidation products, but there are other important controls. It was found that jasmonate, a product of the lipoxygenase/dehydrase pathway, increases lipoxygenase itself and the other major branch of the lipoxygenase pathway, lipoxygenase/hydroperoxide lyase.

1637 WOUND INDUCIBLE EXPRESSION OF 4-COUMARATE CoA LIGASE IN TRANSGENIC PLANTS. M. Ellard and C. Douglas. Dept. of Botany, U. British Columbia, Vancouver, B.C. V6T 2B1.

Stress or pathogen invasion evokes a number of defense responses in higher plants some of which occur systemically and others which are limited to the site of stimulation. One example of such a localised response is the rapid accumulation in parsley leaves of transcripts of 4-Coumarate CoA Ligase (*Pc* 4CL-1) at wound sites. We are using transgenic tobacco and *Arabidopsis* plants to study the molecular basis of this response. A 4CL-1 promoter fragment containing sequences to -600 base pairs is capable of confering wound inducibility on GUS in transgenic plants. We are using 4CL-1 promoter deletions fused to GUS in order to further localise the regulatory sequences responsible for this response. Ethylene is a candidate as a second messenger in the pathway from the wound stimulus to the genetic regulatory apparatus. Exogenously applied ethylene can induce both the entire 4CL-1 gene and the 600bp promoter-GUS fusion in transgenic plants. We are now screening for mutants in *Arabidopsis* which are deficient in these responses. In this way we hope to genetically identify components of the transduction pathway mediating the wound response.

1638 EXPRESSION OF HSP70 AND UBIQUITIN GENE FAMILIES IN POTATO. Timothy Rickey, Joan Garbarino, David Rockhold, & William Belknap. WRRC, ARS, USDA, Albany, CA 94710

HSP70 (70-kD heat shock protein) and ubiquitin are both highly conserved proteins that are encoded by multigene families. Some members of each gene family are expressed constitutively, while other members are expressed in response to stress (heat shock, heavy metals). Northern analysis using heterologous cDNA probes for HSP70 and ubiquitin genes revealed that individual members of these two gene families are expressed differently in response to heat shock and mechanical injury in potato tubers. We have isolated several HSP70 and ubiquitin genomic and cDNA clones from potato. These clones have been used as copy-specific probes in northerns in order to associate the individual clones with the expression of specific mRNAs. Two different HSP70 clones encode transcripts that increase in response to injury, but not heat shock. One of these genes appears to encode a protein with an amino-terminal extension. The exposure of tubers to 23 ppm ethylene induced the expression of both of these HSP70 genes, suggesting a role for ethylene in the regulation of these genes. Another HSP70 clone was induced only by heat shock, while a fourth gene was constitutively expressed.

1639 IDENTIFICATION OF CIS-ELEMENTS MEDIATING COUMARATE REGULATION OF A CHALCONE SYNTHASE GENE PROMOTER IN ELECTROPORATED PROTOPLASTS. Gary J. Loake[1], Ouriel Faktor[2], Christopher J. Lamb[2], and Richard A. Dixon[1]. [1]The Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, OK 73402. [2]Salk Institute for Biological Studies, Plant Biology Laboratory, 10010 N. Torrey Pine Road, La Jolla, CA 92037.

Chimeric constructs containing promoter sequences from the bean CHS15 gene fused to a CAT reporter gene were introduced into alfalfa protoplasts by electroporation. CAT activity was determined for a series of 5' deletions, linker - scanner mutations and co-electroporations, following treatment of the protoplasts with fungal elicitor and a range of concentrations of the phenylpropanoid para-coumaric acid. Two different motifs have been defined which act cooperatively to mediate the promoter specific transcriptional stimulation observed with para-coumaric acid. Furthermore, these motifs can convey coumarate responsiveness to a previously unresponsive heterologous promoter.

1640 ETHYLENE AND POLYAMINES REGULATE WOUND-INDUCIBLE TOMATO FRUIT ACC SYNTHASE. N. Li, B. L. Parsons, D. Liu and A. K. Mattoo. Plant Mol. Biol. Lab, ARS/BARC, Beltsville, MD 20705.

We have studied regulation of the wound-inducible 1-aminocyclopropane-1-carboxylic acid (ACC) synthase transcript in tomato fruit using a oligonucleotide probe homologous to published cDNA sequences. The probe hybridized to a 1.8 kb transcript that increased upon wounding in early-red and red-ripe fruit. Ethylene was found to positively regulate the level of this transcript using several approaches. First, the transcript level was found to increase as tomato fruit ripened, which paralleled the rise in ethylene production. Second, an inhibitor of ethylene perception, 2,5-norbornadiene, inhibited the ethylene-induced accumulation of the transcript. Autocatalytic ethylene production may thus result from induction of ACC synthase gene. We also found that polyamines, putrescine, spermidine and spermine, that have anti-senescence properties and also inhibit the development of ACC synthase activity, inhibit the wound induction of the ACC synthase transcript. The inhibition by spermine was greater than that caused by putrescine or spermidine. These data indicate that polyamine-mediated inhibition of ethylene biosynthesis involves suppression of ACC synthase transcript level. Further, we obtained a 1.6 kb tomato DNA fragment when oligonucleotide sequences from 3' and 5' ends of published tomato ACC synthase cDNA sequence were used as primers to amplify homologous sequences from total tomato (cv. Pik-Red) RNA using polymerase chain reaction. The 1.6 kb fragment was cloned into a pCR 1000 vector and expressed in INVαF' *Escherichia coli*. The soluble extracts of transformed cells contained considerable ACC synthase activity (46-64 units per h per mg protein). The ACC synthase expression was further substantiated by the detection of 73-79 nmol of ACC per ml of the extracellular medium.

POSTER ABSTRACTS

1641 GENE EXPRESSION AT SITES OF WOUNDING AND PATHOGEN INVASION. John Draper, Simon Warner, Robert Darby, Joy Wilkinson Kulaveerasingham Harikrishna, Simon Firek, Aideen Gill, Karl Deacon and Rod Scott. University of Leicester, Department of Botany, Leicester, LE1 7RH UK

We have evaluated a novel system which allows the facile isolation of proteins and mRNA found specifically at wound site cells and has enabled the construction of cDNA libraries enriched for genes expressed at the wound surface and sites of pathogen invasion in both monocots and dicots. Analysis of total extracted protein and *in vitro* translated polypeptides indicated massive qualitative changes in the protein content and mRNA population in our wound-site cells. At least 4 novel wounding upregulated cytosolic Cu/Zn superoxide dismutase enzymes have been recognised which show peak activity as wound respiration rises. Attempts to obtain cDNA clones coding for these enzymes are underway. Around 20% of the transcripts in our initial cDNA library are differentially expressed between seedlings and wounded/pathogen-infected/elicitor-induced cells. Sequence analysis of a number of clones has revealed that a diversity of novel genes are upregulated at wound sites. One particular cDNA (AWIG) codes for a PR1-like protein with homology to certain pollen allergens and is massively and rapidly induced by wounding, its expression persisting for at least one week. A 1.1 kb upstream region of the AWIG gene was fused to the GUS coding region and transferred to tobacco and potato. Histochemical analysis of transgenic plants has indicated that GUS activity is rapidly induced in the locality of the wound surface and GUS activity is found also in maturing pollen and pigmented regions of the flower. Short distance signal transduction pathways responsible for the activation of plant 'defence' genes at sites of wounding and fungal invasion are not understood. We hope to use our wound-site specific cell system to investigate the wound/pathogen attack signalling mechanism in the future.

1642 ISOLATION, CATALYTIC ACTIVITIES AND MOLECULAR CLONING OF TOBACCO LYSOZYMES. A. Stintzi, D. Bersuder, M. Legrand and B. Fritig. Louis Pasteur University, Institute of Plant Molecular Biology, Strasbourg, France

Upon infection by pathogens plants accumulate a large variety of the so-called Pathogenesis Related (PR) proteins. In tobacco many PRs have been shown to be hydrolytic enzymes with chitinase, lysozyme or ß-1,3 glucanase activity. From TMV-infected tobacco leaves 7 different chitinases/lysozymes have been isolated which fall in 3 categories. The first category includes PR P and Q which have only chitinase activity. The second one comprises 3 basic proteins which display both chitinase and lysozyme activities. The 2 enzymes of the 3rd class have very high lysozyme activity and hydrolyze also chitin. These 2 proteins (Lys1 and Lys2) constitute a new group of serologically related lysozymes/chitinases of tobacco. The amino acid sequences of the intact Lys1 and Lys2 and of peptides isolated following trypsin digestion was determined. Oligonucleotides derived from these sequences were used in a polymerase chain reaction to prime the synthesis of DNA from a cDNA library constructed with RNA isolated from TMV-infected tobacco leaves. The PCR products were then used as probes to isolate lysozyme cDNA clones from the library. No significant homology was found between these clones and the acidic and the basic chitinases of tobacco, thus confirming that Lys1 and Lys2 represent a new class of lysozymes/chitinases of tobacco.

1643 RESPONSE OF DORMANT PEANUT SEED TO WOUNDING. G.Gopala Krishna, P.Sudhakar, Latha Sarathy, Adeel Ahmad and B.Gopal Singh*. Biophysics Laboratory, Physics Department, Nizam College, Hyderabad-500001,. India.*Department of Plant Physiology,APAU Hyderabad,India.

Dormancy is the inability of seed to germinate even under favourable conditions. Virginia runners of peanut (Arachis hypogaea L.) generally, show an afterripening period (dormancy) around 40-60 days after physiological maturity. A single cut or removal of a portion in cotyledons of dormant peanut seed (Var.K-3 and TMV-3), with a fine razor resulted in removal of dormancy and increased the germination percentage (90 per cent) compared to uninjured dormant seed (10 per cent). Similarly, injured seeds when held together with their excised portions in contact with a fine thread in combinations, namely, dormant + dormant, non-dormant + non-dormant, dormant + non-dormant seeds, showed more influence on germination and seedling vigour, compared to injured and uninjured dormant seeds. Removal of dormancy by simple physical injury can be ascribed to a change in the electrical double layer configuration, which in turn ennances metabolic activity and proton transfer processes necessary for activation of enzymes. Enhanced electrical conductivity and water uptake in injured dormant seeds as compared to uninjured seeds could be attributed to the operation of such processes, resulting in the removal of dormancy in peanut seeds.

1644 Functional dissection of the rice basic chitinase RCH10 gene promoter
Qun Zhu and Christopher J. Lamb
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A 1.5 kilobase promoter fragment from the rice (*Oryza sativa L.*) RCH1C gene, which encodes a basic endochitinase, was translationally fused to the ß-glucuronidase (GUS) reporter gene and transferred to tobacco by *Agrobacterium tumefaciens*-mediated leaf disc transformation. Wounding of leaves induced GUS activity from low basal levels, and addition of fungal elicitor to the wounded tissue caused a further marked activation of the gene fusion. During vegetative development high levels of GUS activity were observed in roots and moderate levels in stems. Histochemical analysis indicated that the promoter was active in vascular and epidermal tissue, and the root apical tip. In flowers, high levels of GUS activity were observed in stigmas, ovaries and pollen-containing anthers, but only low levels in sepals and petals. The promoter 5' deleted to -160 exhibited the same pattern of expression in floral organs, and was also strongly induced by wounding and elicitor, but GUS activity was almost lost in vegetative organs. More detailed 5' deletions showed that a floral element was located between -160 and -137, a silencer region was located between -137 and -104, and a stress responsive element was located between -74 and -30. These data indicate that the rice RCH10 promoter is highly compact and that signal pathways for wound and elicitor induction are conserved between monocotyledonous and dicotyledonous plants.

1645 A WOUND-INDUCED MONOCOT PROMOTER DRIVES GUS REPORTER EXPRESSION IN TRANSGENIC TOBACCO
Simon Warner, Rod Scott and John Draper, Botany Department, Leicester University, LE1 7RH, U.K.

A wound-induced cDNA derived from a library constructed from message purified from an *Asparagus officinalis* mechanically separated cell suspension was used to design oligonucleotide primers to facilitate the cloning of the putative promoter by the inverse PCR technique. Sequence analysis and nuclease-S1 transcript mapping has been used to confirm the validity of this approach to clone the promoter. Translational fusions with the GUS reporter gene were constructed. Transformed tobacco plants show wound-inducibile expression of the reporter enzyme in leaf, stem and root tissue. The response is limited to cells adjacent to the wound site. Induction of the reporter gene construct is rapid and persistent with the GUS enzyme being detectable from three hours to several days after wounding. This temporal expression pattern contrasts with that seen for many other wound-induced genes in transgenic plants which are generally induced less rapidly. There are however similarities in respect to the spatial expression observed for some of these wound induced genes. Treatment with 4mM salicylic acid results in super induction of GUS activity. Cell specific expression is also observed in the pigmented areas of the petals and in mature pollen grains. Tobacco plants transformed with a fusion construct containing a deleted version of the promoter loose wound-inducibility of GUS but retain activity in pollen. The predicted protein encoded by the cDNA shares homology with a ubiquitous new class of conserved pathogenesis-related proteins including pollen allergens as described by Walter *et al.* (1990). Mol. Gen. Genet. 222:353-360

1646 BIOCHEMICAL AND GENETIC ANALYSIS OF THE AGGLUTINATION PHENOTYPE IN *PSEUDOMONAS PUTIDA*.
C. R. Buell and A.J.Anderson. Utah State University, Department of Biology and Molecular Biology Program, Logan, Utah 84322-5305. Cells of the beneficial fluorescent pseudomonad, *Pseudomonas putida* isolate Corvallis, are able to agglutinate in the presence of a root surface glycoprotein, termed an agglutinin. The agglutination phenotype has been correlated with increased binding and colonization of root surfaces as well as plant growth promotion and disease suppression. Biochemical analyses of the agglutination phenotype in *P. putida* Corvallis suggests periplasmic and cell envelope proteins rather than lipopolysaccharides are involved in generation of the phenotype. Genetic analyses of the agglutination phenotype in *P. putida* Corvallis has indicated at least two distinct loci, *aggA* and *aggB*, are involved in the agglutination phenotype. The *aggA* locus resides on a 2.7 kbp EcoRI-HindIII fragment in *P. putida* Corvallis. Merodiploid analysis revealed the *aggA* locus complements agglutination and binding to root surfaces to wild type levels in the *P. putida* aggA::Tn5 nonagglutinable mutant 5123. The *aggB* locus, as identified through Tn5 insertion and marker exchange analyses, resides on a 23 kbp EcoRI fragment in *P. putida* Corvallis and contains a repetitive DNA sequence which spans at least 14 kbp. Southern analysis has revealed that : *aggA* and *aggB* loci are restricted in their distribution primarily to agglutinable pseudomonads and the *aggB* locus is further restricted to a unique subset of agglutinable pseudomonads. Regulation of agglutination phenotype in *P. putida* Corvallis is observed *in vitro* under various nutrient conditions. Potential regulation of the *aggA* locus *in vitro* and *in planta*, as detected through reporter fusions, will be ascertained.

POSTER ABSTRACTS

1647 RECOGNITION OF BENEFICIAL ROOT COLONIZING PSEUDOMONADS BY ROOT SURFACE ARABINOGALACTAN PROTEINS C. Heck and A. J Anderson. Department of Biology, Utah State University, Logan Utah 84322-5305.

Plant defense against pathogens may depend in some instances upon the maintenance by the plant of populations of bacteria which display antagonism to the pathogen. We are resolving the events involved in root colonization by bacteria with this suppressive potential. Certain of the beneficial, root colonizing pseudomonads are agglutinated by root surface glycoproteins. Agglutination promotes rapid binding of the bacteria to the root surface and increases the competitiveness of colonization. Agglutination is detected in root washes of intact plants of all species examined. Purification from washes of bean, *Phaseolus vulgaris* of the activity for a *Pseudomonas putida* isolate indicates that several fractions of distinct charge are involved. A neutral high molecular weight agglutinin has hydroxyproline and galactose and arabinose as the major glycosyl residues. These fractions possess ß lectin activity because they react with Yariv's antigen. The agglutinin preparations are recognized by monoclonals generated by Roberts et al. which are specific for arabinogalactan structures.

1648 TOMATO EXTENSIN AND GLYCINE-RICH PROTEIN GENE EXPRESSION. Adnan D. Butt, Sunghan Kim and Allan M. Showalter. Ohio University. Department of Botany. Molecular and Cellular Biology Program. Athens, Ohio 45701, USA

In a recent publication (Plant Molecular Biology 16: 547-565, 1991), Showalter et al. described the isolation and initial characterization of fifteen extensin and extensin-like tomato cDNAs. These cDNAs were determined to fall into five distinct classes; class I and II clones encoded extensins, class III and V clones encoded glycine-rich proteins (GRPs), and class IV clones encoded a portion of a GRP sequence on one DNA strand and encoded a portion of an extensin sequence on the other DNA strand. In this poster, a more detailed analysis of the expression of these cDNA classes was performed using RNA gel blot hybridizations with respect to wounding in various tomato organs, development, kinetics of the wound response, and the localization of mRNA accumulation in wounded tissue. In addition, the influence of ethylene treatment, abscisic acid (ABA) treatment, and drought stress on extensin and GRP mRNA levels was determined in a similar manner. In general, extensin gene expression was readily detected in stems and roots, but not in leaves. With both class I and II extensin cDNA probes, wound-induced accumulation of mRNA in stems was first detected between 4 and 8 hours after wounding with maximal accumulation occurring 12 hours after wounding. Moreover, extensin mRNA accumulated locally at the wound site but not systemically. Expression of the class III GRP was largely limited to wounded stem tissue. Initial detection and maximal accumulation of this GRP mRNA in response to wounding was identical to that of the extensins. Moreover, abscisic acid treatment and drought stress resulted in the marked accumulation of the class III GRP mRNA in tomato stems. In contrast, expression of the class V GRP occurred in stems and roots and was down-regulated by wounding.

1649 WOUND-INDUCED EXPRESSION OF POPLAR PROTEINASE INHIBITOR-LIKE GENES. Jay B. Hollick, Harvey D. Bradshaw Jr and Milton P. Gordon. Department of Biochemistry, SJ-70, University of Washington, Seattle, Washington 98195. A large number of new mRNA species are systemically accumulated in the leaves of poplar trees in response to mechanical wounding. Genomic and cDNA cloning has led to the identification of several large multi-gene families whose members are expressed in a wound-induced manner. One such family, (*win3*), contains members whose putative protein coding regions are hypervariable in comparison to the untranscribed flanking sequences and share a 20% amino acid identity to legume Kunitz-type trypsin inhibitors. Transcriptional run-off experiments demonstrate that the wound induction is regulated primarily at the transcriptional level. We have isolated a promoter region from one of the poplar *win3* genes which confers wound-inducible expression on the reporter gene beta-glucuronidase in transgenic tobacco plants. When one leaf from such a plant is mechanically wounded, the GUS transgene is expressed throughout the plant. Expression of this transgene is also found in pollen and seeds produced from the primary transformants suggesting that the *win3* promoter may also contain elements which respond to developmental cues. These findings demonstrate the feasibility of utilizing an herbaceous species to analyze the DNA elements which regulate gene expression in trees.

1650 A WOUND-INDUCIBLE GLYCINE-RICH PROTEIN FROM DAUCUS CAROTA WITH HOMOLOGY TO SINGLE-STRANDED NUCLEIC ACID-BINDING PROTEINS. Arnd Sturm, Friedrich Miescher-Institute, P.O. Box 2543, CH-4002 Basel, Switzerland.

A cDNA clone was isolated that encodes a glycine-rich protein from carrot consisting of two polypeptide domains. The N-terminal domain has a normal glycine content and contains about an 80 amino acid-long sequence, RNP_{80}, conserved in many eukaryotic RNA-binding proteins. The C-terminal domain has about 70% glycine residues with interspersed aromatic and charged amino acid residues. A data bank search reveals the presence of homologous proteins in maize and sorghum and significant homologies to RNA-binding proteins. In the carrot cell suspension culture WOOIC the gene encoding the glycine-rich protein was constitutively expressed and mRNA accumulated to a high level. In mature carrot roots only a low basal level of the mRNA was present which markedly increased upon wounding with maximal expression after 12 hours. In carrot seedlings sprayed with the plant hormone abscisic acid (ABA) the already high level of mRNA increased 3 to 5-fold with maximal expression after 6 hours. Homology of the glycine-rich protein from carrot with the A1 protein of the heterogeneous ribonucleoprotein (hnRNP) complex lead to the proposal that the polypeptide may play a role in biosynthesis and processing of heterogeneous nuclear RNA and in the maturation of specific mRNAs in response to wounding and ABA.

1651 HEVEIN, A LECTIN LIKE GLYCOPROTEIN FROM HEVEA BRASILIENSIS (RUBBER TREE) IS INVOLVED IN THE COAGULATION OF LATEX. Anil Kush, Tan H.L., Gidrol X.,* Tan C.T. & Nam Hai Chua‡. Institute of Molecular & Cell Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511; *Lab of Toxicology, Harvard School of Public Health, 665 Huntington Avenue, Massachusetts 02115; ‡The Rockefeller University, 1230 York Ave, New York 10021-6399.

Latex in the rubber tree (Hevea brasiliensis) is produced in highly specialized cells known as laticifers. We have isolated various laticifer specific genes (1) and one of them, hevein, was found to be expressed predominately in micro-vacuoles called lutoid. We have previously reported the cloning, expression and regulation of hevein from Hevea. Hevein has been shown to have homology both to wheat germ agglutinin as well as some of the wound inducible genes (2). We have further analysed the possible physiological role of hevein in the rubber tree. Lutoid particles were isolated from latex and hevein was purified using gel filtration and affinity chromatography. Lutoid serum ws found to enhance coagulation of the purified rubber particles and antibodies against the hevein completely inhibited the coagulation. N-acetylglucoasamine, the specific sugar of wheat germ agglutinin was found attached to a 23Kd glycoprotein bound to the surface of the rubber particle. We found direct correlation between the coagulation index of various clones of Hevea brasiliensis and the expression of hevein mRNA. We are analysing the genomic clones of hevein and the results relevant to its regulation in transgenic plants shall be presented.

References: 1. Kush et al (1990) PNAS 87: 1787-1790.
2. Broekaert et al (1990) PNAS 87: 7633-7637.

1652 IDENTIFICATION OF WOUND-INDUCED SUPEROXIDE DISMUTASE IN *ASPARAGUS OFFICINALIS*

Joy Wilkinson, Simon Warner, Gary Whitelam and John Draper. University of Leicester, Botany Department, Leicester, England. Mechanically isolated cells provide an ideal system for studying wound-induced genes since a homogeneous population of synchronously wounded cells is obtained. We have shown that in *Asparagus officinalis* cells many genes become transcriptionally activated after this wounding process. Cell respiration increases to a maximum 3-5 days post-wounding. Protein was isolated from one highly upregulated group of genes. N-terminal sequencing of this protein revealed 86% similarity with superoxide dismutase 2 (SOD2) from maize. In-gel assays of *Asparagus* protein isolated from varying days in cell culture showed the presence of 5, possibly 6, constitutive forms of SOD and one induced form that was actively present from days 4-5. We have recently obtained the maize SOD2 clone which we are using to screen an *Asparagus* library made from cell culture RNA. We hope to isolate the clone for the induced SOD and study its expression further in mechanically isolated cell systems and relate this to the possible oxidative stresses found in wounded tissue.

POSTER ABSTRACTS

1653 Purification, characterisation and cloning of tobacco cinnamyl alcohol dehydrogenase. Wolfgang Schuch[1], Mary Knight[1], Claire Halpin[1], Alain Boudet[2], Jaqueline Grima-Pettenati[2], [1]ICI Seeds, Plant Biotechnology Section, Jealott's Hill Research Station, Bracknell RG12 6EY, UK and [2]Centre de Physiologie Vegetale NRS/UPS University Paul Sabatier, 118 Rte de Narbonne, 31062 Toulouse, France.

Lignin, one of the products of the phenylpropanoid pathway in plants, is one of the most abundant polymers found in the plant kingdom. The biosynthetic pathway leading to synthesis of the precursor alcohols used for polymerisation into lignin is well understood. We have developed a purification protocol for the isolation of one of the enzymes of the lignin-specific branch pathway of the phenylpropanoid pathway, cinnamyl alcohol dehydrogenase (CAD). We have purified CAD from 6 week old tobacco stems. The protein has a Km of 12 uM for coniferyl alcohol and 0.3 uM for conifgery aldehyde. The protein is composed of two electrophoretically distinct subunits. These have been separated and partial peptide sequence has been generated. Degenerate oligonucleotides have been designed and used to probe a tobacco stem cDNA library. This has led to the identification of two classes of CAD cDNA clones. Expression of these clones during tobacco development has been studied.

1654

Endogenous proteinase inhibitors in tobacco are wound-induced: consequences for tests of insect resistance with transgenic plants

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Thus far reports have suggested that wounding does not induce endogenous proteinase inhibitors in tobacco. This has been one of the reasons to use tobacco as the host organism for transferring inhibitor genes to study insect resistance. During our experiments with transgenic proteinase inhibitor plants we found that plants infested with larvae for one week turned out to contain very high levels of endogenous inhibitors of trypsin and chymotrypsin. We investigated how these inhibitors were regulated in response to a simulated insect attack. Inhibitor levels were determined using an Radial Inhibitor Diffusion Assay. We found that local systemic induction of inhibitors in tobacco was a factor 4-15 more pronounced than distant systemic induction. Continuous wounding gave a transient inhibitor response. The very high levels of endogenous, unknown inhibitors in wounded leaves correspond to roughly 3.3% CpTI-units and to 10% PI2-units of the total soluble protein. Transgenic tobacco plants with constitutively expressed inhibitor levels of 1-2% of the total soluble protein have been shown to be effective at reducing insect growth. A well known feature of inhibitor action is the need for a certain threshold level for them to become effective against insects. Thus far this threshold has appeared to be roughly five times lower in transgenic plants than on artificial diets. We suspect that this is an artefact of the fact that endogenously induced inhibitor levels are never measured but contribute to overall inhibitor levels. We observed no significant differences in wound response between cultivars.

1655 PHYSICALLY MAPPING GENES AND RFLP IN CEREALS: PROBLEMS AND PROSPECTS. J.P. Gustafson[1], J.E. Dille[2], G. Butnaru[3], H. M. Daud[4], C. Taylor[5], K. Houchins[1]. [1]USDA-ARS, Department of Agronomy,Univ. Missouri, Columbia 65211, [2]Department of Biology, Winthrop College, Rock Hill, South Carolina 29733, [3]Institutul Agronomic, Timisoara, Romania, [4]MARDI, Kuala Lampur, Malaysia, [5]Waite Institute, Glen Osmond,South Australia.

Non-radioactive labelling *in situ* hybridization techniques utilizing biotin and chemilluminescence were evaluated for their potential to physically map genes and restriction fragment length polymorphisms (RFLP) in the cereals *Triticum*, *Oryza*, *Secale*, and *Hordeum*. It was established that the detection of DNA segments as small as .7kb using biotin-antibody complex is possible from cereal root tips if the cellulose has been removed using a protoplast technique. The present *in situ* hybridization technique can be used to physically map genes and linkage groups as to their location on chromosomes. In addition, it can be used to map small alien inserts stemming from wide crosses. The chemilluminescence is useful in detecting highly and moderately repeated DNA sequences, but at the present time is not useful for detecting smaller unique sequences. The main problems that exist involve the very low levels of detection, from 0 to 10%, which make for slow laborious analysis and the levels are even lower when utilizing two probes simultaneously The inability of chemiluminescence to work well with probes less than 1kb in size is yet another problem to overcome.

1656 TOWARDS A MOLECULAR MARKER MAP OF ONION. Hans Sandbrink, Ria Jongerius and Pim Lindhout. Centre for Plant Breed. and Reprod. Research (CPRO), PO Box 16, 6700AA Wageningen, The Netherlands.

Onion (*Allium cepa*) is worldwide one of the major horticultural crops. The culture suffers from various pests and diseases. Resistance genes are available in related species. The introduction of these genes could be efficiently carried out by indirect selection of linked molecular markers. For this purpose preliminary attempts are undertaken to develop a molecular marker map of onion.

The DNA content of the onion genome is very high (33.5 pg per 2C-nucleus). This restricts the use of RFLPs. Alternative molecular markers, based on PCR, are available. In this study the potentials of RAPDs (Random Amplified Polymorphic DNA) for genetic studies in onion were investigated. The detection of an RAPD is based on the variation in amplified DNA with a random 10-mer as single primer for the synthesis of both strands of DNA.

The species *A. cepa*, *A. roylei*, the F_1 (*A. roylei* x *A. cepa*) and the F_2 progeny (van der Meer & de Vries, 1990, Euphytica 47, 29-31) were investigated on the variation of amplified DNA with a set of ten 10-mers. The experimental conditions were identical as for tomato (Klein-Lankhorst et al., 1991, Theor. Appl. Genet. in press).

With each of the primers a number of DNA fragments were amplified. The amplification of some fragments depended strongly on the experimental conditions, others were less sensitive. Some fragments were species-specific amplified, the amplification of others varied within and between species. Most bands of both parents were detected in the F_1. A minority of the fragments segregated in the F_2 as a single dominant locus. These RAPDs can be used for constructing a genetic map of onion.

1657 RAPD, A NOVEL SOURCE OF MOLECULAR MARKERS FOR THE GENETIC STUDY OF TREE SPECIES. Martin Michaud, L. Savard, G. Laflamme, A. Roy, F.M. Tremblay et J. Bousquet. Université Laval , Departement des Sciences Forestières , Centre de Recherche en Biologie Forestière, Ste-Foy, Québec G1K 7P4, Canada.

The study of genetic diversity at the DNA level in tree species has been slowed down by the restricted availability of molecular markers and the limited number of individuals that could be screened. Techniques using restriction fragment length polymorphism, satellite DNA or M13 DNA fingerprinting require large amounts of material and cannot process large numbers of individuals. Randomly amplified polymorphic DNA (RAPD) (Williams and Welsh NAR, 18:6531-6535, 1991) were recently shown to be powerful for the detection of polymorphism at the intraspecific level and has the advantage of requiring small amounts of material since it relies on polymerase chain reaction (PCR). Random primers from 9 to 11 bases have been successfully used to amplify DNA from different tree species in the genera *Alnus*, *Betula* and *Picea*. DNA was extracted using a CTAB minipreparation procedure from mg of *in vivo* or *in vitro* plant material. The amount of DNA isolated from single extraction was sufficient for numerous amplifications. Using single primers, the DNA patterns obtained, were polymorphic and allowed us to distinguish single individuals within species. The reproductibility of the DNA patterns has also been tested and it seemed that two sequential amplifications with the same primer in excess tended to produce more reproducible patterns with minor forms better amplified. The simplicity with which DNA profiles are obtained with these primers confers to RAPD a great advantage over a wide range of other molecular markers as far as time and amounts of material are concerned. Besides fingerprinting purposes, the information can further be used to establish linkage maps, study natural population structure and assess the genetic stability of *in vitro* produced plants.

1658 RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS FOR SASKATOON BERRY GENOTYPE IDENTIFICATION. Brian J. Weir[1], Ravindra N. Chibbar[2], Richard G. St.Pierre[1], and Kutty K. Kartha[2]. [1]Department of Horticulture, University of Saskatchewan, Saskatoon, CANADA. [2]Plant Biotechnology Institute, National Research Council Canada, Saskatoon, CANADA.

A reproducible technique for genotype identification of a woody species has been standardized in our lab. Genotypes of saskatoon berry (*Amelanchier alnifolia* Nutt.) cannot be easily identified as micropropagated, juvenile, or mature material. However, genomic fingerprinting of saskatoon berry genotypes, based on polymorphisms generated by amplification of random DNA segments using single, arbitrary nucleotide primers (9- or 10-mers), is now possible. These random amplified polymorphic DNA (RAPD) markers[3] are generated by the polymerase chain reaction (PCR), an enzymatic reaction requiring sufficiently pure, high molecular weight DNA. Thus, three methods (CTAB, SDS, and CTAB-SDS) for extraction of DNA from leaves were used to determine which could yield suitable DNA. The concen-tration of $MgCl_2$ and Taq DNA polymerase, primer length, and cycling parameters have been standardized to optimize the reaction. Preliminary screening of DNA from seven saskatoon berry genotypes, using twenty arbitrary primers, indicates that enough polymorphism exists to distinguish each genotype.

[3]Williams et al. 1990. Nuc. Acids Res. 18:6531.

POSTER ABSTRACTS

1659 AN ASSESSMENT OF RFLP IN THE OIL PALM (ELAEIS SPP.). Suan-Choo Cheah, Rahimah A. Rahman, Leslie C.L. Ooi and Maria Madon. Palm Oil Research Institute of Malaysia, Bangi, Selangor, Malaysia.

Genetic variability between Elaeis guineensis and E. oleifera as well as within the guineensis species has been demonstrated using restriction fragment length polymorphic (RFLP) probes derived from complementary DNA (cDNA) clones. Fifteen of these clones were assessed for ability to detect DNA polymorphism. Twelve clones detected variability both between and within species. One clone revealed RFLP within the guineensis species only while two failed to reveal any polymorphism. In comparing the efficacy of nineteen restriction enzymes for uncovering RFLP, it was found that enzymes with six-base pair recognition sites revealed more polymorphism than the four- and five-base cutters. Of the 190 probe/enzyme combinations examined, 31% showed RFLP. For nine of the probes investigated, polymorphism was detected with more than one restriction enzyme. This observation suggests that majority of the RFLPs revealed was due to DNA rearrangements rather than base changes.

1660 CONSTRUCTION AND CHARACTERISATION OF A 3 GENOME EQUIVALENT MAIZE YAC LIBRARY. Keith Edwards, Helen Thompson, David Edwards, Wolfgang Schuch and Antione de Sauzeu., I.C.I. Seeds, Jealott's Hill, Bracknell, Berkshire, RG12 6EY. UK.

The recent developments in Yeast Artificial Chromosome (YAC) systems capable of cloning DNA fragments several hundred kilobases in size, opens up the possibility of isolating and characterising agronomically important sequences via chromosome walking. Using high molecular weight DNA prepared from protoplasts of an elite inbred line, we have constructed a maize YAC library containing >75,000 clones with an average insert size of 150kb. This library has been prepared as an ordered array in microtitre plates and has been further processed to produce DNA pools for rapid screening using the PCR. We will present data on the construction of the library together with preliminary results on the characterisation of individual YAC clones.

1661 DEVELOPMENT OF AN RFLP AND RAPD LINKAGE MAP IN ALFALFA. Craig Echt, Kim Kidwell*, Tom Osborn* and Tom McCoy. Montana State University, Dept. of Plant & Soil Science, Bozeman MT 59717 and *University of Wisconsin, Dept. of Agronomy, Madison WI 53706.

In an effort to pursue detailed genetic analyses in alfalfa, we are constructing a molecular marker linkage map using diploid derivatives of the tetraploid crop. The initial map will be made from 78 backcross progeny of highly heterozygous parents. Innovative mapping strategies will be needed to integrate the diverse linkage data being generated. Included in this map will be RFLP markers from *Sau* 3A and *Xba* I genomic clones, RFLP markers from flower petal and root cDNA clones, and RAPD markers, some of which identify repetitive sequences. Given the nature of the various markers used, this map is expected to have a minimum of bias for unique and repetitive coding and non-coding sequences throughout the genome. Initial examination of our mapping population, and of other *Medicago* cultivars and species, revealed the RFLP and RAPD markers to be highly polymorphic. 78% of the *Sau* 3A clones revealed polymorphic alleles when the mapping parents were screened with one enzyme, *Eco* RI, and 90% of the root cDNA clones revealed polymorphic alleles among 5 backcross progeny when screened with *Eco* RI, *Eco* RV and *Hind* III. For the RAPD markers, we found 76% to segregate in our mapping population as dominant Mendelian traits, giving an average of ca. 1.5 useful markers per single PCR primer. Information from this map will be used in future studies on comparative recombination frequencies between diploid and tetraploid states, on the rates of introgression from interspecies hybrids into cultivated germplasm, and on some of the genetic components of heterosis in alfalfa.

1662 A PROMOTERLESS REPORTER GENE AS A TOOL FOR THE ISOLATION AND CHARACTERIZATION OF TRANSCRIPTIONAL CONTROL ELEMENTS IN PLANTS.

B. Z. Rubinfeld, J. S. Beckmann, M. Soller* and Y. Gafni. Inst. of Field and Garden Crops, A.R.O., The Volcani Center, P.O.B 6, Bet-Dagan, * The Hebrew University, Dept. of Genetics, Jerusalem, Israel.

Two promoterless vectors were constructed. In both constructs the promoterless ß-glucuronidase (GUS) gene with the nopaline synthase polyadenylation site (Nos-ter) was attached directly to the right border of the T-DNA of a binary vector. The promoterless GUS gene was used as a reporter gene for identification of transcriptional elements from the plants' genome. In one vector the selection for transformant plants was done with the neomycin phosphotransferase gene (NPT II) fused to the Nos-promoter and Nos-ter. In the second vector the selection was done with the Hygromycin B phosphotransferase (HPT) gene fused to the CaMV 35S promoter and polyadenylation site. Transgenic tobacco plants were generated after leaf disk transformation. Statistical evaluation of the transgenic plants expressing the promoterless GUS gene will be presented. Based on the results of the study, we will explore the theoretical potential of using promoterless vectors as a means of cloning genes which show no specific phenotype after the T-DNA insertion to their sequence occurred. The case of quantitative trait loci will be discussed.

1663 Comparison of RFLP and RAPD technologies for analyzing genetic diversity in open-pollinated maize varieties.

Miriam Fischer[1], Susana Azpiroz[2], and David Hoisington[1]

[1]Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico and 2Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Mexico.

Restriction Fragment Length Polymorphisms (RFLPs) have been used by several investigators to study the genetic diversity within and between species. RFLPs have also proven to be useful in locating qualitative and quantitative trait loci; and strategies for their use as indirect selection tools in breeding programs proposed. Recently, molecular markers based on Randomly Amplified Polymorphic DNAs (RAPDs) have been proposed as an alternative to RFLPs for fingerprinting germplasm and for developing linkage maps in any species.

In developing countries, open-pollinated varieties of maize, as opposed to hybrids, are widely grown by farmers and used in breeding programs. We undertook a study to investigate whether RFLPs or RAPDs would be most useful in determining the diversity between five open-pollinated varieties of maize produced in two different environments. We will present the results of the diversity study and discuss the two techniques used in terms of feasibility, realiability, costs and appropriateness for use in developing countries. In addition, a non-radioactive method utilizing chemituminescence for RFLP analysis in maize and wheat has been optimized and the protocol will be presented.

1664 GENETIC LINKAGE OF MOLECULAR MARKERS IN *CITRUS*. G.A. Moore, R.E. Durham, P.C. Liou, and F.G. Gmitter, Jr. University of Florida, Fruit Crops Dept., Gainesville, FL 32611

Genetic linkage analysis is being performed using two segregating populations of *Citrus*. One population arose from an intergeneric backcross of *Citrus grandis* (L.) Osb. cv. Thong Dee and *Poncirus trifoliata* (L.) Raf. cv. Pomeroy, using the former as the recurrent female parent. The other population came from an interspecific backcross of *C. reticulata* Blanco cv. Clementine and *C. paradisi* Macf. cv. Duncan, using the former as the recurrent male parent. Thus far, a total of 11 isozyme and 58 restriction fragment length polymorphisms have been identified that segregate in a monogenic fashion in one or both populations. Linkage analysis has revealed that 62 of these loci can be mapped to 11 linkage groups, while 7 loci segregate independently. Gene order is highly conserved between the maps generated from the two divergent populations.

POSTER ABSTRACTS

1665 DEVELOPMENT OF AN RFLP MAP IN *TRIPSACUM DACTYLOIDES*, A WILD RELATIVE OF MAIZE. C. A. Blakey*, C.L. Dewald§, E. H. Coe, Jr.•, and J. Gardiner*. *University of Missouri, Department of Agronomy, •USDA-ARS, Columbia, MO 65211; §USDA-ARS, Woodward, OK. The development of a restriction fragment length polymorphism (RFLP) map for *Tripsacum dactyloides* L. ssp. *dactyloides* (Eastern gamagrass) allows for the examination of homology of *T. dactyloides* and *Zea mays* ssp. *mays* (maize) genomes. Information on the genetic similarities and differences between these two species will aid in the use of *T. dactyloides* as a genetic resource for maize improvement. The *T. dactyloides* genomic probes and RFLP linkage map are being applied to the analysis of *Tripsacum*/maize hybrids, addition lines, and introgression lines. Examination of synteny between *T. dactyloides* and maize loci for genes of known function or sequence has revealed rearrangements for some of these loci. The *Tripsacum* molecular markers also provide an alternative source of genomic probes for the existing maize RFLP map.

1666 DETECTION OF DNA SEQUENCE POLYMORPHISMS AMONG WHEAT VARIETIES. Shichuan He, Sally Mackenzie and Herb Ohm. Purdue University, Department of Agronomy, West Lafayette, IN 47907

Perhaps the greatest barrier to use of RFLP technology for marker-based selection in plant breeding is the lack of DNA polymorphism in most important crop species. This is a particularly difficult problem in the case of cultivated wheat, *Triticum aestivum*, a self-pollinated hexaploid crop. To circumvent this problem we have developed a marker detection strategy that allows the rapid, efficient resolution of high levels of polymorphism within closely related lines of wheat. In our study, the technique of randomly amplified polymorphic DNA (RAPD) is combined with a denaturing gradient gel electrophoresis system (DGGE) to explore polymorphisms among different genotypes of wheat. This process does not require use of ^{32}P or of extraction of genomic DNA from plant tissues. Of 65 combinations of primers used for polymerase chain reaction amplification, over 38% of them produce readily detectable and reproducible DNA polymorphisms between a spring wheat line, S0852, and a winter wheat variety, Clark. The high level of polymorphism is observed among a number of commercial varieties and breeding lines of wheat, suggesting that this procedure will allow a wheat breeding program to use marker-assisted selection, previously impossible in wheat. We have tested this procedure to detect DNA fragment polymorphisms in a developed recombinant inbred population segregating for a number of important phenotypic traits in order to test the feasibility of using this rapid screen to facilitate mapping. The polymorphisms visualized using this approach provide a novel source of molecular markers for construction of a high density genetic map of wheat. Of perhaps greater importance, the ready detection of polymorphisms within breeding lines without the need for ^{32}P radioisotope or DNA extraction should provide wheat breeders with the opportunity to utilize DNA markers in their selection process.

1667 GENETIC LINKAGE MAPS FOR INDIVIDUAL TREES FROM HAPLOID DNA USING RAPD MARKERS. Lomas Tulsieram, Jeffery Glaubitz, and John E. Carlson, University of British Columbia, The Biotechnology Laboratory and Department of Forest Sciences, Vancouver, British Columbia, Canada V6T 1Z3.

Williams et al. (1990. Nucl Acids Res. 18(22): 6531-6535) have established a new approach to genome mapping based upon amplification of random DNA segments with single primers of arbitrary nucleotide sequence. RAPD (Random Amplified Polymorphic DNA) markers have the advantages of universality, simplicity, sequence tagging, speed, and a theoretically unlimited number of markers. We have optimized reaction conditions with template DNA from coniferous trees so that 10 base primers yield reproducibly clean and specific amplification products for use in genetic linkage mapping studies. Constructing genetic linkage maps for conifers is complicated by the large size of conifer genomes (4-5 times the complexity of the maize genome), lack of pedigrees beyond the second generation, and long generation times (15-20 years to sexual maturity). Dominance of RAPD markers adds another possible complication. Such problems can be overcome by working with haploid material. A good source of haploid cells is the strictly maternal megagametophyte tissue of conifer seeds. Use of 1N megagametophyte DNA permits segregation and recombination events to be followed among open-pollinated seeds from an individual tree. We are using the RAPD marker system with megagametophyte DNA prepared from seeds of interior spruce (*Picea glauca* x *P. engelmanii* hybrid complex) to quickly construct tree-specific genetic linkage maps. This approach will also be valid for angiosperm plants for which haploid cell cultures or dihaploid plants can be readily obtained from gametes.

1668 USE OF THE POLYMERASE CHAIN REACTION TO IDENTIFY POLYMORPHISMS IN *LYCOPERSICON*. Christie E. Williams & Dina A. St. Clair, Department of Vegetable Crops, University of California, Davis, CA 95616-8746.

L. esculentum germplasm, especially modern tomato cultivars, shows a low level of diversity when subjected to isozyme and restriction fragment length polymorphism (RFLP) analysis. An increase was observed in the detection of polymorphisms when genomic DNA was subjected to polymerase chain reaction using synthetic primers to amplify random sequences (i.e. RAPD markers). Additional polymorphisms were detected when DNA was cleaved with restriction enzymes prior to amplification. The application of this strategy to various accessions within *L. esculentum* resulted in polymorphisms with up to 50% of the primers that were tested, with many primers generating multiple polymorphisms. The combination of restriction digests with PCR acted to a) uncover sequence polymorphisms within monomorphic PCR bands and b) enhance the amplification of DNA that resulted in low molecular weight bands which often yielded distinguishable polymorphisms.

1669 GENERATION OF THE *BRASSICA OLERACEA* COMPOSITE RFLP MAP Shahryar F. Kianian and Carlos F. Quiros. Univ. of California, Dept. of Vegetable Crops, Davis, CA. 95616

A composite linkage map of *Brassica oleracea* was developed from maps of four different populations, derived from 108 DNA, isozyme and morphological loci covering over 747 centimorgans in 11 linkage groups. Of these linkage groups, eight were assigned to their respective chromosomes. Distortions in segregation ratios increased with the level of divergence of the parents and were attributed to differentiation of parental chromosomes. Individual maps demonstrate that the *B.oleracea* genome undergoes frequent chromosomal rearrangement, even at the subspecies level. Small inversions were the most frequent form of aberration followed by translocations. The former type of aberration could occur without a noticeable effect on meiotic behavior of chromosomes or on pollen fertility. The obvious deduction from the composite map is that a large fraction of the *B.oleracea* genome is duplicated, falling into three classes: randomly-dispersed, linked-gene families, and blocks copied onto non-homologous chromosomes. The genealogy of chromosomes sharing duplicated segments was formulated and indicates that *B.oleracea* is a secondary polyploid species derived from ancestral genome(s) of fewer chromosomes.

1670 SYNTENIC RFLP MARKERS AND HOMOEOLOGOUS CHROMOSOME SETS IDENTIFIED USING OAT ANEUPLOIDS. Shahryar F. Kianian, William L. Rooney, Bai C. Wu, Ronald L. Phillips and Howard W. Rines*. Univ. of Minnesota and USDA-ARS*, Dept. of Agronomy and Plant Genetics, St.Paul, Minnesota 55108.

DNA probes producing three to several RFLP bands were used for a detailed analysis of oat (*Avena sativa*, 2n=6x=42) aneuploid stocks. Absence of an RFLP band(s) in nullisomics and ditelosomics or reduction in intensity relative to other bands in monosomics locates the respective sequence to the deficient chromosome. Ditelosomic lines are used to locate the missing sequence to chromosome arm. Syntenic groups, defined as a number of markers mapping to the same chromosome, are useful in identifying homoeologous chromosome sets as well as potential linkage groups. Based on syntenic relationships, several sets of homoeologous chromosomes have been defined. Linkage groups identified by this method are being used in the study of genetic recombination in inter- and intraspecific crosses of oat. Reduced recombination in the interspecific hybrids may prove useful in demonstrating linkage among and between otherwise loosely linked RFLP markers and QTLs.

POSTER ABSTRACTS

1671 REPETITIVE DNA SEQUENCES IN THE NUCLEAR GENOME OF GRAPEVINE. Mark R. Thomas, Shogo Matsumoto and N. Steele Scott. CSIRO Division of Horticulture, GPO Box 350, Adelaide, SA 5001 Australia

The development of repetitive nuclear DNA sequences as markers for grapevine (Vitis) identification offers an objective and rapid technique as an alternative to ampelography. These DNA markers would also prove useful in studies of genome mapping and organisation. The nuclear genome of grapevine is small with a 2C value of 0.7 pg, but is composed of a variety of repeated sequences. The microsatellite sequences or simple sequence repeats are present with copy number dependent on the particular repeated motif. Minisatellite sequences similar to the tandem repeat in M13 phage are also present. Less dispersed repeated sequences like the ribosomal RNA genes have also been characterised. Ribosomal DNA repeat units of a single size class of 9.14 kb have been cloned from V. champini, and four sizes ranging from 11.05 to 12.15 kb were obtained from V. vinifera cv. Cabernet Sauvignon. The differing size classes between and within Vitis species are due to different lengths of the large intergenic spacer region. DNA polymorphisms of repeated sequences have been found in species and cultivars using either length polymorphisms of restriction fragments or PCR products.

1672 COMPARISON OF RAPD AND RFLP MARKERS USING RANDOM cDNA AND GENOMIC PROBES IN A GRASS SPECIES. Rex L. Smith, S. C. Schank, J. C. Seib, M. E. Schweder and G. F. Lorens. Department of Agronomy, University of Florida, Gainesville, FL 32611.

RFLP genetic markers using both random cDNA and genomic probes, and random amplified polymorphic DNA (RAPD) have been developed in the biomass grass species, Pennisetum purpureum (L.) Lam. (elephantgrass). Elephantgrass is a very high yielding biomass species often producing in excess of 40 Mg ha^{-1} of biomass in the Southeastern U. S. region and has a high potential as a forage species. Consequently, elephantgrass has been targeted for genetic improvement and an RFLP marker program has been initiated to assist that program. The species had no genetic data base, no genetic stocks or inbred lines and is an allotetraploid. We shortened the time required to establish predictable segregating progenies for linkage and mapping studies to one generation by using P. purpureum X P. glaucum interspecific hybrid progenies that give expected segregation ratios of 1:1 for monogenic inheritance. We have used the genetic markers to 'fingerprint' genotypes, study genetic relationships among 25 elephantgrass introductions and related species. The markers have been instrumental in developing efficient hybridization methods and the use of stored frozen pollen in the hybridization program. A main objective of the marker program is to map quantitative trait loci. To date 64 markers have been found associated (linked) with 26 plant traits. Data will be presented comparing development and use of RFLPs generated using random genomic probes from a PstI-generated P.I. 300086 genomic library, random cDNA probes from P.I. 300086 leaf cDNA library cloned in Lambda Zap, and RAPD markers.

This research was partially funded by the Gas Research Institute, Chicago, IL.

1673 GENETIC ANALYSIS OF LATE FLOWERING/VERNALIZATION RESPONSE IN NATURAL ISOLATES OF *ARABIDOPSIS THALIANA*. Jonathan H. Clarke and Caroline Dean. The Cambridge Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UJ.

Different ecotypes of *Arabidopsis thaliana* vary widely in their flowering time and responsiveness to a vernalization treatment. Previous analyses by Napp-Zinn has shown that at least 4 or 5 loci were responsible for the late flowering and vernalization sensitive phenotype of the winter annual, ecotype, Stockholm (St). One locus from St, when introgressed into a vernalization insensitive, early summer annual ecotype, Limburg 5 (Li-5), conferred late-flowering and vernalization sensitivity. This locus is named *FRI* and the St allele is dominant. The Li-5 line carrying the St *FRI* locus is termed H51. We are addressing whether the *FRI* locus is allelic to any of the mutagen-induced late-flowering loci in *Arabidopsis*. We are mapping the *FRI* locus in F_2 families from a cross between H51 and a vernalization insensitive, early summer annual ecotype, Landsberg *erecta* (*Ler*) relative to a number of RFLP markers. We then intend to determine close linkage with induced late-flowering loci mapping in the region of *FRI*. Segregation data from 50 F_2 families gave a ratio of 20 early flowering : 27 segregating : 3 late flowering families, suggesting the presence of two loci with a number of possible epistatic interactions. Both loci will be mapped with respect to the RFLP markers.

The *FRI* locus represents only a fraction of those responsible for the late flowering phenotype of St. To dissect the multiple genetic factors underlying flowering in this ecotype a quantitative trait loci analysis is currently being carried out utilizing the wide difference in flowering time between the ecotypes *Ler* and St.

1674 USING RANDOM-SEQUENCE DEOXYRIBONUCLEOTIDES AND THE POLYMERASE CHAIN REACTION FOR CONSTRUCTION OF A GENETIC MAP OF PAPAYA. John J. Stiles*[1], Suresh Sondur[2], Maimunah Morshidi[1,2], and Richard Manshardt[2]. Departments of Plant Molecular Physiology[1] and Horticulture[2], University of Hawaii, Honolulu, HI 96822.

DNA-based genetic maps are playing an increasingly important role in plant breeding programs. These mapping techniques allow analysis of genomes at a level of precision not previously possible. We are using a polymerase chain reaction (PCR)-based technique designated *R*andomly *A*mplified *P*olymorphic *D*NA (RAPD) to produce a DNA-based genetic map of papaya. RAPD uses single random-sequence synthetic deoxynbo-nucleotides 10 bases in length and the PCR to produce products primed from randomly chosen segments of the genome. A series of primers of different sequence will amplify different regions of the genome and can be used to construct a genetic map in a manner analogous to restriction fragment polymorphisms. The advantages of this system are that PCR requires much less DNA than RFLP analysis and is much faster. The limitation is that most markers are dominant. RAPD technology is being applied to a F2 population from a cross of the Hawaiian commercial cultivar Sunrise and C356, a Central American variety. C356 was chosen because it is believed to be distantly related to Sunrise, is much more tolerant of papaya ringspot virus and flowers at a lower node (ie earlier) than Sunrise, whereas Sunrise has higher TSS, better *Phytophthora* tolerance and red flesh color. Ninety-six 10-mers of random sequence, except that all are between 40% and 60% GC content, have been synthesized and are being screened for polymorphisms. Generally about 10 fragments are amplified from each primer, one of which, on average, shows polymorphism although different primers range from 0 to several polymorphic fragments. All of these fragments appear to be dominant with the recessive allele being null. Analysis of several primers indicate that the sequences detected segregate in a normal Mendelian fashion in the F2 population. A map is being constructed utilizing the MAPMAKER computer program. RAPD is also being used to characterize the relationship between commercial and wild lines to assess the genetic variability within cultivated papayas and across the species.

1675 RFLP MAPPING OF GENES FOR RESISTANCE TO PUCCINIA CORONATA IN AVENA. P.John Rayapati[1], Michael Lee[1] and Roger Wise[2]. [1]Iowa State University, [2]Department of Agronomy, [2]USDA-ARS, Ames

The first RFLP linkage map for diploid oats representing the A genome was constructed. An accession of Avena strigosa (CI 2630) that is resistant to 35 races of crown rust (Puccinia coronata) was crossed with an accession of Avena nudibrevis (CI 9009) that is susceptible to the same races. Genetic linkages between RFLP loci and loci for resistance to crown rust will be identified. Molecular markers from the diploid map will be used to identify resistance loci introgressed from wild hexaploid oat (A. sterilis) into the cultivated hexaploid oat (A. sativa). A hexaploid line containing an additional chromosome pair from the diploid (A. strigosa) will be used to begin integration of the genetic and physical maps of oat.

1676 RANDOM CDNA SCREENING AS AN APPROACH FOR IDENTIFYING MAIZE GENES Barry Feigelman, Carlyn Keith, Xiu Lowe, Mary Nelson, Henry Thai and Chris Baysdorfer. Calif State Univ, Dept Bio Sci, Hayward, CA

We are interested in evaluating techniques for the rapid identification of plant genes. In the present study we isolated at random 100+ cDNAs from a library constructed from maize leaves. Plasmids containing the inserts were excised from the vector (Unizap, Stratagene) and amplified. The inserts were then partially or totally sequenced. Database comparisions allowed us to identify 3 separate CAB clones and a NADP-malic enzyme clone. We have tentitively identified a number of other clones based on their sequence similarity to the following genes: bovine proteolipid protein of H^+-ATPase, yeast anthranilate synthase component 2 (indole-3-glycerol phosphate synthase/ TRP3); and barley thiol protease. In addition we isolated a clone that is very similar to, but not identical with, the ORF 1 of the maize cin4 transposible element. We are currently confirming the identity of each of these clones. To provide further information on clones not identifiable by sequence data alone, we used PCR to prepare digoxigenin labeled probes for each clone. We are currently analyzing gene copy number, level of expression (northern analysis), and tissue specificity (tissue printing and in situ hybridization) for each clone. We believe these techniques will prove useful in a program for the rapid identification of plant genes.

POSTER ABSTRACTS

1677 Identification of an Isozyme Marker, Est I-2, Linked Tightly with Semidwarf(sd-1) Character in Rice

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For the selections of in vitro markers based on finding tight linkages with genes governing agronomically important traits, several crosses were made, and combined segregations for morphological characters and isozymes were analysed.

In the F2 populations fo combinations between semidwarf testers and some tall cultivars, such as Kasalath, DA 16, DV 85, ARC 10239, and Chaing Roneal, and between Shiokari and its sd-1 isogenic line, culm length was well segregated into 3 tall and 1 short(sd-1) ratio. The result indicated that culm lenght was controlled by a single recessive gene.

A tight linkage relationship was found between semidwarf(sd-1) gene and Est I-2 locus. Recombination values between sd-1 and Est I-2 were calculated to be less than 1% in the F2 and F3 generations. Three allozymes, two active bands and a nulic one, have been found in Est I-2locus. The tight linkage was also observed even in some combinations which culm lenght segregation was transgressive and showed normal distribution curves. The result indicates that Est I-2 locus is located in next to sd-1 gene on Chromosome 1, since semidwarf (sd-1) gene was known to be located in Cnromosome 1.

1678 RIBOSOMAL DNA VARIATION IN OIL PALM

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Plant rDNA consists of a tandemly repeated unit, composed of a gene for 25S, 5.8S and 18S RNA, a transcribed spacer region and a nontranscribed region. In plant species, the number of ribosomal RNA gene repeats is normally in the order of 10^4 per genome. The rDNA repeat unit may be heterogenous in length due to the nontranscribed spacer regions or the copy number can vary from a few hundred to several thousand. This paper reports the investigation of rDNA in oil palm populations. Oil palm consists of two main species Elais oleifera and E. guineensis. The E. guineensis can be divided into three fruit forms, tenera, dura and pisifera. The species E. guineensis, tenera is grown or a large commercial scale. Results show the presence of variability in the length of the intergenic region in oil palm rDNA.

1679 SELECTION OF A CORE BREEDING POPULATION FOR QUINOA USING RAPD MARKERS. W. R. Andersen, D. J. Fairbanks, L. R. Robison and P.J. Maughn. Brigham Young University, 197 WIDB, Provo, Utah 84602

A primary goal of our breeding program in quinoa (*Chenopodium quinoa* Wild) is to develop a core breeding population by representative specimens: (1) of accessions from representative origins; (2) by screening within and among accessions using characterization data such as DNA polymorphism, morphological and biochemical markers and taxonomic data; (3) selected on the basis of agronomic traits. An efficient method for accurately determining DNA genotypes is needed to establish genotypic diversity of the core selections and provide a logical basis for elimination of genetic duplications from the core. In this study, our purpose was to test the reliability of the RAPDs (random amplified polymorphic DNAs) (Williams, Tingey and Rafalski, DuPont) technique for identification of genetic groups within a potential breeding pool using twenty RAPD primers which reveal over 30 polymorphic loci in quinoa. One hundred forty four accessions of quinoa were screened for genetic diversity using RAPDs DNA analysis. DNA pollymorphism was compared with other biochemical data where possible for similarities in clustering of genotypes. Clustering of genotypic similarities for RAPDs and other markers was measured among the quinoa accessions using parsimony analysis (PAUP).

1680 A DNA TRANSFORMATION-COMPETENT *ARABIDOPSIS* GENOMIC LIBRARY IN *AGROBACTERIUM*. Gerard R. Lazo, Pascal A. Stein, and Robert A. Ludwig Biology Dept., Sinsheimer Laboratories, Univ. of California, Santa Cruz, CA. 95064

We have constructed a nuclear genomic library from the cruciferous plant *Arabidopsis thaliana* ecotype Columbia in a cosmid vector, pLZO3, and a host organism, *Agrobacterium tumefaciens* AGL1, which can directly DNA-transform the parent organism, *Arabidopsis*. The broad host range cosmid pLZO3 carries a gentamicin acetyltransferase gene as bacterial selective marker and tandem, chimeric neomycin and streptomycin phosphotransferase genes as plant selective markers *Agrobacterium* AGL1 carries the hypervirulent, attenuated tumor-inducing plasmid pTiBo542 from which T-region DNA sequences have been precisely deleted, allowing it to optimally DNA-transform many dicotyledonous plants. *Agrobacterium* AGL1 also carries an insertion mutation in its *recA* general recombination gene, which stabilizes the recombinant plasmids. The *Arabidopsis* genomic library consists of some 21,600 clones gridded onto 96-well microtiter dishes and, if random, carries at least three genomic equivalents. When probed for the presence of several *Arabidopsis* low copy-number genes, the genomic library seems representative. As with the unicellular organisms *Escherichia coli* and *Saccharomyces cerevisiae*, this DNA transformation competent genomic library ought to expedite gene isolation, by gene rescue, in multicellular organisms like *Arabidopsis*.

1681 COMPARISON OF DIFFERENT RESTRICTION ENZYMES AND PROBE SOURCES IN DETECTING RESTRICTION FRAGMENT LENGTH POLYMORPHISM IN CASSAVA

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The construction of a detailed genetic map of cassava (Manihot esculenta Crantz), a tetraploid species, depends on the ability of cloned sequences to detect polymorphisms. As a first step in developing this, 96 cloned nuclear sequences from five different genomic libraries (Bam HI, Hind III, Eco RI, Pst I and Xba I) were hybridized to total DNA from three different lines of cassava. Ten different restriction enzymes were employed in this study (Bam HI, Eco RI, Eco RV, Hind III, Dra I, Pst I, Xba I, Hpa II, Hae III, Taq I). Polymorphism was detected less frequently with Bam HI probes than with Pst I, Eco RI, Xba I and Hind III probes. No relationship was found between clone size (ranging from 0.3 to 3.5 Kb) and polymorphism. DNA digested with Hpa II, Dra I and Taq I displayed significant less polymorphism while DNA digested with Eco RI, Eco RV, Hind III and Bam HI displayed more polymorphism as found in lettuce and rice (1,2). Four cutter restriction endonucleases containing a CpG dimer in their recognition sequences displayed less frequency of polymorphism when compared to the other enzymes. No polymorphism was found with Dra I indicating that regions rich in adenine and thymine may not be spots from mutation in cassava. Four lines involving three controlled interspecific crosses are being screenid for polymorphism in order to generate a molecular map of cassava.

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1682 RFLP ANALYSIS OF QUANTITATIVE POWDERY MILDEW RESISTANCE IN BARLEY. Manfred Heun. Department of Plant Breeding & Biometry, Cornell University, Ithaca, NY 14853-1902

Based on the genetic analysis of a diallel cross with eight spring barley (*Hordeum vulgare* L.) cultivars differing in their response to infection with *Erysiphe graminis* f.sp. *hordei* Marchal (Heun 1987), 'Proctor' and 'Nudinka' were selected for further analysis based on their good quantitative resistance. The breeding history of these cultivars is different -- 'Proctor' is an English malting barley registered in 1953, whereas 'Nudinka' is a naked barley bred in Germany for human/feed consumption registered in 1975 -- leading to the assumption that the involved resistance genes might be different, too.

From the F_1 of these cultivars a doubled haploid (DH) population was produced by anther culture (Heun and Jager-Gussen, submitted). Recently, this population has been used to construct a low density restriction fragment length polymorphism (RFLP) map (Heun et al. 1991). To map the quantitative resistance genes, the RFLP mapping data are now being combined with the resistance data of these DH lines. The results will be discussed in respect to plant breeding toward higher levels of quantitative resistance.

POSTER ABSTRACTS

1683 THE USE OF MOLECULAR MARKERS FOR DETERMINING INTROGRESSION PATTERNS AND EVOLUTIONARY RELATIONSHIPS BETWEEN TWO PICEA SPECIES. Anita Klein, Mike Bobola and Dirk Smith. Biochemistry Department and program in genetics, University of New Hampshire, Durham, NH 03824.

Picea rubens (red spruce) and P. mariana (black spruce) are two closely related spruce species sympatric in New England. They are morphologically indistinct and are capable of forming hybrids. Although red and black spruce are considered unique species, there is considerable debate concerning the degree of hybridization and introgression between the two species. We have examined the nuclear ribosomal RNA genes from P. rubens and P. mariana through restriction mapping and sequencing. The repetitive nature of the rDNA genes are useful when investigating hybridization and potential introgression between species. Five polymorphic restriction sites were identified in the rDNA repeat unit. No species specific polymorphisms were discovered and the sites were found to be polymorphic among repeat units within an individual genome. The frequencies of the polymorphic restriction sites did vary within individuals in a species-specific pattern. Polymorphisms were found in mitochondrial and chloroplast genes that were closely associated with species. Using the plastid markers with controlled-cross hybrids, the inheritance patterns of the organelles were deduced for these species. The frequencies of polymorphic restriction sites for the nuclear rDNA repeat and plastid markers have been used to derive a multivariate model capable of correctly identifying red spruce, black spruce, red X black hybrids and black X red hybrids. Natural stands have been examined using the molecular markers to determine the frequency of hybridization and the direction of hybridization. Sequence information from the first internal transcribed spacer (ITS 1) of the nuclear ribosomal repeat has been used to delineate phylogenetic relationships between these and other species of Picea. Cohesive evolution seems to operating to maintain strong homology within species. Analysis of interspecific sequence comparisons suggests a close affiliation between red and black spruce and European Picea species. Knowledge of these evolutionary relationships along with the frequency and direction of introgression, assists in understanding the genetic hobnobbing between these species. Supported by NH AES project MS 27.

1684 CHARACTERIZATION OF RADIATION HYPERSENSITIVE MUTANTS IN *ARABIDOPSIS*. Corinne S. Davies and Nora Wong. McGill University, Department of Biology, Montreal, Québec, Canada H3A 1B1

Intensive efforts are currently underway to produce detailed recombination and physical maps for the genomes of major crop plants. Current emphasis is on the development of methodology to identify the map location of the numerous loci which govern quantitative traits (QTL's). An additional benefit of these efforts will be the resolving power they will provide for study of the recombination system. A theoretically unlimited supply of RFLP or RAPD markers can be used to study in detail the distribution of crossover events. Alignment of physical and recombination maps will identify hot spots for recombination, thus allowing detailed and unprecedented study of the genetic, developmental, and environmental effects on this process. We have initiated a genetic study of homologous recombination in plants by isolating mutants that are hypersensitive to ionizing radiation. By analogy with yeast and bacteria (phenotypes of the *Saccharomyces RAD52* epistasis group and *E.coli, recA* genes), some of these mutants could be defective in recombination and/or mutagenesis. We have also developed a visual assay for meiotic recombination and somatic mutations. The assay is being used to characterize the hypersensitive mutants and to identify additional mutants, environmental shocks, and developmental features that can alter meiotic and mitotic recombination rates. Altered recombination systems will be characterized in detail using molecular markers

1685 MICRODISSECTION OF CHROMOSOMES OF *BRASSICA NAPUS* AT 4000 x MAGNIFICATION WITH A UV LASER MICROBEAM AND STABLE TRANSFORMATION OF HIGHER PLANTS.

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Manipulating the genome of higher plants requires identification and isolation of genes localized in the nucleus on individual chromosomes. Cloned DNA has to be introduced into cells for analysis of its function. Unless a gene product is well characterized and/or synthesized in large quantity there are considerable problems in cloning a gene although its genetics as well as its location on a specific chromosome segment might be well known. Chromosome segment-specific DNA libraries avoid to screen the bulk of irrelevant DNA encountered when using genomic libraries. Therefore, chromosomes of synchronized cells of *Brassica napus* (oilseed rape) were identified at 4000 x magnification employing an computer assisted image-analysis system. With pulses of a UV laser microbeam they were dissected with submicrometer accuracy for preparing chromosome segment-specific DNA libraries. A chromosome (3μm length) could be separated into 4-5 segments, the width of the cuts being 300 nm or less. Analyzing the function of cloned DNA or even of a whole library requires introducing DNA into cells. Large quantities of DNA (10^5 molecules or more) were introduced into selected individual cells with a laser microbeam. Transgenic plants were regenerated from single cells of *Nicotiana tabacum* with a frequency of 4×10^{-3}. The application of laser microbeams for manipulating the plant genome will be discussed.

1686 HIGHLY REPEATED TELOMERIC ARRAYS ARE HYPERVARIABLE IN TOMATO. Pierre E.Broun,Martin W.Ganal,Steven D.Tanksley.Cornell University, Department of Plant Breeding and Biometry, Ithaca, New York 14853

Self-pollinated plant species often contain less genetic variation than their outcrossing counterparts, making it more difficult to distinguish individuals and races even at the DNA level. As a result, while it has been relatively easy to establish unique DNA fingerprints for outcrossing crop species such as maize and potato, it has been difficult to do so for self-pollinated crops such as wheat, soybean and tomato. Recently we have identified and physically mapped two tandemly repeated sequences in tomato (*Lycopersicon esculentum* L.) that comprise the outermost portion of the chromosomes. We report that these sequences are hypervariable when separated on pulsed field gels. They detect, on average, at least 16 times more genetic variation than M13 or single copy sequences and up to 7 times more genetic variation than the hypervariable human minisatellite probes 33.6 and 33.15. These telomeric sequences may therefore be useful for fingerprinting closely related plant varieties. Moreover, most polymorphisms are inherited in a mendelian fashion and might also serve as genetic markers for telomeres in mapping experiments

1687 THE Phaseolus vulgaris LINKAGE MAP. Ney S. Sakiyama, Christine D. Chase and C. Eduardo Vallejos. University of Florida, Department of Vegetable Crops, Gainesville, FL 32611. We have constructed a genetic linkage map of the common bean nuclear genome. "Mapmaker" was used for the linkage analysis of a backcross progeny between 'XR-235-1,' a breeding line with some P. coccineus introgression, and the Andean cultivar 'Calima.' The segregating loci included 9 isozymes, 11 seed proteins, and 230 RFLP markers. These marker loci have been assigned to 11 linkage groups (P. vulgaris 2n=22). The source of probes was a PstI genomic library enriched for single copy sequences. Only 2.5% of the probes tested had the hybridization pattern of repetitive DNA, and 66% of the probes detected polymorphisms between the parental lines with at least 1 of 4 restriction enzymes (DraI, EcoRI, EcoRV, and HindIII). A survey conducted with 18 random probes detected 33% polymorphism between a sample of mesoamerican and andean cultivars.

1688 MOLECULAR ANALYSIS OF BACTERIAL BLIGHT RESISTANCE IN RICE. Pamela C. Ronald, Kun-sheng Wu, Steven Tanksley. Dept. of Plant Breeding, Cornell University, Ithaca New York, 14853.

Bacterial blight disease of rice caused by *Xanthomonas campestris* pv. *oryzae (Xco)* is one of the most destructive diseases of rice in Asia. Genetic resistance has been the most effective and economical control for bacterial blight. Seventeen genes conferring resistance to specific races of *Xco* have been identified by classical genetic analysis. Only one gene, *Xa21*, confers resistance to all races tested. We have begun to characterize this locus by genetically and physically mapping the region with the goal of cloning the gene by chromosome walking techniques. Two methods were used to obtain markers that are linked to *Xa21*. In the first method, 100 RFLP markers were selected from the RFLP map of rice and used as hybridization probes to Southern blots containing DNA extracted from nearly isogenic lines. One marker (RG103) was discovered that showed polymorphism between the isogenic lines. This marker was tighly linked to *Xa21* in an F2 population segregating for *Xa21*. In the second method, 2 RAPD (Randomly Amplified Polymorphic DNA) markers were isolated that also showed close liinkage to *Xa21* in the F2 population. From these results we are able to assign *Xa21* to chromosome 11. Physical mapping of this region using pulsed field gel electrophoresis showed that two of the markers are within 125 kbp from one another. Finally we will present results on the construction of a yeast artificial chromosome library prepared from DNA from a rice cultivar containing the gene *Xa21*.

POSTER ABSTRACTS

1689 TOMATO Cu, Zn SUPEROXIDE DISMUTASES ARE DEVELOPMENTALLY REGULATED AND RESPOND TO LIGHT AND STRESS. Rafael Perl-Treves and Esra Galun. The Weizmann Institute of Science, Rehovot, Israel

The expression of the two Cu, Zn superoxide dismutase (SOD) genes of tomato was followed at the transcript and enzymatic activity levels. The cDNA clones used as probes code for the chloroplast Cu, Zn SOD (clone T1) and the cytosolic Cu, Zn SOD (clone P31). The two genes display distinct expression patterns. The T1 transcript was rare or absent from roots, stems and ripening fruits, whereas the P31 transcript was very abundant. Shoot tips, flower buds, seedlings and young leaves contained high levels of the two mRNAs. During leaf expansion, the levels of both transcripts diminish markedly. Despite this, SOD activity levels of the corresponding isozymes accumulated and were sustained throughout leaf expansion. In non-photosynthetic organs, the SOD-3 (cytosolic) isozyme contained most of the activity, while in the expanded leaf the SOD-1 (chloroplast) isozyme was more abundant. Light regulated accumulation of both the P31 transcript and the T1 transcript was observed upon light-exposure of etiolated seedlings. However, only SOD-1 activity was observed to increase, after a lag of a few hours. The levels of both transcripts increased in response to paraquat and mechanical wounding. The level of the cytosolic transcript and the respective isozyme activity increased dramatically during prolonged drought stress while the chloroplast transcript remained unaffected. The expression of both genes was enhanced by spraying tomato plants with ethephon.

1690 IN SITU HYBRIDIZATION OF LILIUM WHOLE MOUNT SYNAPTONEMAL COMPLEX CHROMOSOMAL PREPARATIONS. Clare A. Hasenkampf. Life Sciences, U.T.-Scarborough, 1265 Military Trail, Scarborough, Ontario, Canada M1C 1A4.

Whole mount meiotic preparations of the synaptonemal complex complement of Lilium have been used for in situ hybridization experiments. A digoxigenin-labeled probe of the maize ribosomal DNA gene cluster has been successfully hybridized to the lily preparations. Three strong signals, corresponding to the three known lily nucleolus organizer regions, have been seen in most of the chromosome preparations. In situ hybridization experiments using meiotic preparations should be useful for identifying specific chromosomes, and for investigating the role of particular DNA molecules important to meiotic chromosome function. Because of the decondensed state of the meiotic prophase chromatin, in situ hybridization of whole mount synaptonemal complex preparations offers the potential for improved sensitivity and resolution. The technique may thus prove useful in gene mapping and chromosome identification.

1691 A GENETIC MAP FOR BRASSICA NAPUS BASED ON RFLP MARKERS. BENOIT S LANDRY[1,2], NATHALIE HUBERT[1], TAKEOMI ETOH[1,3], JOHN HARADA[4] AND STEVE LINCOLN[5] [1] Agriculture Canada Res Sta, 430 Blvd Gouin, St-Jean, Québec, CANADA, J3B 3E6 [2] Universite de Montreal, 4101 Sherbrooke, Montreal, Quebec, CANADA, H1X 2B2. [3] Vegetable Crops Lab, Kagoshima University, 21-24 Korimoto 1, Kagoshima 890, JAPAN [4] Dept Botany, University of California, Davis, CA95616, USA. [5] MIT, The Whitehead Institute for Biomed Res, 9 Cambridge Center, Cambridge, MA02139, USA.

DNA Restriction Fragment Length Polymorphisms (RFLPs) were used to construct a detailed genetic map of Brassica napus. Random cDNA and seedling-specific cDNA clones were used as markers. The 134 loci so far mapped in B. napus covered 1413 cM. No clustering of markers in specific areas of the genome (as we saw with the same markers in B. oleracea) was observed in this B. napus cross. This possibly reflects the higher homology between the genome of the two parental lines we used to map the B. napus genome. This map will be useful in several breeding applications such as tagging important agronomic traits and fingerprinting breeding lines and cultivars of B. napus, a major oilseed crop. In addition, this study is providing informations on genome organization and complement our RFLP mapping effort in B. oleracea. We will describe the current status of our ongoing strategy to saturate the B. napus genome with DNA markers. We are now using this map to tag several agronomic traits.

1692 CONSTRUCTION OF A DETAILED RFLP-BASED GENETIC MAP AND TAGGING OF RESISTANCE GENES TO RACE 2 OF CLUBROOT DISEASE IN BRASSICA OLERACEA

BENOIT S. LANDRY[1,2], NATHALIE HUBERT[1], TAKEOMI ETOH*[1,3], RENÉ CRETE*[1], MORGAN CHIANG*[1], JOHN HARADA*[4] AND STEVE LINCOLN*[5] [1] Agriculture Canada Research Station, 430 Blvd. Gouin, St-Jean, Quebec, CANADA, J3B 3E6 [2] Universite de Montreal, 4101 Sherbrooke, Montréal, Québec, CANADA, H1X 2B2 [3] Vegetable Crops Lab., Kagoshima University, 21-24 Korimoto 1, Kagoshima 890, JAPAN. [4] Dept. Botany, University of California, Davis, CA95616, USA. [5] MIT, The Whitehead Institute for Biomed. Res. 9 Cambridge Center, Cambridge, MA02139, USA.

In this study, we have constructed a RFLP-based genetic map of Brassica oleracea and mapped specific agronomic traits. Random cDNA and seedling-specific cDNA clones were used as DNA markers. The current map consists of 202 genetic markers and covers 1137 cM. The chromosomal location of two disease resistance genes to Race 2 of clubroot has been determined. One physiological and one morphological traits have also been analyzed and mapped. This map is also providing insight on the chromosomal organization within this species, it is being utilized in combination with the B. oleracea monosomic alien chromosome lines to assign linkage groups to specific chromosomes. Several of these cDNA markers are also mapped into the B. napus genome and are generating a valuable information on the evolution of the "C" genome within a diploid and a tetraploid species.

1693 DEVELOPMENT OF AN OVERLAPPING SET OF YAC CLONES FOR THE TOP HALVES OF CHROMOSOMES 4 AND 5 OF *ARABIDOPSIS THALIANA*. Renate Schmidt, Gerda Cnops, Ian Bancroft and Caroline Dean. IPSR Cambridge Laboratory, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK.

We are participating in the international collaboration to construct an overlapping library of the *Arabidopsis thaliana* genome, ecotype Columbia, cloned in yeast artificial chromosomes (YACs) (EG library: Grill and Somerville 1991, MGG in press; EW library: Ward and Yen 1990, PMB 14, 561 - 568). To link up the YAC clones that cover the top halves of chromosomes 4 and 5 we are taking advantage of the RFLP markers that were mapped previously to these regions (Chang *et al.* 1988, Proc. Natl. Acad. Sci. USA 85, 6856 - 6860; Nam *et al.* 1989, The Plant Cell 1, 699 - 705). All available RFLP markers were hybridized to the EG and the EW libraries using the technique of yeast colony hybridization developed by Coulson et al. (1988, Nature 335, 184 - 186). These hybridization experiments were performed with yeast colonies plated at high density (768 colonies per 8 x 11 cm nylon membrane). Corresponding YAC clones for all 35 RFLP markers tested have been identified and the results have been confirmed by Southern blots. On average 5 YAC clones were identified per RFLP marker. It should be noted, however, that YAC clones could not be identified for each of the RFLP markers in each library. Taking into account the size of the region, the number of YAC clones identified and the average contig size around each RFLP marker, we estimate that we have mapped YACs for at least 30 % of the region. To date, we have identified one large YAC contig of approximately 600 - 700 kb on chromosome 4. This contig spans 6 cM, and is being extended with chromosome walking experiments. To identify overlaps between YACs, we are applying the IPCR technique (Ochman *et al.* 1988, Genetics 120, 621 - 623) to generate end-specific probes from the YAC inserts. A protocol has been developed to achieve this using total yeast DNA.

1694 LONG-RANGE PHYSICAL MAPPING OF THE *α-AMYLASE-1* (*α-Amy-1*) LOCI ON HOMOEOLOGOUS GROUP 6 CHROMOSOMES OF WHEAT. Wing Y. Cheung, S.Chao and Mike D.Gale. Cambridge Laboratory, Colney Lane, Norwich, NR4 7UJ, U.K.

Long-range physical maps of the small multi-gene family of the malt α-amylase genes (*α-Amy-1*) located on the long arms of wheat chromosomes 6A (the *α-Amy-A1* locus) and 6B (*α-Amy-B1*) were generated by pulsed-field-gel electrophoresis (PFGE) analysis. By using three methylation-sensitive rare-cutting restriction endonucleases, NotI, NruI and MluI, and an *α-Amy-1* cDNA probe and four gene-specific genomic probes from the *α-Amy-B1* locus, the size of the *α-Amy-A1* locus is estimated to be about 700kb and of the *α-Amy-B1* to be about 4300kb. These two maps indicate clustering of GC-rich and C-methylation-sensitive restriction enzyme recognition sites. At least five regions reminiscent of 'CpG islands' are apparent in *α-Amy-B1*, and three in *α-Amy-A1*. Correlation between recombination frequency and physical distance within the *α-Amy-B1* locus suggests that 1cM approximates to 1 megabase (Mb) in physical distance.

POSTER ABSTRACTS

1695 **Construction and screening of Yeast Artificial Chromosome (YAC) libraries of tomato.** Raymond A.J. van Daelen, Wilbert van Workum, Ronald Roepman and Pim zabel. Dept. of Molecular Biology, Agricultural University, Wageningen, The Netherlands.

In plants obvious candidates for the reverse genetics approach are disease resistance genes. We are interested in the nematode resistance gene (Mi) of tomato. This gene, inhereting in a dominant fashion, confers resistance against several nematodes of the genus Meloidogynae. It is located on chromosome 6, position 35, and closely linked (less than 1 cM, ie less than 500 Kb) to an allele of the acid phosphatase-1gene (Aps1[1]). In addinon to this isozyme marker, a highly saturated RFLP map of the Mi region is currently available. The Mi-gene is therefore a good candidate for cloning via flanking markers.

Here we describe the construction, characterization and screening of YAC libraries, made from tomato DNA digested with several rarely cutting restriction enzymes.To obtain a library consisting of overlapping clones, several enzymes were used. Moreover the enzymes used differ in their restriction preferences in respect that some predominantly cut within (NotI, SstII) and some outside (MluI, SalI, ClaI) CpG-islands. The libraries were screened for the presence of RFLP markers and subsequently used for the construction of a physical map of the region flanking Mi.

1696 **MOLECULAR MARKERS FOR DISEASE RESISTANCE GENES IN PEA.** G.M. Timmerman, T.J. Frew, A.L.M. Harding, W.A. Jermyn and J.E. Lancaster. DSIR Crop Research, Private Bag, Christchurch, NEW ZEALAND.

Resistance genes are being studied for two diseases of peas, *er* for powdery mildew (*Erysiphe pisi*) and *sbm-1* for pea seed-borne mosaic virus. Precise genetic map locations are being determined by examining linkage with RFLP and RAPD markers. The progeny of two genetic crosses segregating for the *sbm-1* allele are being examined. The *sbm-1* allele originates from germ plasm accession PI 193586. One of these two crosses is also segregating for resistance to powdery mildew. Data on map locations and linked molecular markers will be presented.

1697 **RFLP ANALYSIS AND LINKAGE MAPPING IN BETA VULGARIS.** Ellen Barzen,* Rudolf Jansen, Wolfgang Mechelke and Francesco Salamini.* Max-Planck-Institut für Züchtungsforschung* 5000 Köln 30, FRG in collaboration with KWS Kleinwanzlebener Saatzucht AG, 3352 Einbeck, FRG.

A collection of 50 diploid sugarbeet lines was analysed for restriction fragment length polymorphisms (RFLPs) by using four cutter restriction enzymes and a gelsystem which allows the detection of RFLPs of a few nucleotides. With these data a phylogenetic analysis will be done. A segregating population of 50 plants from a cross involving one pair of these lines which already segregates for some interesting agronomical traits was chosen to construct a linkage map for sugarbeet. Until now 90 different random genomic probes are analysed and 9 linkage groups for Beta vulgaris are defined. A few relevant traits segregating in this cross have been tentatively mapped to the 9 RFLP linkage groups.

1698 **STS FACILITATED PCR FOR BARLEY GENOME MAPPING.** Tragoonrung S., Blake T.K., and Hayes P.M. Montana State University, department of Plant and Soil Science, Bozeman, MT 59717

Cost, efficiency, and safety considerations have led many genome mapping projects to favor polymerase chain reaction sequence amplification (PCR) over Southern blotting. However, the source of primer sequences can be a limiting factor in PCR-based mapping. An alternative to random primers (RAPD approach) is the sequence-tagged-site (STS) approach. Short primer sequences can obtained from published sequences or by sequencing portions of DNA clones from genomic or cDNA libraries. These primers were used to screen for polymorphisms in the progeny of a winter x spring barley cross. Two types of polymorphisms were detected using these primer sets: (i) insertion/deletion events that could be read directly from agarose gels and (ii) point mutation events. The latter were identified using polyacrylamide gel electrophoresis of identical size PCR products cut with recognition endonucleases (four base cutters). Four base cutter detected a higher frequency of polymorphisms in this cross than did six base cutters on southern blots. Cost, convenience, efficiency, and the amount of information generated favor the use of STS facilitated PCR in genetic analysis of this cross.

1699 **EVOLUTIONARY RELATIONSHIPS AND ORGANIZATION OF NUCLEAR RIBOSOMAL RNA GENES IN WILD SPECIES AND CULTIVARS OF THE GENUS *SOLANUM*.** Nikolay Borisyuk, Ludmila Borisyuk and Vera Hemleben University of Tübingen, Germany

Phylogenetic relationships between 5 cultivated, 12 wild species and 9 cultivars of potato were characterized by RFLP analysis using rDNA sequences as hybridization probes. All plants analyzed possess rDNA repeats between 9.1 and 9.4 kb in length. The only remarkable exception is *S. neorosii* with rDNA repeats of 10 kb. Nevertheless, the genotypes analyzed could be clearly distinguished by RFLPs due to slight variation in length of the intergenic spacer (IGS) region or/and presence/absence of restriction enzyme sites. rDNA restriction maps were constructed for 12 potato species with different ploidy level between 2n=24 and 5n=60: *Solanum acaule, S. andigena, S. canasense, S. curtilobum, S. demissum, S. gourlayi, S. neorosii, S. phureja, S. sparsipilum, S. stenotomum, S. tuberosum cv. Lorch, S. tuberosum ssp.tuberosum*. The data obtained on the organization of rRNA genes of potatoes are discussed with respect to phylogenetic relationships of the species within the genus *Solanum* compared to the recently published results derived from RFLPs of ctDNA and single copy sequences. In addition we have sequenced the intergenic spacer of potato (*Solanum tuberosum cv Lorch*). The structure of potato rDNA spacer is compared with the published tomato IGS nucleotide sequence.

1700 **LOCATING CENTROMERES ON THE RICE RFLP MAP USING SECONDARY TRISOMICS.** DS Multani, SR McCouch and GS Khush, International Rice Research Institute, PO Box 933, Manila, Philippines

Centromeres are mediators of chromosomal behavior during meiosis and serve as a reference point for both physical and genetic maps. Locating centromeres on the RFLP map of rice is possible based on the availability of secondary trisomic stocks. These trisomics (2n+1) are plants in which the extra chromosome is an isochromosome (one of the arms is duplicated). Analysis of DNA from secondary trisomics using mapped RFLP markers confirms the identity of the isochromosome as identified at pachytene stage of meiosis and allows delimitation of the position of the centromere based on dosage (1 (disomic):2 (secondary trisomic)) effect. Following this approach, DNA from secondary trisomics for the long and the short arms of chromosome 6 were hybridized with several RFLP markers previously mapped to this chromosome. The centromere for this linkage group has been located. Confirmation of centromeric position for all 12 linkage groups will represent an important contribution toward the completion of the molecular genetic map. Further use of these secondary trisomics to locate genes controlling agronomic, morphological and isozyme characters in relation to RFLP markers will allow integration and unification of all genetic maps of rice. In addition, knowledge of centromere position enhances evolutionary studies of Oryza and related genera, and facilitates attempts to correlate genetic and physical distance critical in gene cloning efforts.

POSTER ABSTRACTS

1701 A PARTIAL MAP OF THE PEA GENOME INCORPORATING RFLP, FINGERPRINTING, DISEASE RESISTANCE AND MORPHOLOGICAL MARKER LOCI.

E. Dirlewanger[1], P. Isaac[2], R. Cousin[3] and D. de Vienne[1]. [1]Station de Génétique Végétale, Université Paris Sud/INRA, La Ferme du Moulon, 91190 Gif-sur-Yvette, France. [2]The Nickerson Seed Company, Cambridge Science Park, Milton Road, Cambridge CB4 4WE, UK. [3]Station de Génétique et d'Amélioration des Plantes, CRA 78026 Versailles Cedex, France.

An F_2 population of pea (*Pisum sativum* L.) consisting of 184 plants was produced from a cross between two lines differing for various resistance and morphological genes : *er* (powdery mildew), *Fw* (fusarium wilt race 1), *mo* (pea common mosaic virus), *Ascochyta pisi* race C gene, *r* (wrinkled seed), *l* (green seed) and *af* (afila). Using two restriction enzymes, 30 out of 120 genomic *PstI* clones revealed polymorphism between the parents. Thirty eight RFLP loci were identified. Fingerprinting with oligonucleotide probe $(GAA)_6$ allowed 13 polymorphic bands to be obtained, which provided 5 additional marker loci. Using *MAPMAKER* package we constructed a partial map consisting of 7 linkage groups covering more than 550 cM, while 11 loci remained unlinked. Four of the 5 $(GAA)_6$ loci appeared to be linked within a 35 cM cluster. An RFLP locus mapped at 10 cM of *er*, while *Ascochyta pisi* locus was found in a large linkage group, located on chromosome 1, including *af* and *l*.

1702 USE OF ALFALFA RFLPs TO DETECT HYBRIDS IN CROSSES OF SELF-INCOMPATIBLE CLONES. S. M. Koehler, G. R. Bauchan, and T. A. Campbell. USDA/ARS, Soybean & Alfalfa Research Lab., Beltsville, MD 20705

Ten to fifteen percent increase in forage production can be obtained in hybrid alfalfa, but production of hybrids using male-sterile lines is inefficient due to nonpreference of bees for the pollen-deficient male steriles. Alfalfa clones exhibiting a high level of self-incompatibility (SI), acceptable pollen viability, and female fertility are being developed as an alternative method of obtaining hybrids. Ten self-incompatible alfalfa clones selected from two broad based multiple pest-resistant lines are being evaluated. Due to the lack of other easily scorable morphological or isozyme markers in this elite germplasm, RFLP analysis is being used to determine the percentage of hybrid seed produced in crosses of these clones. Southern blots of *Eco*RI, *Eco*RV, *Hind*III, and *Bam*HI digested genomic DNA were hybridized with random-primed labelled inserts amplified using PCR from random alfalfa leaf cDNA clones. More than 85% of the inserts hybridized to low copy number sequences, and all of the probes have detected RFLPs in these clones. Out of 24 probe/enzyme combinations scored, *Eco*RV and *Eco*RI digests produced the highest number of polymorphisms, but *Eco*RI RFLPs were easier to score due to a lower average size. Cluster analysis was performed on simple matching coefficients for all visible bands scored to determine the degree of genetic similarity of the SI clones. Three clones, not all from the same parent population were at least 93% similar whereas the other clones were less than 75% similar to each other and to the other cluster of closely related clones. The percent of hybrid progeny resulting from bee pollination of clones which fall into different, less related clusters are being determined using several RFLPs unique to each parent clone. The copy number for each unique RFLP is being evaluated in the S_1 population for each clone chosen. A larger boost in heterosis might be expected if parents selected for hybrid production were more genetically diverse. Data is being analyzed to determine if forage yield of F_1 hybrids are correlated with genetic diversity of parents based on RFLP analysis, however QTLs for this trait may provide a more powerful tool for predicting hybrid performance

1703 GENETIC LINKAGE ANALYSIS OF RFLP LOCI IN LOBLOLLY PINE. M. Devey, K. Jermstad, T. Fiddler, C. Tucker, and D. Neale. Institute of Forest Genetics, Pacific Southwest Research Station, 2480 Carson Rd., Placerville, CA 95667.

We are reporting the inheritance and genetic linkage relationships of the first 50 restriction fragment length polymorphism (RFLP) loci detected in loblolly pine (*Pinus taeda* L., 2n = 24), an important timber species in the Southeastern U.S. A three-generation outbred pedigree, consisting of 4 grandparent, 2 parent and 96 F_2 progeny, has been used to evaluate segregation of loci detected by loblolly pine cDNA and genomic DNA probes. A large amount of genetic variability is present in this species. *Hind*III is the only restriction enzyme used for these assays, and the majority of probes detecting single- or low-copy sequences have been polymorphic between the two parents. As many as 5 alleles have been observed at a single locus in grandparent and parent DNAs, and it is common to observe segregation of 3 or 4 alleles/locus in the progeny. An average of 1.5 loci have been detected per probe; 4 or more loci/probe have been noted but the segregation patterns of these are often uninterpetable. We will discuss approaches to analyze the data using MAPMAKER.

1704 CAN RAPD POLYMORPHISMS BE GENETICALLY MAPPED? Mitrick A. Johns. Northern Illinois University, Dept. of Biological Sciences, DeKalb, IL 60115

The RAPD technique uses PCR with a single, short (10-mer), arbitrary primer to amplify a small number of bands from genomic DNA. Thus, an amplifiable sequence is one where two identical 10-mer sites are in inverted orientation within about 2 kb of each other. Polymorphisms, bands present in one line but not another, are common, and Williams et al. (1990; NAR 18:6531) showed that these polymorphisms can be genetically mapped as dominant alleles. However, it may be that different lines will contain different polymorphisms with the same primer, and that these polymorphisms will not map to the same location. A theoretical argument addressing this issue is presented. In essence, there are 30 times as many sites which are 1 base change away from being amplifiable as there are actual amplifiable sequences. It seems unreasonable to expect that independently arising polymorphisms will always affect the same site. Thus, it may be that genetic maps based on RAPDs are line-specific rather than species-specific. Data from recombinant-inbred maize lines bearing on this question, and on the nature of the sequences being amplified, are presented.

1705 MICROSATELLITES AMPLIFY HIGHLY POLYMORPHIC DNA BANDS IN SPAR OF PLANT DNA. Manju Gupta and Philip Filner. Correlation Genetics Corporation, 2050 Concourse Drive, San Jose, CA 95131.

Short repeats (15-20 bases) of di-, tri-, tetra- and pentanucleotides, the microsatellites, were used in single primer amplification reactions (SPAR, patent pending) with thermostable Taq polymerase to amplify genomic DNA of pine, grape, lettuce, tomato and maize. Such SPAR would amplify DNA sequences flanked by the microsatellites of inverse configurations in the genome. In all the five plant species tested representing evolutionarily diverse categories, one gymnosperm, three dicots and one monocot, all three dinucleotide repeats tested showed only smears and failed to amplify any discrete bands. Of nine trinucleotide repeat primers tested, only one, $(TCC)_6$, showed polymorphisms in grape and maize. However, a few of the ten tetranucleotide repeat primers tested, especially $(ACTG)_4$, $(GACA)_4$ and $(GATA)_4$, amplified highly polymorphic DNA bands in all the species tested. The products amplified by only pentanucleotide repeat primer tested, $(GACAC)_4$, were polymorphic in 4/5 species. The polymorphisms in grape were useful in distinguishing two vegetatively propagated clonal lines. A family of pine trees showed that polymorphisms amplified by microsatellites were inherited in the progeny. The results show that the same microsatellites are associated with polymorphisms in diverse plant species, indicating evolutionary conservation of the mechanism by which certain microsatellites become associated with length polymorphisms. The use of microsatellites described here in SPAR has a great potential in: 1. distinguishing closely related lines and clones, and 2. making molecular genetic maps of a genome in which other polymorphisms are scarce.

1706 COMPARISON OF RANDOM PRIMERS AND RFLP MARKERS IN DETECTING INTROGRESSION IN A ORYZA MINUTA X O. SATIVA CROSS. Sarat Sahu[1], Rebecca Nelson[2], Norman Oliva[2], and Hei Leung[1]. [1]Washington State University, Department of Plant Pathology, Pullman, WA 99164, USA, [2]International Rice Research Institute, Division of Plant Pathology, Philippines.

Genes conditioning resistance to blast and bacterial blight were introgressed from a tetraploid rice Oryza minuta (Om) to O. sativa (Os) by embryo rescue and backcrossings (Bordeos et al. submitted). In an effort to identify molecular markers closely linked to the introgressed resistance genes, we compared the relative efficiencies of using random primers and RFLP markers for the detection of introgressed Om DNA sequences. Ten-base random primers were used to detect introgressed Om DNA in a polymerase chain reaction with genomic DNA from blast-resistant BC_2F_2 and BC_2F_3 progeny. Of 100 random primers tested, 29 showed discrete polymorphisms between Om and the Os recurrent parent IR31917. Six of the 29 primers showed Om DNA fragments in BC_2F_2 and BC_2F_3 progeny, suggesting introgression. Of 106 mapped RFLP markers tested (provided by S. Tanksley), 92 showed polymorphisms between Om and Os and five showed introgression. The five RFLP markers were on Os chromosomes 1, 2, 4, and 6. The ability to detect introgression appears to be similar with random primers (6%) and RFLP markers (4.7%). A main advantage of the random primers method is its simplicity, allowing rapid detection of introgressed DNA. However, unlike the use of mapped RFLP markers, additional segregation analyses are needed to locate PCR-amplified markers on specific chromosomes. A combination of PCR-amplified and RFLP markers may expedite the detection of markers closely linked to resistance genes. (Research supported by grants from the Rockefeller Foundation)

POSTER ABSTRACTS

1707 GENOME ORGANIZATION OF A WILD WHEATGRASS: A LOOK AT REPETITIVE DNA FAMILIES. Brian Bournival, Mohamed Obanni, Herb Ohm, and Sally Mackenzie. Dept. of Agronomy, Purdue University, West Lafayette, IN 47907

Due to the rapid depletion of disease resistance genes derived from close relatives of wheat (*Triticum aestivum*), wild wheatgrasses such as *Thinopyrum ponticum* are an important alternative source of these genes. *Triticum* and *Thinopyrum* chromosomes do not pair normally, making the transfer of useful genes difficult without the transfer of additional large unwanted chromosome segments. A better understanding of the genome organization of *Th. ponticum* may result in more efficient exploitation of this important genetic resource. Two repetitive sequence families specific to wheatgrass species have previously been identified. Our laboratory has identified a third family of species-specific repeats. These elements have been characterized and compared. Evidence regarding level of sequence conservation, expression, dispersal and organization in the genome, and polymorphism among *Th. ponticum* accessions will be presented.

1708 GENETIC VARIATION DETECTED BY DNA FINGERPRINTING IN FLOWERS. A. Vainstein(1), J. Hillel(2), U. Lavi(3) and G. Tzuri(1). The Hebrew University of Jerusalem, Departments of Horticulture (1) and Genetics (2), Rehovot, Israel, and Agricultural Research Organization (3), Bet-Dagan, Israel.

Minisatellite and microsatellite DNA probes hybridize to many loci in rose, gerbera and carnation DNA, thus allowing cultivar identification in these ornamentals. The level of polymorphism revealed by DNA fingerprints is dependent upon the probe/genus combination. Jeffreys' human derived probe 33.6 for example, yielded DNA fingerprints in all genera analyzed, while in gerbera, Jeffreys' probe 33.15 yielded no detectable signal. On average, 83 genotype-specific bands were detected by phage, cattle and human derived probes. To assess the limitations of the DNA fingerprinting approach to genotype identification, a cross was made between two very genotypically similar "standard" carnation cultivars. DNA fingerprint analysis of the progeny revealed 36 parental-specific loci out of the 186 detected in parental genotypes. Only two pairs of these loci were found to be linked. No allelic pairs were detected in the analyzed progeny. The probability of two offspring from the crossing of such similar genotypes having identical DNA fingerprints is 1.8×10^{-5}. Genetic relatedness between carnation genotypes belonging to the phenotypical categories "standard", "spray" and "dwarf" was studied by DNA fingerprint analysis. A comparison of genetic relationships within and between categories based on known genetic history, to genetic relationships deduced from DNA fingerprints, revealed a perfect match. It is apparent from our results that the application of DNA fingerprinting methodology to the plant kingdom can greatly assist in cultivar identification, breeding programs and evolutionary research.

1709 THE USE OF FINGERPRINTING WITH THE OLIGONUCLEOTIDE (GATA)4 IN EVOLUTIONARY GENETICS. A.W. van Heusden, W.H.J. van Houten, J. Rouppe van der Voort and K. Bachmann.

University of Amsterdam, Hugo de Vries Laboratory, Kruislaan 318, NL-1098 SM Amsterdam.

Natural populations of the Chilean autogamous annual *Microseris pygmaea* and its nearest Californian relatives, *M. bigelovii* and *M. elegans* are assayed for genetic variation. Inbred strains from representative populations of all 3 species are used to reconstruct their morphological and physiological evolution. The genetics of the strain differences are analyzed in interstrain hybrids. Various techniques revealing nuclear RFLPs are essential tools for this analysis. Recently, we have tested the applicability of fingerprint loci as markers for strain comparison and genetic analysis. Banding patterns are easily obtained with the labelled synthetic oligonucleotide (GATA)4 directly in agarose gels. The applicability of these patterns differs even among closely related species. In *M. pygmaea*, only a few major bands corresponding to 3 segregating loci are reliably demonstrated, and these are hypervariable. In *M. elegans*, a large number of shorter, evolutionary more stable bands can be used for an unequivocal reconstruction of haplotype phylogeny. Currently we are also investi-gating the possibility of using RAPD (Random Amplified Polymorphic DNA) markers for cladistic analysis on the subspecific level.

1710 A HIGH RESOLUTION LINKAGE MAP OF THE NEMATODE RESISTANCE GENE REGION IN TOMATO. V. M. Williamson[1], J. Y. Ho[1], H. M. Ma[1], R. Weide[2], R. Klein Lankhorst[2], T. Liharska[2] and P. Zabel[2]. [1]University of California, Department of Nematology, Davis, CA and [2]Agricultural University, Department of Molecular Biology, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

Root-knot nematodes (*Meloidogyne* spp) are obligate endoparasites that cause extensive damage to many crops. A single, dominant gene, *Mi*, confers resistance to the major root-knot nematode species in cultivated tomato (*Lycopersicon esculentum*). This gene has been introgressed into many modern tomato cultivars from the wild species *L. peruvianum*. *Mi* has been mapped near the centromere of chromosome 6, in tight linkage to the acid phosphatase gene, *Aps-1*. We have identified several RFLP and RAPD markers located on the introgressed *L. peruvianum* region containing the *Mi* gene. Initial marker selection was made by screening nearly isogenic lines that differ in their nematode resistance. Linkage was confirmed by analysis of DNA from tomato plants segregating for resistance. By analyzing RFLP patterns, we have identified a number of tomato lines with genetic crossover events within the introgressed region. This data was used to construct a high resolution linkage map of the region of chromosome 6 containing *Mi*. The construction of a corresponding long range physical map is in progress. These maps constitute major steps toward cloning this important disease resistance gene.

1711 USE OF RAPDs IN BROCCOLI AND CAULIFLOWER VARIETY IDENTIFICATION. Carlos F. Quiros and Jinguo Hu. University of California, Department of Vegetable Crops, Davis, California 95616.

RAPD (Random Amplified Polymorphic DNA) markers generated by 4 arbitrary 10-mer primers, discriminated 12 broccoli and 14 cauliflower cultivars by banding profiles. The size of the amplified DNA fragments ranged from 300 to 2600 base pairs. 28% of the markers were fixed in both broccoli and cauliflower, whereas 4% were specific to either crop. The rest were polymorphic in either of both crops. The markers generated by two and three primers were sufficient to distinguish each of the broccolis and cauliflowers, respectively. The average difference between broccoli and cauliflower was 14.5 markers, 5.8 between two broccoli and 7.9 between two cauliflower cultivars. Higher differences for each crop was found between cultivars of different seed companies than within the same company. RAPD markers provide a quick and reliable alternative to identify broccoli and cauliflower varieties.

1712 Subtelomeric Sequences as Polymorphic Markers
Ed Weck and David Farrar, Molecular Genetics, Northrup King Co., Highway 19, Stanton, MN 55018-9662

Telomeres are end-specific sequences which allow genetic maintenance of chromosomes. Specific telomeric sequences, repeated thousands of times, confer stability on chromosomes by protecting them from degradation. We used an Arabidopsis telomer-specific oligonucleotide (TTTAGGG)$_4$ as a primer in PCR with maize inbreds and observed a smear of bands from 0.5 to 14 kb in size. This would be expected if a sequence were tandemly-repeated thousands of times in a genome. Subtelomeric sequences in humans have been shown by in situ hybridization to map to positions genetically indistinguishable from the ends of chromosomes. One simple use of these sequences is to define the ends of linkage maps. We used oligonucleotides designed from human subtelomeric sequences as PCR primers and observed discrete polymorphic bands in a number of corn inbreds. It should be possible to map these polymorphisms in segregating populations and determine if they are located at the ends of chromosomes.

POSTER ABSTRACTS

1713 DETERMINATION OF GENETIC RESEMBLANCE AMONG ECOTYPES OF ARABIDOPSIS USING RESTRICTION FRAGMENT LENGTH POLYMORPHISM DATA. Charles E. Hussey[1], James Nienhuis[2], Gretchen King[1]. [1]NPI, Salt Lake City, Utah 84108, [2]University of Wisconsin, Dept. of Horticulture, Madison, Wisconsin.

We are interested in studying the genetic resemblance among a wide range of Arabidopsis ecotypes by RFLP (restriction fragment length polymorphism) analysis to elucidate the relationships within the species and to determine the opportunity to select for variability among various ecotypes. We selected 28 diverse Arabidopsis ecotypes to be used in this study. The DNA was extracted, digested with restriction enzymes and hybridized after Southern blotting to 27 mapped RFLP clones (a gift from the lab of Dr. Elliot Meyerowitz). Each polymorphic hybridization band was scored separately and weighted equally. The coefficient of relatedness (CR) was calculated between pairs of genotypes as follows: CR = (C-D)/(C+D) in which C equals the number of concordant and D equals the number of discordant RFLP states. The matrix of CR values was subjected to multivariant analysis. The plot of the first principal component versus the second principal component revealed several subgroups among the ecotypes. It has been postulated that self fertilizing species such as Arabidopsis exhibit a low level of sequence polymorphism. To explore this sequence variation and to determine the source of this variation between related and distantly related ecotypes (as determined by principal component analysis), several polymorphic regions from six ecotypes were PCR amplified and sequenced. The nature of this sequence variability will be discussed.

1714 RAPID DETECTION OF DNA SEQUENCE POLYMORPHISM IN PLANTS. Donna M. Shattuck-Eidens, Russell N. Bell and Jeffrey T. Mitchell. NPI, Salt Lake City, Utah 84108

A rapid and simple method has been developed to detect DNA sequence polymorphism in plants. The method uses allele-specific primers in polymerase chain reaction (PCR) amplifications to simultaneously amplify and discriminate between polymorphic allelic variants. Allelic variants can be distinguished in PCR by choosing oligonucleotide primer sequences appropriately. The success of this technique relies on the specific failure of PCR to amplify with primers that do not perfectly match the target sequences of allelic variants. By coupling rapid DNA extraction with allele specific amplification, it is possible to screen 100 plants per day for a set of predetermined DNA sequence polymorphisms. This procedure has been used successfully in detecting polymorphisms at several maize and sugar beet loci. Those results will be presented. The possible application of this method to other plants, including onion and tomato, will be discussed.

1715 IDENTIFICATION OF TUNGRO RESISTANT GENES IN RICE. H. B. Hamat and H.B. Hashim

Malaysian Agricultural Research and Development Institute(MARDI) P.O. Box12301, GPO50774, Kuala Lumpur, Malaysia. Several rice varieties have been screened and evaluated by the method of ELISA against Rice Tungro Speherical Virus (RTSV) and Rice Tungro Bacilliform Virus (RTBV) . A few varieties namely Phankari 203, Utrimerah, Utrirajapan, Kataribouq, and Basmati 370, were found to be resistant to RTSV but only Utrimerah and Utrirajapan showed a low level of resistance to RTBV. The resistant and the susceptible varieties to both the viruses were also distinguished using RFLP markers. Crosses were carried out involving each of the resistant variety to the susceptible variety TN1. The F1, F2 and F3 generations of these crosses have been raised and their segregating patterns obtained using ELISA and RFLP. Our ELISA results, using 20 F1 and 400 F2 plants together with their respective parents, show that the resistance to RTSV is controlled by a single recessive gene. However, more F3 and RFLP data are required to confirm our observation. We also found out that the resistance to RTBV is most likely controlled by several genes.

1716 FLOW KARYOTYPING, SORTING AND MICRO-CLONING OF TOMATO CHROMOSOMES. K. Arumuganathan, G. B. Martin, J. P Slattery†, S. D. Tanksley, and E. D. Earle. Department of Plant Breeding, †Flow Cytometry/Video Microscopy Facility, Cornell University, Ithaca, NY 14853, USA.

Chromosome-specific libraries would have great value for many aspects of plant genome studies, but very little work has been done in this area. We are interested in constructing chromosome-specific libraries of tomato using sorted chromosomes. We have prepared suspensions of metaphase chromosomes from tomato cell cultures (Arumuganathan et al., 1991, Theor. Appl. Genet., in press). The isolated chromosomes were stained with chromomycin A3 and Hoechst 33258 and analyzed on an EPICS 753 flow cytometer, using a uv laser to excite Hoechst 33258 fluorescence and a 458 nm laser to excite chromomycin A3 fluorescence. Chromosomes from well resolved peaks were sorted onto nitro-cellulose membrane filters for spot-blot analysis. The filters were processed and hybridized with chromosome-specific repetitive DNA probes. In this way tomato chromosome 1 and chromosome 2 were assigned to peaks in the bivariate flow karyotype. Large numbers of chromosomes were collected from these peaks. DNA specific to these fractions was amplified by using PCR and several oligonucleotide primers of random sequence. Amplified products were prescreened to eliminate the sequences with highly repetitive elements. The PCR-amplified products with low copy sequences were selected for mapping. We are now mapping these sequences in the tomato genome to determine the extent to which the fractions are enriched for specific chromosomes. We are also attempting to assign other chromosomes to peaks on the bivariate flow karyotype. We will report our progress in assignment of chromosome(s) to peaks, micro-cloning, and mapping. (Funded by Cornell Biotechnology Program; US Dept. of Agriculture.)

1717 RFLP ANALYSIS OF SOYBEAN BREEDING POPULATIONS: GENETIC MAPPING OF MARKERS AND QTL'S. Paul Keim, William Beavis, James Schupp, Robert Freestone, Mahmood Vahedian, David M. Webb. Department of Biology, Northern AZ University, Flagstaff AZ 86011-5640 and Pioneer HiBred, Int. 7250 N.W. 62nd Street, Johnston IA 50131.

Soybean RFLP mapping has been developed using exotic germplasm with little effort devoted to adapted genetic lines. However, RFLP markers must be identified among adapted germplasm, if they are to be important in soybean improvement. We have analyzed 16 genotypes from a commercial breeding program with ca. 750 RFLP markers. This required screening over 1000 recombinant DNA probes with 5 different restriction enzymes. The RFLP genetic distances among these lines followed expectations from pedigree relatedness (R=0.83). Two of the 16 lines were used to produce a population of F_5-derived recombinant inbred lines (320 genotypes) which was evaluated with over 200 RFLP markers. These same R.I. genotypes were evaluated at eight locations in each of two years for complex traits such as yield, protein and oil content. We will report the analysis of this data which identifies linkage among markers, and between markers and trait variation.

1718 A SIMPLE DNA MICRO-EXTRACTION PROCEDURE SUITABLE FOR PCR AND RAPD ANALYSES OF CANOLA (*BRASSICA NAPUS*). Jean-Marc Deragon and Benoit Landry, Agriculture Canada, 430 Blvd. Gouin, St-Jean-sur-Richelieu, Québec, Canada. J3B 3E6.

Recently, a new type of genetic markers called RAPD for Random Amplified Polymorphic DNA, evolved from the application of PCR technology to rapidly map genomes of higher eukaryotes. RAPD markers can potentially be used as a diagnostic tool for plant selection in a breeding program since it is non destructive and fast and does not require large amount of plant tissues that can be collected from young seedlings. Such early testing can facilitate and accelerate actual and future plant breeding programs. This poster describes our current analytical system and its application to canola breeding. We developed a rapid and simple DNA micro-extraction method suitable for RAPD or other PCR-based analyses. Briefly, 0.5 cm leaf disks are collected from two weeks-old seedling using a standard paper punch and incubated immediately in an enzymatic solution to produce protoplasts. The protoplasts are then washed, lysed and the DNA is extracted directly from the crude lysate. Up to 100 plants can be treated in a day and the procedure can be automated. Our optimized protocol provides enough DNA for at least 10 RAPD reactions per extraction. We compared the amplification products generated with DNA prepared by this quick procedure with those obtained from CsCl-purified DNA; the crude DNA preparation is not altering the RAPD reaction. This micro-extraction could also be very useful to other PCR based protocols since it eliminates time-consuming large scale DNA isolation.

POSTER ABSTRACTS

1719 TOWARDS AN INTEGRATED MOLECULAR/CLASSICAL LINKAGE MAP OF CHROMOSOME 6 OF TOMATO

R. Weide, R. Klein Lankhorst, T. Liharska, M. van Wordragen, M. Koornneef and P. Zabel, Agricultural University, Dept. Molecular Biology and Genetics, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

In tomato (*Lycopersicon esculentum*), most polymorphic DNA markers have been localized on a molecular (RFLP-) linkage map using segregating populations of *L.esculentum* x *L.pennellii* crosses. The map position of many agronomically important genes, however, has been established by linkage analysis involving *L.esculentum* x *L.esculentum* crosses. Integration of both maps is far from what is needed to choose DNA probes closely flanking the target gene to be cloned. Our objective is to construct an integrated molecular/classical map of chromosome 6 of tomato. To this end, a set of chromosome 6-specific RFLP and RAPD markers was collected using a substitution line in which the *L.esculentum* chromosome 6 was replaced by chromosome 6 from *L.pennellii*. From a *L.esculentum* chromosome 6 marker line and the substitution line, a F2 population was made which was segregating for six phenotypical markers from chromosome 6. RFLP linkage analysis using 33 recombinants (representing a segregating population of over 200 individuals) enabled us to construct an integrated map combining a variety of polymorphic DNA markes (anonymous tomato and potato genomic and cDNA clones, RAPD's, T-DNA flanking sequences and transposed Ac flanking sequences) and classical genetic markers. Special emphasis was given to the region carrying the root knot nematode resistance gene Mi.

1720 RECOMBINATION BETWEEN HOMOEOLOGOUS CHROMOSOMES IN SYNTHETIC *BRASSICA NAPUS*.

Derek Lydiate, Andrew Sharpe, Deborah Keith and Rene Ruiter.
AFRC Cambridge Laboratory, Colney Lane, Norwich, NR4 7UJ, United Kingdom.

Brassica napus is an amphidiploid species with two main crop types, oilseed rape and swedes. *B. napus* (n=19) can be "resynthesised" from sexual hybrids between *B. rapa* (n=10) and *B. oleracea* (n=9) and much of the genetic diversity, in both simply-inherited and quantitative traits, which is available for oilseed rape improvement resides in these two diploid *Brassica* species. Cytological studies of *B. napus* have suggested that the homoeologous *B. rapa* and *B. oleracea* chromosomes might interact with one another at meiosis. We have used molecular markers to analyze meiosis in normal and resynthesised *B. napus* genetically. Fifty F1 progeny from a cross between a resynthesised *B. napus* plant and a doubled-haploid oilseed rape plant were scored for the presence of over eighty alleles from each parent using isozymes and RFLP probes. 60% of the alleles from the resynthesised parent were lost from at least one of the F1 plants; these alleles were normally absent from 2-8% of the F1 plants and duplicate copies of these alleles were often found in other F1 plants. No allele from the oilseed rape parent was absent from any of the F1 plants. Detailed analysis of the genotypes of the F1 plants strongly suggests that the loss of markers from gametes from the resynthesised parent is due to recombination between homoeologous chromosomes leading to large translocations which separately involve the overwhelming majority of *B. napus* chromosome arms. These translocations result in gametes where a duplicated chromosomal segment has replaced a segment of an homoeologous chromosome. Chromosomal recombination only occurs between homologous chromosomes in oilseed rape but recombination occurs between homologous and homoeologous chromosomes in resynthesised *B. napus*, the genes which stabilise homologous chromosome pairing in oilseed rape are being investigated.

1721 EVALUATION AND CHARACTERIZATION OF RAPD MARKERS FOR GENOME MAPPING.

Norman F. Weeden[1], Gail M. Timmerman[2], Minou Hemmat[1], Fernando Loaiza-Figueroa[1], and Jiang Lu[1]. [1]Cornell University, Department of Horticultural Sciences, Geneva, New York 14456 and [2]Department of Scientific and Industrial Research, Crop Research, Christchurch, New Zealand.

The mapping of several hundred Random Amplified Polymorphic DNA (RAPD) products in species from three dicotyledonous families indicates that linkage maps can be routinely and efficiently generated despite certain complicating factors. The reproducibility of the technique is particularly sensitive to the purity of the template DNA. A relatively low yielding extraction procedure generating high quality DNA is much preferable to many higher-yielding procedures. Even with relatively pure DNA there appears to be an overall error rate of about 5%. A significant proportion of these errors may be recognizable by screening for double recombinants among tightly linked loci. This approach has worked well in pea and apple linkage mapping. In pea, apple, and pepper, the sequences generating RAPDs tend to occur in clusters, indicating that, at least in certain species, there may be a limit to the degree of saturation to which RAPDs can map a genome.

1722 PHYSICAL MAPPING OF THE ARABIDOPSIS GENOME WITH ANCHORED YACs.

E. Matallana[1], C. J. Bell[1], P. Dunn[1], W. Ewens[1,2], R. Reiter[3], P. Scolnik[3] and J. R. Ecker[1]; [1]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania; [2]Department of Mathematics, Monash University, Victoria, Australia; [3]Central Research and Development Dept., E.I. du Pont de Nemours & Co. Wilmington, Delaware.

Using a six genome equivalent *Arabidopsis* YAC library with an average insert size of 0.25 Mbp (Ecker, J.R. *Methods* 1, 186-194, 1990), anchor points with the *Arabidopsis* genetic map are being established by hybridization of RFLPs and RAPDs to the arrayed YAC library. As part of our effort to construct a physical map of the genome of *Arabidopsis thaliana*, we have made a mathematical analysis of our experimental approach of anchoring yeast artificial chromosome clones with genetically mapped RFLPs and RAPDs (Ewens *et al.* submitted). The details of this analysis and their implications for mapping of the *Arabidopsis* genome will be discussed. In order to test these predictions, we are focusing on chromosome 1 as a "model" genome. Assuming a size of 20 Mbp for chromosome 1 and using all of the available chromosome 1 anchors (at least 100), our calculations predict that a mean number of 36 YAC islands should be generated. A directed approach towards joining of chromosome 1 islands using vectorette PCR end-probes will be initiated.

The *Arabidopsis* genome contains a number of large (0.1-1.3 Mbp) EcoRI restriction fragments that can be observed in ethidium bromide-stained CHEF gels of genomic DNA. YAC clones that contain homologous sequences have been identified. A number of these YACs contain large arrays of α-satellite-like repeats that map to para-centromeric sites on all *Arabidopsis* chromosomes. These long stretches of tandemly repeated DNA may be a component of *Arabidopsis* centromeres. Further analysis of these YAC clones is in progress. (Supported by the National Center for Human Genome Research - HG00322).

1723 MAPPING VIRAL DISEASE RESISTANCE GENES IN PEPPER.

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Three devastating diseases affecting pepper are cucumber mosaic virus, tobacco mosaic virus, and three closely related potyviruses, potato virus Y, pepper mottle virus, and tobacco etch virus. We have populations segregating for resistance to each of the above viruses. We are constructing low density restriction fragment length polymorphism maps in each population with the same set of clones chosen at approximately ten-centimorgan intervals from a previously constructed medium-density map in order to locate the genes conferring resistance to each disease. More detailed mapping will be done in regions of the chromosomes that show a large effect on our resistance phenotypes. Previous work has shown that there are at least two genes operating in our populations for cucumber mosaic virus resistance. Resistance to tobacco mosaic virus also involves at least two genes. The potyvirus resistances are simply inherited and recessive. Data on the location and number of genes in each case and the simultaneous development of near-isogenic lines will assist in the rapid transfer of multiple virus resistance and in the development of material for studies of the mechanisms of virus resistance.

1724 MAPPING AND PROGRESS TOWARDS CLONING OF THE Tm2a GENE ON CHROMOSOME 9 OF TOMATO.

Martin Ganal, Gregory Martin, James Giovannoni, Ramon Messeguer and Steven D. Tanksley. Department of Plant Breeding and Biometry, Cornell University, 252 Emerson Hall, Ithaca, NY 14853, USA.

The Tm2a gene of tomato confers resistance to tobacco mosaic virus (TMV) and is located near the centromere of chromosome 9. Virtually nothing is known about its mode of action or its gene product. In an attempt to characterize this gene, we are developing techniques to clone the Tm2a gene solely based on its map position on a genetic map. Until now at least 15 RFLP markers have been mapped to the respective region of chromosome 9 and found to be cosegregating or very tightly linked to the gene. At the moment, we are in the progress of constructing a high resolution RFLP map for this region with a resolution of less than 1 centiMorgan. Simultaneously, nearly isogenic lines are being screened for additional markers in this region using RAPDs. Pulsed field gel electrophoresis is used to determine the physical distance of some of the Tm2a linked markers and to get an estimate of the physical size of the region. Finally, we have started to screen a tomato genomic library in yeast artificial chromosomes (YACs) with some of the markers linked to the Tm2a gene. To date we have isolated two YACs for two different clones and are in the progress of isolating more. These YAC clones might serve as a starting point for a chromosome walk to the Tm2a gene.

POSTER ABSTRACTS

1725 RAPD MAPPING IN TOMATO. Tsvetana Liharska, Rob Weide and Pim Zabel, Agricultural University, Department of Molecular Biology, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.

Recently, a new genetic mapping technique has been described by Williams et al.,1990 (Nucl.Acids Res. 18/22 pp 6531-6535) based on a modification of the polymerase chain reaction (PCR) in which synthetic primers are used to amplify random sequences from genomic DNA. The amplified DNA fragments which are generated from one individual but not from another are called RAPD markers. These markers are inherited in a Mendelian fashion and are very useful in the construction of genetic maps.

The feasibility of the RAPD assay for rapid identification of genetic markers is demonstrated in tomato (*Lycopersicum esculentum*). Using a substitution line in which the *L.esculentum* chromosome 6 was replaced by chromosome 6 from *L.pennellii* and a collection of commercially available 10-mer primers, chromosome 6-specific RAPD markers were identified. Their chromosomal position on the molecular map of tomato was established by RAPD and RFLP analysis of DNA from a number of tomato lines and recombinants that differ for regions on chromosome 6.

In addition, we have studied several molecular characteristics of the amplified products, including their homogeneity, their copy number and distribution in the genome.

The potential of RAPD markers as diagnostic tools in distinguishing different tomato cultivars is demonstrated.

1726 HETEROGENEITY AND SEQUENCE ORGANIZATION OF THE RIBOSOMAL DNA INTERGENIC SPACER IN ERUCA SATIVA. Malathi Lakshmikumaran, Tata Energy Research Institute, 90 Jor Bagh, New Delhi 110 003, India.

The Eruca sativa rDNA has an unit length in the range of 8.5 to 9.5 kb. There are at least two classes of rDNA in one of the cultivars of E. sativa. The variation in the two classes of rDNA is due to length heterogeneity of the intergenic spacer (IGS) of the 18s-25s ribosomal RNA genes. The major variant is also larger in size as compared to the minor variant. The EcoRI fragment containing the IGS was cloned from two different variants. The EcoRI fragment length of the minor IGS variant is 3kb whereas the size of the major variant is 3.9kb. The 3kb IGS fragment has been completely sequenced. Restriction enzyme mapping of the two IGS clones indicate that the variation is due to an insertion of approximately 1kb DNA sequence in the middle of the IGS. There are 3 types of repeated DNA sequences present in the IGS. A 30bp tandem repetitive DNA sequences is repeated 22 times just upstream of the 18s RNA coding region. The other 2 types are also tandem repeats but present in the middle of the IGS. The characterization of the 4kb IGS by sequencing is in progress. Sequence comparison of E. sativa IGS (3kb) with that of the entire Raphanus sativus large spacer revealed a significant sequence homology but the organization pattern was clearly different from each other. The 30bp tandem repeat present in E. sativa is absent in R. sativus. The sequence analysis of this IGS will be discussed.

1727 RFLP ANALYSIS OF RICE (*O. sativa*) INTORGRESSION LINES. Kshirod K. Jena*, Gurdev S. Khush** and Gary Kochert* *University of Georgia, Department of Botany, Athens, Georgia 30602,**International Rice Research Institute, P.O. Box 933, Manila, Philippines

The wild species of *Oryza* are a rich source of useful genes for rice improvement. One of the species, *O. officinalis* has CC genome and is resistant to all the known biotypes of brown planthopper (BPH), white backed planthopper (WBPH) and bacterial leaf blight (BB). Fifty-two introgression lines (BC2F8) derived from crosses between *O. sativa* and 5 accessions of *O. officinalis* were analyzed to identify the introgression of *O. officinalis* chromosome segments. DNA from the parents and introgression lines was digested with five restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI and *Pst*I) and analyzed with 177 RFLP markers located at approximately 10 cM intervals over the rice chromosomes. RFLPs between the parents were detected by most probe/enzyme combinations. Of the 174 informative markers, 28 identified putative *O. officinalis* introgressed chromosome segments in one or more of the introgression lines. Introgressed segments were found in 11 of the 12 rice chromosomes. In most cases of introgression, *O. sativa* RFLP alleles were replaced by *O. officinalis* alleles, consistent with introgression by reciprocal recombination. Some of the introgressed segments show correlation with known BPH resistance genes but there was no absolute correlation for all the introgressed lines.

1728 APPROACHES TO TRANSFORMATION AND TRANSPOSON TAGGING IN VOLVOX CARTERI. Rüdiger Schmitt,[1] Heribert Gruber,[1] Andreas Lindauer,[1] Bernd Schiedlmeier,[1] Gerd Weber,[2] and David Kirk.[3]

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Volvox carteri is a green alga with many features that recommend it as an object for molecular genetic analysis of development. Mutants with developmental defects are readily recovered and analyzed by classical genetics, however, their molecular genetic analysis has been precluded by the absence of methods for transformation or gene tagging. We have recently cloned and sequenced the single 6-kb nitrate reductase structural gene (*nitA*) of *V.carteri*. Its potential utility as a homologous transformation marker was tested using a novel technique for DNA delivery that involves UV-laser microbeam injection into plasmolyzed gonidial cells. Moreover, *nitA* was used as a "transposon trap" to identify transposable elements that might become useful for transposon tagging and cloning of various loci of developmental interest.

1729 EN/SPM AS A TOOL FOR GENE TAGGING IN TRANSGENIC PLANTS. Guillermo H. Cardon, Monika Frey, Heinz Saedler and Alfons Gierl. Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, FRG.

Since its molecular characterization, the maize transposable element En/Spm has been successfully used to isolate several maize genes. Unfortunately, endogenous transposons have not yet been isolated from many other species preventing the development of transposon tagging strategies in those species. The autonomous element En-1 has been shown to transpose in heterologous species such as tobacco, potato and Arabidopsis. An excision essay system for En/Spm in transgenic tobacco developed recently helped to define the two En/Spm encoded trans-acting functions, TNPA and TNPD, required for excision. In this system, excision and reinsertion of a dSpm element occurs without alteration of the element. A dSpm element marked by the insertion of an antibiotic resistance gene is also capable of excision. Since the dSpm elements are inserted between the promoter and the coding sequences of the GUS, BAR or Spt genes, excision can be monitored either by hystochemical staining or by resistance to PPT or streptomycin. Germinal excisions have been found and experiments are in process to determine germinal excision and loss frequencies. Timing and excision frequency are being manipulated by expressing the trans-acting factors with different promoters. The present studies are currently extended to Petunia and Arabidopsis, diploid plants in which a transposon tagging program is intended.

1730 A GENERALIZED GENE TAGGING SYSTEM FOR HIGHER PLANTS USING AN ENGINEERED MAIZE *Ac*. Wayne P. Fitzmaurice, Long V. Nguyen, William F. Thompson, Earl A. Wernsman and Mark A. Conkling. North Carolina State University, Departments of Botany, Crop Science, and Genetics, Raleigh, NC 27695-7614, USA.

A generalized transposon tagging system will be useful in the isolation of genes having mutant phenotypes that are readily identifiable. It will be especially useful for those genes which produce low abundance mRNAs or for which no biochemical function is known. An ideal transposon tagging system would have a number of characteristics. 1) It would be applicable to a variety of plant species. 2) It would contain a dominant selectable marker allowing the researcher to easily assay the co-segregation of the transposon with the mutant phenotype. 3) The transposon tag would be unable to catalyze its own translocation. Transposition would be achieved by a second defective transposon which would provide an immobilized *trans*-acting transposase. 4) The DNA flanking the transposon tag would be easy to isolate as a probe to use in cloning the wild-type gene. We have developed a series of transposon tagging vectors for dicotyledonous plants based on the maize transposable element *Ac*. This binary system includes the transposase (*Ts*) and the tagging element (*Ds*) on separate T-DNA vectors. The *Ts* elements include versions in which transcription is driven either by the endogenous *Ac* promoter or by the cauliflower mosaic virus (CaMV) 35S promoter. The *Ds* tagging elements include a gene conferring methotrexate resistance for selection and a *supF* gene to facilitate cloning of tagged sequences. The *Ds* element is flanked by a CaMV 35S promoter and the ß-glucuronidase (GUS) coding sequence so that GUS expression occurs upon excision of the element. We have transformed these *Ts* and *Ds* elements into tobacco and *Arabidopsis* and demonstrated that the transposase is functional with either promoter (tobacco) or with the 35S promoter (*Arabidopsis*), and that the artificial *Ds* elements are capable of high rates of transposition over several generations.

POSTER ABSTRACTS

1731 **TRANSPOSITION OF THE MAIZE ELEMENT Ds CO-TRANSFORMED WITH THE Ac TRANSPOSASE GENE IN TRANSGENIC RICE.**
S. Iida, H. Hashimoto*, T. Izawa*, C. Miyazaki, R. Terada*, K. Itoh*, Y. Inagaki and K. Shimamoto*. Dept. Biol. Sci. & Tech., Sci. Univ. Tokyo, Yamazaki, Noda-shi 278, *Plantech Res. Inst., Kamoshida, Midori-ku, Yokohama 227, JAPAN

The Ac/Ds family of the transposable elements in maize is one of the best studied elements in eukaryotes. The autonomous element Ac encodes an active transposase, whereas most of the non-autonomous Ds elements carry internal deletions and they can transpose only when the Ac transposase is provided in trans. To develop an efficient gene tagging system in rice, we have constructed two plasmids. One of them carries the 35S promoter of cauliflower mosaic virus (CaMV) and a Ds element at the untranslated leader sequence of the hph gene for hygromycin B resistance (Hm^1). Another plasmid contains the Ac transposase gene fused with the CaMV 35S promoter. These two plasmids were co-transformed into rice protoplasts by electroporation. Upon excision of the introduced Ds element, the hph gene was reactivated to produce Hm^r transformants of rice cells. Southern blot analysis of these Hm^r transformants and transgenic plants derived from them indicated that the introduced Ds element is trans-activated by the co-transformed Ac transposase gene. Mode of transmission of the transposed Ds elements in the next generation of the transgenic plants is also examined.

1732 **CHARACTERIZATION OF THE MAIZE TRANSPOSABLE ELEMENT, *Mu*3.**
Al B. Agellon[1], J. Kevin Haney[1], Carol C. Mahaffey[1], Karen K. Oishi[1,2]. University of Arizona, [1]Dept. of Molecular and Cellular Biology, [2]Dept. of Plant Sciences, Tucson, Arizona, 85721. Robertson's maize transposable element family, *Mutator* (*Mu*), exhibits high levels of somatic and germinal transposition. Robertson's strains are estimated to contain a very high copy number (300-400 copies) of *Mu* elements. Since the maize genome is large, approximately $5X10^6$ kilobase pairs of DNA, this copy number does not account for the observed high mutation frequency ($1x10^{-3}$-$1x10^{-5}$), if *Mu* inserts randomly. Thus, it has been hypothesized that *Mu* elements have preference for the single copy regions. There are several *Mutator* family members (*Mu1*-*Mu10*) that have now been described; *Mu*3 is a 1.8kb element which exists in approximately 15 copies in *Mu* active lines. It is also the only *Mu* element transposed into a TATA box (*ADH1*). To determine whether *Mu*3 has specificity for the promoter region, a genomic library was constructed from a *Mu* active line (*KO*287-8) displaying new *Mu*3 germinal insertions. Twenty clones containing *Mu*3 were isolated. Southern analysis of one plasmid clone (*pKA*33) indicated that *Mu*3 transposed into a single copy region. We are currently analyzing all the *Mu*3 insertion sites generated by amplification of the flanking sequences.

1733 MUTANT SELECTION FROM NICOTIANA PLUMBAGINIFOLIA PLANTS TRANSFORMED WITH THE AC/DS TRANSPOSABLE ELEMENT.
Annie Manon-Poll, Nicole Houba-Hénn, Véronique Pautot, Nathalie Bonnefoy and Michel Caboche. Laboratoire de Biologie Cellulaire, INRA, Route de Saint Cyr, 78026 Versailles Cedex, France.

Nicotiana plumbaginifolia transformants containing the Ac/Ds maize transposable elements were obtained either by Agrobacterium transformation of haploid leaf disks, or direct gene transfer into protoplasts. Agrobacterium transformed plants carried an autonomous Ac element inserted in the leader region of a kanamycin or streptomycin resistance gene (pKU3 and 1951A constructs obtained from B. Baker and J. Jones respectively). Most of the plants were diploid and either homozygote or heterozygote for the T-DNA insertion. Approximately one half of the pKU3 transformants segregated only kanamycin resistant plants due to an early Ac excision, the other half showed either no excision or a low excision frequency. Ac excision in these last transformants occurred late in plant development since Southern and IPCR analysis of the F1 progeny showed mainly new independent transposition sites, indicating that Ac can be used as a mutagen. Screening of F2 plants for mutant phenotypes is under way. Haploid or heterozygote nitrate reductase-deficient protoplasts were co-transformed with two plasmids: a plasmid encoding a truncated Ac transposase and a second plasmid carrying a NPTII labeled Ds element inserted in the leader sequence of the GUS gene. Ds excision, measured by restoration of the GUS gene activity, has been observed early after protoplast transformation. Under these conditions an increased frequency of chlorate-resistant calli was observed, compared to transformation with the Ds plasmid alone. After regeneration of kanamycin and chlorate resistant calli, the cosegregation of the two markers in the progeny will be investigated.

1734 MAIZE dSpm INSERTION MUTATIONS WHICH DISPLAY LEAKY SUPPRESSION BY Spm IN TRANS.
Victor Raboy, Gregory Bunkers and Oliver E. Nelson. USDA-ARS, Montana State University, Bozeman, MT 59717-0312.

The Suppressor-mutator (Spm) family of maize transposable elements consists of autonomous Spm elements and non-autonomous defective Spm (dSpm) elements. One characteristic of this family is that the insertion of dSpm elements into a structural gene often permits some level of structural gene expression in the absence of Spm activity, and this structural gene expression is suppressed in trans by Spm activity. The Spm's subterminal repetitive regions (SRRs) contain several interaction of a 12 bp repeat motif. It had been proposed that binding of an Spm-encoded protein to these repeat motifs blocks structural gene transcriptional readthrough, thus suppressing gene expression. The bz-m13 allele of the bronze-1 locus contains a 2.24 kb dSpm insertion in the second exon of the bz allele. In the absence of Spm activity, bz-m13 permits substantial gene expression, and this expression is fully suppressed in trans by Spm. Four intra-dSpm deletion derivatives are described which are "suppression-deficient;" suppression in trans by Spm is leaky, resulting in a diffuse bronze-1 expression. Each suppression-deficient dSpm retains at least 12 SRR repeat motifs. Thus the presence per se of these repeat motifs is not sufficient to guarantee complete suppression by Spm in trans. Some second factor such as secondary structure or element size must play a role.

1735 THE MAIZE TRANSPOSABLE ELEMENT ACTIVATOR IS METHYLATED IN ARABIDOPSIS THALIANA.
M.A. Van Sluys, K.C. Scortecci J. Tempe[1] and N.V. Fedoroff[2]. Depto de Botânica-IBUSP - CxP11461, Cidade Universitária, 05499, São Paulo/SP, BRAZIL. [2]Dept of Embriology Carnegie Institution of Washington, USA. [1]ISV-CNRS, Gif-sur Yvette France.

Gene tagging using transposable elements in plants has been demonstrate in maize and Antirrhinum majus. In order to broaden the spectrum of clonable genes, transposable elements isolated from both plants have been introduced in other plants species to see whetter they could be used as gene-tags in these heterologous systems. After co-transformation using Agrobacterium vectors, the maize transposable element Activator (Ac) has been introduced in Arabidopsis thaliana, where it was shown to transpose in root-derived calli (Van Sluys et al, 1987-EMBO J. 6: 3883-3889). Mobility of Ac was not detected in further analysis in the descendence of regenerated plants. Methylation of the 5'-untranslated leader sequence of the Ac mRNA is Reponsible for its transcriptional inactivation in maize (Kunze et al,1988, MGG, 214: 325-327). We investigated the methylation State of Ac in the S1 progeny of Regenerants where it was previously shown to be active. Our results indicate that the overall methylation in the Ac-T-DNA and in Ri-T-DNA is increased after regeneration as well as the specific methylation-sensitive region in Ac mentioned above. We are now trying to correlated this methylated state with the inactivation Ac.

1736 THE BEHAVIOUR OF *AC* IN *ARABIDOPSIS*.
Emily J.R. Lawson, Steve Scofield, Christina Sjodin, Jonathan D.G. Jones, and Caroline Dean. Cambridge and Sainsbury Laboratories, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK.

As part of the development of a transposon tagging system we are characterising the behaviour of the maize transposable elements *Ac* and *Ds* in the *Arabidopsis* genome. Using the non-lethal streptomycin resistance assay developed by Jones *et al* (1989, Science 244, 204-207), where *Ac* is inserted into the untranslated leader of a 35S:streptomycin phosphotransferase gene fusion, we are able to visualise both somatic and germinal excision events. The wild-type *Ac* element transposes only at a very low frequency in the *Arabidopsis* genome, giving an average of 0.11% germinal excisions at T2. However a *NaeI* deleted element, which has 537bp of the unusually long, CpG rich leader sequence of the transposase removed, shows a significantly increased level of excision, giving an average of 2.85% germinal excision at T2, with one transformant reaching 6.9% germinal excisions in this generation. Excision levels increased in subsequent generations, where for example one progeny plant from a single copy transformant gave a 30% level of germinal excision in the T3 generation. Extensive genetic and molecular analyses have shown that this difference is not due to higher copy number of the T-DNA insertions in the *NaeI* deleted transformants. The orientation of *Ac* in the gene fusion has been shown to have no effect on excision frequency, the deleted element giving 75% somatic excision in one orientation, and 88% in the other (percentages express number of seedlings showing any activity with respect to total number of seedlings scored). Southern blots show that approximately 70% of the progeny of plants selected as carrying a germinal excision event have new *Ac* bands. Data investigating further the effect of the *NaeI* deletion, and details of screens for tagged genes will be presented.

POSTER ABSTRACTS

1737 A HIGH FREQUENCY OF SECONDARY TRANSPOSITIONS OF Ac IN PETUNIA. Timothy P. Robbins, Matthew Jenkin and Neal Courtney-Gutterson. DNA Plant Technology Corporation, Oakland, CA 94608. Research sponsored by Florigene.

A wild type Ac transposable element has been introduced into Petunia hybrida by Agrobacterium mediated tranformation. The Ac element is located in a streptomycin (STR) resistance gene to allow cell autonomous analysis of Ac activity. Sectors of STR resistant tissue in seedling cotyledons provided evidence of somatic excision. However, the frequency of germinal excisions was low as judged by the number of fully STR resistant seedlings (generally <1%). This result was confirmed by molecular transposition screens for two transformants, indicating that only 1-2% of progeny carry transposed copies of Ac. In contrast, progeny of confirmed germinal transpositions carry, in addition to the primary transposed copy, additional transpositions to unique sites in up to 50% of self progeny. These data indicate that Ac transposes at a much higher frequency after the initial transposition out of the T-DNA environment. Implications of this result for heterologous tagging strategies will be discussed.

1738 BEHAVIOR OF *Ac/Ds*-DERIVED CHROMOSOME BREAKING STRUCTURES IN HETEROLOGOUS SYSTEMS. J. English, K. Harrison, J. Jones. Sainsbury Laboratory, John Innes Center for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK.

Chomosome breakage was the first manifestation of transposable element activity detected by McClintock. When the element *dissociation (Ds)* was located on a chromosome arm with dominant marker genes, the loss of these genes was observed dependent on the presence of a second element, *activator (Ac)*, in the genome. Two different *Ac/Ds*-derived structures associated with chromosome breakage in maize have been characterized at the molecular level: double *Ds (DDs)* has been described by Doring and Starlinger and coworkers and fractured *Ac (FAc)* has been described by Ralston, English and Dooner. To find out more about the behavior of these structures, a number of constructions resembling *DDs* and *FAc* have been introduced into tobacco and tomato by *Agrobacterium*-mediated transformation. If chromosome breakage occurs, it will make the following approaches possible: (i) we may gain insight into the mechanism of chromosome breakage, (ii) stocks with chromosme breaking structures at known loci could be used in mapping strategies, (iii) "targeted" chromosome breakage may be used to address whether the action of certain genes is cell autonomous.

The tobacco system is based on the loss of marker genes included on the T-DNA. Streptomycin phosphotransferase, beta-glucuronidase and *Agrobacterium rhizogenes rol C* have been used. Marker genes adjacent to a *DDs*-like structure are unstable somatically, dependent on *Ac* activity. A series of constructions has been made to dissect the *DDs* structure in order to determine which components are responsible for the instability. The tomato system depends on the loss of endogenous marker genes for monitoring breakage. Five T-DNA insertions carrying potential chromosome breaking structures have been mapped on the tomato genome. Molecular, genetic and cytological approaches are being undertaken

1739 THE ADH-1 GENE OF TOMATO AS "TARGET" FOR THE IDENTIFICATION OF ENDOGENOUS TRANSPOSABLE ELEMENTS. Ellen Wisman, M.S. Ramanna*, M. Koornneef# and P. Zabel. Depts. of Molecular Biology, Plant Breeding* and Genetics#, Agricultural University, Wageningen, The Netherlands.

In tomato "mutable" lines are known in which the genetic instability of various distinct mutant alleles is likely to be caused by the activity of transposable elements. As none of the mutable loci has been cloned we have chosen the Adh-1 gene as target for insertion of the transposon, using allyl alcohol selection of seeds and pollen.

In the first approach, the "mutable" lines (Adh-1$^+$/+) were crossed to an Adh-1 null mutant line. Among the F_1 population of approximately 130.000 heterozygous Adh-1$^+$/0 seeds, one Adh-1 null mutant was identified

In the second approach allyl alcohol-treated pollen from the "mutable" lines was applied to 3000 wild type flowers. Among the 761 F_1 plants derived, one Adh-1 null mutant was identified.

These two Adh-1 null mutants are currently being screened for the presence of an transposon insertion by Southern blot analysis using genomic Adh-1 clones which encompass the coding and 5'upstream regulatory region.

1740 A BINARY TRANSPOSON TAGGING SYSTEM IN *Arabidopsis* Long V. Nguyen, Wayne P. Fitzmaurice and Mark A. Conkling. North Carolina State University, Dept. of Genetics, Raleigh, NC 27695, USA.

We have established a binary transposon tagging system in *Arabidopsis* using the maize transposable element *Ac*. A general description of this system appears in another Abstract (Fitzmaurice *et al*). This binary system is composed of a *Ts* element containing the *Ac* transposase and a *Ds* element containing the *Ac* border sequences, a selectable marker gene, and a *supF* gene. Transcription of the transposase is driven either by the CaMV 35S promoter (35S-*Ts*) or by the endogenous *Ac* promoter (Ac-*Ts*). Ds excision can be monitored by assaying GUS (β-glucuronidase) activity. We have introduced these *Ts* and *Ds* constructs into *Arabidopsis thaliana* using *Agrobacterium*-mediated transformation of root explants. Data from F1 crosses of *Ts* and *Ds* transformants have shown that 35S-*Ts* catalyzes significant higher excision frequencies than Ac-*Ts*. The F2 (F1 selfed) population exhibits both somatic and germinal excision events. We are currently analyzing the reintegration of the excised *Ds* elements and its recovery using *supF* facilitated cloning.

1741 USE OF MAIZE STREAK VIRUS AS A VECTOR TO INTRODUCE A TRANSPOSABLE ELEMENT INTO MAIZE. Wen-Hui Shen and Barbara Hohn. Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

Maize streak virus (MSV) is a leafhopper-transmitted geminivirus containing one circular single-stranded DNA of about 2.7 kb. We used agroinfection as a means to test MSV mutants and possibilities to use MSV as a vector to introduce DNA into maize. An insertion mutation in the coat protein gene abolished MSV infectivity. However, the small intergenic region (SIR) was found to tolerate small insertions or deletions without abolishing MSV infectivity. PCR analysis revealed that some oligonucleotide-insertion mutants were completely stable while others suffered deletions. Deletion rearrangements were shown to depend on the sequence of the inserted oligonucleotide.

The maize transposable element *Ds1* was inserted in the SIR of MSV. Excision of *Ds1* from the MSV genome was detected only when *trans*-functions from the transposable element *Ac* were supplied either endogenously by the recipient maize plant or by co-transformation with *Agrobacterium* carrying a genomic *Ac* clone. The junction sequences left on the MSV genome after excision revealed "footprints" typical of transposition as described for maize. Thus, the use of MSV replicon provides a rapid and simple tool for the investigation of the excision of tansposable elements in whole maize plants.

1742 TRANSPOSITION OF THE AC ELEMENT IN POTATO. Whitty P., J. Lyon, S. Howard and A. Kumar. Scottish Crop Research Institute, Invergowrie, Dundee, Scotland.

Genes encoding unknown products have been isolated from maize and Antirrhinum by gene tagging using well characterized endogenous transposable elements. Transposable elements have not been sufficiently well characterized for gene tagging in the majority of crop plants. However, Ac transposon of maize has been shown to transpose in other plants including potato (Knapp et al. 1988).We have introduced Ac transposon into potato using the Ti plasmidconstructs of Baker et al. (1986). In construct pKU3 Ac lies between the kanamycin resistance gene, NPTII, and a promoter region such that NPTII expression is prevented. Excision of Ac leads to restoration of a kanamycin resistant phenotype. Characterization of transformed plants by the polymerase chain reaction (PCR) using different primer pairs enables us to demonstrate transformation with pKU3, excision of Ac from the pKU3 T-DNA and the continued presence of Ac in the genome following excision. We have observed Ac excision in around 95% of pKU3 transformed plants but less than 50% of tested plants show the continued presence of Ac transposon in the genome after excision.

Baker B., J. Schell, H. Lonz and N. Fedoroff. (1986). Proc. Natl. Acad. Sci. USA. 83 4844-4848.

Knapp S., G. Coupland, H. Uhrig, P. Starlinger and F. Salamini. (1988) Mol. Gen. Genet. 213 285-290.

POSTER ABSTRACTS

1743 T-DNA INSERTIONAL MUTAGENESIS IN ARABIDOPSIS: SPECTRUM OF MUTANTS OBSERVED. Kenneth A. Feldmann, Nancy Forsthoefel and Yewen Wu. University of Arizona, Dept. of Plant Sciences, Tucson, AZ 85721.

More than 12,000 transformants of Arabidopsis have been generated using the Feldmann and Marks (1989) seed infection/transformation procedure. These transformants contain 1-4 inserts, with an average 1.4 inserts per transformant, as assayed on kanamycin. More than 8,000 of these transformed lines have been screened, under greenhouse conditions or on vertically-oriented agar plates, for visible alterations in phenotype. More than 1,000 putative mutants were observed. These mutants fall into several, somewhat overlapping, classes including, seedling-lethal, size variant, embryo-defective, reduced-fertility, pigment, dramatic (true developmental), and physiological. Genetic analysis of a number of the mutants indicates that if the line is segregating 3:1 (wild-type:mutant) there is a >80% probability that the altered phenotype is due to a T-DNA insert. If, however, there is a deficiency of mutant plants among the T3 family (x<3:1) there is a greatly reduced probability that the mutation will cosegregate with a T-DNA insert. The genetics in these latter lines indicates that there is some type of somaclonal mutation in the transformed plant such that only a sector is transformed and thus few mutant seeds are generated. A summary of how many transformants have been characterized, how many tagged (and untagged), and our analysis of how many transformants will be required to reach saturation will be presented in the poster. We will also show some of our latest mutants.

1744 TRANSPOSITION EVENTS IN TRANSGENIC DATURA INNOXIA MILL. HARBOURING DIFFERENT Ac CONSTRUCTS. Martin Meixner, Thilo Schmidt-Rogge, and Otto Schieder. Freie Universität Berlin, Institut für Angewandte Genetik, Albrecht-Thaer-Weg 6, 1000 Berlin 33, GERMANY

Transposition of various "jumping genes" especially Ac/Ds elements is currently intensively investigated in different plant species with the goal of targeted transposition of these elements (transposon tagging), e. g. in Lycopersicon esculentum (Yoder et al. (1990) Horticult. Biotech. 11, 85-94), Arabidopsis thaliana (Schmidt et al. (1989) Mol. Gen. Genet. 220, 17-24), Nicotiana tabacum and Petunia hybrida (Haring et al. (1989) Plant Mol. Biol. 13, 189-201).

In our recent work we demonstrated, that the Ac element from Zea mays is also able to transpose in Datura innoxia. This was done by showing NPT II-activity after exision of the transposable element from constructs, carrying the Ac element in the untranslated leader of the neomycin phosphotransferase gene (Baker et al. (1987) EMBO J. 6, 1547-1554) as well as by Southern hybridization. In the hybridization experiments we confirmed that Ac is able to excise from the genome of transgenic Datura plants. In further experiments we were also able to show reintegration of the transposon into the host genome at different sites.

In a second set of experiments we studied the behaviour of the Ac element in transgenic Datura lines during long time growth in tissue culture. We found that Ac in all lines under investigation is very stable after reintegration in long time tissue culture as well as in regenerated plants. Therefore our conclusion is , that somatic excision is a very early event in this special plant/transposon interaction system and that transposition is stopped at the level of protoplast regeneration or first division cycles of regenerating plant cells.

1745 TRANSACTIVATION OF *Ds* BY *Ac*-TRANSPOSASE FUSIONS IN HETEROLOGOUS PLANT SPECIES. Cajus Rommens, H John Nijkamp and Jacques Hille, Department of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands. The maize transposable element *Ac* can excise throughout development of transgenic tobacco plants. This feature of *Ac* may complicate the generation of stable and independent insertions which are required for gene tagging experiments. To regulate transposition, the transposase coding region of *Ac* has been fused to several well defined promoters isolated from *Petunia hybrida*. Transactivation of *Ds* by these transposase fusions was compared to transactivation by the regular *Ac* element and by CaMV35S/transposase. For our studies, a phenotypic assay based upon *Ds* excision restoring hygromycin resistance was used. The transposase fusions induce excision of *Ds* on callus level, indicating functioning of the constructs. With an element containing the flower specific chalcon synthase A (CHS A) promoter, the frequency of transactivation is comparable to that with CaMV35S/transposase and twice as high as with *Ac* itself. With an element containing the chalcon isomerase -B (CHI B) or -A_2 (CHI A_2) promoter, specific for anthers and pollen grains respectively, the frequencies are reduced considerably. On plant level, transactivation of *Ds* can be observed with CHS A/transposase and CHI B/transposase. The somatic excision frequency in crosses of transgenic tobacco plants is increased in case of CHS A/transposase and reduced in case of CHI B/transposase. Indications were obtained suggesting an "acceleration" and "delay" respectively in the timing of excision. The germinal excision frequencies are comparable to those with *Ac*. The element with the CHI A_2 promoter does not induce somatic or germinal excision of *Ds*. Our results imply that by modifying transposase expression, the frequency and timing of *Ds* excision can be altered. The lack of complete tissue specificity may be due to low concentrations of transposase molecules sufficient to induce transposition. At high transposase expression levels, however, transactivation does not reach 100%, suggesting that transposase might not be the only limiting molecule in the transposition process.

1746 ANALYSIS OF PLANT GENE EXPRESSION USING TRANSPOSABLE ELEMENTS IN TOMATO. M.J.J. van Haaren, Department of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

To study the mechanism of transposition of the maize Ds-element in Tomato, we introduced a marker gene in the non-autonomous transposable element, Ds. The coding region of this marker gene (GUS) is proceeded by a short (-90) CaMV-35S 5'-region and contains a complete nos 3'-region. This short promoter will not induce expression of the GUS gene when introducedin the plant genome. However, since this gene already contains a TATA-box, its expression will be readily induced when inserted near a plant promoter region. Because this gene with its truncated promoter is present within the Ds-element, it will be possible to obtain a great number of different independent insertions in the plant genome.

Using this strategy we hope to obtain more information on the integration sites of transposable element like Ds. Furthermore by *in situ* analysis of the obtained GUS expression patterns, plant promoters with an interesting tissue and/or developmental expression pattern can be isolated. Transposable elements have been used for the isolationof insertional mutants in plants like *Anturrhinum majus* and *Zea mais*. We started experiments in which we use the non-autonomous transposable element Ds in tomato, for the isolation of mutants induced by trans-activation of gene expression. To enhance the expression of genes in the plant genome the transposable element carries a strong promoter (Big Mac) or enhancer element (35S-enhancer). Upon insertion of the transposable element into a locus of the plant genome, transactivation of thegenes present next to the insertion site will be induced. In this way we hope to obtain a number of interesting mutants that can not be isolated by insertional mutagenesis, because the mutation would either not show or be lethal.

1747 TRANS-ACTIVATION OF A DS ELEMENT IN ARABIDOPSIS Lluís Balcells and George Coupland. Cambridge Laboratory, John Innes Centre for P.S.R., Colney Lane, Norwich NR4 7UJ, England, U.K.

A transposon tagging system using the maize *Ac* element is being developed in *Arabidopsis thaliana*. In order to increase the very low transposition frequency of the native element in *Arabidopsis* and make any mutations stable, a two component system is being used. The transposase is provided by the *Ac* ORF under the control of different promoters. The *Ds* element was inserted in the leader region of a Streptomycin resistance (Str^R) gene. When *Ds* excises, the Str^R gene becomes active and these somatic excision events are visualised as green sectors on the cotyledons (variegated phenotype) of seedlings growing on a medium containing streptomycin. When an excision event takes place in the cell lineage leading to gametes, full green plants (germinals) are found among the offspring of that plant. We have tested the Ac promoter (nt 32-355 fused to 736-Right end of *Ac*). 5 independent transformants containing this fusion were crossed to a *Ds* transformant, to introduce both constructs in the same genome. In all cases, *Ds* excises in the F1, albeit at very different levels: 2.3 and 23.6 % of individuals variegated being the two extremes. Moreover, among individuals with the same genotype there are highly and low variegated. Preliminary results with one transformant show that F1 individuals highly and low variegated yield F2 plants with very similar % of variegation. Some of the F1 variegated plants analysed segregated germinals in the F2. A thorough F2 analysis, investigating somatic and germinal excision frequencies and frequency of *Ds* reintegration is under way.

1748 TOWARDS TRANSPOSON-TAGGING IN POTATO Karin M. Blok, Arjen v. Vark, Nanne M. Faber and Jacques Hille -Free University, Dept. of Genetics, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

As there is no genetic map of potato, and many interesting genes have not been localised on the existing RFLP-maps yet, the choice of methods to isolate genes from potato is limited, if the geneproduct is not known. To isolate genes by transposon-tagging, no knowledge of either the gene-product, or the location of the gene is necessary, so we chose this method to isolate disease resistance genes from potato.

Because there is no well-characterised endogenous potato transposon available, we make use of the Ac/Ds transposons from maize. For our tagging experiments we prefer to use the non-autonomous Ds-element, as it can not transpose without transactivation by the Ac-tranposase. This gives us the possibility to regulate transposition, by the way and timepoint the transposase is supplied (see abstract of C. Rommens et al.).

The transposon-tagging construct contains a selectable marker for transformation, one for excision of the transposon, and a marker on the Ds-element itself. This allows us to follow the Ds-element genetically after transposition. Using *Agrobacterium tumefaciens* over 400 independent diploid potato transformants were obtained, that expres the correct markergenes. In maize and tomato Ds-elements are known to transpose mainly over short distances on the same chromosome. Assuming the same behaviour in potato, transformants with a T-DNA (and thus the transposon) inserted close to a resistance gene will be selected first, to enlarge the chance that the tranposon "hits" the gene. This selection is done by both RFLP analysis and classical segregation analysis of the marker on the T-DNA and the gene of interest. In transformants that show such linkage, the non-autonomous Ds-element will be activated by bringing in the Ac-transposase via crossing or retransformation. By selection on markers, excision and reintegration of the Ds-element can be confirmed, and plants can subsequently be tested for inactivation of the resistance gene, due to insertion by the transposon.

If an insertion-mutant is found, the construction of the Ds-element we use allows rapid cloning of the plant-DNA that flanks the transposon (containing the sequence of the resistance gene), using iPCR or electroporation of *E. coli*. This cloned sequence can be used to isolate the intact plant gene from a genebank.

POSTER ABSTRACTS

1749 *Ac/Ds* TRANSPOSON TAGGING SYSTEM IN *ARABIDOPSIS*.
Candace S. Waddell, Mary A. Honma and Barbara Baker.
Plant Gene Expression Center, USDA-ARS, Albany, Ca. 94710.

We have introduced a transposon mutagenesis/tagging system into the model plant, *Arabidopsis thaliana*. Transposons are powerful genetic tools. Our system is based on the well characterized maize transposon family *Activator/ Dissociation* (*Ac/Ds*) and consists of two elements: a stable *trans*-activator, Ac^{st}, and a responsive element, *Ds*. *Ds* transposes to other sites when *trans*-activated by Ac^{st} but is stabilized when Ac^{st} segregates away from *Ds*. Consequently, insertions are created that act as stable genetic and physical markers in the genome. Stable Ac^{st} *trans*-activators contain neither a functional 5' nor 3' terminus. In addition, the endogenous *Ac* promoter has been replaced with one of three strong heterologous promoters: the CaMV 35S promoter or the promoter from the *Arabidopsis rbcS* or *CHS* genes. The Ac^{st} T-DNA carries *DHFR*, encoding resistance to methotrexate, and *GUS*. *Ds* has an internal deletion of the transposase coding region and carries a mutant *Arabidopsis ALS* gene that confers resistance to the sulfonylurea herbicide, chlorsulfuron. *Ds* resides in the 5' untranslated region of a chimeric 1' *NPTII* gene such that *Ds* excision results in the expression of kanamycin resistance. The *Ds* T-DNA carries hygromycin and nopaline genes. The use of selectable and screenable markers allows us to genetically monitor both the presence of Ac^{st} and *Ds*-bearing T-DNAs, as well as the *Ds* excision and reintegration events. All T-DNA constructs were introduced into *Arabidopsis* utilizing methotrexate, chlorsulfuron or hygromycin for selection. Segregation analysis of selfed progeny using these same selective agents demonstrated that most transformants contain T-DNA insertions at a single locus. Two independent *Ds* lines have been crossed to lines carrying the Ac^{st} elements. Progeny from these crosses will be characterized to assess the ability of the Ac^{st} elements to *trans*-activate *Ds*. Results from this analysis will be presented.

1750 VARIABLE PATTERNS OF TRANSPOSITION OF THE MAIZE ELEMENT ACTIVATOR IN TOBACCO.
Janis Keller, Elizabeth Harper, Edward Ralston and Hugo Dooner.
DNA Plant Technology Oakland, CA 94608

The strategy to be followed in a transposon tagging experiment will be largely determined by the transposition pattern of the transposon in question. With a view to utilizing the maize element Activator (Ac) as a transposon tag in heterologous systems, we have investigated the pattern of transposition of Ac from six different loci in transgenic tobacco. We have isolated germinal revertants from plants carrying mutable alleles of the antibiotic resistant gene streptomycin phosphotransferase (SPT) and mapped the location of the transposed Ac (trAc) elements relative to the donor SPT gene. A comparison of the distributions of trAcs among the six loci reveals that although the receptor sites for trAcs tend to be linked to the donor locus, the pattern of Ac transposition in tobacco displays surprising locus-to-locus variation. Some trAc distributions show the same tight clustering around the donor locus previously seen in maize, while others are more dispersed.

1751 DEVELOPMENT OF TRANSPOSON MUTAGENESIS SYSTEM IN *Lactuca sativa*.
Chang-hsien yang and Richard W. michelmore. University of California. Davis. Department of Vegetable crops. Davis. CA 95616

It is difficult to clone genes, such as disease resistance genes, whose products and biochemical and physiological process are unknown. Recently, transposable genetic elements have been used to identify and tag genes. We are developing a system for transposon mutagenesis in lettuce, *Lactuca sativa*, by using the transposable element, *Ac* from corn. Two constructs contained *Ac* from maize *waxy-m7* locus have been transformed and tested in lettuce. No *Ac* transposition could be detected in either construct. However, by using modified constructs of *Ac*, 1) pKU4-8 in which the activator (putative transposase cDNA) expressed from the T-DNA 2' promoter was separated from the mutator (*Ds*) which interrupted the neomycin phosphotransferase II (NPT II) gene; 2) pKU3 which contained a *Ac* interrupted the neomycin phosphotransferase II (NPT II) gene, excision was observed in transformed lettuce calli by using a phenotypic assay based on kanamycin resistance. The observed excision frequency increased with time and reached about 82% in pKU4-8 and 42% in pKU3 transformed calli four weeks after cocultivation. The excision events were further confirmed by using PCR amplification of the excision site and DNA sequencing. Excision and reintegration also have been confirmed by using Southern analysis. Therefore *Ac* is capable of excision in lettuce callus and that the cDNA expressed from the 2' promoter results in higher rates of excision of the *Ds* element. In the future, we will use different constructs to control transposition by separating the transposing and transposase activities and will use this system to clone genes for resistance to *Bremia Lactucae*, the fungal pathogen causing lettuce downy mildew.

1752 *MU9*, A POTENTIAL AUTONOMOUS *MUTATOR* ELEMENT.
Jane Hershberger, Christine A. Warren and Virginia Walbot. Dept. of Biological Sciences, Stanford University, Stanford, California 94305-5020.

The *Mutator* family of transposable elements causes a very high level of forward mutation in *Zea mays*, making it a useful tool for transposon tagging and for the generation of new mutants. The regulation of *Mutator* activity is poorly understood, however, because the autonomous element controlling the excision and reinsertion of the *Mutator (Mu)* elements has not yet been identified. At least eight different types of *Mu* elements have been isolated thus far, but none of these has been shown be an autonomous element or to encode a transposase. These elements, named *Mu1* through *Mu8*, have unique central regions flanked by conserved ~215 nt terminal inverted repeats. We isolated *Mu9* after it transposed into *Bronze-2*, creating a mutable allele that has a phenotype resembling that of other *Mu*-induced mutations. The 4.7 kb *Mu9* element has the characteristic *Mu* terminal inverted repeats, and it is flanked by 9 bp host duplications. *Mu9*-hybridizing transcripts are present only in active *Mutator* lines, not in inactive *Mutator* lines or in non-*Mutator* lines. In addition, *Mu9* hybridizes to a fragment of its own size in DNA from individuals of the *a1-Mum2* line that have been shown to contain a genetically defined *Mutator* regulatory element; this fragment is absent from their siblings that do not contain the regulator. *Mu9* elements are present in multiple copies in *Mutator* lines and are absent from most or all of the standard inbred lines. We have cloned and sequenced the entire *Mu9* element, and we will present information on its expression, molecular characterization, and the proteins it encodes.

1753 INSTABILITY AT THE TMV RESISTANCE LOCUS *N* IS ASSOCIATED WITH LOSS OF A TIGHTLY LINKED MARKER.
Reinhard Hehl and Barbara Baker. Plant Gene Expression Center, USDA-ARS/UC Berkeley, Albany, CA 94710.

Tobacco mosaic virus resistance is mediated by the *N* locus in tobacco. The *N* locus is a dominant monogenic trait and its presence is directly correlated with the ability of the plant to produce a hypersensitive response (HSR) upon TMV infection. We have determined that the *N* locus is unstable, not unlike other dominant plant resistance loci. We have investigated the instability of the *N* locus and found that instability may be attributed to deletions or chromosome loss in the vicinity of the *N* locus. Tobacco cultivars Petite Havanna (SRI, nn) and Samsun NN were crossed and among 93000 heterozygous F1 plants screened for loss of *N* function 49 *N*- plants were obtained. No reversion to *N*+ was observed in large F2 populations obtained through selfing and out-crossing of *N*- plants indicating that loss of *N* function is stable. Analysis of five target DNA fragments of transposed *Ac* elements in tobacco revealed that one of them detects an RFLP between tobacco cultivars Samsun NN and Petite Havanna (SRI, nn). This DNA fragment hybridizes to distinct RFLPs in *Nicotiana sylvestris* and *N. tomentosiformis* the progenitor species of tobacco and in *N. glutinosa* the source of the *N* locus in cultivar Samsun NN. While cultivar Petite Havanna (SRI, nn) harbors only the *sylvestris* and *tomentosiformis* RFLPs cultivar Samsun NN harbors RFLPs specific for *sylvestris* and *glutinosa*. Genetic segregation analysis established that the *glutinosa* specific RFLP is tightly linked to the *N* locus (<1CM). No recombinants were detected. Furthermore, 23 of 31 *N*- plants isolated from Nn heterozygotes lost the *glutinosa* specific RFLP while a control population of 41 Nn plants not selected for loss of *N* function retained the *glutinosa* RFLP. The impact of these results on strategies for isolating *N* will be discussed.

1754 ANALYSIS OF THE ACTIVITY OF THE MAIZE TRANSPOSABLE ELEMENT*Ac* IN *ARABIDOPSIS*.
Janis Keller, Eda Lim, Douglas James, and Hugo K. Dooner. DNA Plant Technology Corp., Oakland, CA 94608

The *Ac*:SPT assay system developed by Jones et al. (Science, 1989) is being used to study *Ac* activity in *Arabidopsis* and to determine how useful transposon tagging will be for gene isolation in this important model system. In the assay, seeds are germinated on streptomycin and bleach unless *Ac* excises from the SPT gene. Green variegation against a white background indicates somatic *Ac* activity and fully green seedlings arise as a consequence of germ-line excision events. Eighty independent transformants have been generated and characterized for *Ac* copy number and *Ac* activity. In general we find that single copies of the transposon have very low somatic activity and rarely exhibit germinal excision events. Multiple copies of Ac give more activity both somatically and germinally. About 60% of the germinal excisions result in reinsertions as determined by DNA blot analysis. However, early premeiotic transpositions are observed such that more than one green progeny from an individual may arise from the same excision event. Our efforts to isolate genes by Ac tagging and to increase Ac activity in Arabidopsis will be discussed.

POSTER ABSTRACTS

1755 *CHAMELEON*: A NOVEL CLASS OF MOBILE ELEMENT IN RICE. Thomas E. Bureau, *Gurdev S. Khush and Susan R. Wessler. University of Georgia, Department of Botany, Athens, GA 30602 *International Rice Research Institute, P.O. Box 1099, Manila, Philippines

Two novel mobile elements were found to be associated with the unstable *waxy* mutations resulting from a wide hybrid cross between the domesticated *Oryza sativa* and its wild relative *O. officinalis*. Both elements appear to be mutable in the waxy and revertant lines, and are moderately repeated in the rice genome. We suggest that a mechanism such as gene conversion may account for this mutability. Furthermore, we are pursuing the notion that the mutability is responsible for the observed changes in *waxy* gene expression. The elements were named *chameleon* (*cha* 1 and 2) due to this mutable characteristic. *Cha* 1 is located within an intron of the rice *waxy* gene, while *cha* 2 is located upstream of the *waxy* transcription start site. Both are 122 bp in length, share 75% nucleotide identity and are flanked by 16 bp direct repeats that appear to be target site duplications rather than element termini. The elements lack other structural features of transposons (i.e. inverted repeats) or retrotransposons (i.e. long terminal repeats, *gag*, *pol*, etc.). There is evidence for site specific insertion, since the *cha* 1 and 2 target sites share nucleotide homology with one another. Wild species of the *Oryza* genus, including representatives from all genome types, appear to lack one or both of these elements. This suggests that *cha* 1 and 2 were inserted into *waxy* relatively recently. Finally, we are investigating the possibility that *cha* 1 and 2 may be involved in other unstable phenotypes isolated from the progeny of this wide hybrid cross.

1756 CHARACTERIZATION OF *Ds* INSERTION SITES IN TRANSGENIC TOMATO Caius Rommens, Paul Dijkwel, George Rudenko, Bert Overduin, H John Nijkamp and Jacques Hille, Department of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

To study transposition in tomato, a *Ds* element was constructed which contains the GUS gene and the chloramphenicol resistance gene and the origin for replication of pACYC184 between 0.5 kb *Ac* terminal sequences. The GUS gene allows *Ds* to be genetically followed after transposition. The bacterial plasmid sequences enable *Ds* and flanking plant DNA sequences to be rescued from genome. Four transgenic tomato plants containing a single copy of *Ds*, mapped on either chromosome I or -III, were selected for our experiments. *Ds* was mobilized by crossing the *Ds* plants with "tester" plants containing an active *Ac* element. New *Ds* insertions are being analyzed with plasmid rescue. This technique can efficiently be carried out by electrotransforming *E.coli* cells with fragmentized and subsequently circularized genomic DNA of F1 plants. Up to 10 kb Ds flanking DNA fragments have been recovered from the plant genome. The data that will be presented refer to specificity of *Ds* insertion and distance of transposition.

1757 TRANSPOSITION OF Ac,Ds IN ARABIDOPSIS: UTILIZATION OF A TWO-ELEMENT SYSTEM INCLUDING MARKED Ds ELEMENTS. Robert Masterson, Christoph Grevelding, Verena Fantes, Albrecht von Menges, Elke Kemper, Marius Pfefferle, Detlef Becker[1], Peter Starlinger[1] and Jeff Schell. Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG; [1]Institut für Genetik, Universität zu Köln, D-5000 Köln 41, FRG.

We have developed and introduced an Ac/Ds transposable element system into Arabidopsis for the purpose of insertional mutagenesis. The system consists of two parts: transgenic plants containing T-DNA constructs including Ac transposase and separate constructs which harbor marked Ds elements. The Ac constructs include a tandem CaMV 35S promoter followed by a TMV translation leader sequence and different truncated coding regions of Ac. A hygromycin resistance gene is located adjacent to the Ac sequence on the same T-DNA, thus transgenic plants which are resistant to hygromycin cosegregate with Ac. The marked Ds element (Ds-DHFR) contains a dihydrofolate reductase gene, driven by a CaMV 35S promoter, and is located in the leader sequence of an NPTII gene. We have previously demonstrated that Ds-DHFR elements transpose efficiently in Arabidopsis and tobacco. Excision and reinsertion events can be monitored by selection with methotrexate (Ds-DHFR) and kanamycin (excision).

1758 RETROTRANSPOSONS ARE UBIQUITOUS IN PLANTS. Hirohiko Hirochika, Atsushi Fukuchi and Rei Hirochika. Department of Molecular Biology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki 305, JAPAN

The general method to clone plant retrotransposons have been developed and used for the cloning of rice retrotransposons (see another presentation by Hirochika et al) This method was applied to the cloning of retrotransposons in Arabidopsis thaliana The presence of about 200 copies of retrotransposons was suggested on the Arabidopsis genome. Structure of two families of retrotransposons (Tat1, Tat2) was analysed The partial sequencing of reverse transcriptase domain of Tat2 revealed the presence of amino acid sequence homologous to the corresponding regions of plant copia-like elements Tal-3 and Tat1, and copia itself. By using highly conserved amino acid sequences in this domain, we have developed the method to amplify the sequences of unidentified retrotransposons by PCR This method was used to probe the presence of retrotransposons in different plant species Thirty plant species including a moss, gymnosperms and angiosperms were examined and shown to have retrotransposons with no exception.

1759 RETROTRANSPOSONS AS MAJOR TRANSPOSONS IN RICE REVEALED BY A NEW CLONING METHOD. Hirohiko Hirochika, Atsushi Fukuchi, Kazuhiko Sugimoto and Yuichi Katayose. Department of Molecular Biology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki 305, JAPAN.

The general method to clone plant retrotransposons have been developed. This method is based on the sequence conservation at the primer binding sequence for reverse transcription. By using this method, cloning of three families of retrotransposons (Tos1-Tos3) was succeeded in rice plant. Southern blotting analysis showed that the sequences homologous to Tos1-Tos3 are distributed in wild rice species as well as cultivated rice species. The copy number and genomic location of the families vary in different strains of some wild rice species, suggesting that these elements are still active. The PCR and Southern blotting analyses showed that the transposition of Tos3 occurred after or during the Indica-Japonica differentiation, and that the transposons were inactivated by structural alteration and/or DNA methylation after that time. The pol (reverse transcriptase) region of each Tos3 transposon is displaced by a unique unknown, probably cellular, sequence. The structure and expression of these sequences are under investigation The total copy number of retrotransposons was estimated to be about 1000 on the rice genome. Preliminary data suggested that rice plant has about 30 different families of retrotransposon. These results indicate that the retrotransposons are major transposons in rice as in yeast and Drosophila.

1760 DIVERGENCE OF THE RICE *WAXY* GENE BY INSERTION OF MOBILE DNA ELEMENTS INTO INTRONS. Masaaki Umeda, Hisako Ohtsubo and Eiichi Ohtsubo Institute of Applied Microbiology, University of Tokyo, Tokyo 113, Japan

The *waxy* (*wx*) gene of a rice strain, *Oryza glaberrima*, was cloned, and its nucleotide sequence was determined. A waxy mutant of *O. glaberrima* was found to contain a nonsense codon in exon 10 of the *wx* gene, which may be responsible for the reduction or lack of the *Wx* protein in endosperm Comparison of the *wx* sequences between *O. glaberrima* and *O. sativa* revealed that there were differences in a few bases with substitutions and one-bp insertions/deletions. The most striking difference between the two sequences was a 139-bp deletion (or insertion) in intron 10 of *O glaberrima* (or *O. sativa*). In *O. sativa*, 125-bp of the 139-bp sequence is flanked by direct repeats of 14-bp. A sequence homologous to this was found in intron 1 which is also flanked by 14-bp direct repeats. The sequence was interspersed in the rice genome with some sequence heterogeneity, suggesting that they are SINEs (short interspersed elements) in the plant system. We also identified a transposon-like DNA sequence in intron 13 of both *O. glaberrima* and *O. sativa*: the sequence has long terminal inverted repeats and is flanked by direct repeats of four nucleotides, and was present in multiple copies in the rice genome, indicating that it is the transposable element in rice. This and the above results suggest that mobile DNA elements have diversified the rice *wx* gene.

POSTER ABSTRACTS

1761 A TRANSPOSABLE DNA ELEMENT OF *Solanaceae*. Yasushi Yoshioka, Shogo Matsumoto*, and Yasunori Machida. Department of Biology, Nagoya University, Chikusa-ku, Nagoya, Japan. *Biochemical Research Institute, Nippon Menard Cosmetic Co., Ltd., Ogaki, Gifu-ken, Japan

We found a new family of repetitive sequences which are dispersed in the genome of *Nicotiana tabacum*. Its copy number was several thousands per haploid genome. Homologous sequences to this family also exist in the genomes of *N. glauca, N. langsdorfii*, tomato, and petunia but do not exist in the genomes of maize, wheat, rice, and *Arabidopsis*. We have cloned three independent repetitive sequences of this family which are adjacent to unique regions in chromosomes of *N. tabacum*. Sequence analyses of these revealed that length of each varies between 35 and 158 bp, and it was flanked by a direct duplication, the size of which is variable between 4 and 10 bp. These repetitive sequence has neither open reading frame nor terminal inverted repeats but have the TTG track of variable size (TTG)n at one of termini. By computer data base search, we found that the repetitive sequences are highly homologous to the 123 bp insertion sequence found in the 5' upstream region of the pathogenesis-related 1c protein gene of *N. tabacum* (EMBL accession no. X17681) which was also flanked by a direct duplication of 14 bp and a intron of tomato ATP-dependent protease gene (EMBL accession no. TOMCD4A). Both of them also have the (TTG)n sequence at one of termini. Structural comparison of these repetitive sequences showed that their boundaries adjacent to the (TTG)n sequence were nearly identical, whereas the another boundaries varied from one repeat to another. From these results, we suppose that these repetitive sequences are members of a transposable DNA element which exists in the genomes of a wide variety of *Solanaceae* plants

1762 DEVELOPMENT OF A GENE TAGGING STRATEGY IN HIGHER PLANTS USING A YEAST TY ELEMENT Yongbiao Xue[1], Brian S. Cox[1], Cathie Martin[2] and Philip M. Mullineaux[2]. [1] department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, U.K. and [2] John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, U.K.

Yeast Ty elements are retrotransposons that are similar to retroviral provirus. Retrotransposition is a replicative process involving reverse transcription of Ty mRNA and integration of Ty cDNA into the genome. It has several advantages over DNA transposons for gene tagging, for example, the retrotransposition rate is inducible and transposition events seem to be random.

A yeast Ty element, Tyl-15, was placed under control of the CaMV 35S promoter after removing its own promoter. An expression cassette containing a selectable marker gene was inserted just upstream of the 3' LTR in the reverse orientation as Ty transcription. The coding region of the marker gene was disrupted by an intron from a tobacco *rbs* gene cloned in the same orientation as the Ty transcription. After retrotransposition the intron would be spliced out and the function of the selective marker restored. The modified Ty element was introduced into a plant transformation binary vector. The binary construct was used to transform tobacco mediated by *Agrobacterium tumefaciens*. Results of primary transformant analysis will be presented. Possible further modifications for Ty element in order to make the element active in plants will also be discussed.

1763 PRESENCE OF THE RETROTRASPOSON Tnt1 IN SOME NICOTIANA Spp PLANTS AND THEIR TISSUE CULTURE. °Chiara Geri, *Edi Cecchini, *Agnese Vivaldi and *Mauro Durante.
°Institute of Mutagenesis and Differentiation, Italian Res. Council, Pisa, Italy
*Department Agr. Plant Biol., Genetics sect., University of Pisa, Italy

After the isolation of the first tobacco transposable element, Tnt1, and its characterization, its presence has been demonstrated by Southern blot analysis in Nicotiana plumbaginifolia and in many Solanacee species.(Grandbastien M.A. et al., 1989, Nature 337, 376). Therefore we analysed the Tnt1 presence and copy numbers in a tumorous amphidiploid hybrid between N. glauca and N. langsdorfii, its non tumorous mutant and the parental strains. There are not clear differences among the four strains in the restriction enzymes patterns, nor during the cell cycle in culture, thus confirming the idea that retrotrasposons are known to excise at very low frequencies. It is not known whether the Tnt1 is active in plants throughout the life cycle. But striking differences between the restriction patterns of DNA extracted from differentiated tissues as leaves, and from cells in culture, especially as far as the enzymes AccI, BamHI, XbaI are concerned, have been observed, therefore supporting the suggestion that Tnt1 might have been activated by tissue culture, as reported for Ac in maize. The purpose of this work is to determine if the transposon activity could be related to the normal and abnormal differentiation patterns "in vivo" and especially "in vitro", that is tipical of the above mentioned Nicotiana species.

1764 ISOLATION OF ECERIFERUM (*CER*) MUTANTS OF *ARABIDOPSIS THALIANA* BY T-DNA INSERTIONAL MUTAGENESIS. [1]John McNevin, [2]Kenneth Feldmann and [1]Bertrand Lemieux, [1]Department of Biology, York University, Toronto, M3J-1P3 Canada and [2]Department of Plant Science, University of Arizona, Tucson, Arizona, 85721 USA.

We have isolated 5 *Arabidopsis thaliana* mutants with deviating epicuticular wax layers (i.e., *cer* mutants) by screening 7,100 transformed lines produced by the seed transformation method. In 4 of the 5 families studied, all the individuals with the *cer* phenotype were found to be kanamycin resistant. In accordance with this result, we found that all the mutant individuals of these 4 lines had T-DNA insertions after probing Southern blots of EcoRI digests with a right border specific probe (H23). Three *cer* mutant individuals from the fifth line (2227) were not kanamycin resistant and were found not to have a T-DNA insertion. Complementation crosses with the 20 known *cer* mutant lines indicated that our 4 tagged lines correspond to the *cer1* (2824), *cer2* (7083), *cer3* (1096) and *cer6* (5627) loci. By using the pBR322 replication origins and the ampicillin resistance markers located within the T-DNA, we were able to rescue plasmid clones of the right and left borders of the T-DNA insertions which will be used to isolate cDNA and genomic clones of the **CER-1, CER-2, CER-3** and **CER-6** genes. We will compare the inheritance of RFLP markers revealed by the genomic clones with that of the *cer* phenotypes. We have also analyzed the chemical modifications of the *cer* mutant wax layers relative to that of wild-type plants. As the epicuticular wax layer is the plant's first line of defense against insects, we feel that *cer* genes will be useful as molecular probes in RFLP assisted plant breeding programs aimed at increasing insect pest resistance as well as to the plant viruses the latter carry. This work was supported by a grant from NSERC to B. Lemieux, we acknowledge the DuPont company for providing the transgenic plants.

1765 CHARACTERIZATION OF THE *Ac2* TRANSPOSABLE ELEMENT OF MAIZE. John C. Osterman and Mei Kuang Lu. University of Nebraska-Lincoln, School of Biological Sciences, Lincoln, NE 68588

We have been characterizing a newly arisen transposable element called *Ac2*. The *Ac2* element was identified by its action on the *Ds* element inserted at *bz2-m*. The *Ac2* element causes transposition of this *Ds* element to occur late in development resulting in small dots of color on a bronze background. Two doses of *Ac2* are required to see transposition and three doses increase the frequency of transposition. Of the *Ds* elements tested, only the element in *bz2-m* responds to the presence of *Ac2*. We have examined lines segregating for *Ac2* by Southern blotting using an *Ac* specific probe. We have been unable to detect a fragment that cosegregates with the mutability of *bz2-m* that we use detect the presence of *Ac2*. In order to obtain a clone of *Ac2* we have screened for mutants at several loci. Heritable mutants have been obtained at *A, C, Sh* and *Bz*. Most of these mutants are stable showing no sign of mutability. Our analysis has concentrated on a mutable *c* allele. This allele is nonautonomous but responds to *Ac2*. Small sectors of color on a colorless background are observed with a single dose of *Ac2*. Germinal revertants occur at a frequency of 10^{-4}. We are in the process of analyzing this allele using a clone of the *C* as a probe.

1766 REPEATED SEQUENCES IN PINE. M. Carol Alosi, Michael Devey, Claire S. Kinlaw. Institute of Forest Genetics, U.S. Forest Service, Berkeley, CA 94701.

Pines, like other coniferous plants, have large genomes (10-20 pg/haploid genome). The mechanisms by which conifer genomes have evolved are unknown, but amplification events that produce high copies of similar sequences may have contributed substantially to the present genomes. We are beginning a characterization of the genome of *Pinus taeda* to identify classes of repeated sequences. Using the *P. taeda* genomic clone, p1C2, we have identified a non-ribosomal, non-organellar sequence that appears to be present as a conserved fragment in thousands of copies in the pine genome. The sequence shows similar copy numbers, but slightly different restriction fragment length polymorphisms among various pine species. Distinctly different restriction enzyme fragments and hybridization signal intensities are detected in DNA from more widely-diverged gymnosperm species. The sequence also appears to be present in the woody angiosperm *Populus*. The DNA sequence appears to be transcribed. On Northern blots of *P. taeda*, p1C2 detects two non-polyadenylated transcripts of 1.2 and 3.5 kilobases. We are currently analyzing the sequence of p1C2 for its relationship to other known repeated sequences.

POSTER ABSTRACTS

1767 INTRODUCTION AND TRANSPOSITION OF MAIZE CONTROLLING ELEMENT " Ac " INTO *Nicotiana tabacum* AND *Petunia hybrida*. Shin-Je Kim, Wha-Jin Cho, Byung-Dong Kim. Seoul National University, Department of Horticulture, Suwon, KOREA 441-744. Yong-Pyo Lim and Kwang-Tae Choi, Korea Ginseng and Tobacco Research Institute, Taejon, Korea

Maize controlling element *Ac* was introduced into *Nicotiana tabacum*, cv. NC82, and *Petunia hybrida*, cv. Petunia White Madness (PWM), which is widely cultivated in Korea. *Agrobacterium*-mediated transformation method was applied to transfer the *Ac*, and the transformants were regenerated to whole plant. After 2-3 weeks of inoculation of the leaf disc with *Agrobacterium* containing the TAc7 plasmid, the callus was formed in callus inducing media supplemented with 100 mg/l kanamycin. The shoots were regenerated from calli by shoot inducing media supplemented with 100 mg/l kanamycin. The petunia leaves mostly produced multi-shoots in shoot inducing media supplemented with 100mg of kanamycin. Transformation of the regenerated plants were confirmed by checking the stability on media supplemented with kanamycin. Integration, copy number, and transposition of the *Ac* element in the transgenic plants were confirmed by Southern blot analysis. Southern blot analysis confirmed the integration and transposition of *Ac* element in transgenic plants.

1768 INSERTION MUTAGENESIS IN *ARABIDOPSIS THALIANA* USING AN *AC*-DERIVED TRANSPOSABLE ELEMENT SYSTEM. T. Altmann, G. Felix, A. Jessop, P. Morris and L. Willmitzer
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The small and simple organisation of the *A. thaliana* genome makes this flowering plant an ideal subject for gene isolation. To obtain a tool for the identification of genes only defined by a mutant phenotype, a two component transposable element system has been developed. It consists of an immobilized *Ac* element devoid of one of its terminal repeats ("Ac clipped wing", Ac_{cl}) as a source of transactivating transposase and a non-autonomous "Ds" element, Ds_A. A chimaeric neomycin phosphotransferase gene used as excision marker allows to differentiate between germinal and somatic excision events of Ds_A when activated by Ac_{cl}. As reinsertion of excised Ds_A elements was detected in about 50% of the excision events, a scheme could be developed, in which plants with a high rate of somatic excisions serve as sources of new germinal Ds_A transpositions. Thus the selection of a large number of independent Ds_A insertions has been made possible. Progeny of plants selected for germinal Ds_A excisions were screened for mutant phenotypes. The broad screening program included growth in soil and on synthetic media. Plants with reduced growth, reduced fertility, aborted seeds and bleached primary leaves have been identified. Progress on genetic and molecular analysis of these putative Ds_A insertion mutants will be presented.

1769 CLONING OF A NOVEL INSERTION SEQUENCE ASSOCIATED WITH ENVIRONMENTALLY INDUCED HERITABLE CHANGES IN FLAX. Richard G. Schneeberger and Chris A. Cullis.
Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106.

Several varieties of flax demonstrate heritable changes after growth for a single generation in environmentally stressed conditions. The environmentally induced plant lines termed genotrophs differ from the progenitor line in terms of morphological, biochemical and genetic characters. Numerous differences from the progenitor line have been documented in the copy number and organization of most of the repetitive sequences in the genome. However, most of these copy number differences are not associated with induction of a particular genotroph. Recently we demonstrated that specific 5S rRNA gene DNA rearrangements are associated with different phenotype genotrophs. Here we describe the characterization of a low copy number, genotroph associated RFLP. Comparison of the cloned genotroph allele and the progenitor allele shows the presence of an approximately 5.0 kb insertion sequence with transposable element characteristics. Characterization of this sequence in the progenitor line and independently induced genotrophs will be described.

1770 REGULATION OF A TRANSPOSING *Ds* -YAC VECTOR VIA A MICROSPORE-SPECIFIC PROMOTER. Gary D. Foster, Michael R. Roberts, Robert P. Blundell, Amar Kumar+, Rod Scott and John Draper.
Botany Department, University of Leicester, Leicester LE1 7RH, U.K.(+)SCRI, Invergowerie, Dundee, Scotland.

The isolation of a plant gene of interest for which the product is unknown but a mutant phenotype has been observed, is classically carried out by either chromosome walking or by transposon tagging. An improvement on both of these approaches might be achieved by a combination of both processes carried out *in vivo*. By placing the essential components of a Yeast Artificial Chromosome (YAC) vector into the maize transposable element *Ds*, a gene of interest may be tagged by close linkage to the transposing YAC vector and large flanking regions rescued as a YAC vector into yeast. In subsequent generations the preference for *Ds* to transposase to linked sites could be utilised to capture limited contiguous YAC clones to a specific region of the chromosome, and thereby allow for a limited chromosome walk *in planta*. These constructs will be stabilised by expressing the transposase from the *Ac* element *in trans* via a microspore-specific promoter, thus allowing one transposition per generation to occur. This promoter, isolated from *Arabidopsis thaliana* via microspore-specific cDNAs from *Brassica napus*, gives no expression in green tissues, stems or roots of tobacco, *A.thaliana* and *B.napus*, but activity was detected in anthers. Activity appears in the microspores shortly after their release from the meiotic tetrads, and was maintained until the interphase after first pollen mitosis. It is now being tested whether this promoter when used to express transposase from *Ac*, will increase the frequency of germinal transpositions of the trans-activated element, *Ds*. Excision reconstitutes a streptomycin gene, thus allowing screening for transposition in the F1 generation, with reinsertion being monitored by a hygromycin gene within the *Ds* . This is a feature highly desirable in transposon tagging, where the generation of large numbers of plants with unique stable inserted elements is required.

1771 SPECIFIC EXPRESSION OF THE TOBACCO Tnt1 RETROTRANSPOSON IN PROTOPLASTS. S.Pouteau, E.Huttner, M.Caboche and M-A.Grandbastien.
Laboratoire de Biologie Cellulaire, INRA, 78026 Versailles Cedex, France

The tobacco Tnt1 retrotransposon was isolated after its transposition into the nitrate reductase (NR) gene, through selection of in vitro NR-deficient lines. Tnt1 is a member of the Ty1-copia family of retrotransposons, is 5.3 kb long, and contains a single ORF of 1328 aminoacids and two long terminal repeats of 610 bp. We have analysed Tnt1 expression in tobacco, by measuring Tnt1 RNA levels, and by testing the ability of the LTR to promote the expression of a translational LTR-beta-glucuronidase fusion in tobacco, both in transient assays and in transgenic tobacco plants. We show here that two RNA species, 5.2 kb and 6.5 kb long, are detected in tobacco. Our results suggest that the 6.5 kb RNA is a chimaeric RNA initiated in a gene in which Tnt1 has inserted. The 5.2 kb RNA, however, has the expected size for a full length Tnt1 transcript, and is initiated in Tnt1 LTR. Tnt1 LTR can promote the transient expression of a translational LTR-beta-glucuronidase fusion at high levels. The 5.2 kb RNA and the LTR-GUS fusion of transgenic tobacco plants are specifically expressed in protoplasts derived from leaf mesophyll, but not in leaf tissues. The presence of the 5.2 kb transcript was investigated in other tissues, but could be only be detected at low levels in roots. Our results suggest therefore that the 5.2 kb RNA is a Tnt1 specific transcript, and that Tnt1 expression is strongly regulated in tobacco. The 5.2 kb RNA is rapidly induced during the first steps of protoplast isolation, and we attempted to determine which steps of the isolation procedure are involved in the RNA induction. Neither wounding nor plasmolysis seems to be directly responsible for the activation, but the presence of cell wall hydrolases always result in the 5.2 kb RNA expression. Separation of the different hydrolases sources shows that induction correlates with their ability to release protoplasts. For the first time in plants, Tnt1 expression features thus provide strong evidence that the very first step of tissue culture via protoplasts is involved in the activation of a transposable element.

1772 HIGH FREQUENCIES OF SOMATIC AND GERMINAL EXCISION OF Ds IN *Arabidopsis*. J. Swinburne[1], LI. Balcells[1], K. Ingle[1], S. Scofield[2], J. Jones[2] and G. Coupland[1]
Cambridge Lab.[1] and Sainsbury Lab.[2], JI Centre for Plant Science Research, Colney Lane, Norwich.

We have introduced into Arabidopsis thaliana Landsberg erecta 8 constructs carrying fusions of various plant promoters to the single open reading frame of the maize transposable element Ac. Here we will only discuss the fusion of the CaMV35S promoter. This will not transpose itself as in creating the fusion essential terminal sequences of Ac were deleted. We have also introduced a construct carrying a Ds element (an Ac with the ORF inactivated by a frameshift mutation) inserted within a streptomycin (SPT) resistance gene. The Ds will transpose, thereby restoring activity of the resistance gene, only in the presence of the fusion to the intact Ac ORF. 5 independent transformants homozygous for the CaMV35S fusion at a single locus were crossed to a line homozygous for the Ds. The hybrid seeds were sown on SPT containing medium. Between 50% and 93% of the seedlings showed large numbers of clones of SPT resistant cells on their cotyledons. These variegated seedlings were grown to maturity and selfed. Between 10% and 61% of the F2 progeny were fully SPT resistant and were assumed to have inherited germinal excision events. Hybridisation of an SPT resistance gene probe to DNA extracted from 12 of these SPT resistant individuals confirmed this. The frequencies of somatic and germinal excision detected are significantly higher than those previously reported in Arabidopsis. These data will be discussed along with their significance for transposon tagging in Arabidopsis.

POSTER ABSTRACTS

1773 EXTREME HETEROGENEITY OF COPIA-TY FAMILY RETROTRANSPOSONS IN PLANTS. A. Kumar, D. Smith* and A. Flavell*. Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland; * Biochemistry Department, Dundee University, Dundee DD1 4HN, Scotland

Retrotransposons have recently been discovered in flowering plants, and three of these are members of the Copia-Ty family. We have used the polymerase chain reaction to analyse copia-Ty family retrotransposons of flowering plants. All eight species studied contain reverse transcriptase fragments from copia-Ty family retrotransposons. Sequence analysis of 31 subcloned fragments from potato reveals that each is different from the others, with predicted amino acid diversities between individual fragments varying between 5% and 72%. Such sequence heterogeneity within a single species contrasts strongly with the limited diversity seen in such retrotransposons in yeast and Drosophila. The fragments from the other seven plant species are also heterogeneous, both within and between species, showing that this is a general property of this transposon family in plants. We suggest that this heterogeneity is at least partially due to the high copy number of these elements in the plant genomes studied, which is itself a consequence of the large sizes of the genomes containing them. Phylogenetic analysis of all these sequences reveals that many of them fall into subgroups which span species boundaries, such that the closest homologue of one sequences is often from a different species. We suggest that both vertical transmission and horizontal transmission of copia-Ty family retrotransposons have played major roles in the evolution of copia-Ty family retrotransposons in flowering plants.

1774 PLANT RETROTRANSPOSONS. Andrzej Konieczny[1], Daniel Voytas[2], Michael Cummings[3], Steven Rodermel[2] and Frederick Ausubel[1]. [1]Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114; [2]Dept. of Botany, Iowa State University, Ames, IA 50011; [3]Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138.

We have identified a superfamily of *Arabidopsis thaliana* retrotransposable elements that consists of at least ten related families. The nucleotide sequences of the reverse transcriptase regions of ten element families place them in the category of copia-like retrotransposons and phylogenetic analysis of the amino acid sequences suggests that horizontal transfer may have played a role in their evolution. Degenerate oligonucleotides were synthesized based on conserved amino acid sequences shared by the plant reverse transcriptases and used as primers in polymerase chain reaction. This led to the identification of related retrotransposon families throughout the plant kingdom, including all major crop species. In maize we have identified two distinct retrotransposon families, one from maize cDNA library. We hope further characterization of the plant retroelelements will offer insight into the role retrotransposons play in mutation and plant genome change.

1775 A FAMILY OF REPETITIVE DNA RESTRICTED TO THE GENUS *ZEA* MAY CONSTITUTE A NEW TYPE OF RETROPOSONS. Regina Raz, Carlos M. Vicient, Pere Puigdomènech and José A. Martínez-Izquierdo. Dep. Genética Molecular, CID-CSIC, Jordi Girona, 18, 08034 Barcelona (Spain).

The initial isolation of a new family of repetitive DNA sequences, 253 bp long on average, has allowed to find a putative new family of retroposons, several kbp long. The short repetitive elements are interspersed in the genome and the show the same genomic organization pattern and similar copy number in all the *Zea* species examined. Independent clones from a genomic library of the teosinte, *Zea diploperennis* have been isolated and the short repetitive sequences, termed ZEAR, have been sequenced. These repeats, flanked by *Hae*III sites, are more than 70% G+C rich and show 78% similarity to each other. ZEAR are in a highly cytosine-methylated context and they present some features resembling those of coding sequences. In fact, when sequencing flanking regions of one ZEAR, ZEAR 270, an ORF including ZEAR is revealed. This ORF codes for a protein of 157 residues, rich in glycine, serine and aspartic and glutamic acids. The ORF is flanked by a TATA box and three putative polyadenylation sites. The ZEAR probe hybridizes with RNA extracted from different tissues of maize and from teosinte, indicating that these repeats or similar ones are present in transcribed sequences. Indeed, one cDNA, 1.8 Kbp long, fished out with a ZEAR probe from a maize cDNA library, contains the ORF corresponding to the genomic one, and shows 77.8% similarity over 1,6 kbp with the teosinte genomic sequence. Preliminary results, suggest that the larger ZEAR-containing repetitive elements belong to the superfamily of retroelements. These results will be discussed in relation to the similarities observed with retroelements such as retrotransposons and retroposons.

1776 TRANSPOSON TARGETING AND RFLP MAPPING OF RESISTANCE GENES IN POTATO. W.J. Stiekema, A. Pereira, W. te Lintel Hekkert, S. van Agtmaal, J.M.E. Jacobs, C.M. Kreike, J.R.A. de Koning, B. Verkerk-Bakker. Department of Molecular Biology, Centre for Plant Breeding and Reproduction Research CPRO-DLO, P.O. Box 16, 6700 AA Wageningen, Netherlands.

Transposon targeting strategies utilizing the maize Ac-Ds system and RFLP mapping are practicised to isolate genes in diploid potato. A visual assay in minitubers to monitor excision from a GBSS (Granule Bound Starch Synthase) reporter gene transformed into an amf/amf (waxy) genotype has been developed. Data on frequency of excision and correlation to copy number/position will be presented. The cell autonomous 'waxy' variegated phenotype has been exploited for target practice transposon mutagenesis to monitor Ac integration in the Amf locus. Ac cDNA expression controlled by different promotors is being compared for efficiency in producing germinal excision events. Different marker genes are being used to select for excision events. A library of transformed lines with mapped Ds elements is being generated so that by recombination a Ds element can be linked to a gene of interest. Controlled insertional mutagenesis can then be carried out to isolate the targeted gene. For mapping of Ds elements and interesting genes an integrated potato RFLP linkage map is being constructed. Mapping of loci providing resistance to the potato cyst nematodes *Globodera rostochiensis* pathotypes Ro1 and Ro4, and *G.pallida* pathotypes Pa2 and Pa3 derived from *Solanum vernei* and *S.spegazzinii* resp. is in progress. Preliminary results show no linkage between the Ro1 resistance locus from *S.vernei* and the Fb locus from *S.spegazzinii*, which provides also pathotype Ro1 resistance and has been mapped by Barone et al. (1990) Mol. Gen Genet. 224, 177.

1777 THE R173 FAMILY OF RYE-SPECIFIC REPEATED SEQUENCES
P. Rogowsky, J.-Y. Liu, S. Manning and P. Langridge.
Waite Institute, Glen Osmond, South Australia 5064

The R173 family of repeated DNA sequences consists of approximately 15,000 individual copies per diploid rye (*Secale cereale*) genome and it is distributed over all 7 rye chromosomes in a dispersed manner. In contrast, the copy number in wheat and barley is <20 copies per diploid gneome, thus making the R173 family rye-specific. The R173 family shows no homology to any other published repeated DNA sequence of rye and is rarely (<2%) linked to those sequences on genomic λ clones. Individual copies of the R173 family vary in size between 3 and 4 kb, are generally not arranged as tandem repeats and are flanked by both multi copy and single copy sequences. Preliminary sequence data from three individual copies show strong homology to a 400 bp long region of a proposed wheat retrotransposon. However, no long terminal repeats (LTRs) or open reading frames for a reverse transcriptase or integrase could be detected. The three sequenced copies were also mapped to chromosome regions by PCR, a prerequisite for studies concerning the transposition of individual copies.

1778 Ac-Ds TRANSPOSABLE ELEMENT : A PROTOPLAST APPROACH FOR GENE TAGGING ? Nicole Houba-Hérin. Astrid Post, Yvan Larondelle. Institut fur Genetik, Uni-Koln, Weyertal 121, D-5000, Koln 41, Germany

Natural Ds elements exist as internal deletions of the transposable element Ac of Zea mays L. They are able to transpose only when an Ac element (or its encoded product) is present in trans. Artificial Ds can be created by restriction digests and labeled with marker genes. The excision of the transposable element from the resident DNA is one step of the transposition mechanism. We have shown that an engineered Ds is properly excised very early after co-transformation of Petunia protoplasts with two plasmid DNA : the Ds-carrying plasmid and a transposase encoding plasmid (MGG,1990,224:17-23). Because the engineered Ds has been cloned in the untranslated leader sequence of the GUS gene, its excision restores the activity of the gene. Therefore the excision frequency can be measured. The same experiments have been carried on with Petunia and tobacco protoplasts by using a NPTII-labeled Ds.A higher number of kanamycin resistant calli (as well as a higher excision frequency) was obtained when providing in trans a plasmid carrying a truncated version of the putative transposase gene (the 3.3kb AccI genomic fragment of Ac under the control of the 2'promoter). Kanamycin resistant tobacco plants have been regenerated and their DNA is submitted to Southern analysis. While the interpretation of the Southern profiles is often difficult because of the process of direct DNA transformation by entire plasmid DNA or pieces thereof, it seems however that labeled Ds elements have jumped into the plant genome. The analysis is pursued. PCR experiments are planned in order to check if the 8 base pair duplication of the host DNA is present at the site of insertion.

POSTER ABSTRACTS

1779 TRANSPOSON MUTAGENESIS IN *ARABIDOPSIS THALIANA*. Arne Speybrouck, Jack K. Okamuro*, Mieke Van Lijsebettens and Marc Van Montagu. Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium; *University of California, Santa Cruz, Department of Biology, Santa Cruz, California 95064

The *As/Ds* transposon system has been successfully exploited for gene tagging and subsequent gene isolation in maize. It has also been shown that *Ac/Ds* is active in heterologous plant species. We adapted the *Ac/Ds* system for gene tagging in *Arabidopsis thaliana*. More than one hundred transgenic lines were generated bearing *Ds* elements marked with the phosphinothricin (*bar*) gene which confers resistance to the herbicide Basta® (Hoechst). This allows us to follow the *Ds* elements genetically in subsequent generations. Mapping of original *Ds* insertions is going on using classical as well as inverse polymerase chain reaction/restriction fragment length polymorphism mapping techniques. Transactivation was achieved by transformation using an *Ac* transposase cDNA with a $2'T_R$ promoter. We could show that *Ds* excision and transposition occur upon transactivation. Currently, F1 progenies are analyzed for the occurrence of transposon-induced mutations

1780 BIS 1, A RETRO_TRANSPOSON LIKE SEQUENCE IN BARLEY: Inari Manninen and Alan H. Schulman. University of Helsinki, Institute of Biotechnology, 00380 Helsinki, FINLAND

Plant transposons can be divided into two broad categories: elements like Ac or Tam which carry short terminal inverted repeats and transposase function in internal ORFs; and retro-transpsons with long terminal direct repeats (LTRs) and internal regions homologous to retroviral functional regions such as gag or reverse transcriptase. In our search for transposable elements in barley, we have isolated a genomic clone with very high homology to the wheat insertion sequence Wis-2 5' LTR and also with homology to the Ta-1 transposon of Arabidopsis. We name this Bis-1, for Barley Insertion Sequence 1, the first such element identified. The junction of the Bis-1 LTR with the surrounding DNA carries the 5' TG 3' shared with other retro-transposons such as the plant Wis-1, Cin-1, yeast Ty-1, copia, and various mammalian retroviruses. Genomic Southern hybridizations show that Bis-1 is present in multiple copies. We have screened a genomic library from barley for clones homologous to Bis-1, isolated and subcloned a set of these selected clones, and are now in the process of obtaining a complete (LTR to LTR) sequence of a Bis-1 insertion. Functional and evolutionary aspects of the Bis-1 family of elements will be presented.

1781 cDNA SEQUENCE, mRNA, AND GENES FOR NUCLEAR-ENCODED TOBACCO CHLOROPLAST RIBOSOMAL PROTEIN L27. Gasmalla A. Elhag and Don P. Bourque. University of Arizona, Department of Biochemistry, Tucson AZ (USA) 85721

A tobacco (*N. tabacum cv. Petite Havana*) leaf cDNA library was constructed in the expression vector gt11. Using immunological and nucleic acid hybridization procedures, several cDNAs encoding a protein homologous to *E. coli* ribosomal protein L27 were isolated. One full-length cDNA (L27-1) is 882 nucleotides long, has 104 bp (base pairs) of 5'-non-coding sequence, a 51 codon putative transit peptide sequence, a 128 codon predicted mature L27 polypeptide and 241 bp of 3' downstream non-coding sequence, including the poly(A)$_{29}$$^+$ tail. The proposed mature L27 protein has 64%, 61%, 56%, and 47% sequence identity with the L27-type ribosomal proteins of *E. coli*, *Bacillus subtilis*, *Bacillus stearothermophilus*, and yeast mitochondria (MRP7), respectively, in the homologous overlapping region. The putative transit peptide is both typical of described chloroplast transit peptides, as well as being homologous to the amino-terminal leader sequence of yeast mitochondrial ribosomal protein MRP7. Northern blot analysis revealed a single band of mRNA corresponding in size (0.85-0.90 kb) to full-length L27 cDNAs. Two full-length cDNAs for tobacco ribosomal protein L27 were identical in coding sequence, but differed in 3' non-coding sequence. Existence of multiple genes coding for tobacco L27 was confirmed by hybridization of L27 probes to restriction fragments of tobacco genomic DNA and to unique, cloned genomic fragments.

1782 EVOLUTIONARY AND STRUCTURAL DIVERSITY OF DINOFLAGELLATE RIBULOSE 1,5 BISPHOSPHATE CARBOXYLASE. T.Nadeau, J.Chesnick, R.A.Cattolico University of Washington, Dept. of Botany, Seattle, WA 98195 USA

Dinoflagellates are virtually unstudied with respect to Calvin cycle enzymes even though this taxon is second only to diatoms in its contribution to oceanic primary production. These algae are phylogenetically clustered in the Chromophyta, whose members encode both large and small subunit genes of Rubisco in the chloroplast; rhodophytic algae display a similar chloroplast localization of the Rubisco operon. This gene arrangement contrasts sharply with the chloroplast and nuclear localization of *rbcL* and *rbcS*, respectively, in the Chlorophyta. Moreover, the *rbcS* of several chromophytes and rhodophytes show a much greater sequence similarity to that of *Alcaligenes eutrophus*, a chemolithotrophic bacterium, than to those of Cyanobacteria or Chlorophyta. To further our understanding of Rubisco structural diversity among photosynthetic organisms, the putative *rbcL/rbcS* operon from three dinoflagellate species, *Peridinium inconspicuum*, *Wolosszynskia* sp., and *Amphidinium* sp., was amplified using the polymerase chain reaction (PCR). PCR primer design was based upon available chromophytic and rhodophytic algae, and *A. eutrophus* Rubisco sequence data. Conserved regions 418 bp into the *rbcL* gene (forward) and 146 bp into the *rbcS* gene (reverse) were used. In each case, an approximately 1.4 kb DNA fragment was amplified, which is correct in size. The identity of these amplification products was confirmed by hybridization analysis using the *Olisthodiscus luteus* (chl-a,c) *rbcL* and *rbcS* genes as probe. These data demonstrate that the *rbcL/rbcS* gene arrangement of all three dinoflagellates is similar to that found in other chromophytic algae. Nucleotide sequence analysis and phylogenetic comparison of these amplification products is in progress.

1783 EVOLUTION OF SUGARCANE (*Saccharum* sp.). Bruno W.S. Sobral, Rhonda J. Honeycutt, Michael McClelland, and James E. Irvine*. California Institute of Biological Research, La Jolla, CA 92037. *Texas Agricultural Experiment Station (Texas A&M), Weslaco, TX 78596.

The term "*Saccharum* complex" was coined to refer to the genera that can interbreed in the wild and are believed to be involved in the evolution of the *Saccharum* genus and "noble" sugarcanes (*Saccharum officinarum* L.). The term *Saccharum* complex is not a formal taxonomic unit, although it is interesting to note that all species and genera currently recognized as part of the complex have been classified as *Saccharum* by taxonomists at one time or another. The currently accepted genera that belong in the complex are: *Saccharum*, *Erianthus* sect. *Ripidium*, *Sclerostachya*, *Narenga*, and *Miscanthus* sect. *Diandra*. We have collected clones that represent various members of the *Saccharum* complex from diverse geographic locations. We have constructed primers that we are using to investigate phylogeny within the complex. We are investigating the nuclear and cytoplasmatic genomes of various sugarcane clones using the arbitrarily-primed polymerase chain reaction (AP-PCR) and standard PCR. Our long-term goal is to further understand evolution of this complex polyploid group of species.

1784 INTRONS SHARED BY BACTERIA AND CHLOROPLASTS. Maria G. Kuhsel and Jeffrey D. Palmer. Indiana University, Department of Biology, Bloomington, Indiana 47405 USA.

Introns within chloroplast genes are mostly regarded as eukaryotic features, since until recently no introns have been found in eubacteria. However, a land plant chloroplast tRNA-Leu (UAA) intron has recently been discovered at the same gene position in chloroplasts from all major lineages of algae (1) as well as in the progenitors of chloroplasts, the cyanobacteria (1, 2). This group I intron seems to predate the origin of chloroplasts. We now report the presence of this intron within the tRNA-Leu genes of diverse eubacterial lineages including ancient thermophilic bacteria. Likewise, the group II intron of the land plant chloroplast tRNA-Val (UAC) gene is present in identical positions in all chloroplast lineages and in diverse eubacteria. The presence of both the tRNA-Leu intron and the tRNA-Val intron within *Thermotoga*, possibly the most ancient eubacterium so far described, implies that these introns are at least as ancient as the eubacteria and have been stably maintained during eubacterial and chloroplast evolution. In addition, we have evidence for the presence of both introns within archaebacteria and protists. Therefore, they might possibly have been present in the progenote.

1. M. G. Kuhsel et al., Science 250, 1570 (1990);
2. M.-Q. Xu et al., Science 250, 1568 (1990)

POSTER ABSTRACTS

1785 MOLECULAR DIFFERENTIATION OF DONG-XIANG WILD RICE AND ITS MEANING IN EVOLUTION AND BREEDING OF RICE Shao Qiquan ,Y. sano , Yi Haoxiong , and S. Iyama Genetics Institute , Academia Sinica , Beijing 100101 China

Eleven lines of Dong-xiang wild rice have been systematically studied by morphological, cytologically , genetically and molecular methods. The frequencies of rDNA sl variation in seven Oryza species carrying the AA genome in comparison with Dong-xiang wild rice shows that Dong-xiang wile rice carried the specific sl band at size (kb) 4.50 specific for japonica rice . The role of Dong-xiang wild rice in evolution and origin of japonica rice is speculated. Dong-xiang wild rice could be concerned as direct evidence for earlier differentiation of rice on japonica and indica ,before they had been cultivated . The results of offsprings from crossing of Dong-xiang wild rice with different varieties of cultivated rice shows great potentiality of Dong-xiang wild rice in breeding of new male sterility of rice . By this way , the farmers will got the completely new sources of male sterility of rice then currently widely used very uniform material in rice production of hybrid rice in China and others.

1786 MOLECULAR EVOLUTION OF CHLOROPHYLL *a+c*-CONTAINING PLASTIDS Klaus V Kowallik, Maria G. Kuhsel, Carola E.W. Leitsch and Peter G Pancic. University of Düsseldorf, Department of Botany, Universitatsstr. 1, D-4000 Düsseldorf 1, Germany

The evolution of chlorophyll *a+c*-containing (chromophytic) plastids and the prokaryotic ancestors of these plastid types are widely unknown or controversely discussed. In order to obtain more information about the phylogeny of chromophytic plastids, we have characterized several plastid genomes from different chromophytic lineages. These genomes resemble those of chlorophyll *a+b*-containing (chlorophytic) plastids in size and structural organization, but differ in gene content and gene order which is highly scrambled even among closely related genera. Gene mapping and nucleotide sequencing reveals that most genes coding for structural and soluble polypeptides, which are involved in photosynthesis in chlorophytes, are also present in chromophytic plastid genomes. In addition, genes that are nuclear encoded in chlorophytes, map within transcriptional units in chromophytic plastid genomes at the same position as in cyanobacterial gene clusters. This, together with other cyanobacterial characters, such as a 15 base pair (bp) overlap between psbD and psbC, a one or four bp overlap between atpF and atpD, as well as the use of GTG instead of ATG as the presumed translation initiation site of psbC, indicates that chromophytic plastids most probably derived from cyanobacterial ancestors. Further differences in comparison to chlorophytic plastid genomes include the lack of intron sequences in pet- and atp-genes and nucleotide exchange rates which at least are ten times higher than in chlorophytes. These structural differences may have resulted from secondary eukaryotic/eukaryotic endocytoses that are considered to have led to the individual chromophytic lineages.

1787 CHLOROPLAST DNA FROM OIL PALM SPECIES Farida H. Shah, Genetics Department, Universiti Kebangsaan Malaysia, 43600 Bangi, MALAYSIA.

Chloroplast DNA has proven to be a powerful tool in the elucidation of phylogenetic relationship in many plant taxa. Analysis of ctDNA has been successfully applied in studies on interspecific variation because it is highly conserved. In same cases, intraspesific variation have also been observed which provides information on intraspescific differentiation. Chloroplast DNA from various fruit types of oil palm species Elaeis guineensis, tenera, dura and pisifera, and their related species, E. oleifera have been characterised by restriction enzyme digestion analysis. Although the two species E. guineensis are almost similar in size (116kb an 121kb respectively) restriction digestion analysis with Hind III, Eco RI and Sac I showed clear differences. The physical map of the chloroplast DNA from E. guineensis dura is constructed by southern blotting and hybridisation using wheat chloroplast DNA, and this map is used to compare chloroplast genome within species and between species.

1788 A MOLECULAR SURVEY OF CHLOROPLAST DNA IN 9 PINYON PINE SPECIES (SUBSECTION CEMBROIDES). Eric S. LaHood, and Paul Keim. Northern Arizona University, NAU Box 5640, Flagstaff, Arizona 86011

The phylogeny of the pinyon pines has been based on morphological and biochemical factors with little attention to molecular genetics. We have characterized the chloroplast DNA of 9 species of pinyon pine. Our goal was to better understand chloroplast DNA evolution in conifer species and to compare the molecular phylogeny with that derived by more classical criteria. Southern analysis of total genomic DNA was used on a total of 80 individual trees. DNAs were digested with three restriction enzymes: *Eco*RI, *Hin*DIII, and *Dra*I. Hybridization probes from Douglas fir cpDNA, representing 80% of the genome, were used to reveal RFLPs. We have observed variation both within and among species that is informative about the systematic relationships in this group. Phylogenetic trees generated using maximum parsimony algorithms will be presented. In order to understand the molecular mechanism generating these RFLPs, we have constructed a restriction map which allows us to differentiate point mutations from major rearrangements.

1789 STRUCTURE OF A MAIZE MITOCHONDRIAL RNA REPLICON. Mingda Zhang and Gregory G. Brown, McGill University, Department of Biology, Montreal, Quebec, Canada H3A 1B1

Several single and double stranded RNA species in the mitochondria of maize carrying S-type male-sterile cytoplasm have been found to replicate in a DNA-independent manner and have hence been classified as RNA plasmids[1]. We are analyzing the nucleotide sequences of these molecules in order to determine those features that enable them to replicate autonomously and to investigate their evolutionary origin. The most abundant of the RNA plasmid species, S/RU RNA *b*, was purified by agarose/urea gel electrophoresis and partial sequences of it's 5'- and 3'-ends were obtained by direct enzymatic RNA sequencing. Corresponding oligonucleotides were then used to prime and amplify cDNA corresponding to the RNA sequence. The sequence of a cDNA clone representing >95% of the S/RU RNA *b* has been determined. The RNA is predicted to have an extensive and novel secondary structure configuration, consistent with it's solubility in 2M LiCl. Preliminary database searches have failed to identify significant similarity between S/RU RNA *b* and any known sequence. Results from other experiments indicate that S/RU RNA *b* shares sequence homology with at least two other large ssRNA species in maize mitochondria.

[1] Finnegan, PM and Brown, GG (1986). Proc. Natl. Acad. Sci. USA, 83:5175-79

1790 ORGANIZATION OF coxII GENE AND ITS POSSIBLE CORRELATION WITH THE EVOLUTION OF ANGIOSPERMS. C.De Benedetto, L.De Gara*, M.Albrizio, O. Arrigoni* and R. Gallerani. Università di Bari, Dipartimento di Biochimica e Biologia Molecolare ed Istituto di Botanica*, Bari, Italia.

We are currently studying the organization of the coxII gene among higher plants by hybridization and PCR analysis. The hybridization analysis is carried out using specific sunflower coxII probes corresponding to the first exon, the intron region common to the maize gene, and the insertion sequence common to the rice, wheat, sunflower and petunia genes. The PCR analysis is carried out using two oligonucleotides of 18 mer corresponding to highly conserved regions of coxII gene (the 3' terminus of first exon and the 5' terminus of the second). The hybridization analysis revealed a wide distribution of composite introns (similar to those of rice, wheat, sunflower and petunia) among monocot plants as well as dicot plants. The results we obtained from studying the distribution of composite introns suggest a relationship between the evolutionary positions of each system and the organization of coxII introns. Phyletic lines proposed by different authors, based on different parameters, are confirmed by our molecular approach.

The PCR analysis reveals the existence of introns longer than those of systems already sequenced in some species where composite introns have been detected. The amplification of the intron region of the coxII gene of theosinte (a wild ancestor of maize) reveals absolute identity with the maize intron. Sequencing analysis of introns of specific systems, having relevant positions in the phylogenetic tree of Angiosperms, is currently in progress in our laboratories.

POSTER ABSTRACTS

1791 THE SMALL AND LARGE SUBUNITS OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE (RUBISCO) ARE ENCODED IN THE PLASTID OF THE RED ALGA *PALMARIA PALMATA*: SIMILARITY WITH CHEMOAUTOTROPHIC BACTERIUM *ALCALIGENES EUTROPHUS*. Rama K. Singh NRC-Institute for Marine Biosciences, Halifax, N.S., Canada.
In the land plants, as well as in the chlorophytic algae examined to date, the large subunit of Rubisco is chloroplast-encoded, whereas, the small subunit is nucleus-encoded. Using heterologous probes for the small and large subunits of Rubisco from *Cryptomonas Φ*, these genes were shown to reside on the plastid DNA of *P. palmata* by Southern hybridization. A 6.2 kb *EcoRI* fragment, which gave a positive hybridization signal with both probes, was cloned in λ-ZapII. The rescued phagemid from this clone was used in generating physical map of the 6.2 kb insert and in obtaining ssDNA for sequencing. The small subunit gene is 417 bp, encoding a protein of 139 aa residues. It is found 89 bp downstream of the large subunit gene and is co-transcribed with it. The small subunit protein is similar to *A. eutrophus* in having a 7 codons deletion at amino terminus and an extended carboxy terminus, relative to cyanobacteria and chlorophytes. The large and small subunit Rubiscos of *P. palmata* show a higher percent identity to those of *A. eutrophus* (68%; 59%) than to those of cyanobacteria (55%; 40-44%). These results clearly demonstrate that both subunits of Rubisco are plastid-encoded and are co-transcribed in *P. palmata*.

1792 A 14 KB REGION OF THE PLASTID GENOME OF PORPHYRA UMBILICALIS ENCODES AT LEAST FIVE GENES NOT LOCATED ON THE PLASTID GENOME OF GREEN PLANTS. Michael Reith and Janet Munholland. National Research Council, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada.
Partial DNA sequence analysis of four contiguous EcoRI clones from the plastid genome of Porphyra umbilicalis has revealed the presence of at least five genes not encoded on the plastid genome of green plants. These genes encode an hsp70 (dnaK) homolog, ferredoxin, the psaE gene product, a serine tRNA (CGA anticodon) and acetolactate synthase. In addition, an unidentified open reading frame (ORF 234) is present in this region of the genome. Interspersed between these unusual genes are genes present on the plastid genomes of green plants: psbK, petG, rps14, psbB, psbN, psbH, trnG (GCC), trnT (GGU), trnY (GUA), ORF62, ORF31. The psbB - ORF31 - psbN - psbH gene cluster occurs in the same organization as found in green plants; however, the petB and petD genes that are co-transcribed with these genes in green plants are not contiguous with this operon. Upstream from the hsp70 gene, a ribosomal protein gene operon is found. While not fully sequenced, this operon includes many of the genes found in the ribosomal protein operon at one end of the large single copy region of land plant plastid genomes as well as at least one gene (rpl6) not encoded on green plant plastid genomes. These data suggest that the plastid genomes of red algae have retained significantly more genes than those of green plants.

1793 ORGANELLAR AND NUCLEAR RIBOSOMAL GENE SEQUENCES WITHIN ONAGRACEAE:PHYLOGENETIC AND EVOLUTIONARY CONSIDERATIONS. Carol J. Bult and Elizabeth A. Zimmer. Smithsonian Institution, Laboratory of Molecular Systematics, Washington, D.C. 20560
The primary structure for regions within the small subunit ribosomal RNA gene from nuclear, chloroplast and mitochondrial genomes is presented for species within the plant family, Onagraceae. The methods for rDNA gene amplification via the polymerase chain reaction and direct sequencing of the double-stranded PCR product are presented. The sequence data are used to assess the congruence of nuclear and organellar rDNA-based phylogenies within Onagraceae and reveal fundamental aspects of the pattern and process of rDNA evolution on a fine time scale.

1794 MOLECULAR PHYLOGENETIC ANALYSIS OF PYRRHOPHYTA INFERRED FROM THE SEQUENCE OF THE 24S rRNA CONSERVATIVE AND DIVERGENT DOMAINS. Michel Herzog and Guy Lenaers. University of Grenoble I, Department of Plant Molecular Biology, BP 53X, F-38041 Grenoble, France
The sequence of the large subunit ribosomal RNA (LsuRNA) gene of the dinoflagellate *Prorocentrum micans* (Pyrrhophyta) has been determined. The conserved portions of the molecule (1900nt) have been aligned with the corresponding sequences from various eukaryotes. The topology of the trees inferred with distance and parsimony methods suggests close relationship between dinoflagellates, ciliates and yeast. This was confirmed by analysing the 5' end sequence of LsuRNA from various dinoflagellates which were determined by primer extension using total RNA as template. Data are provided confirming that dinoflagellates represent a monophyletic group. To investigate phylogenetic relationships within this highly diversified and taxonomically controversial group of unicellular algae, two divergent domains (D1 and D8) of LsuRNA were analyzed by sequence comparison. A coherent and convincing evolutionary pattern was obtained for the dinoflagellates which provides new perspectives on proposed evolutionary trees based on morphology, biology and fossil records. In addition, the emergence of heterotrophic species preceded that of photosynthetic species.

1795 DNA SEQUENCE ANALYSIS OF THE MAIZE CHLOROPLAST GENOME: STRUCTURE AND EXPRESSION OF rpo GENES. Gabor Igloi, Rainer Maier, Axel Acker, Novrus Achmedov, Kurt Appel, Kai Neckermann, Patric Zeltz and Hans Kossel. Institut für Biologie III, Universität Freiburg, Schänzlestr. 1, D - 7800 Freiburg, Germany.
The aim of our research effort is the determination and analysis of the complete 139 kb DNA sequence of the maize chloroplast genome. We are complementing the 80 kb of sequences already published by several groups, including our own, by cloning the DNAs corresponding to large gaps into vectors suitable for creating exonuclease III mediated nested deletions while closing small (<600bp) gaps with specifically synthesised primers. The rate of sequence data acquisition has been enhanced by using a fluorescence-based automated DNA sequencer of the EMBL design permitting a throughput of 2500 bases per 6 h run. A total of 30.000 bp of new sequence have been determined in this way, including approximately 2000 bp not found in the otherwise closely related rice plastome, allowing a significant update in the gene map of the maize plastome.
A comparison of the now available tRNA gene sets from the monocots rice and maize with those from the dicots tobacco and liverwort shows a number of differences in the localisation of the genes due to genomic rearrangements, while confirming the conservation of the primary structures, to a large extent. Greater variations are seen when comparing tRNA pseudogenes, even between rice and maize.
The structure and deduced sequence motifs of the genes rpo A, B, C_1 and C_2 coding for the subunits α, ß, ß' and ß" of RNA polymerase have been analysed extensively. Plasmids for overexpression in E.coli of the subunits α, ß, and ß' have been constructed and the overexpressed peptides were used for raising subunit specific antisera. The latter allowed us to demonstrate in vivo expression of the respective rpo genes.

1796 DISEQUILIBRIUM BETWEEN NUCLEAR LOCI AND cpDNA GENOTYPES IN BARLEY. Q. F. Zhang and M. A. Saghai Maroof Biotechnology Center, Huazhong Agricultural University, Wuhan, China and Department of Crop and Soil Environmental Sciences, VPI & SU, Blacksburg, VA 24061, USA. Associations between alleles at 9 nuclear loci and 3 chloroplast (cp) DNA genotypes were assessed in a sample of 247 accessions of the wild barley Hordeum vulgare ssp spontaneum collected from Israel and Iran. Alleles at two of the 9 nuclear loci are marked by length variations in the intergenic spacer region of ribosomal DNA, and those of the other 7 loci are well characterized isozymes. The 3 cpDNA genotypes are marked by RFLP resulting from 3 polymorphic restriction sites. The analyses were performed by dividing the 9 loci into a series of two locus subsets, and log-linear models were constructed to characterize the associations between subsets of two nuclear loci and the cpDNA genotypes. It was found that 6 of the 9 loci demonstrated significant associations with the cpDNA genotypes, either individually as pairwise correlations, or interacted with another locus to form three variate complexes. These results also suggest that multiple nuclear loci are involved in association with cpDNA genotypes, although the sample size of the present study did not allow for characterization of such higher order structures. The observed cyto-nuclear associations are likely to be the results of interplay among a number of evolutionary forces including predominant selfing, differentiation of population gene pools and adaptation of barley genotypes to specific environmental conditions.

POSTER ABSTRACTS

1797 THE CHONDRIOME OF THE ALGA PROTOTHECA WICKERHAMII IS CLOSELY RELATED TO PLANT MITOCHONDRIA

Gabriele Wolff, Gertraud Burger *, Franz Lang * and Ulrich Kück. Lehrstuhl fur Allgemeine Botanik, Ruhr-Universität Bochum, FRG, * Department de Biochimie, Universite de Montreal, Canada

The unicellular heterotrophic alga Prototheca wickerhamii is a suitable organism to study algal mitochondrial genomes. The heterotrophy implies that this alga requires functional mitochondria for energy releasing respiratory chain reactions. A physical and genetic map of the 53kb circular mtDNA genome was constructed using restriction and hybridization data. Sequence analysis of the genes for the small subunit rRNA (SSUrRNA) and for the subunit I of the cytochrome c oxydase (coxI) provides the necessary data for phylogenetic comparisons. A phylogenetic tree based on coxI sequences from various origins supports the close relationship between plant and Prototheca mitochondria. This confirms the results from SSUrRNA sequence analysis, which have already suggested a common ancestor for plant and Prototheca mitochondria (Wolff and Kück 1990). The mosaic coxI gene from Prototheca contains three intronic sequences, showing homology to introns of coxI genes from fungal mitochondria. In addition, transcript patterns of several protein coding genes of the Prototheca mtDNA will be presented.

Wolff G, Kück U (1990) Curr Genet 17:343-351

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1798 HIGHER PLANT PHYLOGENY RECONSRUCTION ON RIBOSOMAL RNAs

A.V.Troitsky,Y.F.Melekhovets*,G.M.Rakhimova,V.K.Bobrova,K.M.Valiejo-Roman and A.S.Antonov. Belozersky Laboratory,Moscow State University,119899 Moscow,and *Institut Bioorganic Chemistry, 220067 Minsk, USSR.

Complete or partial nucleotide sequences of five different rRNA species, coded by nuclear (18S, 5.8S and 5S) or chloroplast genomes(5S, 4.5S) from a number of different groups higher plants were determined. The phylogenetic dendrograms were built by two methods,maximum parsimony and compatibility. The topologies of the trees for different rRNA species are not fully congruent but they share some common features.The taxon Pteridophyta, incliding ferns, clab mosses and horsetails , is not monophyletic group. The data obtained suggest that the divergence of all the main groups of extant gymnosperms occurred after the branching off of the angiosperm lineage and that both gymnosperms and angiosperms are monophyletic groups.As the time of divergence of at least some of these gymnosperm taxa is traceable back to the early Carboniferous, it may be concluded that the genealogical splitting of gymnosperm and angiosperm lineages occurred before this event, at least 360 million years ago, i.e. much earlier that the first angiosperm fossils were dated. Ancestral forms of angiosperms ought to be searched for among Progymnospermopsida.Judging by the rRNA trees,Magnoliales are the most ancient group among dicotyleodons. The most ancient lineage among monocotyledons leads to modern Liliaceae.

1799 *SecY*, PART OF THE BACTERIAL PROTEIN EXPORT COMPLEX, FROM *CYANOPHORA PARADOXA* CYANELLES.

Wolfgang Löffelhardt[1], Ralf Flachmann[2], Christoph Neumann-Spallart[1], Christine B. Michalowski[2] and Hans J. Bohnert[2]. [1]Institut für Biochemie und Ludwig-Boltzmann-Forschungsstelle für Biochemie, Universität Wien, Wien, Austria, and [2]Department of Biochemistry, The University of Arizona, Tucson, AZ, U.S.A.

The plastid (cyanelle) chromosome of the alga *Cyanophora paradoxa* is distinguished from the higher plant chloroplast DNAs by a largely different gene complement. An ORF492 which is not encoded by higher plant chloroplast DNAs shows high sequence homology to the well-characterized *secY* gene from *E. coli*. SecY in association with SecE is considered to make up the core of the bacterial protein export pore to which chaperone-associated proteins are delivered. We will present *in vitro* expression and localization data and results on the insertion and orientation of cyanelle *secY* in *E. coli*. Increasing evidence indicates that protein insertion into the eukaryotic endoplasmatic reticulum proceeds along a pathway that is similar to bacterial protein export. Supportive of this concept is that*Cyanophora paradoxa* nuclear DNA contains two genes with homology to cyanelle *secY*. These might resemble the eukaryotic SecY-like proteins identified in yeast. Cyanelles are surrounded inside the "host cell" by a peptidoglycan wall and an outer membrane. Efforts have been directed towards an understanding of this presumed cyanelle outer membrane (OM). *In-vivo* labelling and cyanelle fractionation studies indicated a proteinaceous layer located outside the peptidoglycan layer. The tentative cyanelle OM is composed of proteins that are translated by both cytosolic and cyanelle ribosomes, indicating the need of an export machinery in the cyanelle. Supported by the Austrian Research Council, the Deutsche Forschungsgemeinschaft, and the National Science Foundation.

1800 ANALYZING THE STRUCTURE OF PLANT MITOCHONDRIAL GENOMES BY PULSED FIELD GELS.

Charles P. André, Avi A. Levy and Virginia Walbot. Department of Biology, Stanford University, Stanford Ca., 94305. We have used Eckhardt and pulsed-field gel electrophoresis to examine the *in vivo* structure of plant mitochondrial chromosomes. We have mapped the recognition sites for all available rare-cutting restriction sites on cosmid clones of the N cytotype. In a maize tissue culture line (Black Mexican Sweet variety), we have identified and characterized several different circular chromosomes. In this line, there appears to be little or no DNA in the "master chromosome" configuration; rather most or all of the DNA is present in smaller subgenomic circles. We have also employed these techniques to examine the mt chromosomes from seedlings of various varieties of maize.

1801 A REARRANGED *trnK-psbA* REGION IN THE CHLOROPLAST GENOME OF A PINE, *Pinus contorta*: EVIDENCE FOR A TRANSCRIPTIONAL FUSION, IDENTIFICATION OF RECOMBINATION SITES AND A MODEL FOR THE REARRANGEMENT EVENTS.

Jonas Lidholm and Petter Gustafsson, Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden.

The chloroplast genome of *Pinus contorta* contains two copies of the *psbA* gene, which encodes the reaction center D1 polypeptide of photosystem II. The duplicated segment on which *psbA* is located was found to be 1969 bp long and to end 19 bp downstream of the *psbA* stop codon. The translocated copy of the segment was found to be inserted immediately upstream of the split *trnK* gene, 2.4 kb from its original position. A northern blot analysis revealed that a transcriptional fusion has occurred between the translocated *psbA* gene copy and the *trnK* gene. As a result the transcription of *trnK* is strongly induced by light and *trnK* transcripts accumulate to a level which is approximately 30-fold higher than in *Pinus sylvestris*, a pine species which lacks the rearrangement. The duplicated segment carries, in addition to the *psbA* gene, the 3' exon of *trnK*, which is partially included in a 124 bp direct repeat within the large duplication. The translocated segment is followed by a repeated 41 bp stretch from the *psbA* coding region. Sequence comparisons of the duplicated segment and the flanking DNA to the corresponding regions of *P. sylvestris* made it possible to identify the recombination sites in *P. contorta* and to derive a model which accommodates the conversion of an ancestral, *P. sylvestris*-like *trnK-psbA* locus into the rearranged structure found in *P. contorta*. The rearrangements appear to result from recombinations between 3-9 bp homologies.

1802 ANALYSIS OF MITOCHONDRIAL DNA IN GENUS *ORYZA*: INDIRECT EVIDENCE FOR A CASE OF ITS INTERSPECIFIC RECOMBINATION IN NATURE.

Gérard Second*, Zhenyuan Wang and Steve Tanksley. Cornell University, Department of Plant Breeding, NY 14853 Ithaca, USA. *(From ORSTOM, France. Now at International Rice Research Institute, PO Box 933, Manilla Philippines).

The mitochondrial genomes of most cultivated and wild species of rice (Genus *Oryza*) have been charactenzed for RFLP. Genetic distances between individual plant mitochondrial genomes were generated and compared with similar distances obtained from nuclear and chloroplast DNA. Species with a similar nuclear genome grouped closely together at mitochondrial DNA level. Generally, distances between these genome-defined species groups agreed well with each other, whether the distances were derived from mitochondrial, single copy nuclear or chloroplast DNA, and assuming a usual maternal inheritance of cytoplasmic organelles. A particularly interesting case, however, was that of the allotetraploid "CD" genome species: they showed the same levels of similarity to both the widely divergent genomes C and E, and also RFLP bands unique to them. Interpretation of this pattern of variation through divergence in evolutionary time is not compatible with the general pattern of relationships observed in the genus *Oryza*. It is compatible with a hypothesis of recombination in mitochondrial DNA following occasional biparental inheritance of mitochondria in the formation of these allotetraploids.

POSTER ABSTRACTS

1803 **RNA MEDIATED TRANSFER OF THE GENE *COX*II FROM THE MITOCHONDRION TO THE NUCLEUS.** Nugent, J. M. and Palmer, J. D. Department of Biology, Indiana University, Bloomington, Indiana 47405.

Southern blot hybridization results suggest that mitochondrial gene content has been highly conserved during flowering plant evolution. However, an interesting exception to this involves the gene *cox*II (encoding subunit II of the cytochrome c oxidase complex). The gene *cox*II, normally present in the mitochondrion, is absent from the mitochondrial DNA of two legume species within the genus *Vigna*; *V. unguiculata* (cowpea) and *V. radiata* (mungbean). Nuclear DNA from cowpea and mungbean give positive hybridization signals when probed with *cox*II suggesting that a functional *cox*II gene is now resident in the nucleus of these two species. By screening a cowpea cDNA library we obtained a polyadenylated cDNA clone containing an open reading frame with 85% sequence similarity to the soybean mitochondrial *cox*II gene. Using primers complementary to the cDNA sequence we have PCR amplified and sequenced the cowpea nuclear *cox*II gene. The nuclear *cox*II gene sequence more closely resembles edited mitochondrial *cox*II transcripts than it does the mitochondrial genes encoding these RNAs. Hence, the gene transfer event appears to have involved reverse transcription of an edited RNA intermediate. Phylogenetic analysis of *cox*II nucleotide sequences suggests that transfer of *cox*II to the nucleus occurred between 60 and 200 million years ago, after the monocot/dicot divergence but before the emergence of the legume subfamily Papilionoideae; loss of *cox*II from the mitochondrion occurred much more recently, being restricted to a single genus within legumes. Most legumes have *cox*II in both the nucleus and the mitochondrion, however, no evidence is found for simultaneous expression of *cox*II in both compartments. The nuclear gene encodes a putative transit peptide and contains an intron at or near the junction of this sequence and the mature protein coding sequence. Exon shuffling, via recombination within the intron, may have played a role in attaching expression and targeting signals upstream of the transferred gene.

1804 **A NOVEL FUNCTION OF REPEATED DNA SEQUENCE:AS PROKARYOTIC PROMOTER-LIKE ELEMENT.** Tang Shaojun, Hou Yongming, Zhong Ling, Zhang Suming, Liu Liangshi Biotech. Research Center,Zhongshan University, Guangzhou 510275, China

The most puzzled question about the repeated DNA sequences in eukaryotic genome is whether these stuff have their specific functions in cellular life procedure or genomic evolution. We report here a novel repeated DNA sequence harboring an active prokaryotic promoter . This repeated sequence was isolated from rice Keta Nangka (O. Indica) genomic DNA by standard reassociation method and cloned into a promoter selection vector pkk 175-6, in which it endue the downstream promoterless Tetracycline (Tc) gene with the ability of transcription and thus endow the host bacteria with the resistance to Tc. The maximum Ic resistance of this Clone is up to 80 ug/ml. The complete sequence, 467 base pairs , of the repeatitive DNA fragment was determined . An interesting character of this sequence is , under relative loose criteria (homologous ratio >= 4/6) , the fragment itself contains several homologous regions to -10 and -35 conserved boxes of prokaryotic promoter and many inner repeated elements . Using this fragment as probe, we performed Southern hybridization and in situ hybridization with several completely digested DNA samples of different rice variaties and the genomic library of rice respectively. The former experiment showed a typical hybridization pattern of repeatitive DNA and the later result told that there was about several hundreds to a thousand copies of this sequence in this rice genome , which might represent a middle repeated DNA sequence family. Basing on these results, we proposed that some repeatitive DNA sequence can function as promoter. In addition , by using the same strategy and suitable vector, it will be possible to probe if there are some repeated DNA sequence can act as other modules of gene expression, such as enhancer.

1805 **REPEATITIVE DNA CLONES FROM RICE (O.sativa) AND THEIR FUNCTIONAL IMPLICATION.** Tang Dongjiang, Xu Liping, He Mulan, Lin Jiandong Biotech. Research Center,Zhongshan University, Canton, China 510275

By the application of appropriate vectors together with DNA renaturation kinetic method, repeatitive DNA sequences of different Cot value have been cloned from rice genome. We have also used two promoter selection plasmids, pkk 175-6 and pkk 232-8, as cloning vectors, which contain promoterless tet or cat gene respectively. Escherichia coli cells harboring pkk 175-6 or pkk 232-8 are resistant to ampicillin but sensitive to tetracycline or chloramphenicol, while recombinant clones with rice repeated sequence inserts have been selected on Tc or Chl containing plates, demonstrating that they acquire additional resistence to Tc or Chl. The resistant levels of these clones are not identical, from 12.5 ug/ml to 80 ug/ml Tc or 30 ug/ml to 260 ug/ml Chl. Growth curves in the presence of the antibiotics of defferent concentrations have been determined for some clones. The results show that the clones differ in their growth types, this even happens between two clones, pRRD 3 and pRRT 25, which have the same resistant strenth --- both can be resistant to Tc at a concentration as high as 80 ug/ml. Difference in insert structure may contribute to the differences in resistant level and growth type, and more insights can be gained through DNA sequencing. Dot-blot confirmes that these clones are of rice genome origin, using 1-2 kb random genomic DNA of rice as probe. For a number of clones, pRRD 3, pRRD 8, pRRD 9, pRRD 107 and pRRD 117, southern hybridization with digested rice genomic DNA have been carried out which show a typical pattern of repeatitive sequence, and DNA sequencing is undergoing. All these studies implicate that some repeatitive DNA sequences from rice may play a functional role, as in this case, they carry some promoter-like activities. We are also doing cross-hybridizations to study whether all these clones are from the same family of repeated sequences or not.

1806 ANDROGENESIS LED TO SPECIFIC rDNA CHANGE IN *NICOTIANA SYLVESTRIS*. Hervé Levesque, Rosine De Paepe and Fernand Vedel.
Génétique Moléculaire des Plantes, URA115, UPS, Orsay F-91405

Three rDNA repeat length classes were found in leaves of a botanical line of *N. sylvestris* : class I has a length of 9.8 kb and about 2.600 copies, class II 10.1 kb and 2.000 copies and class III 10.3 kb and 900 copies. Length heterogeneity was located in the 5'region of the intergenic spacer. No difference could be detected between the rDNA organisation of any plants after five generations of selfing of the original line. In contrast, rDNA changes were observed in leaves from haploids and double haploids (D.H.) regenerated from successive cycles of androgenesis. This DH change consisted in the loss of 200 to 1200 copies of class II rDNA units. The loss occured as early as the first androgenetic cycle, increasing over the following cycles. Genetic studies showed that DH change was inherited through sexual generations but could segregate or reverse. Loss of class II rDNA copies was found in pollen vegetative nuclei from which androgenetic plants generally arose. This result suggested that class II-rDNA change did not originate from *in vitro* culture.

1807 GENETIC AND PHYSIOLOGICAL ANALYSIS OF TRANSGENIC TOBACCO CONTAINING HETEROLOGOUS PAL GENE, EXHIBITING SUPPRESSION OF ENDOGENOUS PAL GENES AND ABNORMAL PHENOTYPES. Yonatan Elkind, Ribak Ofer and Christopher J. Lamb*. Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76-100, Israel. *Plant Biology Laboratory, Salk Institute for Biological Studies, San Diego, CA, USA.

Transgenic tobacco plants containing a heterologous (bean) phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) gene modified by inclusion of cauliflower mosaic virus 35S enhancer sequences in its promoter, exhibited an unusual phenotype. Aberrations included localized lesions, altered leaf shape, reduced lignification in the xylem, stunted growth and reduced pollen viability. This phenotype was associated with reduced levels of PAL activity and soluble phenylpropanoids. Genetic analysis of T2 transgenes revealed a 1:2:1 segregation of reduced PAL activity, indicating a single, partially dominant gene. Accumulation of transcripts encoded by endogenous tobacco PAL genes was suppressed, while high levels of the bean PAL transcript were observed. Following three generations of selfing, T2, T3, T4 and T5 plants homozygous to the insert were grown simultaneously and analyzed for PAL activity and phenotype. The PAL activity in plants homozygous to the inserted gene was 1%, 23%, 31% and 78% of the non-transgenic plants for the various generations, respectively. The corresponding phenotype-effect indexes were 4.8, 1, 0 and 0, respectively, on a scale of 0 to 5. The physiological and genetic mechanisms involved in the perturbation of PAL activity in transgenic plants and the gradual decrease of the PAL2 transgene effect in subsequent generations are under investigation.

1808 DEVELOPMENT AND APPLICATION OF DNA PROBES SPECIFIC TO THREE RICE SUCROSE SYNTHASE ISOZYME GENES. Ju-Wei Huang and Jong-Ching Su. Department of Agricultural Chemistry, National Taiwan University, Taipei 10764. Republic of China.

We have shown that different sucrose synthase (SS) isozymes are expressed in different tissues of rice plant at different growth stages (Plant Physiol 94: 1456) and that at least three types of SS mRNA's are present by the cDNA cloning and sequencing (submitted to Plant Mol Biol). Recent intensive purification work has also yielded results proving unambiguously the presence of at least three SS isozymes in the rice plant (unpublished data). Since the expression of these isozymes are tissue and growth stage specific, the physiological roles played by them in the life span of the rice plant is an interesting problem to study.

The cDNA sequencing data showed that the coding regions of three types of SS cDNA had high homology but the long 3'-ends from the stop codon up to the polyadenylation signal are highly divergent. We selected about 160 base long segment of each SS cDNA and synthesized it by the PCR method. The radiotracer labeled DNA's have been proved to be highly specific toward the cDNA clones and are useful in analyzing the respective mRNA. Some results showing the tissue and growth stage specific expression of the isozymes will be presented.

(This work has been supported by grants from the National Science Council, Taiwan, Republic of China.)

POSTER ABSTRACTS

1809 THE AMOUNT OF INPUT DNA AFFECTS PHENOTYPIC VARIATION IN TOBACCO TRANSFORMED BY THE PARTICLE GUN. Bruce Drummond, Paula Beck, Martich, Sandi Lane, Margit Ross, Debi Blair, Tim Fox, and Ben Bowen. Pioneer Hi-Bred, P.O. Box 38, Johnston, IA 50131. Microprojectile bombardment is an efficient DNA delivery system for generating large numbers of stably transformed plants. Results from analyzing approximately 2500 independent Kan^R tobacco transformants derived by this method will be presented. Our first goal was to determine co-transformation frequencies for one, two, and three non-selected markers (GUS, BAR, and AMVcp), using levels of input DNA which are optimal for transient gene expression (approximately 1μg per bombardment). In subsequent dose-response experiments, we discovered that these levels of input DNA are saturating for stable integration. When the input DNA amount is reduced to 1ng per bombardment, the level of transient gene expression decreases by 500-fold, but stable transformation frequency decreases by only 50%. One effect of increasing the plasmid dose above 1ng per bombardment is a dramatic increase in the proportion of GUS^- co-transformants which exhibit a mosaic or "spotty" histochemical staining pattern. Currently, we are performing Southern hybridizations to determine if this phenotype is associated with aberrant integration patterns and/or methylation of the transforming DNA.

1810 CHROMOSOME PAIRING IN PROGENY FROM AN INTERSPECIFIC SOMATIC HYBRID OF *SOLANUM*.
Christie E. Williams and John P. Helgeson. University of Wisconsin. Dept. Plant Pathology, Madison, WI 53706

Segregation of RFLP loci was monitored to determine the degree of intergenomic pairing and recombination in a somatic hybrid that resulted from protoplast fusion between the cultivated potato, *Solanum tuberosum*, and its distant relative *S brevidens*, which harbors several traits for disease resistance. The hexaploid somatic hybrid, A206, was crossed to a tetraploid potato cultivar to generate a segregating population of pentaploid progeny. Although *S tuberosum* was highly polymorphic, the diploid *S. brevidens* was homozygous at all but three of the 70 RFLP loci that were tested. Thus, intergenomic recombination could be detected only when homeologous chromosomes paired and segregated so that the *S. brevidens* homologs were distributed into separate gametes. A bias toward intragenomic pairing was observed for all 12 chromosomes. Five chromosomes participated exclusively in intragenomic pairing, which resulted in each of 25 progeny containing all *S. brevidens* - derived RFLP markers from chromosomes 4,7,8,9 and 10. The remaining seven chromosomes paired with their homolog(s) about twice as often as expected if hexaploid pairing were completely random. Where detectable, intergenomic recombinations occurred at a frequency of 1.8 per chromosome and recombinational hot-spots were observed for chromosomes 1, 2 and 6. Somaclonal variation at the DNA level was observed in an *S. brevidens* -derived chromosome of hybrid A206 and several other hybrids from the same fusion parents. A specific small deletion in chromosome 4 was detected with 12 out of 14 somatic hybrids representing 13 independent fusion events. However, the deletion occurred in only one of two plants that regenerated from a single callus, indicating that the loss occurred in culture after fusion had taken place.

1811 USE OF THE ROLC GENE AS A MARKER GENE TO VISUALIZE T-DNA *trans* INTERACTION. Thomas Schmülling, Silke Pilz and Jeff Schell. Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, FRG

Expression of the *rolC* gene of *Agrobacterium rhizogenes* T-DNA under the transcriptional control of the 35S RNA promoter of cauliflower mosaic virus establishes in transgenic tobacco plants dominant pleiotropic phenotypic alterations such as male sterile flowers, reduced apical dominance, and light green leaves. The reduced pigment content in leaves is limited to cells that actively express the *rolC* gene. Crosses of kanamycin resistant P_{35S}-*rolC* transgenic plants with hygromycin resistant tobacco plants expressing a *rolC* antisense mRNA leads to a partial suppression of the *rolC* induced alterations in the uniform F1. Backcrosses of F1 plants with wildtype plants resulted in the inactivation of both the kanamycin and hygromycin marker genes carried by the two different T-DNAs. The formation of variegated light and dark green areas on leaves of the F2 generation visualizes the inactivation of the T-DNA encoded *rolC* sense and antisense genes, thus providing a tool to easily monitor the pattern of this process in leaves. Investigations on factors influencing these unexpected phenotypes, probably due to *trans*-interactions between different T-DNAs, will be discussed.

1812 ELUCIDATING PINE RIBOSOMAL GENE ORGANIZATION USING A P1 PHAGE LIBRARY. Susan W. Gorman, Robert D. Teasdale and Christopher A. Cullis. Case Western Reserve University, Biology Department, Cleveland, OH 44106. [1]Griffiths University, Brisbane, Queensland, Australia.

The organizaton of the ribosomal gene family in Monterey pine (*Pinus radiata*) has beeb difficult to determine using conventional cloning techniques. Data obtained from EMBL phage clones containing rRNA gene homology suggested a size in excess of 28 kilobases for the 18S - 5.8S - 25S repeat unit. However, the construction of a repeat unit from overlapping clones was unreliable since a significant amount of heterogeneity existed between the phage clones. *In situ* hybridization of the EMBL phage clones to mitotic chromosomes generated unique, dispersed patterns for some of the clones thereby confirming the heterogeneity within the rRNA gene family. In addition, CHEF gel analysis gave no indications that the rRNA genes are organized into long tandem arrays. In order to obtain an entire repeat unit and to better determine the organization of this gene family in pine, we constructed a genomic library using the P1 bacteriophage cloning system. Recombinant clones containing inserts averaging 80 kilobases in size and having homology to the 18S - 5.8S - 25S rRNA genes of flax have been identified. The relationship between these P1 clones and the EMBL clones and their respective placements within the genome is described.

1813 A UNIQUE GENE ENCODING A DEVELOPMENTALLY REGULATED β-TUBULIN IS PRESENT IN BOTH SOYBEAN AND TOBACCO GENOMES. Ian N. Jongewaard and Donald E. Fosket. University of California, Department of Developmental and Cell Biology, Irvine, California 92714

Tubulins are encoded by small multigene families in both soybean (*Glycine max*) and tobacco (*Nicotiana tabacum*), and some of these genes have been cloned and sequenced. A gene-specific probe was constructed from the 3' transcribed, but predominantly untranslated region of a cloned and sequenced soybean β-tubulin gene (sβ-1), which recognized a single band in diagnostic digests of soybean genomic DNA in southern blots. This probe was used to investigate the tissues and organs of the soybean plant in which the sβ-1 gene was expressed. We demonstrated that the sβ-1 gene is developmentally regulated, with strong expression primarily in etiolated hypocotyls and internodes. Analysis of tobacco genomic DNA and poly (A)+ RNA strongly suggested that this species contained a homologous expressed β-tubulin gene. Southern blots of tobacco genomic DNA probed with the sβ-1 gene specific probe exhibited a single band of hybridization at high stringency, with restriction patterns almost identical to those seen with soybean genomic DNA. Eleven independent lambda clones were isolated from a tobacco genomic library screened with the sβ-1 gene specific probe. Sequencing will determine the degree of homology between these two β-tubulin genes.

1814 GENOMIC AFFINITIES BETWEEN APOMICTIC AND SEXUAL SPECIES OF *Pennisetum*. Peggy Ozias-Akins and Edward L. Lubbers. University of Georgia Coastal Plain Experiment Station, Department of Horticulture, Tifton, Georgia 31793

The genus *Pennisetum* contains over 36 named species of which at least 6 are known to be obligate apomicts. Introgression of the trait for apomixis into the cultivated pearl millet (*P. glaucum*) has only been successful in complex crosses with apomictic *P. squamulatum*. Molecular characterization of an apomictic introgression line has led us to further study the molecular relationship between *P squamulatum* and 15 other apomictic and sexual species within the genus plus one species in a closely related genus, *Cenchrus ciliaris*. This has been accomplished using genomic clones from *P. glaucum* to survey for common restriction fragment lengths in other species, and by the isolation of specific repeated DNA sequences from *P. glaucum* and *P squamulatum*

POSTER ABSTRACTS

1815 PROPERTIES OF HOMOLOGOUS DNA RECOMBINATION IN PLANTS.
H Puchta, S. Kocher, P. Swoboda, S. Gal and B. Hohn. Fnedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

Homologous DNA recombination most often is analyzed with the help of marker genes used for selection and counterselection. We set up a system which allows monitoring of extrachromosomal recombination (ECR) in plant cells by a transient assay of the enzyme activity of the recombined gene. Overlapping deletion mutants of the β-glucuronidase (GUS) gene were transfected into plant protoplasts. We found a positive correlation between the length of overlapping homologous GUS sequences and the recombination rates. A significant drop of recombination efficiency was observed when the overlap was reduced to 456 basepairs or less. The requirement for such a long stretch of homology might ensure the stability of the rather repetitive plant genome. The flanking sequences of the recombination partners were found to be exchanged during ECR. This finding indicates that ECR in plant cells might proceed via "single strand annealing" of the recombination partners.

These approaches are in the process of being complemented by analysis of intrachromosomal and interchromosomal recombination (ICR) employing the GUS gene in transgenic plants. Recombination events *in planta* will be visualized by the wellknown histochemical assay.

1816 CONTROL OF THE Spm TRANSPOSABLE ELEMENT BY METHYLATION IN TRANSGENIC TOBACCO. M. Schlaeppi, P. Masson, D. Smith, and N. Fedoroff. Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland, 21210.

The maize Suppressor-mutator (Spm) transposable element has been transformed into tobacco cells and a visual assay for Spm activity has been developed.[1,2] Callus lines exhibiting different frequencies of Spm excision ranging from high Spm activity to null activity have been obtained from initially highly active callus. This phenomenon resembles the epigenetic inactivation of Spm in maize, correlating with differences in methylation of sequences immediatly upstream and downstream of the element's transcriptional start site.

The present work will discuss differences in the methylation of the Spm promoter in transgenic tobacco in relation with the element's activity. Different Spm derived cDNAs have been retransformed into transgenic tobacco with no or intermediate Spm activity. The ability of Spm-encoded gene products to promote the element's reactivation will be discussed.

[1]P. Masson and N. Fedoroff (1989) PNAS 86, 2219-2223

[2]P. Masson, M. Srem, and N. Fedoroff (1991) The Plant Cell 3, 73-85

1817 INTER-MOLECULAR HOMOLOGOUS RECOMBINATION USING CAULIFLOWER MOSAIC VIRUS. Susannah Gal, Barbara Pisan, Thomas Hohn, Nigel Grimsley and Barbara Hohn. Friedrich Miescher-Institut, P.O Box 2543, CH-4002 Basel, Switzerland, and INRA-CNRS, Castanet-Tolosan, France.

A non-selective method for scoring intermolecular recombination events in whole plants was set up. The genome of the cauliflower mosaic virus (CaMV) was separated into two parts. Either part alone is defective, but when the two parts are inoculated together, viral infection is produced. One part contained the information for gene VI of the virus, while the other contained genes I through V. Each part was placed separately into the genome of *Brassica napus* plants. The transgenic plants were provided with the complementing viral information either by inoculation with *Agrobacterium* containing the sequences in the T-DNA, or by crossing these plants with other transgenic plants containing the complementing information. Detection of the successful intermolecular recombination event was by analysis of DNA, by infectivity of leaf extracts on turnips or by visual appearance of viral symptoms on the transgenic plants. In two separate experiments where gene VI plants were inoculated with *Agrobacterium* containing genes I through V, CaMV was detected. In experiments where the two pieces of viral information were provided in plants that were crossed, one set showed virus in the resulting progeny plants. These viruses have been cloned and sequenced to discern by what mechanism the intermolecular recombination events took place.

1818 HOMOLOGOUS RECOMBINATION IS INFLUENCED BY GENOMIC CONTEXT. Peter Swoboda, Susannah Gal and Barbara Hohn. Friedrich Miescher-Institut, P.O Box 2543, CH-4002 Basel, Switzerland.

A non-selective method for scoring genomic recombination events in whole plants was set up. Defective partial dimer sequences of Cauliflower Mosaic Virus (CaMV) were placed into the genome of *Brassica napus* plants. Provision of repeated homologous regions within the CaMV-sequences allows the virus to recombine out of the genome, to amplify and to produce viral symptoms, thus serving as the recombination marker.

Transgenic plants containing a single locus with respect to the recombination substrate (CaMV-sequences) were used to investigate the time in the development of the testplant at which the recombination event takes place. Analysis of three transgene loci revealed different recombination efficiencies, the highest one being in the order of 10^{-7}/cell division. This may have precluded detection of recombination events early in plant development (cotyledons). Alternatively, recombination events absent from cotyledons may be interpreted as developmental regulation. Additionally, recombination efficiencies were also dependent on the ploidy level of the transgenes.

The system presented here will be used in the future to elucidate whether plant internal (developmental) or external (environmental) factors have an effect on recombination.

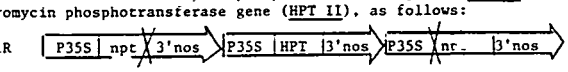
1819 CLONING AND SEQUENCING OF GENOME SPECIFIC REPETITIVE DNA SPECIES OF *SOLANUM NIGRUM*. P.K. Mallik, D.K Mukhopadhyay, A. Dutta, A. Banerjee and Rajat K. Chaudhuri. Calcutta University, Department of Botany, 35 B C Road, Calcutta 700 019, India.

Solanum nigrum having a very small genome (0.3 pg) is under intensive study at this laboratory. Three cytotypes of the species, namely diploid, tetraploid and hexaploid, show variability in habit, stem, leaf and flower morphology. At DNA level however they show some degree of homology. By comparing the ribosomal RNA genes it is apparent that the genes have identical tandem reapeats in three forms, they their copy numbers vary. A 300 basepair Hind III repeat was cloned in pUC vector. The clone show clustered identical pattern in diploid and hexaploid forms but is absent in tetraploid cytotype. In scrutiny it is found to be genome specific. After sequencing, the repeat is found to contain controlling element like short repeats. Results will be presented.

Financial assistances of CSIR project, UGC and Rockefeller Foundation (project no 48) are acknowledged.

1820 DUPLICATED TRANSGENES IN ARABIDOPSIS: RECOMBINATION AND GENE-SILENCING. Farhah F. Assaad & Ethan R. Signer. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA

Repeated sequences in plants are targets for recombination and also for homology-dependent, repeat-induced gene inactivation (gene silencing). To monitor the behavior of repeated sequences in plants we have introduced duplicated selectable marker genes into the plant genome. Our construct consists of two mutant neomycin phosphotransferase genes (npt II) flanking a hygromycin phosphotransferase gene (HPT II), as follows:



Recombination between the two npt deletions may yield an active NPT gene that confers kanamycin resistance on regenerated protoplast colonies (tobacco) or seeds (Arabidopsis). Recombination events are observed at frequencies of 10^{-4} in Arabidopsis seedlings and 10^{-5} to 10^{-6} in tobacco protoplasts on kanamycin selection. Recombinants are characterized by Southern analysis. We observe silencing of the duplicated NPT gene among selected kanamycin-resistant progeny of the recombinant Arabidopsis plants. Different recombination events appear to yield different degrees of gene silencing. In addition, the HPT gene, which is not duplicated but expressed from a P35S promoter as are the NPT genes, is also occasionally inactivated.

POSTER ABSTRACTS

1821 INTERACTION AMONG MEMBERS OF THE R FAMILY IN MAIZE. G.Gavazzi, A.Ronchi, G.Consonni, M.A.Mereghetti and C.Tonelli. Dipartimento di Genetica e di Biologia dei Microrganismi, Università degli Studi di Milano, via Celoria 26, 20133 MILANO,Italy

The genetic system under investigation is defined by two major components: a gene, Sn conferring tissue specific anthocyanin accumulation in different plant regions and another gene, R, lying two units Sn proximal and required for pigment accumulation in tissue not of Sn competence. Attention will be focused on *Sn:bol3*, an accession characterized by strong pigmenting potential in the mesocotyl and partially constitutive expression in specific plant tissues. A feature of *Sn:bol3* not shared by the other *Sn* accessions so far tested, is the recovery of nonparental *Rr-g* seedlings in the progeny of *RrSn:bol3* females crossed to homozygous *r-g* males, exhibiting the *Sn* phenotype in their mesocotyl as if the heterozygous association in the femal parent would promote passage of *Sn* from the *r* marked (donor) chromosome to the *R* marked (recipient) homologue. This apparent passage is not associated to outside markers recombination, is negatively affected by chromosomal rearrangements and varies in its frequency according to the *R* constitution of the recipient chromosome. Two tests were devised to ascertain the *Sn* constitution of the nonparental *R* marked chromosome recovered in the exceptional progeny. The first one, referred to as functional *Sn* assay, relies on the observation that introduction of *Pl* on *Sn* bearing genotype leads to constitutive, light indipendent, pericarp pigmentation while the second test is based on the Southern analysis of DNA extracted from individual progeny seedlings obtained in testcrosses of *Rr Sn:bol3* heterozygotes and probed with specific *Sn* probes recognizing either the 5' or 3' region of the gene. Surprisingly both tests are consistant in showing that *R* progeny seedlings exhibiting an *Sn* phenotype in their mesocotyl do not carry *Sn* in their genome. We believe that the *Sn* phenotype is due to activation of an inactive component present on the *R* bearing chromosome becoming expressed by association with *Sn bol3* in the previous generation. We refer to the acquisition of the *Sn* phenotype without physical transfer of DNA as *Sn-p* and to the inductive process as reversed paramutation.

1822 5-METHYLCYTOSINE CONTENT IN HOMOSPOROUS FERNS. J. Mitchell McGrath and Eran Pichersky. University of Michigan, Department of Biology, Ann Arbor, MI 48109.

Homosporous pteridophytes have high chromosome numbers relative to other vascular plant groups. The high chromosome numbers could have resulted from polyploidization. However, genetic analyses of isozyme expression in ferns (specifically those with the lowest chromosome number within genera) indicates an equivalent number of loci as seen in diploid angiosperms. The observations of diploid patterns of gene expression and high chromosome numbers indicative of polyploidy could be reconciled if gene silencing of duplicate loci in an ancient polyploid lineage has occurred. In support of this hypothesis, the majority of chlorophyll a/b binding protein (CAB) genes recovered from the fern *Polystichum munitum* appear to have suffered various mutations and are thus unlikely to be expressed. In contrast, defective CAB genes have been rarely identified in angiosperms. Another mechanism of gene silencing may involve DNA methylation; often, expressed genes are hypomethylated. We have examined the level and distribution of 5-methylcytosine nucleotides in three homosporous pteridophytes: *Ceratopteris richardii* (n=39), *Polystichum munitum* (n=41) and *Psilotum nudum* (n=52). Each species shows an overall level of 5-methylcytosine similar to angiosperms; i.e. 29 mol %, 34 mol % and 30 mol %, respectively. Restriction endonuclease digestion with methylation sensitive/insensitive isoschizomers *Hpa* II and *Msp* I (CCGG) or *Eco* RII and *Mva* I (CCWGG, where W is A or T) indicate both CG and CWG sequences are extensively modified in fern genomic DNA. Digestion with the isoschizomer *Apy* I (CCWGG), which requires 5-methylcytosine for activity, cleaves *Eco* RII resistant genomic DNA to a pattern similar to that seen with *Mva* I, confirming the predominant modification is 5-methylcytosine. Genomic blots indicate that a minority fraction of fern ribosomal RNA genes are methylated. In contrast, the majority of signal detected from CAB gene sequences appears in methylated regions.

1823 CLONING AND CHARACTERIZATION OF RICE β-AMYLASE GENES. Iychian Chen, Eva Cheng, Wei-Ling Lue, Shue-Mei Wang*. Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan. *Botany Dept. National Taiwan Univ. Taiwan

β-Amylase, an exoamylase that hydrolyzes starch to produce maltose, is one of the major amylolytic enzymes found in germinating cereal grains. We have cloned two β-amylase genes from rice, which are sharing high homology to each others and to barley β-amylase gene. From the genomic sequence compared to barley β-amylase cDNA sequence, six introns and seven exons were identified. Carboxyl terminus of barley β-amylase contains 44 amino acids repetitive sequence, which may be processed during seed germination. This repetitive sequence was not found in the predicted rice β-amylase protein sequence. With antibody to β-amylase we found that β-amylase was not synthesized or synthesized of low amount during seed development. In barley β-amylase was synthesized mainly throughout seed development, and was processed from the precursor to active form with smaller molecular weight during seed germination. However, the rice β-amylase was synthesized during seed germination with similar size to the matured barley β-amylase. In rice leaves two forms of β-amylases, the major form with smaller size than the seed β-amylase and the other with similar size to seed β-amylase, could be partially purified by glycogen precipitation Analysis of promoter activity with GUS reporter in transgenic tobacco tissue is underway.

1824 VARIABILITY IN RUBISCO SMALL SUBUNITS CORRELATED WITH CARBOXYLATION ACTIVITY. Hanna Eilenberg, Sven Beer, Shimon Gepstein[1], Nurit Geva and Aviah Zilberstein. Tel Aviv University, Dept of Botany, Tel Aviv 69978; Technion[1], Dept of Biology, Haifa 32000, Israel.

Different morphogenetic patterns are induced by red (R) or blue (B) light in Pteris vittata (a fern) gametophytes. We have shown that these two types of light spectra also induce the appearance of different Rubisco SSUs. Our results show that while Rubisco LSUs and SSUs are present in stoechiometric amounts in both types of gametophytes, the R-induced SSUs are not recognized by polyclonal antibodies raised against SSU from spinach, while the B-induced SSUs are well recognized. Analysis of proteolytic cleavage of R and B SSUs by proteases further confirmed the difference between the two types of SSUs. While no significant difference was detected in the Glu-C (V8) cleavage products on SDS-PAGE, a clear difference was observed with trypsin cleavage. It, therefore, seems that under various light spectra, different rbcSs from the multi-gene family are expressed. Comparison of Rubisco carboxylation activity in extracts from gametophytes grown under blue or red light, revealed a significantly higher enzyme activity in extracts from B gametophytes. An approximately 2-3 fold higher V_{max} value was observed for the B-induced enzyme. We suggest, that a certain correlation exists between SSU primary structure and holoenzyme activity. Amino acid sequence analysis of the first twenty N-terminal amino acids of SSU^B and SSU^R revealed a 65-70% homology to the sequence of higher plants. A much lower homology (40%) was found with cyanobacterial sequences. No difference was found in the sequence of the first twenty amino acids of the B and R SSU. Microsequencing of trypsin and V8 cleavage products of SSU^B and SSU^R are under way.

1825 GENE EXPRESSION OF CARBONIC ANHYDRASE IN CHLAMYDOMONAS REINHARDTII REGULATED BY ENVIRONMENTAL CO_2 AND LIGHT. Hideya Fukuzawa, Akira Tachiki*, Shigetoh Miyachi*
Kyoto Univ., Dept. of Agric. Chem., Kyoto 606-01, JAPAN, *Marine Biotechnology Institute Co., Tokyo 113, JAPAN

Carbonic anhydrase (CA) catalyzes reversible hydration of CO_2, and enhancing CO_2 concentrating mechanisms in plant and algal cells. In unicellular green alga Chlamydomonas reinhardtii, most CA is located in periplasmic space. The enzyme structure was defined to be a heterotetramer consist of two large subunits (35 kDa) and two small subunits (4 kDa) liked by disulfide bonds[1]. Analysis of cDNA clones revealed that these two subunits are cotranslated as a precursor polypeptide with hydrophobic signal peptide[2]. Two tandemly clustered genes (CAH1 and CAH2) were located in the Chlamydomonas genome[3,4]. CAH1 mRNA is induced by lowering environmental CO_2 concentration in the bubbling gas in light. On the other hand, CAH2 mRNA is repressed in low-CO_2 condition and induced by increase of CO_2 concentration. We have isolated CA2, which is CAH2 gene product from high-CO_2 grown cells. These results indicate that two CA isozymes are differentially regulated by environmental CO_2 and light. We are now studying to identify CO_2 responsive cis- and trans- acting elements in Chlamydomonas cells.

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(2) Fukuzawa, H. et al. Proc Natl Acad Sci (1990) 87:4383-4387.
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(4) Fujiwara, S. et al. Proc Natl Acad Sci (1990) 87:9779-9783.

1826 IDENTIFICATION OF NEW GENE (icfA) WHICH IS ESSENTIAL FOR EFFICIENT CARBON FIXATION IN CYANOBACTERIUA SYNECHOCOCCUS PCC7942.
Hideya Fukuzawa and Eiji Suzuki*,
Kyoto Univ., Dept. of Agric. Chem., Kyoto 606-01, JAPAN, *Ibaraki Univ., Dept. of Biol., Mito 310, JAPAN

In order to understand the nature of inorganic carbon concentrating mechanisms in Cyanobacteria, several high-CO_2 requiring mutants have been isolated[1,2]. By using DNA transformation techniques genomic regions have been cloned and some open reading frames have been identified. We have cloned a genomic DNA, which complement a high-CO_2 requiring temperature sensitive mutant C3P-O[3]. Nucleotide sequence analysis of the genomic region revealed that an open reading frame could be a gene essential for efficient carbon fixation in Cyanobacteria. We have done insertional mutagenesis at several restriction sites around the open reading frame and identified that this ORF is essential for carbon fixation. Therefore we named this ORF as a gene icfA. We discuss the relationship between this gene icfA and carbonic anhydrase, which catalyzes reversible hydration of CO_2.

(1) Abe T. et al. (1988) Plant Cell Physiol 29: 1353-1360.
(2) Price GD and Badger MR (1989) Plant Physiol 91: 514-525.
(3) Suzuki E, Fukuzawa H, and Miyachi S. (1991) Mol Gene Genet, in press.

POSTER ABSTRACTS

1827 SITE-DIRECTED MUTAGENESIS OF THE RUBISCO LSU TO SSU INTERFACE. Ralf Flachmann and Hans J. Bohnert, Department of Biochemistry, The University of Arizona, Tucson, AZ, 85721, U.S.A.

The LSU of Rubisco carries all active site residues yet activity in the L_8S_8 holoenzyme is enhanced by a factor of approximately 150 when SSU is assembled with the L_8 core. We are interested in identifying residues which are of importance for the activation step that presumably is mediated via long-range conformational changes following LSU/ SSU assembly leading to stabilization of the active site conformation. A better understanding of the assembly and activation processes by SSU might help explain the evolution of the CO_2/ O_2 specificity factor that distinguishes bacterial, cyanobacterial, and higher plant Rubisco enzymes. Results from two parallel approaches will be presented. We have focussed initially on the mutagenesis of a higher plant-specific peptide in SSU which forms a hairpin loop in spinach holoenzyme. While a number of alterations within the loop are tolerated for the assembly of higher plant Rubisco, replacement of an invariant arginine leads to an assembly-deficient SSU. In a different approach residues in cyanobacterial LSU at the LSU to SSU interface (as judged from structure models of the spinach holoenzyme) were modified. When such altered LSU were assembled with unmodified SSU, several holoenzymes with significantly altered specific activities were observed. Further alterations at the interface of the two subunits will help us understand the role of SSU in activation. Supported by DFG and NSF.

1828 MOLECULAR APPROACHES TO STUDY THE ROLE OF MEMBRANE TRANSPORTERS IN ASSIMILATE PARTITIONING OF HIGHER PLANTS. W. Frommer, U.I. Flügge, S. Hummel, T. Martin , J. Riesmeier, B. Schulz, R. Wöhner, L. Willmitzer. IGF, D1000 Berlin 33, Germany

Assimilates that are produced in chloroplasts of leaves have to cross the plastid envelope to become available e.g. for sucrose synthesis in the cytosol. The export of assimilates is mediated by the phosphate translocator. Sucrose and amino acids are actively loaded into the phloem in the source leaves and transported to sink organs that are dependant on the supply from the sources such as roots, flowers, tubers and seeds. The active loading presumably involves a proton gradient that is energized by proton ATPases and specific transport systems in form of proton cotransporters for amino acids and sucrose where . In order to study and modulate the expression of genes involved in assimilate partitioning we have isolated both cDNA coding for membrane transporters such as the chloroplastidic triose-phosphate-phosphate translocator and the plasmalemma ATPase from potato. We have analysed their expression and made constructs in order to overexpress or antisense-repress expression of the respective genes in transgenic plants.

1829 REDUCTION OF RUBISCO CONTENT BY ANTISENSE RNA REDUCES PHOTOSYNTHESIS IN TRANSGENIC TOBACCO PLANTS. Graham S. Hudson, John R. Evans, Susanne von Caemmerer, Yvonne B.C. Arvidsson and T. John Andrews. Research School of Biological Sciences, Australian National University, Canberra, Australia

Rodermel et al. (1988) have shown that antisense RNA directed against the mRNA for the Rubisco small subunit (SSu) can lower the leaf content of both SSu and LSu polypeptides in transgenic tobacco plants. We have used the same technology to generate tobacco plants with reduced levels of Rubisco to study the effect of this change on the biochemical and physiological properties of their leaves. A cDNA for tobacco SSu was isolated from tobacco and used to construct an antisense gene with a binary plasmid. The antisense gene was introduced into tobacco cells by *Agrobacterium*-mediated transformation, and plants were regenerated for analysis. The mean Rubisco content (as determined by enzyme activity) of 10 anti-SSu plants was 18% of the content in control transgenic plants. The reduction in soluble protein content was consistent with this loss of Rubisco. There was little change in the levels of chlorophyll and another enzyme of the Calvin cycle, phosphoribulokinase. The rate of CO_2 assimilation at high light was reduced, and was limited by the level of Rubisco activity at high and low p(CO_2). By contrast, the rates in control leaves were limited by Rubisco activity only at low p(CO_2). Stomatal conductance was unaffected, indicating some independence of guard cell function and Rubisco activity. This was supported by measurements of carbon isotope discrimination.

Rodermel, S.R., Abbott, M.S. and Bogorad, L. (1988) Cell 56, 673-681.

1830 REGULATION OF EXPRESSION OF THE CYTOSOLIC FRUCTOSE 1,6-BISPHOSPHATASE IN SUGARBEET LEAVES. Eli Khayat and Jaleh Daie, Dept. of Crop Science, Rutgers Univ., New Brunswick, NJ 08903

The high degree of regulation which is presumably required for precise and coordinated control of the enzymes which interplay in carboxylation and in assimilate synthesis, makes the cytosolic fructose 1,6-bisphosphatase (FBPase) an interesting target for biochemical/ molecular analysis. Cytosolic FBPase catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate and Pi. The latter is transported into the chloroplast to feed intermediates of the Calvin Cycle. A rat liver cDNA and antibody against the sugarbeet FBPase were used to determine the regulation of FBPase in developing and during greening of sugarbeet leaves. The expression of cytosolic FBPase gene was temporally coordinated with those encoding the small and large subunits of RuBISCO in developing leaves of sugarbeet. In addition, the synthesis of the protein was repressed in etiolated leaves and could be detected after exposure to approximately 12h of light. The transcriptional expression of FBPase in sugarbeet leaves was coordinated with leaf photosynthetic rates and with the transcriptional expression of RuBISCO genes.

1831 ACCUMULATION OF CARBOHYDRATE LEADS TO A DECREASE OF PHOTOSYNTHETIC PROTEINS. Anne Krapp[1], Monika Brauer[1], Uwe Sonnewald[2], Lothar Willmitzer[2] and Mark Stitt[3]. [1]Universität Bayreuth, Lehrstuhl für Pflanzenphysiologie, Universitätsstr. 30, 8580 Bayreuth, FRG, [2]Institut für Genbiologische Forschung GmbH, Ihnestr.63, 1000 Berlin 33, FRG, [3]Universitat Heidelberg, Institut für Botanik, Im Neuenheimerfeld 360, 6900 Heidelberg, FRG

The "sink" regulation of photosynthesis by carbohydrate has been studied in (1) detached leaves, (2) cell suspension cultures and (3) transgenic tobacco plants expressing invertase in their cell wall. Evidence will be presented that accumulating carbohydrate may lead to a specific loss of photosynthetic proteins. The contribution of transcriptional control will be investigated.

1832 TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION IN THE WHEAT PHOTOSYNTHETIC CARBON REDUCTION CYCLE Alison J. Miles. Christine A. Raines and Julie C. Lloyd University of Essex, Department of Biology, Colchester, Essex, CO4 3SQ

Earlier studies which we have made on the expression of photosynthetic carbon reduction (PCR) genes in wheat suggested that steady-state transcript levels of chloroplast ribulose-5-phosphate kinase (PRKase), fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase) are controlled by light, stage of development and tissue-type. Cloned copies of each of these genes have been isolated from a wheat genomic library and characterised by DNA sequence analysis. We are currently investigating the transcriptional regulation of these PCR genes by examining protein-DNA interactions in their upstream regions which might be involved in regulation. Gel-shift assays are being used to identify protein-DNA interactions between upstream sequences and nuclear protein extracted from light-grown 7 day old wheat leaves. Competition studies are being used to indicate the specificity of binding and have revealed that one interaction is common to both the PRKase and FBPase promoters. We intend to make use of deleted fragments in gel-shift assays and DNA footprinting techniques to identify the cis-acting sequences which are involved in this interaction. Nuclear extracts prepared from dark-grown leaves and other tissues will be used to determine the tissue distribution of the nuclear factor giving this binding reaction.

POSTER ABSTRACTS

1833 APPROACHES TO INFLUENCE SINK / SOURCE INTERACTIONS IN TRANSGENIC PLANTS. B. Müller-Röber, J. Koßmann, L. Willmitzer and U. Sonnewald. Institut für Genbiologische Forschung Berlin GmbH, Ihnestraße 63, 1000 Berlin 33, F.R.G.

Growth and development of plants are dependent upon the energy gained by fixing CO_2 into carbohydrates during photosynthesis and the distribution of newly synthesised metabolites. The primary sites of photosynthesis are mature (source) leaves from where the assimilates are transported via the phloem to sink tissues such as roots, seeds or tubers. The development of sink organs might be dependent on the ability of the sink tissue to use the imported photoassimilates and/or the ability of the source tissue to export photoassimilates efficiently.

Based on known biochemistry of carbohydrate metabolism we have tried to identify key steps controlling carbohydrate metabolism and partitioning. To this end transgenic plants were designed carrying either endogenous genes in the antisense orientation or alien genes coding for heterologous enzyme activities. The following experiments were performed:

1. Chimaeric yeast invertase was introduced into the cell wall in order to interfere with apoplastic sucrose export.
2. *E. coli* inorganic pyrophosphatase was expressed in the cytosol of transgenic plants. The rationale behind this experiment was to remove the cytosolic PP_i in order to push the activities of PFP and UDPglucose pyrophosphorylase towards sucrose synthesis.
3. The influence of sucrose cleavage on the ability of sink tissues to attract photoassimilates was approached via antisense inhibition of sucrose synthase or heterologous (apoplastic or cytosolic) expression of yeast invertase in tubers of transgenic potato plants.

The influence of these alien gene products in transgenic plants will be discussed.

1834 APPROACHES TO INFLUENCE STARCH QUANTITY AND QUALITY IN TRANSGENIC PLANTS. B. Müller-Röber, J. Koßmann, L. Willmitzer and U. Sonnewald. Institut für Genbiologische Forschung Berlin GmbH, Ihnestraße 63, 1000 Berlin 33, F.R.G.

Starch is one of the most abundant carbohydrates in the biosphere. It is transiently synthesised in chloroplasts or for long-term storage in amyloplasts of seeds, tubers and roots. Starch is compost of the linear α-(1,4) glucan amylose and the branched α-(1,4) glucan amylopectin carrying additional α-(1,6) bonds. The key reaction of starch biosynthesis is thought to be the conversion of glucose-1-P to ADPglucose which is carried out by the enzyme ADPglucose pyrophosphorylase (AGPase). ADPglucose is added to primer molecules leading to the synthesis of linear α-(1,4) polyglucans. This reaction is catalysed be the enzyme starch synthase which exists in two distinct, soluble and granule bound, forms. The branch points are introduced into the linear polyglucan via the action of starch branching enzyme (BE). A second possible pathway of starch synthesis is mediated by starch phosphorylase (STP) which uses glucose-1-P as a substrate for the polymerisation reaction. Since the reaction of STP is readily reversible depending on the substrate concentrations the enzyme is thought to be more likely involved in starch degradation.

In order to analyse the transcriptional regulation of key enzymes in starch biosynthesis and to elucidate the possibility of altering the amount and the structure of starch granules we isolated cDNA clones encoding potato ADPglucose pyrophosphorylase and starch branching enzyme. These clones as well as a cDNA clone coding for the granule bound starch synthase were used in antisense experiments in transgenic potato tubers. Furthermore we approached the function of starch phosphorylase in starch biosynthesis and/or degradation using the same strategy.

Results arising from these experiments will be discussed.

1835 IMPROVING THE EFFICIENCY OF GROWTH, PHOTOSYNTHESIS, RuBP-CARBOXYLASE, NITROGENASE AND NITRATE REDUCTASE ACTIVITIES BY TRIACONTANOL IN *SESBANIA PALUDOSA* PRAIN. K. Muthuchelian, School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai. India.

The effect of mixture of long chain aliphatic alcohols, that have been shown to function as growth regulators, (Triacontanol, $CH_3(CH_2)_{28}\ CH_2OH$) was studied on growth, photosynthesis, RUBp-carboxylase, nitrogenase and nitrate reductase activities in *Sesbania paludosa* prain. Triacontanol (2 mg Kg-1) applied as seed soak and foliar spray resulted in the promotion of biomass (208%), nodule biomass (216%), chlorophyll (126%), soluble protein (126%), net photosynthetic rate (230%), nitrate reductase (209%), nitrogenase (144%) and RuBp-carboxylase (225%) activities. These results strongly suggest that treatment with triacontanol enhance the photosynthetic and nitrogen assimilating efficiency results in increased growth and productivity.

1836 HEAT-INDUCED CHANGES IN NET PHOTOSYNTHESIS, PHOTOSYSTEM II AND *in vivo* CHLOROPHYLL *a* FLUORESCENCE IN *CENCHRUS CILIARIS* L. A C4 GRASS. K. Muthuchelian, School of Energy, Madurai Kamaraj University, Madurai 625 021. India.

Heat-induced changes in net photosynthesis, transpiration, electron transport system (PSII) and *in vivo* chlorophyll *a* fluorescence were studied in *Cenchrus ciliaris* L. a C4 grass. Exposure of leaves to 30, 35 and 40°C temperatures did not cause any significant inhibition in net photosynthetic rate. However, exposure to 50°C caused a substantial inhibition of net photosynthesis accompanied by increased stomatal diffusive resistance and decreased transpiration rates. The rate of PS II as monitored by DCPIP reduction increased with the heat treatment upto 40°C and hampered at 50°C. Similarly *in vivo* chlorophyll *a* fluorescence was not affected significantly at 30, 35 and 40°C. Thus it is evident that the energization of thylakoid membrane and the light harvesting chl a/b protein complex from PS II were not affectd appreciably leading to the considerable degree of stability upto 40°C in *Cenchrus ciliaris*.

1837 SUBUNIT STRUCTURE OF POTATO TUBER ADPGLUCOSE PYROPHOSPHORYLASE. Paul A. Nakata, Joseph M. Anderson, Thomas W. Okita, and Jack Preiss. Institute of Biological Chemistry, Washington State University, Pullman, WA. 99164-6340. Department of Biochem, Michigan State University, East Lansing, MI 48824 (J.P.).

The biosynthesis of α-1,4-glucans in both bacteria and plants is controlled primarily by the expression and allosteric regulation of ADPglucose pyrophosphorylase. A near homogeneous ADPglucose pyrophosphorylase was prepared from potato tubers using a modified published procedure together with Mono Q chromatography. In single dimensional SDS-PAGE the enzyme migrated as a single 50 kD polypeptide band. Analysis by two dimensional SDS-PAGE, however, revealed the presence of two different polypeptides distinguishable by their slight differences in net charge, molecular weight, and immunological reactivity to antibodies raised against the individual spinach leaf ADPglucose pyrophoshorylase subunits. This result suggests that the potato tuber ADPglucose pyrophosphorylase is not a simple homotetramer as previously described but is formed of two separate and distinct subunits as observed with the spinach leaf and maize endosperm enzymes. Recently, both cDNA and genomic clones of the potato tuber small subunit have been isolated and their structure elucidated. A comparison of the rice endosperm and potato tuber cDNA's revealed a 74% homology at the nucleotide level and a 91% homology at the amino acid level. Similarly, both rice and potato pyrophospnorylase genes exhibit a complex gene structure as do a number of other genes involved in starch metabolism. Currently, cis acting elements and their involvement in spatial and temporal expression are under investigation. (Supported in part by a McKnight Foundation Graduate Fellowship).

1838 GENE EXPRESSION DURING LEAF DEVELOPMENT: SYNTHESIS OF A CDNA PROBE USING DEGENERATE OLIGONUCLEOTIDE PRIMERS AND THE POLYMERASE CHAIN REACTION. Asmita Patel, Linda L. Walling, Irwin P. Ting. Department of Botany & Plant Sciences, University of California, Riverside, California 92521

Peperomia scandens is a tropical epiphyte that exhibits a shift in carbon metabolism from C_3 to CAM during development. Studies in our laboratory have shown that leaf PEPC activity increases significantly during the ontogenic C_3 to CAM shift. However, Rubisco activity does not change significantly. Based on amino acid comparisons of PEPC protein identified in corn and *Mesembryanthemum crystallinum*, 2 mixed oligonucleotide primers were synthesized (a 5' sense primer and a 3' antisense primer) to two regions that were separated by 2.0 kb. Using these two primers, and first strand cDNA made from CAM-induced leaves of *Peperomia scandens*, a 2.0 kb cDNA fragment was synthesized by the polymerase chain reaction (PCR). The 2.0 kb PCR product was consistently observed under moderate to high primer annealing temperatures (42°C-65°C). This fragment was subcloned into pBluescribe SK. The subcloned PCR fragment (pPSC3) was sequenced to confirm its identity and was used to screen an λ GEM 4 leaf cDNA library of *P. scandens*. Several putative PEPC cDNA clones have been isolated and are being characterized. Northern blot analyses indicate that PEPC transcripts in *P. scandens* are regulated during leaf development which contrasts with the pattern of Rubisco small subunit and Cab PSII transcripts accumulation during leaf ontogeny. The autoradiographic signals from these RNA blots are currently being quantified by laser densitometry. RNA levels when correlated with PEPC protein and activity will allow us to evaluate the contribution of post-transcriptional, translational, and post-translational control to the expression of PEPC.

POSTER ABSTRACTS

1839 A CONTINUOUS FLOW, OPEN PHOTOSYNTHESIS SYSTEM FOR MEASURING ASSIMILATION RATE VERSUS INTERNAL CO_2 CONCENTRATION AT USER-SELECTABLE HUMIDITIES. Kerry Petersen, Dayle McDermitt and Steve Roemer, LI-COR, inc., 4421 Superior Street, Lincoln, Nebraska, 68504.

The measurement of leaf assimilation versus internal CO_2 concentration (AC_i) may be used to draw inferences about the biochemistry of photosynthesis. Typically, AC_i studies have been limited to the laboratory or to bulky and expensive mobile field laboratories. Recently, field measurement of AC_i curves were accomplished using the LI-6200 Portable Photosynthesis System. AC_i measurements were made in a closed-loop configuration while the CO_2 concentration in the chamber was continuously variable. The principle advantage of this approach is the elimination of precision CO_2 tanks. However, under certain conditions it is desirable to maintain steady-state concentrations of both CO_2 and water vapor in the chamber at values either significantly higher or lower than ambient. In this study we describe the LI-610 Portable Dewpoint Generator, a recently released instrument which may be configured with the LI-6200 Portable Photosynthesis System to enable photosynthesis and stomatal conductance studies with user-controlled water vapor and CO_2. Configuring the LI-6200 for continuous, differential measurement of CO_2 eliminates the requirement for precision CO_2 tanks (up to ambient levels), and permits the leaf to remain in the chamber indefinitely in a steady-state condition.

1840 EXPRESSION OF CHLOROPLASTIC AND CYTOSOLIC PHOSPHOGLYCERATE KINASE (PGK) GENES IN BARLEY AND OTHER PLANTS. Nishith Shah and J.William Bradbeer. King's College London, Division of Biosphere Sciences, Campden Hill Road, London W8 7AH, U.K.

We have studied chloroplast development and photosynthesis and isolated the PGK isoenzymes of barley leaves (McMorrow and Bradbeer, Plant Physiol.93,374-383, 1990) but, because of the greater agricultural significance of wheat, antisera raised against barley isoenzymes were used to identify the appropriate clones in a wheat nuclear cDNA library (Longstaff et al., Nucleic Acids Research 17,6569-6587, 1989). Although the corresponding barley cDNAs have yet to be cloned and sequenced the two chloroplast isoenzymes are closely similar to each other, as are the two cytosolic isoenzymes. The similaritieswithin the families of chloroplastic and cytosolic isoenzymes extend through a wide range of vascular plants but barley PGK antisera do not precipitate cyanobacterial PGK. Isoenzyme levels in barley leaves were found to be proportional to the mRNA levels. Both isoenzymes are present in leaves of plants grown in total darkness, the chloroplastic isoenzyme amounting to 70% of total PGK activity. Illumination brings about a 3-fold increase in chloroplastic PGK mRNA and an 8-fold increase in the isoenzyme but has little effect on the cytosolic PGK mRNA and activity, resulting in 91% of total PGK enzyme being in the chloroplasts. Well before the onset of leaf senescence there is a 15-fold increase in cytosolic PGK, but no change in chloroplastic PGK, a development which may be concerned with the export of leaf reserve materials prior to senescence and death.

1841 CELL-SPECIFIC REGULATION OF THE C_4 NADP-MALIC ENZYME. John D. Stubbs[1,2], John Mason[2], Paul Whitfeld[2], Elizabeth Smith[2] and William C. Taylor[2]. [1]Dept of Biology, San Francisco State University, San Francisco, CA 94132 and [2]CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia

Plants using the C_4 pathway of photosynthetic CO_2 fixation are well suited for study of cell-specific and light-regulated transcription. Each C_4 enzyme is present in either mesophyll or bundle sheath cells as a result of cell-specific gene expression. NADP-malic enzyme (NADP-ME) is specifically expressed in BS cells. We have isolated several different genomic clones containing NADP-ME sequences from *Flaveria bidentis*, a dicotyledonous C_4 weed. The gene encoding the chloroplast C_4 isozyme has been identified. Northern blot analysis detects a 2.2 kb mRNA transcribed from this gene which is strongly light inducible. Significant structural differences were found between the *Flaveria* and maize genes encoding the C_4 isozyme of NADP-ME. The maize gene has an intron in the transit peptide sequence and another at or near the transit - mature polypeptide junction (Rothermel, 1990, PhD thesis, Yale University). The *Flaveria* gene is missing both of these introns and has its first intron at about amino acid 120. The promoter region of *F. bidentis* NADP-ME has been sequenced and expression constructs with a GUS-OCS vector have been made. Results of transient expression of *F. bidentis* and tobacco leaves using micro-projectile introduction of vector will be presented. Based on these results, selected constructs will be stably transformed into tobacco and *F. bidentis*.

1842 CELL-SPECIFIC GENE REGULATION IN THE C_4 DICOT *FLAVERIA BIDENTIS*. Elizabeth Smith, Julie Chitty, Chris Chastain, Robert T. Furbank, John G. Mason, Paul Whitfeld and William C Taylor. CSIRO Division of Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia

The C_4 photosynthetic pathway increases the efficiency of photosynthesis by suppressing photorespiration. Cell-specific expression of C_4 photosynthetic genes is critical for the C_4 cycle to operate. To understand how C_4 photosynthesis genes are expressed in a cell-specific manner, we have cloned the gene encoding pyruvate orthophosphate dikinase (PPDK), a key regulatory enzyme in the C_4 pathway, from the C_4 dicot *Flaveria bidentis*. PPDK promoter regions fused to GUS are being tested for cell-specific and light-inducible activity in transient expression assays by microprojectile bombardment of leaves. Selected constructs will be stably transformed into *F. bidentis* using *Agrobacterium* infection of explants. Despite a very low frequency of regeneration from transformed callus, we have recovered over 80 transgenic C_4 plants showing GUS expression from a CaMV 35S promoter. GUS activity in leaves is highest in vascular tissue and approximately equal in mesophyll and bundle sheath cells, showing that the 35S promoter does not respond to the C_4 cell-specific regulatory program. Transgenic mutants with reduced PPDK enzymatic activity are being generated using PPDK antisense and ribozyme constructs to decrease the level of PPDK mRNA. These mutants will be used to evaluate the role of PPDK in regulating flux through the C_4 pathway under a wide range of environmental conditions.

1843 CARBON ASSIMILATION IN LINSEED (LINUM USITATISSIMUM L.) VAR. T-397 UNDER SALT STRESS CONDITIONS. M.G. Shitole and N.J.PINTO. Department of Botany,University of Poona Pune 7 India.

Linseed Var. T-397 was grown at various levels of chloride and sulfate salinizations and rate of 14CO2 fixation, products of 1h light 14CO2 assimilation and photosynthetic enzymes under control and saline conditions were studied. Results revealed that under control condition ratio of RuBP Case to PEP Case was 2.51 suggesting its C_3 nature while photorespiratory ratio and activity of glycolate oxidase was less than a known C_4 plant, Zey mays. Radioactivity in glycine and serine was negligible during one hour light 14CO2 fixation. These results revealed that linseed var T-397 is a C_3 plant without photorespiration. Rate of 14CO2 fixation during 1 h light was more than the control at all levels of chloride (ECe 2.5 to 10 $mScm^{-1}$)and sulfate (Ece 2.5 to 15 $mScm^{-1}$) salinizations which suggested that all levels of both the salts stimulate photosynthesis. During 1h photosynthesis with 14CO2 radioactivity in sugar, amino acid and organic acid fractions was more than the control under both the salinizations. Sucrose was the major pool under control conditions. Sucrose and malate was the major pool at all levels of chloride while sucrose, malate and citrate form the major pool under sulfate salinization. Photorespiratory ratio, activities of glycolate oxidase and catalase increased under saline conditions. In vivo effect of these salts on activities of RuBP Case and PEP Case showed increase at all levels of both salts while In vitro effects revealed that low concentrations (upto 20 mM of NaCl and upto 10 mM of Na_2SO_4) stimulate activity of these enzymes. These results suggest that deviated carbon metabolism under saline conditions was due to altered enzyme activities.

1844 CARBON ASSIMILATION IN SEEDLING AND IN VITRO RAISED PLANTLETS OF NIGER (GUIZOTIA ABBYSSINICA CASS) CV. SAHYADRI. M. G. Shitole and T. D. *Nikam. Department of Botany, University of Poona, Pune 7. India. *Modern College Pune. India.

Rate of $^{14}CO2$ fixation products of 1 h light and dark 14CO2 fixation and and activities of RuBP Case, PEP Case, Glycolate oxidase, Catalase and peroxidase in In Vivo (Seed borne) developed seedlings (age-28 days after germination, 8 leaves) and In Vitro raised plantlets (age-28 days after formation of shoot primordia, 8 leaves) of niger cv. Sahyadri and in fully expanded (3rd) leaf of niger cv. Sahyadri at flowering stage were investigated. Results revealed that rate of 14CO2 fixation was several times more in the cotyledonary leaf and upper leaves (2nd and 3rd) of the seedlings and fully expanded leaf of niger at flowering than the base (positionwise equivallent to cotyledonary leaf) and upper leaves (2nd and 3rd) of the plantlets. It was also more in the cotyledonary leaf of seedlings than the fully expanded leaf of niger at flowering stage. During 1 h light 14CO2 fixation sucrose was the highly lebelled compound in all leaves of seedlings and in the 3rd leaf of niger at flowering stage. While it was phenylalanine in all the leaves of plantlets which suggested poorly developed sucrose synthesizing machinary. Asparagine showed highest lebelling during 1 h dark 14CO2 assimilation in all leaves of seedlings, mature plant and plantlets thereby suggesting that dark carbon assimilation was on similar lines in all cases. Activities of RuBP Case, PEP Case, Glycolate oxidase, Catalase and Peroxidase were less in all leaves of plantlets than all leaves of seedlings and fully expanded leaf of niger at flowering thereby indicating juvenile metabolic status of leaves of plantlets.

POSTER ABSTRACTS

1845 SUBUNITS OF ADP-GLUCOSE PYROPHOSPHORYLASE: SPINACH LEAF SUBUNIT cDNAS AND COMPARISON TO THOSE FROM DIVERSE SOURCES. Brian Smith-White and Jack Preiss, Graduate Program in Genetics and Department of Biochemistry, Michigan State University, E. Lansing, MI.

ADP-glucose pyrophosphorylase, the site of regulation of starch biosynthesis, is an allosterically regulated enzyme comprised of two subunits differing in molecular mass. In spinach leaf, these are 51 kDa and 54 kDa. The cDNAs representing these were isolated from a λ gt11 library of spinach leaf mRNA and the nucleotide sequences determined. Comparison of the deduced primary structures of these to other known deduced primary structures suggests that the enzyme is comprised of a small subunit with a conserved primary structure and a large subunit which has a variable primary structure that differs between photosynthetic and nonphotosynthetic tissue, perhaps to accomodate tissue-specific metabolism. Comparison with the cDNAs representing the subunits from other plants confirms the groupings indicated by analysis of the primary structures and suggests that the large subunit diverged into two groups shortly after the divergence of the small subunit gene and the precursor of the two large subunit genes. Previous work with the enzyme from *E. coli* had identified the regions of the primary structure involved in binding of substrates or allosteric regulators. Comparison of the primary structures of plant enzymes and the *E. coli* enzyme suggests that the regions associated with substrate binding and allosteric activator binding in the *E. coli* enzyme are conserved.

1846 ENHANCEMENT OF STARCH CONTENT OF PLANT CELLS BY EXPRESSION OF A CARBOHYDRATE BIOSYNTHETIC GENE. Kurt P. Timmerman, David M. Stark, Gerard F. Barry, Jack Preiss[1], and Ganesh M. Kishore, Monsanto Company, 700 Chesterfield Village Pkwy., St. Louis, MO 63198 and [1] Michigan State University, Department of Biochemistry, East Lansing, MI 48824.

Starch (alpha, 1-4 glucan) is the major storage carbohydrate in a wide range of plant cells and tissues. During photosynthesis, leaf tissues accumulate a significant amount of starch to serve as a rapidly mobilizable store of carbon for metabolism under dark conditions. Starch is also the principal carbohydrate of many storage tissues such as tubers, roots and seeds. It is generally believed that the biosynthetic reactions involved in starch formation regulate the starch content of the tissue, although the involvement of the degradative reactions in regulating the starch content cannot be ruled out at the present time. To verify this hypothesis, we have expressed in plant cells a gene encoding an enzyme involved in carbohydrate biosynthesis. In order to target the enzyme to the plastid, a fusion containing a chloroplast targeting sequence with the gene was made. The results of the effect of expression of this gene under control of the 35S promoter in plant cells will be presented.

1847 CHARACTERISATION OF cDNA CLONES FOR THE GLYCINE CLEAVAGE SYSTEM OF PEA MITOCHONDRIA. Simon R. Turner, Robert Ireland and Stephen Rawsthorne. Cambridge Laboratory, IPSR, Colney Lane, Norwich, U.K. and Biology Dept., Mount Allison University, Sackville, N.B. Canada

Photorespiratory CO_2 evolution involves a two step reaction in which the enzyme glycine decarboxylase (GDC) catalyses the cleavage of glycine to form CO_2, NH_3, and methyl-tetrahydrofolate, followed by transfer of the methyl group to a second molecule of glycine by the enzyme serine hydroxymethyl-transferase (SHMT). GDC is a large complex composed of non stochiometric amounts of four subunits. Of particular interest is the lipoamide dehydrogenase (L) subunit since a similar subunit is used in two other mitochondrial dehydrogenases (pyruvate (PDC) and α-ketoglutarate (αKGDC)). We have specifically isolated the L subunit from GDC and determined its N-terminal amino acid sequence. This sequence has been used to confirm the identity of a cDNA clone isolated from a pea leaf expression library using an antibody to the L subunit. The amino acid sequence deduced from the sequence of this clone shows remarkable similarities, along its entire length, with PDC L subunit sequences from yeast, human and E. coli. The corresponding mRNA is more abundant in roots than it is in leaves andis encoded by only a single gene. This suggests that GDC and PDC (and probably αKGDC) all share a common L subunit. In leaves the expression of the corresponding mRNA is light-induced in a manner similar to those of other GDC subunits. We have used an antibody raised to the enzyme SHMT to isolate several cDNA clones from a pea leaf expression library. The deduced amino acid sequence from these clones matches the N-terminal amino acid sequence determined directly from the mature protein and shows good homology with the sequence of the E. coli enzyme.

1848 Sucrose Phosphate Synthase, a Key Enzyme for Sucrose Biosynthesis: Protein Purification, Cloning of its cDNA and Expression in Transgenic Plants

Toni A. Voelker [1], Ann C. Worrell[1], Mike Boersig[1], Kristin Summerfelt[1], Bernard Cambou[2], Danielle Lando[2], Jean-Michel Bruneau[2]

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We have purified to homogeneity the protein and isolated a complementary DNA sequence for the enzyme sucrose phosphate synthase (SPS) from corn leaves (Zea mays). The 3509 bp long cDNA codes for a polypeptide of 1068 amino acids in length. The SPS cDNA and specific antibodies were used to monitor SPS mRNA and protein accumulation throughout the plant. The biological activity of the cDNA was confirmed by the ability of the cloned sequence to direct sucrose phosphate synthesis in *E. coli*. No plant specific factors were necessary for enzymatic activity.

When expressed in leaves of transgenic tomato, the corn SPS dramatically increases the endogenous SPS level of leaves and leads to a low leaf starch phenotype. This represents evidence that SPS is influencing the carbon partitioning in the source leaves.

1849 MOLECULAR EVOLUTION OF C4 PHOTOSYNTHESIS IN THE GENUS *FLAVERIA*. Peter Westhoff, Jürgen Hermans, Elke Rosche, Dagmar Börsch, Winfried Poetsch and Karin Ernst. Heinrich-Heine-Universität, Institut für Entwicklungs- und Molekularbiologie der Pflanzen, D-4000 Düsseldorf 1, Germany.

It is commonly accepted that C4 plants have arisen from C3 ancestral species and that this transition must have occured several times independently during the evolution of angiosperms. The differential compartmentation of the enzymes which are required for the establishment of a functional C4 cycle, i.e. PEP carboxylase, pyruvate Pi dikinase and NADP malic enzyme, is one of the key steps in the sequence of C4 evolution. Isoforms of these enzymes are also found in the leaves of C3 species. However, they are functioning in different metabolic contexts. To unravel the key changes these genes may have experienced during the evolutionary transition of C3 to C4 plants, the genus *Flaveria* was chosen as model system. This genus is characterized by C3 and C4 species as well as a large number of C3/C4 intermediates which may be considered as progessing towards C4 plants. Full-size cDNA clones for PEP carboxylase, pyruvate Pi dikinase and NADP malic enzyme have been isolated from the C4 species *Flaveria trinervia* (Rosche et al. 1990; Börsch et al. 1990) and are used to isolate genomic sequences from *F. trinervia* (C4) and the C3 species *F. pringlei*. Southern analysis and the numbers of non-identical clones obtained indicate that probably all the genes are encoded by multi-gene families. In the case of PEP carboxylase the various members of the gene families in the C3 and the C4 plant (Hermans & Westhoff 1990) have been characterized with respect to primary structure and expression patterns. Transformation experiments are in progress to delineate the transcription control regions of these genes and to examine whether their expression profiles are maintained in heterologous plants. (Supported by DFG and the Bundesminister für Forschung und Technologie).

1850 INFLUENCE OF EXPRESSION OF PLANT-TYPE RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE GENES ON METABOLISM IN A CYANOBACTERIUM. Akira Wadano[1], Akiho Yokota[1], Shogo Wada[1], Michio Himeno[1], and Hirokazu Kobayashi[2]. [1]Department of Agricultural Chemistry, University of Osaka Prefecture, Sakai, Osaka 591; and [2]School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka 422, Japan.

The affinity of the substrate CO_2 to nbulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) derived from higher plants is higher than that to the enzyme from cyanobacteria. To reveal the metabolic regulation of photosynthetic CO_2 fixation pathway, we attempted to express genes for RuBisCO from the photosynthetic purple sulfur bacterium *Chromatium vinosum*, which relatively resembles higher plant RuBisCOs in a lower $Km(CO_2)$ value (approx. 37 μM) and a quaternary structure composed of octamers of both large and small subunits (L_8S_8). The 3.6-kilobase pair (kbp) DNA fragment of *C. vinosum* RuBisCO genes (*rbcA–rbcB*) containing its promoter placed downstream of *Escherichia coli lac* promoter, was ligated with an 8.0-kbp *Xba*I–*Xho*I fragment from the shuttle vector pUC303 to construct pUCV1. The cyanobacterium *Synechococcus* 7942 whose RuBisCO has higher $Km(CO_2)$ value (approx. 250 μM) was transformed with pUCV1, and obtained a transformant, MT1, which is resistant to chloramphenicol. Expression of *C. vinosum* RuBisCO in MT1 was examined by immuno-blotting with antibodies against the large and small subunits of *C. vinosum* RuBisCO. The total enzyme activity of *C. vinosum* RuBisCO plus *Synechococcus* RuBisCO in MT1 was 1.4 times higher than the enzyme activity in the wild strain of *Synechococcus*. A maximal rate of photosynthetic oxygen evolution increased 20% in MT1 in comparison with the wild strain. The disruption of *Synechococcus* RuBisCO genes (*rbcL–rbcS*) and the transformation of a mutant deficient in a CO_2-pump are being achieved.

POSTER ABSTRACTS

1851 EXPRESSION AND GENOMIC CHARACTERISATION OF ARABIDOPSIS SEDOHEPTULOSE-1, 7-BISPHOSPHATASE.
Nicola M. Willingham and Christine A. Raines.
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Sedopheptulose-1,7-bisphosphatase is an enzyme unique to the Photosynthetic Carbon Reduction (PCR) cycle. It catalyses the dephosphorylation of Sedoheptulose-1,7-bisphosphate during a series of reactions that brings about the regeneration of the carbon dioxide acceptor molecule, Ribulose-1,5-bisphosphate. Screening of an Arabidopsis cDNA library has led to the isolation of a cDNA clone which has been sequenced and shown to encode the complete precursor protein sequence. This clone is being used as a probe to determine the developmental and photoregulatory effect on the steady-state SBPase mRNA levels in Arabidopsis. In order to understand the molecular factors regulating the expression of SBPase, a copy of the Arabidopsis gene was isolated from a genomic library and the sequence of upstream regulatory regions determined. Analysis of upstream sequences aimed at elucidating the mechanisms underlying SBPase regulation using chimeric promoter fusions is underway.

1852 CLONING AND CHARACTERIZATION OF A PEPC cDNA FROM A C_3 PLANT. Robert F. Whittier, Toshio Sugimoto*, Tsutomu Kawasaki, Daisuke Shibata and Yukio Kawamura*. Mitsui Plant Biotechnology Research Institute, TCI, Sengen 2-chome 1-6, Tsukuba, Ibaraki 305, Japan and *Protein Science Laboratory, National Food Research Institute, Ministry of Agriculture, Forestry, and Fisheries, Tsukuba, Ibaraki 305, Japan

A full-length cDNA clone encoding phosphoenolpyruvate carboxylase (PEPC) has been cloned from soybean, a C_3 plant. The open reading frame present in this cDNA is predicted to encode a protein 967 amino acid (a.a.) residues in length. Comparison to other published plant PEPC sequences indicates 82% a.a. identity with a CAM-type PEPC, and 78% and 71% identity with the C_4 type enzymes from maize and sorghum, respectively. Data will be presented on the tissue expression pattern of this PEPC gene in soybean.

1853 REACTION CENTER DAMAGE-AND-REPAIR CYCLE IN PHOTOSYSTEM-II OF CHLOROPLASTS. A. Melis and P. J. Neale, University of California, Plant Biology, Berkeley, CA 94720.

The research addresses mechanistic details of the damage and repair cycle of PSII reaction centers in chloroplasts which includes turnover of the D1/32 kDa protein (*psb*A gene product). Structural and functional properties of intermediates in the PSII repair cycle have been identified. Damage to PSII occurs in the light in the membrane domain of the grana partition regions. Damaged PSII centers can accumulate in the thylakoid membrane under conditions of chronic photoinhibition. The damaged centers are photochemically inert, they contain the reaction center proteins, the core Chl-protein antenna, and the inner complement of the LHC II. They can not perform a photochemical charge separation. They can be detected by quantitative SDS-PAGE and immunoblot analysis. The repair of the reaction center takes place in the stroma-exposed thylakoids and involves *de novo* synthesis of D1, and removal and replacement of the damaged D1 protein. The newly repaired centers are competent in the photochemical charge separation and reduction of the primary quinone Q_A. However, they are unable to perform the Q_A-to-Q_B electron-transport step. The repair process is inhibited in the presence of chloramphenicol. The activation of the Q_A-Q_B electron-transport step in the reaction center complex is mediated by light and is inhibited at temperatures below 10 °C. The activation reaction is also inhibited by -SH blocking compounds. Sensitive absorbance difference spectrophotometry revealed different optical properties for the semiquinone anion radical (Q_A^-) in newly repaired centers (localized in stroma-exposed membranes) compared to those centers in the grana partition regions. The physiological significance of the photosystem-II repair cycle will be discussed.

1854 **Organization and Subunit Stoichiometry of the Light-harvesting Antenna of Photosystem II in Barley.** Michael Harrison and Anastasios Melis, Dept. of Plant Biology, University of California, Berkeley CA 94720.

The polypeptide composition and copy number in the chlorophyll *a*/*b*-binding light-harvesting antenna of barley photosystem II (LHC-II) has been examined by high-resolution SDS-PAGE and immunoblot analysis. Of four constitutive polypeptides in LHC-II (subunits a, b, c and d) with molecular masses in the range 30-25 kDa, only subunit d was found to occur at an equal copy number per photosystem II reaction centre in wild type barley and in the chlorophyll *b*-less *chlorina* f2 and chlorophyll *b*-deficient *Chlorina* 103 mutants. This polypeptide, of molecular mass 25 kDa, is therefore proposed to bind the residual LHC-II antenna which contains 12 chlorophyll *a* molecules in the *chlorina* f2 mutant and 18 chlorophyll *a* + *b* molecules in the *Chlorina* 103 mutant. On the basis of the determined stoichiometries of LHC-II polypeptide subunits in wild type and mutant thylakoid membrane and in photosytem II preparations, a model for the assembly of the LHC-II antenna of photosystem II is presented.

1855 ANALYSIS OF MUTANTS IN THE EXPOSED LOOP BETWEEN TRANSMEMBRANE HELICES IV AND V OF THE D1 PROTEIN IN PHOTOSYSTEM II. Hadar Kless[1], Lee McIntosh[2] and Marvin Edelman[1]. Dept. Plant Genetics, Weizmann Inst. Science, Rehovot, Israel[1].
D.O.E. Plant Res. Lab., Michigan State U., E. Lansing, MI USA[2]. The D1 protein is a core component of the PSII reaction center and involved with a diverse array of activities on both sides of the photosynthetic membrane. Transmembrane helices VI and V, and the stromal-exposed loop in between, bind several cofactors such as a special-pair chlorophyll, a plastoquinone (Q_B) and a non-heam iron. Several PSII herbicides can substitute for Q_B in this area. Also, the primary cleavage site involved in specific and rapid degradation of D1 is thought to be located here. In order to probe functionally distinct sites, we prepared a series of site-directed mutants which affected residues between Ser-221 and Ser-270 of the D1 protein. Mutagenesis was performed on *Synechocystis* PCC 6803 having only one copy of the psbA gene. Alterations were based on several criteria: sequence homology to the L and M subunits of the bacterial reaction center, phylogenetic conservation of known sequences; functional analogy to similar sequences from other proteins; and possible interaction with Q_B or herbicides. The following general conclusions have been drawn from this mutant series: 1. Mutations which modify charged residues markedly alter the electrophoretic mobility of the mutated protein. The more positive the net charge, the faster the mobility. 2. Point mutations in residues of direct functional interaction suspend photoautotrophy (e.g., Tyr 237 or His 252 - which are thought to be liganded to Q_B). 3. Deletions of 2-5 residues, even in phylogenetically conserved regions, do not necessarily affect basic PSII functions and allow photoautotrophy to proceed, albeit at reduced efficiencies. 4. Several mutants affect herbicide resistance at sites not previously known from natural selection. This may relate to overall conformational changes as well as specific effects on herbicide binding. 5. Unusually rapid turnover of D1, which saturates at very low light intensities, is characteristic of some non-photosynthetic mutants. This may indicate the existance of more then one patnway for D1 degradation

1856 SUPPRESSION OF A NON-PHOTOSYNTHETIC MUTATION IN psbD LEADS TO NOVEL PROTEIN SEQUENCES IN A FUNCTIONAL D2 PROTEIN. Hadar Kless[1], Wim Vermaas[2] and Marvin Edelman[1]. Dept. Plant Genetics, Weizmann Inst. Science, Rehovot, Israel[1]; Dept. Botany, Arizona State U., Tempe, AZ, USA[2]. D1 and D2 are structurally related proteins forming the core of the PS II reaction center. The extended, stroma-exposed loops between transmembrane helices IV and V of the two proteins have been proposed to harbor a contact point between them. In D1, this loop is involved with herbicide binding and protein degradation. For both proteins it is the primary region of trypsin sensitivity in-vitro. We attempted to determine whether the D1 sequence in this region (<30% homologous to that of D2) could be functionally transferred to D2. Using *Synechocystis* PCC 6803, we introduced D1 residues 225-246 into the D2 protein, replacing residues 224-244. The resulting mutant, D2-1, did not evolve oxygen and lost the ability to grow photoautotrophically. Surprisingly, selection for D2-1 suppression on non-glucose containing medium yielded several photosynthetically competent colonies. When direct transformation of D2-1 with PCR-amplified fragments of the psbD gene from the suppressed mutants was checked, a marked increase in efficiency of reversion to photosynthetic competence was obtained. A novel protein sequence for D2 was found in all suppresor mutations analyzed. These suppressors apear to result from two consecutive events: a single nucleotide deletion at a particular 'hotspot' which shifted the reading frame, followed by an insertion of varied length shifting the sequence back to the original frame. The minimal change in D2-1 leading to photosynthetic competence was a deletion of a threonine codon, while the largest change was an insertion of 94 bases. These findings indicate that: 1. in the stroma-exposed loop following transmembrane helix IV, a functional PS II reaction center can bear heavy substitution of D1 residues in the analogous region of D2. However, complete symmetry between D1 and D2 in this region causes total failure of PS II function. 2. Photoautotrophy can be retained despite huge changes in the exposed loop region between helices IV and V of D2.

POSTER ABSTRACTS

1857 **CHARACTERIZATION OF A BARLEY cDNA CLONE ENCODING THE 31kDa LHC IIa (CP29) APOPROTEIN OF PHOTOSYSTEM II.** DARYL T MORISHIGE AND J PHILIP THORNBER. Dept. of Biology, University of California, Los Angeles, CA 90024-1606.

The light-harvesting complex (LHC) of photosystem II is composed of several different pigment-binding apoproteins. We have identified a cDNA clone pLHCIIa-1.2 encoding the 31kDa LHC IIa (CP29, chl *a/b*-P1) apoprotein of barley (*Hordeum vulgare*). Direct protein microsequencing of an internal peptide fragment from the LHC IIa apoprotein has been used to unequivocally identify the cDNA clone as that coding for the LHC IIa protein. Microsequencing of the 28.5kDa LHC IIc protein resulted in a sequence with only minor similarity to the LHC IIa protein, indicating that they are two different gene products. The pLHCIIa-1.2 clone encodes a protein (M.W.=31,308) of 286 amino acid residues, which displays strong similarities to other pigment-binding LHC proteins, and yet contains an additional 42 amino acid residue segment. Two regions of strong intramolecular sequence similarity are also observed. Most probably there is only a single gene coding for the LHC IIa apoprotein. The accumulation of mRNA for LHC IIa (1.2kb) appears to be controlled by phytochrome. We have also examined LHC IIa gene expression during the light-induced biogenesis of the photosynthetic apparatus.

1858 CAROTENOID CONTENT OF THE CAB GENE PRODUCT, LHC IIB, UNDER HIGH LIGHT STRESS. A. Lee, and J.P.Thornber. Dept. of Biology, University of California, Los Angeles, CA, 90024-1606.

Our laboratory is interested in the structure and function of the multiple pigment-protein complexes involved in photosynthesis. These pigment-protein complexes bind various types of carotenoids as well as chlorophylls a & b. I am interested in the role of the various types of carotenoids in photosynthesis. At the moment, I am characterizing the carotenoid content of the cab gene product, LHC IIb, under high light conditions. The oligomeric form of LHC IIb binds the carotenoids neoxanthin, violaxanthin, and lutein. Violaxanthin can be converted to zeaxanthin in a process called the xanthophyll cycle. Zeaxanthin may have a photoprotective role for the plant since the appearance of zeaxanthin during high light stress has been correlated with increased non-radiative energy dissipation. It may be that the violaxanthin associated with the LHC IIb oligomer is the violaxanthin involved in zeaxanthin formation under high light stress. To date, the xanthophyll cycle has only been studied in the whole leaf. Starting with LHC IIb, I am trying to pinpoint in which of the many pigment-protein complexes the xanthophyll cycle occurs. Along with studies of the cab gene itself, we hope to obtain a complete picture of LHC IIb structure and function and to extend this study to other pigment-protein complexes.

1859 ANALYSIS OF THE PLASTOQUINONE Q_B BINDING SITE IN PSII BY SITE-SPECIFIC MODIFICATIONS OF THE D1 POLYPEPTIDE. N. Ohad, Y. Inoue[1] and J. Hirschberg, Department of Genetics, The Hebrew University of Jerusalem, Jerusalem 91904, and [1]The RIKEN Institute, Wako-shi, Saitama, 35101 Japan.

Photosystem II (PSII) is a protein-pigment complex that is localized in thylakoid membranes of chloroplasts and catalyzes the reduction of plastoquinone following light absorption and oxidizing water. The secondary electron acceptor in PSII is a weakly-bound plastoquinone called Q_B. The apoprotein of Q_B is a PSII subunit polypeptide- D1 We have studied the structure-activity relationship of the Q_B-binding domain on D1 by analyzing the effects of specific modifications in the protein on electron transfer to Q_B. Site-specific mutagenesis of *psbA*, the gene encoding D1, was carried out in cyanobacteria. It has been demonstrated that the Q_B binding site on D1 in cyanobacteria is identical to that of higher plants, hence this organism is an excellent model system for PSII in plants. Eight novel mutations were introduced into the gene *psbAII* of the cyanobacterium *Synechocystis* PCC6803 by site-specific *in-vitro* mutagenesis. These mutations resulted in specific amino acid replacements in D1 in the plastoquinone (Q_B) binding domain. Characterization of the mutants included: fluorescence induction, fluorescence relaxation, thermoluminescence, oxygen flash yield, herbicide resistance and D1 degradation in the light.

Three mutations: Y254S, F255W and L271S, abolish photosynthetic growth as a result of loss electron transfer to Q_B. It was concluded that an aromatic side chain (Tyr or Phe) in position 254 of D1 is essential for electron transfer to QB. Leucine at position 271 in the D1 protein interacts with the plastoquinone Q_B similarly to isoleucine 229 in the L subunit of the reaction center of purple bacteria. Replacements of Leu 271 by Val, Ala or Met. affected Q_B binding, but in addition they impaired electron donation to Q_B at the "donor side" of PSII. Rapid light-induced degradation of D1 in the mutants, including in the non-photosynthetic ones, indicates that the initiation of D1 turnover and the degradation process itself occur independently of electron transfer in PSII.

1860 PURIFICATION AND PROPERTIES OF AN ENZYME INVOLVED IN THE PROCESSING OF D1 PROTEN. Shuji Fujita, Noritoshi Inagaki and Kimiyuki Satoh. Department of Biology, Okayama University, Okayama 700, Japan

The newly synthesized D1 precursor protein of PS II reaction center is enzymatically cleaved at the C-side of Ala-344; the cleavage is supposed to be essential to the assembly of catalytic center of water oxidation. The enzyme involved in the cleavage has been extracted with Triton X-100 from spinach grana thylakoids (FEBS Lett. 255, 1-4, 1989) and highly purified in this study by a series of procedures which include ion-exchange, hydroxylapatite, metal-chelating and gel-filtration chromatographies. The purified enzyme consists of a single polypeptide of about 45 kDa with pI value of 5.5, and cleaves a synthetic oligopeptide corresponding to the 29 amino acid residues of the C-terminal end of D1 precursor protein deduced from nucleotide sequence of psbA gene, at the predicted processing site, an *in vitro* translation product of tobacco psbA transcripts and D1 protein of Scenedesmus obliquus LFI mutant. The enzyme is not active in the cleavage of a lumenal precursor of 24 kDa protein of oxygen evolving system of PS II which is translocated across thylakoidal membrane. The enzymatic activity is highly sensitive to the ionic strength of reaction mixture, but is resistant to the prevailing protease inhibitors such as PMSF, o-phenanthroline, thiol-reagents and heavy metal ions. The basic properties as well as the site of location of this enzyme in thylakoids will also be discussed in this presentation.

1861 SITE DIRECTED MUTAGENESIS OF THE CHLOROPLAST *psbA* GENE IN *CHLAMYDOMONAS*. A. Lers[1], P. B. Herfetz[2], E. Gross[2], J. E. Boynton[2], N. W. Gillham[1] and B. Osmond[2]. Duke Univ., Depts. of [1]Zoology and [2]Botany, Durham, NC 27706

We have developed methods for analysis of site directed mutations in the *psbA* gene of *Chlamydomonas reinhardtii* which encodes the PSII reaction center D1 polypeptide. These include techniques for reintroducing the mutagenized gene into the chloroplast genome and for evaluating *in vivo* the physiological consequences of the protein modifications. Wildtype cells of *Chlamydomonas* were cotransformed simultaneously with plasmids containing mutagenized *psbA* genes and selectable antibiotic resistance mutations in the chloroplast *16S* ribosomal RNA genes. A high frequency of the antibiotic resistant transformants also carried copies of the nonselected *psbA* gene. Integration of both donor genes occurred by homologous recombination, resulting in targeted replacement of their wildtype counterparts in an otherwise isogenic background. We are investigating the functional consequences of these cotransformants on photosynthetic performance and growth. To separate the effects of the *psbA* mutations from any phenotypic effects attributable to the presence of the selectable antibiotic resistance markers, we have also evaluated the performance of wildtype cells transformed with the rDNA plasmid alone. Each genotype was grown under a variety of environmental conditions, and exponential growth rate, photosynthetic efficiency and maximum (light-saturated) rates of photosynthesis were measured. Our results indicate that the different antibiotic resistance markers used for cotransformation exert varying levels of phenotypic effects which are additive to those of the *psbA* mutations. Supported by DOE grant DE-FG05-89ER/4005 and an NIH predoctoral traineeship to PH.

1862 ANALYSIS OF PSII ACTIVITY IN MUTANTS OF *SYNECHOCYSTIS* sp. PCC 6803. Neil Bowlby[1], Idah Sithole[1], Gerald T. Babcock[2], John Sinclair[3] and Lee McIntosh[1]. [1]MSU-DOE Plant Research Lab and [2]Dept of Chemistry, East Lansing MI 48824 and [3]Dept. of Biology, Carlton Univ. Ottawa, Canada. Directed mutagenesis of PSII was previously used in this lab to identify Tyr-161 of the D1 protein (*psb*A gene product) as the direct donor to P_{680}^+ and Tyr-160 of the D2 protein (*psb*D gene product) as the species giving rise to the dark-stable EPR signal known as Signal II_{slow}. Several groups are now using this strategy to identify the amino acid residues involved in ligation of the catalytic intermediates during charge-accumulation in the tetranuclear Mn cluster. We have isolated several site-directed mutants of *psb*A which result in slower autotrophic growth and decreased rates of oxygen evolution. Photosynthetic electron transfer in control and mutant cells was analyzed using steady-state and modulated oxygen evolution, EPR spectroscopy, and fluorescence induction kinetics. The results suggest that mutation of specific aspartate, glutamate and histidine residues in the D1 protein leads to slowed PSII electron transfer activity. Experiments are now in progress to more clearly define the role of these amino acids during catalytic turnover of PSII.

POSTER ABSTRACTS

1863 C-TERMINAL DELETION MUTANTS OF THE D2 PROTEIN IN PHOTOSYSTEM II OF *SYNECHOCYSTIS* 6803 Beth Eggers and Wim Vermaas, Department of Botany and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe AZ 85287-1601.

Photosystem II (PS II) is a pigment-protein complex in thylakoid membranes consisting of at least six integral membrane proteins. D2 is one of two major components that form the binding environment for cofactors and prosthetic groups in the reaction center of PS II. Five mutants that produce a C-terminally truncated D2 protein have already been made in the cyanobacterium *Synechocystis* sp. PCC 6803. From characterization of these mutants it appears that a domain within the 16 C-terminal amino acid residues is important to PS II stability and function. Site-directed mutagenesis is being used to delete codons within the region near the 3' end of *psb*D, the gene coding for D2. These deletions are concentrated in the domain between 19 and 11 residues from the C-terminal end. The internal deletions cause the loss of 8, 6, 4, 3, 2, or 1 amino acid residues within the C-terminal region of D2. The mutants are constructed so that a unique restriction site is created together with the deletion. This will allow us to insert the proper number of different amino acids back into the truncated protein. This will help determine if the identity of amino acids in a particular region of the C-terminus is important or if merely the number of residues is vital for PS II stability.

This work is supported by NSF grant DCB 90-19248.

1864 MOLECULAR MECHANISM OF D1 PROTEIN DEGRADATION Hugh Salter, Ivar Virgin, Åsa Hagman, Demetrios Ghanotakis* and Bertil Andersson. University of Stockholm, Dept. Biochemistry, Stockholm, Sweden and *University of Crete, Dept Chemistry, Iraklion, Crete, Greece.

Photoinhibition of PSII in higher plants results in the degradation of the D1 protein, a rapidly turned-over protein that makes up part of the heterodimer in the PSII reaction centre. We have investigated this light-induced degradation *in vitro*.The initial degradation of the D1 protein can be catalysed by a protease which is an integral part of the PSII compex. The light-induced D1 protein degradation reaction can occur in isolated PSII core particles, PSII core particles depleted of the 22kDa and 10 kDa intrinsic polypeptides and even in PSII reaction centres. In the last case, an electron acceptor must be added for degradation to occur. We show that reaction centres can be purifed from core particles illuminated in the cold. These reaction centres degrade when warmed to room temperature, clearly separating the activation phase from the D1 protein degradation phase. The degradative process involves the production of specific fragments with definable kinetics. Cleavage is at identifiable points in the sequence, and the initial cleavage results in fragments of 23 and 16 kDa. The 23 kDa and 16 kDa fragments are derived from the *N* and *C* termini of the protein respectively. The cleavage site may be identified by means of protein sequencing/mass spectroscopy. The half-lives of the these fragments, from isolated core particles, are markedly different. We show that the initial degradation is inhibited by protease inhibitors, specifically those acting against serine proteases. The most effective inhibitor we have found is diisopropylfluorophosphate, clearly categorising the reaction mechanism as involving a serine residue within the PSII complex. Radio-labelled inhibitors are being used in binding studies both to allow the identification of the proteolytic subunit and to elucidate the time at which activation and/or cleavage occurs.These results are considered in terms of the structure-function relationships between the PSII subunits.

1865 STUDY OF DONOR SIDE FUNCTION OF PS2 IN CHLAMYDOMONAS USING SITE-DIRECTED MUTAGENESIS Julian Whitelegge, Tony Maung, Siroun Tahtakran and Jeanne M. Erickson. Department of Biology, UCLA, Los Angeles, CA 90024

We are performing site-directed mutagenesis studies in Chlamydomonas reinhardtii to investigate the role of the D1 polypeptide of Photosystem 2 in electron transport from water to the primary electron donor, P680. Synthetic oligonucleotide primers are used to introduce point mutations into suitable psbA fragments cloned in E.coli, and the mutant DNA is delivered into Chlamydomonas using the biolistic particle gun. DNA recombination results in incorporation of the mutated DNA into the chloroplast genome of some transformants. The use of chloroplast drug-resistant marker genes permits us to select transformants based on their drug resistant phenotype and subsequently screen, by RFLP mapping, for co-transformants which also exhibit the RFLP pattern associated with the site-directed mutation. Biochemical and biophysical analyses of transformants, including the levels of oxygen evolution, fluorescence measurements, and EPR, allow us to correlate the structure of D1 with its function in PSII, and to investigate the role of specific amino acid residues of D1 in the binding of PSII cofactors. Here we report the construction of transformants with single amino acid substitutions in the chloroplast-encoded D1 polypeptide, and the detailed characterization of the resulting PSII phenotypes.

1866 EXCHANGE OF HOMOLOGOUS DOMAINS BETWEEN THE CHLOROPHYLL-BINDING PROTEINS CP47 AND CP43 OF PHOTOSYSTEM II. Julian J. Eaton-Rye and Wim Vermaas. Department of Botany and the Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, Arizona 85287

The chloroplast *psb*B and *psb*C genes encode two Photosystem II (PS II) chlorophyll-binding proteins referred to as CP47 and CP43, respectively. These core antenna proteins exhibit six putative membrane-spanning regions and a large hydrophilic loop between helices five and six. Additionally, the distribution of histidine residues in hydrophobic regions of both proteins is similar. We have shown that His-469 in helix six, and the region from Glu-351 to Thr-365 in the large hydrophilic loop of CP47, are required for the stable assembly of PS II. Experiments are now in progress to replace helix six from CP47 with helix six from CP43. Additionally, the large hydrophilic loop of CP47 will be exchanged with the loop from CP43. These experiments are designed to see if apparently equivalent domains of CP43 can functionally substitute for their counterpart in CP47. These exchanges have been made possible by the introduction of an *Eco*R I site at nucleotide 797 creating mutant L267F (the number refers to the residue in CP47 that has been altered) and by creating a *Hind* III site at nucleotide 882 resulting in the deletion of Gly-295. Both of these mutants are photoautotrophic. These sites are both present in CP43 and a *Sma* I site is present immediately preceeding the putative helix six in both proteins. In this way the desired portion of *psb*C can be easily incorporated into *psb*B. This work is supported by DOE Grant DE-FG02-89ER14031.

1867 THE IMPORTANCE OF CP47 IN PHOTOSYSTEM II AS STUDIED BY DELETION MUTAGENESIS. Elisabeth Haag, Julian Eaton-Rye, Gernot Renger and Wim Vermaas. Botany Dept., Arizona State Univ., Tempe AZ 85287-1601; Max-Volmer Inst., TU-Berlin, Berlin, FRG

In cyanobacteria, algae and higher plants the oxygen evolving complex in photosystem II appears to be closely associated with at least six intrinsic polypeptides: the two reaction center proteins D1 and D2, the two chlorophyll a binding proteins CP47 and CP43 and the two subunits of cytochrome b559, and several external ones, including the hydrophilic 33 kDa protein. It is now well established that all functional redox groups for primary charge separation are in the D1/D2 heterodimer complex. The importance of CP47 and CP43, however, is still a matter of debate apart from their role as core antenna. There are several recent lines of evidence that CP47 might be involved in binding or stabilizing the functional Mn in the catalytic site of water oxidation. The predicted tertiary structure of CP47 exhibits six membrane spanning α-helices with an extraordinary large loop of about 200 amino acids between helix V and VI; this loop (loop E) presumably is exposed to the lumenal side of the thylakoid membrane. In the present study the functional domains of loop E are mapped by the use of oligonucleotide-directed mutagenesis creating short deletions (3-8 codons) in the appropriate region of the psbB gene, encoding CP47. The mutated psbB gene constructs will be used to transform a psbB deletion mutant strain of the cyanobacterium Synechocystis sp. PCC 6803. The resulting transformants will then be characterized for their biochemical and biophysical properties: photosynthetic growth, photosystem II stability, protein composition, herbicide binding, fluorescence emission, electron transport and oxygen evolution.

1868 EFFECTS OF HISTIDINE RESIDUES IN CP47 OF PHOTOSYSTEM II ON LIGHT ENERGY TRANSFER AND PHOTOSYSTEM II STABILITY Gaozhong Shen, Julian Eaton-Rye and Wim Vermaas, Department of Botany and the Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe AZ 85287-1601

Mutations have been introduced at histidine residues in hydrophobic regions of the photosystem II (PS II) chlorophyll-binding protein CP47 in the cyanobacterium *Synechocystis* sp. PCC 6803. In most cases, this leads to destabilization of the PS II complex. The selected histidine residues are possible binding sites for chlorophyll molecules. Thus, chlorophyll binding may have an important function in the stable assembly of PS II. To monitor the effect of the mutations on light-harvesting efficiency of PS II, the light intensity needed for half-saturation of PS II electron transport was measured in mutants and wild type upon excitation with orange (≥570 nm) light (exciting chlorophylls and phycobilins), red (≥665 nm) light (exciting mainly chlorophyll) and at 600 nm (exciting phycobilins). Most mutants needed higher light intensities for half-saturation of electron transfer than wild type upon excitation of either chlorophyll or phycobilin. This could be interpreted to indicate a role of selected CP47 histidine residues in binding of chlorophyll involved in energy transfer pathways. However, other hypotheses cannot be ruled out at the moment. Interestingly, changing certain histidines to asparagines had no effect on PS II activity. This also indicates that asparagine may functionally replace histidine to bind chlorophyll in CP47. This research is supported by DOE grant DE-FG02-89ER14031.

POSTER ABSTRACTS

1869 THE ROLE OF THE LARGE HYDROPHILIC LOOP IN CP43 OF PHOTOSYSTEM II Matthias Kuhn & Wim F.J. Vermaas, Department of Botany and the Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe Arizona 85287-1601

CP43 and CP47, the two major chlorophyll-binding protein in the Photosystem II (PSII) core complex, not only serve to bind antenna pigments, but also appear to play an important role in structure and assembly of PSII, which is a crucial component of the photosynthesis process in higher plants, algae and cyanobacteria. Hydrophobicity plots for both CP43 and CP47 predict 6 membrane-spanning helices together with a rather unique large hydrophilic loop on the lumenal side between helix V and VI. In order to study the importance of the hydrophilic loop in CP43 we used site-directed mutagenesis techniques to modify CP43 in the cyanobacterium *Synechocystis* 6803. In CP43 the predicted lumenal loop stretches approximately from residue N280 to W412 (CP43 is 460 residues long). This region is possibly involved in binding to other PSII components/cofactors on the lumenal side of the membrane. In order to define regions of functional/structural importance within the hydrophilic loop of CP43, the psbC gene has been mutagenized by introducing a number of deletions into the gene-region coding for the hydrophilic loop. These mutants are: ΔQ289/V306; ΔR307/A313; ΔN314/T322; ΔG334/M343; ΔF345/E354; ΔW352/N360; ΔG364/D370 and ΔQ375/A386 (the number refers to the first and the last amino acid of the deletion, respectively). The phenotype of all deletion mutants lacking regions between Q289 and A386 is obligate photoheterotrophic. Future studies will deal with the more refined characterization of the mutants created so far and with the generation of additional deletion mutants in order to cover the entire loop. Parallel to this we will use cassette-mutagenesis techniques to reinsert the deleted part, now carrying desired mutations. This can be done quite easily since the deletion was designed to generate a unique restriction site. This work is supported by a BARD grant to W. Vermaas and I. Ohad.

1870 PREPARATION OF PHOTOSYSTEM II REACTION CENTERS FROM D2 MUTANTS OF THE CYANOBACTERIUM SYNECHOCYSTIS SP. PCC 6803 FOR EPR STUDIES. Jiyang Yu and Wim F.J. Vermaas, Department of Botany, and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe AZ 85287-1601

The integral membrane proteins, D1, D2, CP43 and CP47 are the major components of the Photosystem II (PS II) reaction center complex. Selected residues of the D2 protein are thought to provide ligands to the water-splitting system, the reaction center chlorophyll P680, and the primary electron acceptor Q_A. Site-directed mutations have been created in D2 to elucidate the functional and structural role of this protein. Electron Paramagnetic Resonance (EPR) is one of the spectroscopic techniques used to understand electron transfer processes in PS II. Because of contaminating EPR signals from PS I in the thylakoid membrane, functional PS II reaction center particles must be purified to achieve optimal results with this technique. We have modified and fine-tuned existing methods for isolation of PS II particles from Synechocystis sp. PCC 6803 to yield reasonably large amounts of thylakoid extracts depleted of PS I. Upon treatment with dodecyl-β-D-maltoside (DM) and octyl-β-D-glucoside (OG), PS II is preferentially extracted while PS I remains in the thylakoid fraction. In the extract, PS II electron transport remains active, and the ratio of PS II-to-PS I as measured by 77K fluorescence emission is increased by approximately 20-fold. However, a number of site-directed D2 mutants are more sensitive to the detergent extraction, and we are currently establishing optimal chlorophyll-to-detergent ratios for extraction for each of the mutants.

This research has been supported by NSF (DCB90-19248).

1871 **CLONING AND EXPRESSION OF CAB I-1 GENE ENCODING 20 KD PROTEIN OF LIGHT HARVESTING COMPLEX I IN *Chlamydomonas reinhardtii*.** Seongbin Hwang and David L. Herrin. University of Texas at Austin, Department of Botany, Austin, TX 78713. The light-harvesting complex of photosystem I of *C.reinhardtii* contains 4 proteins of 20-27 kD. We have cloned a full length cDNA for the 20kD protein of LHCI (LHCI20) by immunoscreening of lambda gt11 library. We confirmed the identity of this clone by N-terminal sequencing of LHCI20 and sequencing both strands of cDNA. The cDNA(cabI-1) is 1066 bp long and has a suspected poly A signal(TGTAA) of *Chlamydomonas*. The precursor and matured protein of LHCI20 are 24kD and 21kD respectively, and transit peptide is 29 aa long. LHCI20 contains 2 conserved regions which include 2 of the transmembrane domains, and β-turns amino-terminal to each transmembrane domain. LHCI20 also contains 3 histidines presumed to function in chlorophyll binding. Southern blot hybridization of *Chlamydomonas* nuclear DNA with cabI-1 showed a single band, indicating that cabI-1 is a single gene. Also, northern blot hybridization revealed that expression of cabI-1 is the highest at the middle of light period(L6) and lowest at the middle of dark period(D6), during the light-dark cycle. This pattern of gene expression was maintained even after shifting cells into continuous light indicating a circadian rhythm control of cabI-1 expression.

1872 SITE DIRECTED MUTAGENESIS OF THE PHOTOSYSTEM I REACTION CENTER LEUCINE ZIPPER MOTIF. Pamela B. Gibbs, Scott E. Bingham and Andrew N. Webber Department of Botany and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe AZ 85287-1601.

The photosystem I reaction center polypeptides, encoded by the chloroplast psaA and psaB genes, both contain conserved heptadic repeats of leucine residues similar to the leucine zipper motifs involved in dimerization of DNA binding proteins. These leucine repeats are located adjacent to the cysteine residues that co-ordinate the iron-sulfur center, Fx. We are using site directed mutagenesis to test the hypothesis that the leucine repeats mediate the dimerization of the psaA and psaB gene products. The psaB gene from Chlamydomonas reinhardtii, cloned into M13mp19, has been engineered so that it contains a unique StuI restriction enzyme recognition site. Subsequent rounds of site directed mutagenesis have been used to generate the mutations L534V; L541V; L548V; L534+541+548V. Substituting valine for leucine has previously been shown to destabilize the leucine zipper structure. The mutated psaB gene is currently being introduced into the ac49-2.3 strain of Chlamydomonas using the biolistics technique (see Bingham et al, these abstracts) and transformants selected by either (i) co-transformation with P-183 and selection on antibiotic plates (Newman et al. Genetics 126, 875) or (ii) by restoration of photosynthetic growth. Screening transformants for the presence of the StuI site ensures that all copies of the psaB gene have been replaced. The impact of these leucine mutations on the assembly and function of photosystem I will be presented.

1873 ISOLATION AND CHARACTERIZATION OF A cDNA CLONE ENCODING AN 18-kDa HYDROPHOBIC PHOTOSYSTEM I SUBUNIT (PSI-L) FROM BARLEY. Jens Sigurd Okkels, Henrik Vibe Scheller, Vibeke Skovgaard Nielsen, Birgitte Andersen, and Birger Lindberg Moller. Plant Biochemistry Laboratory, Department of Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark.

Ten different polypeptides have been identified in a photosystem I (PS I) preparation from barley. Four of these polypeptides are encoded by the chloroplast genome and six are nuclear encoded. The chloroplast gene, *psaI*, and cDNA clones for the nuclear genes, *PsaD*, *PsaE*, and *PsaH*, have previously been isolated and characterized from barley. Recently, we isolated a novel nuclear encoded PS I subunit designated PSI-L and a corresponding cDNA clone (*PsaL*). The cDNA clone encodes a 22 kDa precursor polypeptide. The mature polypeptide, with a molecular mass of 18 kDa, is an integral membrane protein and is predicted to have at least two membrane-spanning α-helices. The function of the PSI-L subunit is unknown. A cDNA clone for the barley *PsaG* gene encoding the PSI-G polypeptide reveals a high homology to PSI-G from spinach. Surprisingly, a significant similarity to PSI-K of *Chlamydomonas reinhardtii* is also found. PSI-G and PSI-K may be symmetrically arranged in the PS I complex and thus contribute to the general picture of a symmetrical organization of the polypeptides within PS I. Northern blot analysis of RNA isolated during light-induction of barley plants shows that the expression of all the barley PS I genes have a similar pattern of light induction.

1874 TOPOGRAPHY AND ASSEMBLY OF THE PHOTOSYSTEM I REACTION CENTER FROM SPINACH. J. Steppuhn[1], Y. Cohen[2], R. Nechushtai[2] and R.G. Herrmann[1]

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[2]Department of Botany, The Hebrew University, Jerusalem 91904, Israel

Antisera and λgt11-expression libraries made from greening spinach leaves have been used to isolate full-length cDNA clones for subunits II-VI of the photosystem I reaction center from spinach. The analysis of the deduced transit peptides and *in organello* import of cell-free made precursor proteins into isolated spinach chloroplasts suggest that only the psaF-gene product (subunit III) is located on the luminal side of the thylakoid membrane. All the other subunits appear to be peripheral components exposed to the reducing (stromal) side of the membrane. In order to study assembly processes of PSI-subunits the precursor forms as well as the mature proteins have been produced *in vitro* and incubated with isolated thylakoid membranes. The stromal located subunits II, IV, V and VI assemble efficiently into the PSI-complex as precursors, in a process that seems not to be dependent on the presence of stromal factors nor on external ATP. After addition of stroma fraction, the precursor proteins were found to be processed to the mature polypeptides. The assembly of mature PSI-subunits is substantially less efficient than that of the precursor forms. Only the luminal subunit III needs both, the stroma fraction and external ATP, for translocation into the lumen and processing to the mature form. Supported by the Deutsche Forschungsgemeinschaft (SFB 184).

POSTER ABSTRACTS

1875 DIRECTED MUTAGENESIS OF THE PHOTOSYSTEM I CORE. Lawrence B. Smart, Shawn L. Anderson and Lee McIntosh. Genetics Program and D.O.E.-P.R.L., Mich State Univ, East Lansing, MI 48824

The reaction center of photosystem (PS) I consists of a heterodimer of 82-83 kDa polypeptides, PSA-A and PSA-B, that bind the electron transport components P_{700}, A_0, A_1, and F_X, a [4Fe-4S] center. F_X appears to be coordinated by two cysteines in each of the two core polypeptides. Immediately adjacent to F_X is an apparent leucine zipper that may play a role in the assembly of F_X and in maintaining the heterodimer. Using molecular genetic techniques, we have the ability to analyze the assembly of the PS I complex and to determine the structural and functional roles of particular components in the reaction center. We are studying the cyanobacterium *Synechocystis* sp. PCC 6803, which is easily transformed and can be grown heterotrophically (with glucose in complete darkness, except 5 min of light every 24 h). Cells grown in the dark have approximately three-fold less PS I than in light-grown cells, but have comparable levels of PS II. Data suggest the assembly of PS I and PS II is, in part, post-transcriptionally regulated under these conditions. By selecting under heterotrophic growth conditions, we have inactivated the *psaA* gene. This mutant, ADK9, lacks the PS I core and the peripheral proteins PSA-C and PSA-D, but assembles near wild type levels of functional PS II. Also, we have isolated putative mutants with *psaB* inactivated. We are currently performing site-directed mutagenesis of the proposed cysteine ligands to F_X and of leucines thought to form the leucine zipper. Analysis of these mutants will address questions about electron transport, protein structure, and protein-protein interactions.

1876 MOLECULAR HETEROGENEITY OF PHOTOSYSTEM I COMPLEX IN NICOTIANA SPECIES. Junichi Obokata, Kohki Mikami, Yoshiharu Yamamoto and Nobuaki Hayashida*. Department of Botany, Faculty of Science, Hokkaido University, Sapporo 060, and *RIKEN Institute, Tsukuba 305, Japan.

Nicotiana species appear to have 16 PSI proteins per genome in the range of molecular mass from 3 kD to 80 kD. Partial amino acid sequences were determined for these proteins, revealing that the psaD, psaE and psaF products consist of isoproteins of two, four and two species, respectively. Two isoproteins of the psaD products, PSI-D1 and PSI-D2, have distinct amino acid sequences, indicating that they are encoded by different genes within a genome. Immunoblotting analysis of the psaD products showed that PSI-D1 was abundant in developing chloroplasts, while PSI-D2 was most abundant in mature chloroplasts. Four isoproteins of the psaE products, PSI-E1, E2, E3, and E4, are classified into two types, E1-E2 type and E3-E4 type, by pepetide mapping analysis with lysil endopeptidase and by partial amino acid sequences determined for the proteolytic fragments. This indicate that at least two psaE genes are present in a genome, which is in agreement with that we have isolated two types of psaE cDNAs from Nicotiana sylvestris. Quantitative analysis of the electrophoregrams of the PSI proteins revealed that the psaC, psaD, psaE, psaF and psaH products are integrated into each PSI complex as equimoler. These results indicate that PSI of Nicotiana species consists of several isocomplexes containing different isoproteins, and their differentiation are controlled by the nuclear genes, psaD, psaE, and psaF. Physiological significance of these differentiation will be discussed.

1877 CHARACTERIZATION OF A PEA cDNA CLONE ENCODING THE 21kDa LHC I APOPROTEIN OF PHOTOSYSTEM I. SHIVANTHI ANANDAN, DARYL MORISHIGE AND J. PHILIP THORNBER. Department of Biology, University of California Los Angeles, California 90024-1606.

The light-harvesting complex of Photosystem I, (i.e., LHC I), is thought to be composed of four distinct caroteno-chlorophyll proteins (*Anandan et al. FEBS Lett. 256, 150-154 (1989); Vainstein et al. JBC 264, 4058-4063 (1989)*). These pigment-proteins, termed LHC Ia, b, c and d, are of 24, 21, 17 & 11 kDa, respectively (*Peter and Thornber, Methods in Plant Biochemistry, Volume 5, 195-210 (1991)*). The major xanthophyll- chlorophyll a/b-binding component of LHC I is the LHC Ib pigment-protein complex, which is comprised of at least two polypeptides of 21 and 20.5 kDa in pea. We have purified each of the two LHC Ib apoproteins and have obtained partial N-terminal protein sequences for them. A monoclonal antibody specific to the 21kDa LHC Ib apoprotein was used to isolate a cDNA clone with an insert of approx. 1kb for this polypeptide. The partial N-terminal protein sequence obtained for this polypeptide was used to establish the identity of this clone and to determine the cleavage site of the transit peptide. The mRNA for the 21 kDa protein is present in etiolated leaf tissue, and accumulates with increased exposure to light. Phytochrome regulation of the mRNA during chloroplast development was examined. We have also examined the accumulation of the apoprotein during light driven development of the chloroplast.

1878 TARGETED MUTAGENESIS OF *PSAC* GENE ENCODING PSI-C POLYPEPTIDE OF PHOTOSYSTEM I IN *ANABAENA VARIABILIS*. B. Mannar Mannan and Himadri B. Pakrasi, Plant Biology Program, Department of Biology, Box 1137, Washington University, St. Louis, MO 63130-4899, USA.

The filamentous nitrogen fixing cyanobacterium *Anabaena variabilis* (ATCC 29413) is capable of growth in the dark using fructose as the sole energy source. In addition, targeted gene replacements are feasible in these cells. These features make this organism ideally suited for the mutagenesis of the Photosystem I (PS I) complex. We have used this organism to perform targeted mutagenesis of *psaC* gene encoding PSI-C polypeptide, the apoprotein for two [4Fe-4S] centers, F_A and F_B, which function as the terminal electron acceptors in PSI. An insertion mutation introduced in the *psaC* locus resulted in the creation of a mutant strain T398-1 that lacks the PSI-C polypeptide. In the whole cells of T398-1, HCO_3^--dependent whole chain electron transport (PSII+PSI) could not be detected although partial electron transport (H_2O to DCBQ/FeCN and DAD/ascorbate to methyl viologen) could be detected at significant rates. The mutant cells can grow well in BG11 medium supplemented with 5mM fructose in the darkness. However, when grown in the same medium, T398-1 cells were unable to survive under light intensity of 600 lux which supports normal growth of wild-type cells. Charactenzation of the PSI complex in this mutant is currently under progress. [Supported by funds from NIH to H.B.P.].

1879 SITE-DIRECTED MUTATIONS IN D2 IN PHOTOSYSTEM II OF *SYNECHOCYSTIS* SP. PCC 6803. Cathy Madsen and Wim Vermaas, Dept of Botany, and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe AZ 85287-1601

The photosystem II (PS II) reaction center complex is homologous in many respects to the reaction center from photosynthetic purple bacteria, for which a detailed X-ray structure is available. In spite of the striking homology, there are sufficient important structural and functional differences between the two systems to warrant a detailed investigation of PS II structure/function relationships. In the absence of a PS II X-ray structure, we utilize site-directed mutagenesis to probe the function of selected residues in the PS II complex. One residue of emphasis is His-117 in D2, one of the reaction center proteins of PS II. This residue, located in the presumed second trans-membrane helix of D2, is conserved throughout evolution in plants and cyanobacteria; in D1, a protein with large similarity to D2 and the other major reaction center protein of PS II, a symmetric His residue is also conserved. Preliminary results indicate that mutation of this His residue in D2 generally leads to a loss of photoautotrophic growth, and in some mutants to a drastic decrease in apparent stability of the PS II complex. Another area of emphasis is the role of D2 in water splitting, in particular with respect to providing ligands for Mn involved in oxygen evolution. We have previously shown an important role of Glu-69 in donor-side events. Now we are targeting additional Thr, Ser, Trp, and His residues presumed to be on the lumenal side of D2. Mutation of these residues has not yet led to identification of Mn ligands; however, mutation of His-189 to Leu was found to decrease photoautotrophic growth capacity. The function of this residue is currently under investigation. This work is supported by NSF (DCB90-19248).

1880 PHOTOSYSTEM I - BIOGENESIS, ASSEMBLY AND REGULATION OF ITS EXPRESSION. *R. Nechushtai, O. Almog, Y. Cohen, T. Gilon, Z. Karmona, O. Lotan and D. Michaeli.* Botany Dept., The Hebrew University of Jerusalem, Israel, 91904

Photosystem I functions in the reducting site of the photosynthetic electron transfer to reduce ferredoxin and NADP. Its core complex (CCI) contains 40-60 chlorophyll *a* molecules, 1-5 β-carotens, 2 vitamin K_1, 3[4Fe-4S] clusters and 12 polypeptide subunits encoded both in the chloroplast and the nucleous. Light was found to regulate the expression of the nuclear-encoded subunits of CCI. While their mRNAs accumulated in a two peak pattern, the protein subunits accumulated in the thylakoid membrane lineary in a sequential manner; one after the other. The first subunit to appear in the thylakoid membrane was subunit II which its expression was found to be regulated via the phytochrome photoreceptor. Using an *in-vitro* system of isolated thylakoids, enabled to show that this subunit is inserted into the pigmented-PSI complex prior to its processing. The processing occurs by a stromal protease that was partially purified and characterized. The optimal conditions for its activity were proven to be at pH 8.5 and 30°C. The integration, organization and function of other subunits of CCI (like III, IV and VII) was studied as well by molecular and structural methods. This part of the study was performed with thylakoids and isolated CCI complexes of C-3 and C-4 plants as well as cyanobacteria.

POSTER ABSTRACTS

1881 THE PHOTOSYSTEM I MUTANT *viridis-zb*^{63}: ANALYSIS OF THE PHOTOSYSTEM I SUBUNITS AND TRANSCRIPTS. Vibeke Skovgaard Nielsen, Jens Sigurd Okkels, Henrik Vibe Scheller and Birger Lindberg Møller, Plant Biochemistry Laboratory, Department of Plant Biology, Royal Veterinary & Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark.

The thylakoids of the barley mutant *viridis-zb*^{63} contains no functionally active photosystem I (PS I) complex as evidenced by the absence of a light induced P700$^+$ signal (EPR analysis) and by the lack of PS I polypeptides as analyzed by SDS-PAGE. The mutant contains the normal light-harvesting complexes and a normally functioning photosystem II. The mutation in *viridis-zb*^{63} is located in the nuclear genome. Transcripts of the nuclear genes *PsaD, PsaE, PsaG, PsaH* and *PsaL* and of the chloroplast gene *psaI* are present in the mutant in the same sizes and nearly the same amounts as in wild-type barley. Very low amounts of most of the PS I polypeptides have been detected in mutant thylakoids by Western blotting analysis. The nuclear encoded subunits PSI-D, PSI-E and PSI-F are present in normal sizes in the mutant thylakoids, while the PSI-H antibody recognizes in the mutant a polypeptide with a higher molecular mass compared to the wild-type PSI-H polypeptide. The lack of photosystem I activity in *viridis-zb*^{63} may be associated with (I) a mutation in one of the nuclear encoded photosystem I polypeptides, (II) a translational defect of one of the PS I polypeptides, (III) a defect in the synthesis of an electron transfer component or (IV) a defect in a key factor required for the assembly of a functional photosystem I complex. The rate of synthesis and turnover of PS I subunits in the mutant is being studied by pulse-chase experiments and the uptake of PS I polypeptide precursors by mutant and wild-type chloroplasts is being studied by import experiments.

1882 PARSLEY DNA-BINDING PROTEINS WHICH RECOGNIZE ACGT-CONTAINING PROMOTER ELEMENTS. Gregory A. Armstrong, Bernd Weißhaar and Klaus Hahlbrock. Max-Planck-Institut für Züchtungsforschung, Abteilung Biochemie, D-5000 Köln 30, Federal Republic of Germany.

We have recently described the cloning and molecular characterization of three cDNAs encoding sequence-specific DNA-binding proteins from parsley (Petroselinum crispum) (Weißhaar et al. (1991) EMBO J., in press). These cDNAs encode distinct proteins (CPRF-1, CPRF-2, CPRF-3) which interact with two short ACGT-containing cis-acting elements (Box II, Box III) found in the light-responsive parsley chalcone synthase promoter. Full light inducibility of this promoter requires the presence of Box II. The CPRFs contain bZIP (basic domain, leucine repeat) motifs with homology to other bZIP transcription factors. We describe here the expression of truncated derivatives of the CPRFs using coupled in vitro transcription / translation to demonstrate that these proteins require intact bZIP domains for DNA-binding. Detailed competition experiments with wildtype and mutated Box II and Box III sequences, as well as with other related plant and viral ACGT-containing cis-acting elements (ex. as-1, hex, Emla), identify subtle differences in the binding specificities of the CPRFs. Additionally, we have explored the dimerization potential of these proteins and conclude that they can interact with each other to generate heterodimeric DNA-binding activities. Comparison of the binding specificities of the individual CPRFs with that of parsley nuclear extracts suggests a complex regulatory interplay between members of this family of factors. Functional analysis of various ACGT-containing cis-acting elements in transient expression studies using parsley protoplasts accompanies the DNA-binding data.

1883 DELINEATION OF *CIS*-ACTING ELEMENTS IN THE RICE PHYTOCHROME-A GENE (*phyA*). Wesley B. Bruce[1], Jim Tepperman[2], Randal Hauptmann[1], and Peter Quail[2]. [1]Plant Molecular Biology Center, NIU, DeKalb, IL 60115-2861; [2]PGEC, 800 Buchanan St., Albany, CA 94710.

Phytochrome in its active Pfr form, negatively regulates the transcription of its own *phyA* genes. High Pfr levels repress *phyA* transcription within minutes of irradiation whereas low Pfr levels result in derepression of transcription. Using microprojectile-mediated gene transfer into intact rice seedlings, we have previously defined *cis*-acting elements, designated PE1, PE3 and TATA, required for expression in the oat *phyA*3 promoter. We also demonstrated that a GT element in the rice *phyA* promoter is important for high levels of expression. Here we further analyze the rice *phyA* promoter using this expression system and compare defined elements with those in the oat *phyA*3 promoter. A 5' deletion analysis of a rice *phyA*/CAT construct shows that the 5' boundary of the promoter lies between -217 and -171 bp which encompasses GT-1 and GT-2 sequence elements. Deletion of the TATA element essentially eliminates expression. Linker scan and point mutations in the GT-1 and GT-2 elements show their requirement for high levels of expression in low Pfr cells yet do not show a contribution to photoregulation. A second region, at −165 bp of the rice *phyA* promoter encompasses a sequence that has high sequence similarity to the oat *phyA*3 PE3 element and is also conserved in the maize *phyA* promoter. A linker scan series has confirmed that this rice PE3 element is also required for high levels of expression in low Pfr cells. These results suggest that the *phyA* promoters are modular in structure requiring TATA, a highly conserved PE3 element (possibly limited to *phyA* genes) and an upstream element (PE1 for oat and GT for rice) for high levels of expression in low Pfr cells. We are currently testing the modularity of these promoters by mutual exchange of elements and exchange with heterologous promoters. Attempts to identify the element(s) responsible for the negative regulation of the *phyA* genes will be described.

1884 CIRCADIAN REGULATION OF GENE EXPRESSION IN *ARABIDOPSIS* Marsha L. Pilgrim, Tracy Onega, Karyn Goudie and C. Robertson McClung. Dartmouth College, Department of Biological Sciences, Hanover, N.H. 03755

Rhythmic biological phenomena which recur at approximately 24 h intervals in the absence of environmental time cues are termed circadian rhythms, and their timing is regulated by an endogenous biological clock. Circadian rhythms are ubiquitous among eukaryotes, yet in no biological system is the molecular basis of circadian rhythmicity understood. We have begur to identify nuclear genes controlled by the biological clock in *Arabidopsis* by screening for evidence of rhythmic expression of photosynthetic genes, some of which have been shown to be rhythmically expressed in other plant species. We have shown that steady state mRNA levels for genes encoding the chlorophyll a/b binding proteins (*cab*), Rubisco activase (*rca*), the small subunit of Rubisco (*rbcS*), the α and β chaperonin subunits (*cpn* α and β) and nitrate reductase (*nia*) oscillate in a circadian fashion in plants grown in a light-dark cycle, and continue to oscillate in plants transferred to constant darkness or to constant light. We are currently determining whether this regulation is at the level of transcription using nuclear runoff experiments. We are constructing gene fusions in which elements of the *rca* gene are used to regulate *cat* expression. These constructs are being transformed into *Arabidopsis*. The resulting transgenic plants will be assayed to determine minimal elements required *in cis* for circadian regulation of gene expression.

1885 ORGAN-SPECIFIC PROTEIN-DNA INTERACTIONS WITHIN THE FIVE RBCS PROMOTERS OF TOMATO. Iris Meier, Thianda Manzara and Wilhelm Gruissem. Dept. of Plant Biol., University of California, Berkeley, CA 94720.

The expression of the five genes encoding the small subunit of ribulose-1.5-bisphosphate carboxylase (rbcS) in tomato is organ-specific and light-regulated. Differential expression including expression of three genes in etiolated cotyledons and two genes in immature fruit is caused by differential transcriptional activity of the five promoters, that differ with respect to the presence or absence and the spatial arrangement of a number of conserved sequence motifs. DNAse I footprint analyses were used to determine the functional significance of these sequence motifs and to correlate the binding patterns observed with nuclear extracts from cotyledons, leaves, roots, immature and mature fruits to the transcriptional activity of the respective genes. Organ-specific binding differences were detected that include binding to certain of the conserved sequence motifs as well as to additional, not yet described sequence motifs. Individual binding-sites that show sequence specific protection patterns correlating to the promoter-activity were synthesized as oligonucleotides and tested in mobility shift assays with nuclear extracts from different organs. Certain oligonucleotides showed specific binding only with extracts from organs in which a footprint has been observed. The respective differences in the footprint pattern are thus due to different abundance of the binding activity and not to cooperative interactions with other binding proteins. Progress in cloning the sequences encoding these organ-specific DNA-binding proteins from tomato cDNA expression libraries will be presented.

1886 PHOSPHORYLATION OF THE NUCLEAR FACTOR GBF-1 BY CASEIN KINASE II FROM BROCCOLI. Leszek J. Klimczak, Ulrike Schindler, and Anthony R. Cashmore. Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018

To study the phosphorylation of one of the G-box binding factors from *Arabidopsis thaliana*, GBF-1, we have obtained large amounts of this protein by expression in a histidine-fusion vector in *Escherichia coli*. Bacterial GBF-1 was shown to be phosphorylated very efficiently by nuclear extracts from broccoli (*Brassica oleracea* var. *italica*). The phosphorylating activity was purified by chromatography on heparin-Sepharose and DEAE-cellulose, and was characterized. It showed the essential features of casein kinase II activity: utilization of GTP as phosphate donor, strong inhibition by heparin, preference for acidic protein substrates, salt-induced binding to phosphocellulose, and salt-dependent deaggregation. The very low K_m value for GBF-1 (220 nM compared to ca. 10 μM for casein) was in the range observed for identified physiological substrates of casein kinase II. Phosphorylation of GBF-1 resulted in stimulation of the G-box binding activity and formation of a slower-migrating protein–DNA complex. Current experiments focus on the determination of the phosphorylation sites and attempt to address the possible biological significance of this phosphorylation.

POSTER ABSTRACTS

1887 A LIGHT REGULATED URF FROM COTTON CONTAINING MEMBRANE SPANNING DOMAINS. Aliki Kapazoglou, Francis Sagliocco and Leon S. Dure. University of Georgia, Dept. of Biochemistry, Athens, GA 30602.

Among the genes from cotton that have been isolated and sequenced in our laboratory and that are regulated by light (rbcs, cab, rubisco activase), a nuclear gene with a small unidentified open reading frame was isolated and characterized.

A partial cDNA that was obtained by differential screening of a cotton leaf cDNA library for leaf specific sequences was sequenced and used as a probe for screening a cotton genomic library and isolating the gene. 1332 nt of genomic DNA were sequenced and the sequence was analyzed for open reading frames. The longest open reading frame starts at 789 nt from the beginning of the sequence and contains 315 nt (105 aa) which would code for a relatively small putative protein of about 11 Kd. Analysis of the deduced aa sequence showed that the derived protein contains two regions of 19 aa each which have the characteristics of membrane spanning domains. The domains are flanked by two positive charges each. These observations suggested that this could be a transmembrane protein.

Northern analysis has shown that the mRNA of this gene is very abundant in leaves, less abundant in mature cotyledons, it is not detected in roots, embryos and dry seeds, and it is induced by light in four days old cotyledons.

A search of the most recent protein and nucleotide databanks did not detect any significant homology of this gene with any known sequences.

1888 CHARACTERIZATION OF TRANS-ACTING PROTEIN FACTORS WHICH INTERACT WITH THE LIGHT REGULATED GS2 PROMOTER. G. Tjaden, J.W. Edwards and G.M. Coruzzi. Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021

In *Pisum sativum* glutamine synthetase (GS) is encoded by a multigene family. One of the GS isoenzymes, GS2, is localized to the chloroplast; the three other genes encode cytosolic GS isoenzymes. GS2 plays an important role in reassimilation of photorespiratory ammonia. Expression of GS2 is positively-regulated by light. The light induction of GS2 expression is at least partly mediated by phytochrome. The metabolic state of the chloroplast also affects GS2 levels since growth under photorespiratory conditions results in increased GS2 mRNA levels. In order to characterize the cis-elements important to GS2 expression, transgenic analysis of GS2 promoter deletion mutants is being performed using GS2 promoter-GUS fusion genes in *Nicotiana tabacum*. In addition, we have identified nuclear protein factors which interact with the GS2 promoter. Using a Southwestern screen procedure and a *N. tabacum* cDNA expression library, we have isolated and characterized clones encoding protein factors which bind to cis-elements within the GS2 promoter.

1889 CHARACTERISATION OF A PEA CAB GENE ENCODING A TYPE III LHC II POLYPEPTIDE. Denis Falconet[1], Christian Godon[1], Michael J. White[2], Brian W. Fristensky[3], and William F. Thompson[2]. [1]Université de Paris Sud, Laboratoire de Biologie Moléculaire Végétale, 91405, Orsay, France. [2]North Carolina State University, Department of Botany and Genetics, Raleigh, North Carolina, 27695 USA. [3]Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.

The chlorophyll a/b-binding polypeptides of photosystem II (LHC II) are encoded by at least seven closely related nuclear genes in pea. Five of these genes AB96, AB66, AB80, AB8 whose sequences have been published and AB9 whose sequence has been determined in collaboration with J. Watson and B. Roe, encode a type I polypeptide. We have recently shown that a sixth gene Cab-215 encodes a type II polypeptide. We have now characterized a seventh gene, Cab-315 which encodes a type III LHC II polypeptide. Many amino acids predicted by Cab-315 are common to both type I and type II sequences (overall homology of 75%), while some of them are specific to this sequence and others are characteristic of either type I or type II. At the amino terminus, the sequence is 11 and 7 amino acids shorter than either pea type I or type II sequences, respectively. The transit peptide predicted from the cDNA sequence consists probably of 38 residues and differs considerably from the type I and type II homologous sequences. Evidence that the different types are regulated differentially comes from earlier fluence response and escape from far-red reversibility data on Cab-215 and Cab-315 as compared with total Cab mRNA behavior.

1890 TWO APPROACHES FOR THE ISOLATION OF A XANTHOPHYLL CYCLE MUTANT. S. Lorraine Tausta and Richard B. Peterson, Connecticut Agricultural Experiment Station, New Haven, CT 06504.

One proposed function ascribed to the xanthophyll cycle in higher plants is that of photoprotection. A linear relationship between a leaf's zeaxanthin content and its ability to dissipate excess photochemical energy has been demonstrated. We are interested in pursuing the extent of this relationship by examination of a mutant deficient in operation of the xanthophyll cycle. Two approaches are being undertaken to obtain such a mutant. Seedlings from a population of EMS mutagenized Nicotiana sylvestris seed are being screened for reversal of bleaching upon withdrawal of light. The carotenoids of those plants which exhibit a greener color in the dark are then analyzed using HPLC procedures. Out of a total of 121 light sensitive families tested 61 were able to recover at least some color in the dark. The carotenoid profiles of 30 of these families have been obtained from plants which were grown in the dark and either subjected to a high light treatment or not, immediately prior to pigment isolation. Although none of these displayed a lack of any of the xanthophyll cycle intermediates, a few showed unusual amounts of certain pigments. The second approach is to make a mutant directly by isolating a cloned gene of the de-epoxidase responsible for the conversion of violaxanthin to zeaxanthin.

1891 ISOLATION OF THE CAB-1 PROMOTER BY PCR AND ANALYSIS OF EXPRESSION IN MAIZE TISSUES. K. Budelier, M. Moynihan, K. Lowe, C. Flick and D. Pierce. Pioneer Hi-Bred, Johnston, IA 50131

The Polymerase chain reaction method has been used to isolate the 5' regulatory region of the maize cab-1 gene directly from maize genomic DNA. The cab-1 fragment which was isolated included -839 to +52 bp of the cab-1 upstream region. Chimeric expression cassettes were then constructed which carry the cab-1 promoter region attached to the GUS gene. Particle bombardment was used to test the activity of the cab-1 gene constructs using both transient assays and stable transformation of maize. GUS constructs driven by the 35S promoter were also included in these assays. The cab-1 promoter was found to be active in maize leaves in the transient assay. GUS expression driven by the cab-1 promoter was also seen in green regenerating sectors of transgenic maize callus and in leaves, but not roots, of transgenic maize plantlets. In addition, the cab-1-GUS constructs showed no activity in non-regenerating maize suspension cells and callus. In contrast, the 35S promoter was strongly expressed in leaves and roots of transgenic maize plants and in non-regenerating and regenerating callus or suspensions. These results indicate that the 891 bp upstream fragment of the cab-1 gene when placed in a chimeric construct has maintained at least some of the tissue-specific regulation expected of a light-regulated gene such as cab-1. The effect of intron 6 from the maize Adh1 gene on the activity of the cab-1 and the 35S promoters was also examined in transient leaf assays.

1892 MOLECULAR CLONING, GENE STRUCTURE, AND EXPRESSION OF PROTOCHLOROPHYLLIDE REDUCTASE IN PEA. Zhenghui He, Anthony J. Spano and Michael P. Timko, University of Virginia, Department of Biology, Charlottesville, Virginia 22901 USA

The light-dependent phototransformation of protochlorophyllide into chlorophyllide is catalyzed by an NADPH-dependent protochlorophyllide oxidoreductase (Pchlide reductase EC 1.6.99.1). Antibody elicited against the purified Pchlide reductase protein was used to immunoscreen a cDNA expression library constructed in λgt11 from etiolated pea (*Pisum sativum* L.) leaf poly(A)$^+$. Several near full-length cDNAs encoding the protein were isolated and characterized. A set of nested primers derived from the nucleotide sequence of these cDNAs was subsequently used to clone the 5' and 3' most terminal portions genes by a modified RACE protocol. Cloned cDNA fragments were also used to isolate a corresponding nuclear genomic fragment. The pea Pchlide reductase gene encodes a protein comprised of a mature polypeptide of 313 amino acids and an 87 amino acid transit peptide. The coding portion of the gene is interrupted by three small introns. The deduced primary structure for the dicot protein is similar that previously reported for the monocot enzyme. Steady state levels of mRNA encoding the pea Pchlide reductase do not decrease in leaves illuminated under continuous white light for up to 48 hours. Western blot analysis revealed the presence of two immunoreactive species in extracts of whole leaf tissues, an abundant major form at 38 kDA and a minor form at 36 kDa. The amount of the immunodetectable 38 kDA Pchlide reductase protein decreases to undetectible levels during light induced greening of leaves, whereas the minor 36 kDa forms remains unchanged.

POSTER ABSTRACTS

1893 ISOLATION AND CHARACTERIZATION OF NUCLEAR GENES ENCODING THE AMINOLEVULINATE DEHYDRATASE OF PEA. Jianming Li and Michael P. Timko. University of Virginia, Department of Biology, Charlottesville, Virginia 22901 USA

Aminolevulinate dehydratase (ALA dehydratase) catalyzes the second step of heme and chlorophyll biosynthesis converting ALA to porphobilinogen, the first pyrrole compound in the pathway. We have isolated and characterized an overlapping set of cDNAs from pea encoding most of the mature ALA dehydratase protein. The deduced amino acid sequence of the pea enzyme bears striking similarity over its entire length to other prokaryotic and eukaryotic ALA dehydratases. The pea enzyme contains a highly conserved active site domain centered on LYS. In the pea enzyme the Zn^{2+} binding domain characteristic of other ALA dehydratases has been replaced by a domain based on ASP. This latter observation is consistent with the reported requirement of the plant enzyme for Mg^{2+} rather than Zn^{2+} for activity. Using radiolabeled cDNA probes we have isolated several independent phage from an EMBL4 pea genomic library. Restriction digestion and subsequent Southern blot analysis indicate that these phage contain different genes encoding the ALA dehydratase. The hybridizing fragments have been subcloned into pBluescript vectors for sequence analysis. Additional studies are underway to examine the intracellular localization and expression characteristics of the encoded gene products.

1894 PROTOCHLOROPHYLLIDE REDUCTASE IN PINE: EVIDENCE FOR CONSERVATION IN PROTEIN STRUCTURE BETWEEN ANGIOSPERMS AND GYMNOSPERMS ENZYMES

Anthony J. Spano. Zhenghui He and Michael P. Timko. University of Virginia, Department of Biology, Charlottesville, Virginia 22901 USA

NADPH-protochlorophyllide oxidoreductase (Pchlide reductase) catalyzes the reduction of protochlorophyllide to chlorophyllide, a key regulatory step in chlorophyll synthesis. In contrast to most angiosperm species where protochlorophyllide reduction is a light-dependent process, gymnosperms are capable of light-independent chlorophyll synthesis. A λgt11 expression library constructed from poly(A)$^+$ RNA prepared from dark-grown pine needles was used to isolate phage containing sequences encoding the white pine Pchlide reductase. Two independent phage were identified, λPinPCR300 and λPinPCR900. The nucleotide sequence of the cDNA inserts contained within these phage were determined and shown to encode two distinct expressed forms of the enzyme. The deduced amino acid sequences for the pine enzyme are similar to those previously reported for monocots (oat and barley) and dicots (pea). Southern blot analysis suggests that a small family of genes encode the Pchlide reductase of white and loblolly pine. Radiolabelled cDNAs were subsequently used to screen an EMBL3 library prepared from loblolly pine in order to isolate the analogous nuclear genes. One recombinant phage containing the PinPCR300 analog has been isolated and sequenced. We are in the process of characterizing the expression of this gene as well attempting to identify other members of the gene family. Studies toward the elucidation of the transcriptional/ post-transcriptional mechanisms involved in controlling Pchlide reductase levels are in progress.

1895 NOVEL PHENOTYPES BASED ON LUCIFERASE EXPRESSION IN TRANSGENIC PLANTS. Andrew J. Millar, Sharla R. Short-Russell, Steve A. Kay and Nam-Hai Chua. Rockefeller University, New York.

The accumulated data on patterns of promoter activity in plants have largely depended upon invasive reporter genes, such as CAT and GUS. The destructive assays used to detect such markers limit their use in circadian experiments, compared to non-invasive reporter genes. We have examined the luciferase system for use as a non-invasive reporter in transgenic plants. Our constructs fuse both the bacterial and the firefly luciferases to the *Arabidopsis Cab2* promoter, which has previously been shown to mediate both light/dark- and circadian-regulated transcription of other marker genes. We have tested the fidelity of *Cab::luciferase* regulation at the level of mRNA, protein and luciferase activity *in vitro*. We have further examined the pattern of bioluminescence in intact plants, using "photon-counting" video imaging. We find that the luciferase activity is sufficiently unstable to recapitulate a decrease in transcription from the *Cab2* promoter at the level of whole-plant bioluminescence. This assay system will permit a range of studies based on monitoring gene expression *in vivo* and in real time. The detection of circadian-regulated bioluminescence in seedlings, for example, may permit genetic screening for mutants in the circadian clock.

1896 STRUCTURAL ANALYSIS AND CIRCADIAN EXPRESSION OF THE MAIZE *CAT3* CATALASE GENE. M.L. Ahler and J. G. Scandalios, North Carolina State Univ., Genetics Dept., Raleigh NC 27695-7614

Our lab has recently described the novel circadian regulation of *Cat3* gene expression in maize (Redinbaugh et al. (1990), **PNAS** 87:6853-6857; Acevedo et al. (1991), **Genetics** 127:601-607). We have isolated a genomic clone encoding the *Cat3* catalase gene from a maize leaf genomic library. We sequenced the clone to determine the structure of the *Cat3* gene and located the initiation point of the *Cat3* transcript by primer extension analysis. Results of experiments to characterize the circadian nature of *Cat3* expression in different organs of maize seedlings and in plants subjected to various light regimes will also be discussed. Research supported by Fellowships from NSF and NCBC to MLA and a grant from the EPA to JGS.

1897 LOCALIZATION OF THE *FED-1* LIGHT REGULATORY ELEMENT Lynn F. Dickey, Maria Gallo-Meagher, George C. Allen, Deborah Marin Thompson and William F. Thompson. North Carolina State University, Departments of Botany and Genetics, Raleigh, North Carolina 27695, USA.

The pea ferredoxin I (*Fed-1*) gene is regulated by light acting through the phytochrome system. When dark grown seedlings or dark adapted plants are exposed to light the *Fed-1* mRNA levels increase approximately 5- to 10-fold. Some features of the light response of this gene are strikingly different than other light regulated genes and have led us to hypothesize a post-transcriptional mechanism. Previously, we demonstrated that the light regulatory element(s) is located within the transcribed portion of the gene in contrast to other light regulated genes which are controlled primarily by light through sequences in the promoter regions. We are continuing to define the light regulatory sequences using transgenic plants. The abundance of *Fed-1* mRNA is still light regulated when the *nos* terminator is substituted for the *Fed-1* 3' untranslated region; thus no essential light regulatory elements are located at the 3' end of the message. There is a rapid loss of light regulation as the 5' end is progressively removed, but the *Fed-1* 5' untranslated region alone does not confer light regulation when fused to the *uidA* (GUS) control gene driven by the CaMV 35S promoter. Sequences within the 5' untranslated region are therefore necessary but not sufficient for light regulation. However, light responsiveness is conferred by a combination of the 5' untranslated region and the first third of the coding region when this fragment is fused to the *nos* terminator or to the GUS gene. Thus we believe light regulatory sequences are located in both the 5' untranslated and coding regions, or that one element spans the junction between these two regions.

1898 ORGAN-SPECIFIC AND LIGHT-REGULATED EXPRESSION OF THE PEA FED-1 GENE IN TRANSGENIC TOBACCO SEEDLINGS. Maria Gallo-Meagher, Dolores A. Sowinski, Robert C. Elliott*, and William F. Thompson. Departments of Genetics and Botany, North Carolina State University, Raleigh, N.C. 27695 USA. *Present address: John Innes Institute, Norwich, UK.

Earlier analysis of pea *Fed-1*, the gene encoding ferredoxin-1, revealed that it displays unusual light responses. For example, in pea seedlings *Fed-1* is induced very rapidly by light and appears to require the continued presence of Pfr for expression. In addition, studies using chimeric gene constructs in transgenic tobacco plants determined that the major cis-acting elements required for light regulation of *Fed-1* mRNA levels in green leaves of primary transformants was located within the *Fed-1* transcription unit. We have extended the transgenic work further to include expression in etiolated material. We show that the rapid induction and overall accumulation of pea *Fed-1* mRNA in etiolated transgenic tobacco seedlings exposed to white light closely mimics the response of the native gene in pea seedlings. However, the pea *Fed-1* gene is not as responsive to red light in transgenic tobacco seedlings as in pea. *Fed-1* is expressed in an organ-specific manner in pea and we show here that the promoter is important in determining *Fed-1* organ-specificity in transgenic tobacco. In transgenic tobacco cotyledons, as in green tobacco leaves, a large proportion of the *Fed-1* light response can be attributed to cis-acting elements located within the transcribed region of the gene.

POSTER ABSTRACTS

1899 EXPRESSION OF CHLOROPHYLL a/b PROTEIN GENES IN WILD-TYPE PEA AND SELECTED MUTANTS. Michael J. White, Denis Falconet, Brian Fristensky, Shannon Frances, Ben Horwitz and William F. Thompson. North Carolina State University, Department of Botany, Raleigh, North Carolina 27695, USA.

We are studying the light responses of different members of the pea *Cab* multigene family. Partial or complete DNA sequences are available for seven genes. Five of these (*AB80*, *AB66*, *AB96*, *Cab8* and *Cab9*) encode standard Type I, light-harvesting complex II (LHCII) polypeptides. Recently, a genomic clone for a sixth gene, *Cab-215* has been isolated and sequenced (see poster by Falconet *et al.*). It encodes a Type II, LHCII polypeptide and contains an intron of 355 base pairs in the 5' portion of the coding region. A seventh gene, *Cab-315*, appears to encode a novel LHCII sequence based on analysis of a partial cDNA sequence. A system using cDNA synthesis and PCR has been developed to study the abundance of mRNA produced from these genes under various light regimes. The system is both gene-specific and quantitative. Using this system, the response of these 7 *Cab* genes to very low fluence and low fluence red light has been measured. At least several of these genes are expressed at high levels in the dark in a mutant pea exhibiting a de-etiolated phenoptype (see poster by Frances *et al.*). These genes are also expressed in several chlorophyll b-deficient mutants at levels as high or slightly higher than wild type pea. Differences in the responses of various *Cab* gene family members to light and developmental stage will be discussed.

1900 INITIAL CHARACTERIZATION OF A DE-ETIOLATED MUTANT IN PEA. Shannon Frances, Michael J. White, Michael D. Edgerton[1], Alan M. Jones[1], Robert C. Elliott and William F. Thompson. Departments of Genetics and Botany, North Carolina State University, Raleigh, N.C. 27695, USA. [1]Department of Biology, University of North Carolina, Chapel Hill, N.C. 27514, USA.

A mutant of pea (*Pisum sativum* L. cv Alaska) has been identified which has characteristics of a seedling grown in the light even when grown in complete darkness. Dark grown wild-type peas have a typically etiolated morphology: elongated epicotyls, unexpanded buds and yellow/whitish color with small, undifferentiated cells. The dark grown mutant has a de-etiolated morphology at the gross- and micro-anatomical levels: short epicotyls, expanded leaves and yellow color with developed palisade, mesophyll and stomatal cells. Various assays were performed on mutant and wild-type peas that were grown in the dark for six days then transferred to the light. Chlorophyll accumulation in the mutant has a greatly reduced lag period. Two normally light-regulated genes (*Cab* and *Fed-1*) were also found to produce high levels of mRNA in dark grown mutant seedlings. (For further discussion of *Cab* expression see poster by White *et al.*) The dark-grown mutant has substantially reduced levels of both spectral and immunologically detectable phytochrome.

1901 *IN VITRO* AND *IN VIVO* DETERMINATION OF PEA *FED-1* mRNA TURNOVER. George Allen, Dolores Sowinski, Maria Gallo-Meagher, and William F. Thompson. North Carolina State University, Department of Botany, Raleigh, N.C. 27695 USA

Pea ferredoxin (*Fed-1*) mRNA is light-regulated by *cis*-acting elements within the transcribed region. This and other supporting data have led to the hypothesis that a major portion of the regulation of *Fed-1* mRNA is post-transcriptional. To test this hypothesis we are using two approaches. Nuclear run-on assays show only small changes in *Fed-1* transcription when nuclei isolated from light- and dark-treated transgenic tobacco plants are compared. Comparison of this data with steady state levels of mRNA from plants grown under the same conditions suggest that pea *Fed-1* mRNA has a higher rate of degradation in the dark than in the light. To confirm this possibility, *in vivo* studies using RNA synthesis inhibitors are now in progress. Etiolated pea buds exposed to white light were floated on nutrient solution with or without α-amanitin. Throughout the next 10h *Cab* mRNA levels declined steadily in the presence of α-amanitin whereas *Fed-1* mRNA levels remained unaffected. We are currently studying the feasibility of growing intact seedlings in liquid shake culture to test the *Fed-1* light response in transgenic tobacco. Initial results indicate that the light/dark response under these conditions is rapid and similar to that of pea seedlings. Using this system we hope to study the effect of RNA synthesis inhibitors on chimaeric constructs of *Fed-1* in transgenic tobacco.

1902 CIRCADIAN RHYTHMICITY OF CHL a/b BINDING PROTEIN GENE EXPRESSION. Birgit Piechulla, Jan W. Kellmann, Hartmut Meyer, Eran Pichersky*, Sabine Riesselmann.

Institut für Biochemie der Pflanze, Untere Karspüle 2, 3400 Göttingen, Germany. *University of Michigan, Dept. of Biology, Ann Arbor, MI 48109, USA.

The expression of the chlorophyll a/b binding protein genes (cab genes) is controlled by exogenous and endogenous signals (e.g. light, tissue specificity, 'biological clock') and at different levels (transcription, translation, assembly etc.).

Further characterizations of the circadian rhythmicity of cab mRNA accumulation in tomato seedlings revealed that a) moderate temperature alternations with constant period (simulation day and night temperatures!) at constant illumination can reset the phase, therefore such alternations function as 'Zeitgeber', and b) the growing temperature also influences the amplitude of the oscillations.

Separate determination of transcript levels of fourteen genes of the tomato cab gene family revealed the presence of diurnal transcript oscillation patterns for each cab gene, however with different amplitudes and phasing. In vitro transcription 'run off' experiments support the idea that the control elements of the 'biological clock' interact with the process of transcription. Together, these results suggest similar or identical control mechanisms for all cab genes during their expression despite the fact of dispersed location on the genome and differences in the promoter regions.

In vivo labeling experiments with tomato seeedlings grown under light/dark or continuous dark conditions indicate diurnal synthesis of chl a/b binding proteins.

1903 IDENTIFICATION OF THE CHLOROPHYLL a/b (CAB) POLYPEPTIDES OF PHOTOSYSTEMS I AND II WITH THEIR CORRESPONDING GENES. Beverley R. Green[a], Eran Pichersky[b], Ruedi Aebersold[c];[a]Botany Dept., [c]Biomedical Research Laboratory & Biochemistry Dept., Univ. of British Columbia, Vancouver, B.C. Canada V6T 1Z4;[b]Biology Dept.,Univ. of Michigan, Ann Arbor, Mich. 48109 USA.

Four different Chl a/b (CAB) antenna complexes can be isolated from higher plant and green algal thylakoid membranes: LHCII, CP29 and CP24 (PSII) and LHCI (PSI). Their polypeptides are encoded by an extended family of genes. We have succeeded in resolving all of the tomato CAB polypeptides and obtaining peptide sequences which conclusively link each one to the gene(s) which encodes it. All but one of the tomato genes have been cloned. Tomato LHCII has 4 polypeptides: Type I(27kDa) encoded by 8 closely-related genes, Type II (26kDa) encoded by 2 genes; and two Type III(24-25kDa) encoded by 2 genes. The gene for the 26 kDa CP29 polypeptide (HGG,in press 1991) and two genes for the CP24 polypeptide (P.Mol.Biol.15:157,1990) are more distantly related to the LHCII sub-family. The four LHCI genes are almost as distant from each other as from the LHCII and CP29 genes (FEBS Lett.280:229,1991). All the proteins in this family are predicted to have three trans-membrane helices. The first and third helices plus their preceding stroma-exposed "hooks" (high beta-turn) are highly conserved and related to each other. The shorter middle helix and its connectors are more variable and may have specific structural information for assembly into the correct photosystem. There are not enough conserved H,N,Q to ligate 15 Chls, suggesting that specific Chl attachment sites vary between CAB polypeptides. Since light-absorbing and transfer properties of all complexes are similar, why do plants have such a variety of light-harvesting molecules? (Supported by NSERC, MRC, USDA).

POSTER ABSTRACTS

1904 PHOSPHOENOLPYRUVATE CARBOXYLASE GENE FAMILY IN SORGHUM REGULATION BY LIGHT AND NITROGEN. C. CRETIN, L.LEPINIEC S. SANTI, E. KERYER, J. VIDAL and P. GADAL. URACNRS D1128, Université Paris XI, 91405 ORSAY, FRANCE.

Three to four members of the multigene family encoding for phosphoenolpyruvate carboxylase (PEPC) have been identified in Sorghum vulgare. Three of them, CP46, 28 and 21, have been isolated form a genomic library. Comparison of the DNA sequences with cDNA sequences and other PEPC genomic clones (corn and CAM plant) revealed that the CP46 gene encodes for the C4 type PEPC whereas the CP21 and CP28 genes correspond to the C3 type.These two types of PEPC isoforms show a high level of homology: 75% between the aminoacid sequences. Using specific probes in Northern blot experiments, it could be shown that the accumulation of C4 PEPC mRNA is strictly light-dependent and leaf specific. On the other hand, the expression of the C3 type genes is neither restricted to the leaf tissue nor light regulated but it is induced by nitrogen sources in roots. Promoter sequences analysis is under investigation in order to characterize DNA elements implicated in the specificity of the PEPC genes expression.

1905 ARE THE EXPRESSION OF GENES ENCODING LHC-II INDEPENDENT OF LIGHT IN SCOTS PINE SEEDLINGS? Anna-Karin Lundberg, Stefan Jansson and Petter Gustafsson. Department of Plant Physiology, University of Umeå,S-901 87 Umeå, Sweden.

Darkgrown pine seedlings can be photoactivated rapidly. They have about 10 % of the chlorophyll content present in light grown seedlings and they have the proteins necessary for photosynthesis present in almost the same proportions as in light. One exception is the major light harvesting complex of photosystem II (LHC-II) where about 20 % of the "normal" amount is present in darkness. Still, little is known about how the different photosynthetic polypeptides are regulated.

We have been following the expression of genes coding for type I and II LHC-II chlorophyll a/b binding polypeptides (*cab*-genes) in *Pinus Silvestris L.* during development in darkness and during light induction. In darkgrown pine seedlings the *cab*-genes are highly expressed which agrees well with their photosynthetic capacity and distinguishes gymnosperms from angiosperms.

In early stages of development in darkness there is a high level of *cab*-transcripts which decline to about 30 % of the initial value after 3-4 days with no further decline during the following 10 days. After that most of the seedlings dies. The amount of transcripts from two type I and one type II gene is roughly 10: 2: 1. However, there seems to be no gene-specific pattern of expression from the different *cab*-genes during these conditions.

Preliminary results show that although *cab*-genes are highly expressed in darkness they can be further induced when transferred to light.

1906 RED AND BLUE LIGHT INDUCTION OF HYDROXYPYRUVATE REDUCTASE TRANSCRIPT IN CUCUMBER LEAVES. Steven G. Daniel[1], Brian W. Schwartz[2], Gregory P Bertom[3] and Wayne M. Becker[1]. Departments of [1]Botany, [2]Genetics and [3]Biochemistry, University of Wisconsin-Madison, 53706 Peroxisome localized, NADH-dependent hydroxypyruvate reductase (HPR) is a key enzyme in the photorespiratory glycolate pathway. This pathway salvages some of the carbon diverted from the Calvin cycle via the oxygenase activity of ribulose bisphosphate carboxylase/oxygenase. Previous studies in our lab have demonstrated that HPR transcript levels in both cotyledons and leaves (from etiolated and dark-adapted plants, respectively) are strongly enhanced by light We are now determining the wavelengths of light responsible for this induction. Cucumber plants grown in continuous white light for 12 days were transferred to darkness for 3 days followed by a 3-hour pulse of either white, red or blue light and returned to darkness. Leaf tissue was harvested at various time points up to 24 hours after the light treatments and total RNA was prepared. HPR transcript levels were determined for each treatment by northern and slot-blot analysis. Red light treatment resulted in the largest increase in HPR transcript level, to approximately one-half that of the continuous white light control. Transcript levels were highest immediately following the 3 hour red light treatment and declined to nearly undetectable levels by 24 hours. Blue light treatment induced HPR transcript levels to about one-fourth of continuous white-light levels Transcript was nearly undetectable six hours following treatment. White light induction kinetics were strikingly different from those of red and blue light HPR transcript was nearly undetectable immediately following the white-light pulse, but continued to increase for 24 hours, ultimately reaching about one-fourth continuous white light control levels These results indicate that phytochrome and perhaps a blue-light photoreceptor regulate HPR expression in developing cucumber leaves Supported by NSF grant #DCB-9004013 to WMB and an NIH traineeship to BWS and GPB.

1907 METABOLISM, PHOSPHORYLATION AND ABSORPTION CROSS-SECTION OF THE LIGHT HARVESTING ANTENNA OF *Prochlorothrix hollandica*. K. M. Milbauer[1], A.F. Post[2] & G.S. Bullerjahn[1]. [1]Dept. Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, U.S.A.; [2]Institute of Life Sciences, Hebrew University, Jerusalem 91904, Israel.

Previous studies have identified 3 immunologically related major antenna chlorophyll (Chl) *a/b*-binding proteins in the prokaryote *Prochlorothrix hollandica*. These 35, 33 and 30 kD intrinsic membrane proteins are immunologically distinct from the major LHCII Chl *a/b* antenna proteins in higher plants. Light has been shown to induce the phosphorylation of the 35 kD antenna protein. The phosphorylation kinetics of the 35 kD protein suggest that it is part of a long term adaptation to a change in light conditions. Additionally, the 35 kD antenna protein is tightly coupled to PSI. Current work indicates that the 35 kD protein is phosphorylated at subsaturating to saturating light conditions, and it is the phosphorylated form of this protein that is predominately associated with PSI. In concert with these studies, we show from fluorescence induction measurements that State 1>2 transitions occur under conditions controlling phosphorylation of the 35 kD antenna protein. Moreover, upon the addition of phosphatase inhibitors, the cells are locked in State 2. Monitoring non-photochemical and photochemical fluorescence quenching, the balance of PSII/PSI activity changed following a state transition; thus, the excitation energy is truly redistributed. Additional experiments also show that the 35 kD protein is preferentially degraded within 9 hours after cells are shifted to high (supersaturating) light conditions. Overall, we believe that the phosphorylation state of the 35 kD antenna protein dictates the partitioning of the protein between the two photosystems. We also suggest that the selective degradation of this protein serves to limit the size of the light harvesting antenna as *P. hollandica* adapts to long-term exposure to saturating light intensities.

1908 STRUCTURE AND EXPRESSION OF THE PHASEOLUS VULGARIS *rbcS* GENES. N.A.R. Urwin, T.I. Sawbridge, M.R. Knight, E. Lopez, J. Jardine and G.I. Jenkins. Departments of Biochemistry and Botany, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

The small subunit (*rbcS*) of ribulose 1,5-bisphosphate carboxylase/oxygenase in the French bean *Phaseolus vulgaris* L. is encoded by a small gene family consisting of probably three members. Three *rbcS* genes represented in a light-grown primary leaf cDNA library were characterised by sequencing cDNA clones. The genes are identical in their coding sequences, for both the transit peptide and the mature polypeptide, but divergent in their untranslated sequences. The first amino acid of the mature polypeptide is isoleucine, which differs from the methionine found in other *rbcS* genes. Surprisingly, one of the cDNA clones contains two introns, which are at positions conserved in *rbcS* genes from other species. This cDNA clone presumably resulted from the cloning of an unprocessed transcript. Expression of the individual *rbcS* genes has been investigated using gene-specific oligonucleotide probes. Expression is stimulated by light, although transcripts can readily be detected in dark-grown primary leaves, and the *rbcS-3* gene shows the smallest degree of photoregulated expression. We have found that expression in light-grown primary leaves is strongly dependent on the fluence rate of white light and that this control is mediated by a blue light photoreceptor. DNA sequence elements concerned with photoregulation in the upstream flanking sequences of the *rbcS-2* gene are currently being investigated

1909 ANALYSIS OF A CUCUMBER HYDROXYPYRUVATE REDUCTASE GENE IN TRANSGENIC TOBACCO PLANTS

James S. Sloan[1] and Wayne M. Becker[2], Departments of Genetics[1] and Botany[2], University of Wisconsin-Madison, Madison, WI 53706

The enzyme NADH-dependent hydroxypyruvate reductase (HPR) is involved in the photorespiratory glycolate pathway, and is localized in leaf peroxisomes. Expression of HPR is confined to photosynthetically active tissues. HPR transcript is detectable in both light-grown and etiolated cucumber seedlings a few days after germination. The level of HPR transcript is enhanced by light, and regulation of the gene is mediated at least in part by phytochrome. We have recently isolated cucumber genomic clones corresponding to two HPR alleles. A 6.7-kb region containing one of the alleles, *hpr*-A, has been sequenced. As a first step in conducting a functional analysis of *cis*-acting elements governing the regulation of *hpr*-A, we have introduced a 5.7-kb fragment, which includes the entire gene and 1.1-kb of 5' flanking sequence, into tobacco plants. Six independent transgenic lines containing *hpr*-A have been identified. Three of these lines appear to contain a single copy of the transgene. The *hpr*-A gene is transcribed in all six lines, though at varying levels. Analysis of several of the lines indicates that the gene is regulated by light in the expected manner. This work was supported by NSF Grant #DCB-9004013 to WMB and an NIH traineeship to JSS.

POSTER ABSTRACTS

1910 LIGHT-REGULATED TRANSCRIPTION FACTOR GENES OF ARABIDOPSIS THALIANA. Sjef Smeekens, Nicolette Quaedvlieg, Jan Dockx and Peter Weisbeek. Dept. of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Many plant developmental processes are dependent on and controlled by light. We hypothesize that this control will, in many cases, be exerted by transcription factors which themselves can be light-regulated. This hypothesis is currently being tested by differential screening of an ordered transcription factor library derived from Arabidopsis thaliana. To obtain such a transcription factor library we screened a genomic library using as probes those regions of known transcription factor which encode the DNA-binding motifs. These probes were derived from animal, yeast and plant transcription factor genes which encode different classes of DNA-binding proteins. At present this transcription factor library is being screened with cDNA prepared from mRNA of different stages of light-dependent plant development.

1911 FUCOXANTHIN-CHL A/C PROTEINS OF THE CHRYSOPHYTE ALGA Olisthodiscus (Heterosigma) ARE RELATED TO DIATOM CHL A/C AND HIGHER PLANT CHL A/B PROTEINS. Dion G. Durnford and Beverley R. Green, Botany Dept., University of B.C., Vancouver, B.C. Canada V6T 1Z4.

Olisthodiscus luteus is one of the chromophyte (Chl a/c) algae which have two additional membranes (chloroplast ER) surrounding the chloroplast envelope, and both its Rubisco SSU and LSU genes are encoded by ct DNA. Olisthodiscus thylakoids have three major and two minor polypeptides immunostained by antibodies raised against fucoxanthin-Chl a/c (FAC) proteins of the diatom Phaeodactylum tricornutum. When thylakoid membranes are solubilized with digitonin or octyl glucoside and fractionated on a sucrose gradient, the upper fractions contain fucoxanthin, Chl a, Chl c, and three polypeptides of 18-19 kDa. The FAC bands can be cation-precipitated like LHCII, and two of the bands are immunostained by antibodies raised to barley LHCII. The lower fraction contains PSI and PSII holocomplexes (identified by photochemical assays and immunoblotting with higher plant antibodies) which can be further resolved with dodecyl maltoside to give a fraction enriched in two of the major FAC polypeptides. N-terminal and tryptic peptides sequences have been obtained for the major bands. The corresponding genes are being cloned and sequenced in order to study the evolutionary relatedness of antenna Chl a/c and Chl a/b polypeptide families and determine the process by which these nuclear encoded polypeptide cross four membranes before integration into the thylakoid membrane. (We thank Dr. A. Grossman for probes and antibodies, and NSERC for financial support).

1912 ANALYSIS OF DNA FRAGMENTS INVOLVED IN LIGHT CONTROL OF GENE EXPRESSION IN SYNECHOCYSTIS PCC6803. P. Marraccini, C. Cassier-Chauvat and F. Chauvat, Service de Biochimie et Génétique Moléculaire, Département de Biologie Cellulaire et Moléculaire. Centre d'études de Saclay 91191 Gif sur Yvette cedex France.

Cyanobacteria are attractive organisms to study light regulation of gene expression since they are easily amenable to gene cloning. In these organisms, however, the molecular mecanisms involved in the control by light at the level of transcription initiation, remains largely unknown. We therefore decided to search for light-regulated promoters in *Synechocystis* PCC 6803, using our promoter probe vector, pFF11. This plasmid replicates autonomously in *Synechocystis* PCC 6803. It is based on the reporter *cat* gene encoding the chloramphenicol acetyl transferase (CAT) enzyme which can be easily assayed and promotes Cm^r phenotype. A genomic library of *Synechocystis* PCC 6803 DNA was made in pFF11 and introduced in this cyanobacterium, selecting for constitutive expression of the marker of the vector (Km^r) and light induced expression of the *cat* reporter gene. 34 clones which were isolated, on the basis of their resistance to chloramphenicol in high light (5000 luxes) and sensitivity to this drug in low light (500 luxes). 5 were further studied since they are genetically stable. Their hybrid plasmid keep replicating autonomously without any integration into the cyanobacterial chromosome. Each plasmid contain a different segment of the cyanobacterial genome, as shown by sequencing analysis. Their size range from 85 bp to 1,925 bp, depending on the clone. We will present the data coming out from comparison of these DNA sequences, which reveal several putative open reading frames and many conserved sequences. The latter are presently under investigation using in vitro mutagenesis, in combination to in vivo studies, using Northern blot analysis and CAT activity measurements.

1913 LARGE UNIDENTIFIED OPEN READING FRAME IN PLASTID DNA (ORF2208) IS EXPRESSED IN CHLOROPLASTS. R. E. Glick, B. B. Sears, Michigan State Univ., Botany and Plant Pathology, East Lansing, MI 48824

The chloroplast DNA (cpDNA) encodes genes for components of photosynthesis and the transcription-translational machinery; a number of unidentified open reading frames (ORFs) are also present. To determine whether a large ORF in the inverted repeat of cpDNA of tobacco (ORF2280) encodes a chloroplast protein, a conserved region of the ORF was expressed in E. coli. An antibody against the ORF protein was prepared using the purified fusion protein as an antigen. When incubated with proteins from the soluble fraction of tobacco, spinach and Oenothera chloroplasts, the antibody reacts with relatively labile polypeptides, which have apparent molecular weights of 170-180 kDa. The ORF in tobacco and spinach is large enough to encode a protein of approximately 300 kDa, thus it is possible that post-transcriptional or post-translational processing reduces the size of the expression product in the plastid. Analysis of Oenothera chloroplasts representing four different plastome types revealed restriction fragment length polymorphisms in cpDNA indicative of insertion/deletion events in a region of the cpDNA that shared significant sequence similarity with ORF2280. The ORF2280 antibodies are being used to determine whether the polymorphisms observed in the Oenothera cpDNA result in differences in the ORF proteins in chloroplasts from the four plastome types.

1914 DIFFERENT PROMOTER ARCHITECTURE OF PHOTOSYNTHETIC GENES. R. Oelmüller, K. Flieger, A. Tyagi, T. Lübberstedt, C. Bolle, R.G. Herrmann. Botanisches Institut der LMU, Menzingerstr. 67, 8000 München 19, FRG

One of the most intriguing questions to be addressed concerning the regulation of eukaryotic genes is how the expression of specific sets of genes is controlled within and between cellular components (nucleus/cytosol and plastids). Based on the availability of cDNA clones for 26 components of the thylakoid membrane, we have initiated a comparative study of the expression of nuclear genes for thylakoid proteins. These genes are regulated by various internal and external factors, such as light. Today, we have characterized genomic clones for the genes of subunits II, III and IV from the photosystem I reaction center, of the ATP synthase subunits gamma and delta, of ferredoxin-$NADP^+$-oxidoreductase, plastocyanin and of a member of the *cab*-gene family from spinach. Promoter deletion/GUS fusions have been analysed in transgenic tobacco. Although the RNA patterns are quite uniform, the promoters differ substantially in primary sequence and architecture with regard to light-responsive elements and elements involved in organand tissue-specific expression. Using gel-shift assays, various regions have been identified which bind *trans*-acting protein factors and confer expression upon an inactive (truncated) *cab*-promoter in transgenic tobacco.
Supported by the Deutsche Forschungsgemeinschaft (SFB 184)

1915 COMPETITION BETWEEN PLASTOCYANIN AND A CU-DEPENDENT REGULATOR OF THE CYT C6 GENE FOR INTRACELLULAR CU. Kent Hill, Gregg Howe and Sabeeha Merchant. Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA 90024-1569, USA

Cytochrome c6 catalyzes the transfer of electrons from reduced cyt f (of the b6f complex) to $P700^+$ of Photosystem I. In *Chlamydomonas reinhardtii* however, plastocyanin appears to be the preferred catalyst if Cu is available to support holoplastocyanin synthesis. The expression of cyt c6 is therefore controlled by the amount of Cu available to the cell, such that transcripts encoding cyt c6 are observed only in Cu-deficient cells (approximately 10^2 transcripts/cell) whereas in Cu-supplemented cells cyt c6 messages are not detectable (< 1 message/cell). In determining the sensory threshhold of a putative Cu-responsive cellular factor to medium Cu, the amount of Cu required for the sustained repression of cyt c6 accumulation (9×10^6 per cell) was noted to be of the same magnitude as the ratio of plastocyanin molecules per *C. reinhardtii* cell, *i.e.* 8×10^6. In other words, the number of Cu ions required to repress cyt c6 transcription completely is exactly matched by that required for maintenance of normal holoplastocyanin levels. In fact, the level of expression (*i.e.* % of maximum expression) of the gene encoding cyt c6 is inversely correlated with the amount of plastocyanin (*i.e.* % of maximum) that can be synthesized. The above results suggest either that plastocyanin itself functions as a transcriptional regulator of cyt c6, or, that plastocyanin competes with a distinct Cu-dependent cellular factor (possibly a transcriptional activator or repressor) for intracellular Cu. We favor the latter since *ac-208* (a plastocyanin-less mutant) continues to display Cu-regulated expression of cyt c6. The autoregulatory circuit that describes the Cu-dependent expression of cyt c6 and plastocyanin is analogous to the feedback control of Cu-metallothionein in yeast with the interesting distinction that in our system, competion for the ligand is proposed to occur between the putative transcriptional regulator of the cyt c6 gene and the functional counterpart of the gene product, *viz.* plastocyanin, rather than between the transcriptional regulator (ACE1/CUP2) of the gene (CUP1) and its own gene product, *viz.* metallothionein.

POSTER ABSTRACTS

1916 CU-DEPENDENT REGULATION OF CYT C6 EXPRESSION IN *CHLAMYDOMONAS REINHARDTII*. Jeanette Quinn, Kent Hill and Sabeeha Merchant. Dept. of Chem. and Biochem., UCLA, Los Angeles, CA 90024

Plastocyanin is the most abundant copper protein in plant leaves and is therefore a major contributor to the plant's requirement for copper. Aquatic algae and cyanobacteria are, however, photosynthetically competent even under Cu-deficient growth conditions owing to functional replacement of plastocyanin in photosynthetic electron transfer by a heme-containing, soluble c-type cytochrome -- cyt c6. Whether an organism uses plastocyanin or cytochrome c6 as the electron carrier is determined solely by the presence of Cu in the surrounding medium. If the amount of available Cu is sufficient to support plastocyanin accumulation at the stoichiometry required for efficient photosynthesis, plastocyanin appears to be the preferred *in vivo* electron carrier. However, if the concentration of available Cu is too low to support the synthesis of stoichiometric amounts of functional plastocyanin, cyt c6 synthesis is induced. The Cu-dependent, coordinate and reciprocal regulation of plastocyanin and cyt c6 levels is well-established, and forms the basis of an excellent and unique model system for the study of metal ion regulated gene expression involving the control of essential metalloprotein synthesis. Cyt c6 is encoded by a single nuclear gene in *C. reinhardtii* that is regulated by Cu at the level of mRNA accumulation. A region comprising 2.6 kb was sequenced and found to contain the entire transcribed portion of the *C. reinhardtii* cyt c6 gene, plus 5' and 3' untranscribed regions. Analysis of the sequences upstream of the 5' border reveals only one stretch of limited sequence similarity (8 of 12 matches) to the consensus 12 nucleotide target site of the yeast (CUP2/ACE1) Cu-responsive transcriptional activator. Time course studies indicate that transcription of the cyt c6 gene is turned off within 30 minutes following the addition of Cu to Cu-depleted cells. In contrast to the yeast CUP1 gene, the cyt c6 gene is completely unresponsive to Ag ions. It does however respond to Hg ions, albeit with 10-20 fold less sensitivity. We are presently dissecting the 5' upstream regulatory region of the cyt c6 gene in order to identify *cis*-acting copper-regulated elements with the long term goal of isolating a metal-responsive molecular switch with unique metal specificity.

1917 *CIS*- AND *TRANS*-ACTING ELEMENTS INVOLVED IN PEA PLASTOCYANIN GENE EXPRESSION. Keng-Hock Pwee and John C. Gray Botany School, University of Cambridge, Cambridge CB2 3EA, UK.

Plastocyanin is an essential chloroplast protein involved in photosynthetic electron transfer. A 3.5kbp *Eco* RI fragment derived from a pea genomic clone has been shown to direct tissue-specific and light-induced expression of authentic pea plastocyanin when analysed in transgenic tobacco plants (1).Deletion analysis of the 5' non-coding region of this gene fragment using promoter-GUS (beta-glucuronidase) fusions in transgenic tobacco has uncovered strong positive and negative *cis*-elements within the promoter which modulate quantitative expression. However, the qualitative properties appear to remain intact in even the shortest promoter fusion: GUS is localized in chloroplast-containing cells of mature plants, but is not markedly induced in etiolated seedlings exposed to light.Gel retardation assays of a positive promoter element -444 to -176bp from the translation start have detected a number of protein-DNA complexes. One of these is heat-resistant and sensitive to competition with poly(dAdT).poly(dAdT) but not poly(dGdC).poly(dGdC). This resembles BC1 (Bound Complex 1) from the pea ferredoxin promoter (2) and suggests that a High Mobility Group (HMG) nuclear protein is involved which binds selectively to AT-rich regions in double-stranded DNA (3). DNaseI footprinting studies show that tracts of greater than 6 A/T base pairs are protected within the plastocyanin element by pea nuclear extracts. Such A/T runs are distributed non-randomly within the pea plastocyanin gene sequence and are particularly concentrated in the positive element. Partial purification of pea HMGs using FPLC show that binding activity elutes at about 25 kDa on a Superose 12 column and can be separated by anion exchange into two isoforms on a Mono Q column.

(1) D.I.Last and J.C.Gray (1990) *Plant Mol. Biol.* 14: 229-238
(2) R.C.Elliot *et al.* (1989) *Plant Cell* 1: 681-690
(3) T.J.Pedersen *et al.* (1991) *Plant Mol. Biol.* 16: 95-104

1918 A NEGATIVE SELECTION SYSTEM FOR TRANSGENIC PLANTS. D.P. O'Keefe, S.H. Russell, C. Dean, J.M. Tepperman, K.J. Leto, & J.T. Odell. Du Pont, Central Research & Development and Agricultural Products, Wilmington, DE 19880-0402; and DNAP, Oakland, CA.

Positive selection systems are used routinely to select transformed plants and assay for gene expression. In contrast, a negative selection system allows selection for loss of gene function. With microorganisms, one can use the technique of replica plating to isolate mutants that lack a function; unfortunately, replica plating of whole plants is virtually impossible. We have devised a method for negative selection of plants *in vitro*, using a gene from *Streptomyces griseolus*, which encodes a herbicide-metabolizing cytochrome P450. Maximum functional expression of the bacterial P450 requires that the mature protein is located in the chloroplast stromal space to intercept reducing equivalents from ferredoxin in photosystem II. Metabolism by this enzyme activates a non-phytotoxic sulfonylurea herbicide (R7402) into a phytotoxic compound. Hence, when the gene is expressed in a plant, the growth of the plant is arrested. Nuclear transformation of both *Nicotiana tabacum* and *Arabidopsis thaliana* produced transformants harboring the P450 gene under the control of a constitutive promoter (CaMV 35S) and two light regulated, tissue specific promoters and transit sequences (SSU and Cab) that target the protein to the chloroplast. The linked NOS/NptII gene, which confers resistance to kanamycin, was used to select the transformants. Transformants survive under kanamycin selection, but growth is arrested on R7402. Applications of this system will be discussed.

1919 EXPRESSION OF THE NUCLEAR GENE CODING FOR THE CHLOROPLAST L21 RIBOSOMAL PROTEIN: EVIDENCE FOR TWO TRANSCRIPTION PROMOTERS IN SPINACH LEAVES. T. Lagrange, S. Lerbs-Mache, M. Axelos and R. Mache. Lab. Biol. Mol. Vég., CNRS & Univ. de Grenoble, BP53, Grenoble, France.

A cDNA clone coding for the chloroplast L21 r-protein has been isolated and characterized (Martin et al., Curr Genet.18, 553,1990). One gene per haploid genome has been identified. We now report on the organization of the nuclear *rpl*21 gene and of the 5' upstream non-coding region. The coding sequence is interrupted by 4 introns one of which beeing more than 1.8kb long. The presence of L21 transcripts is detected in leaves and to a much lesser extent in roots and in dry seeds. The accumulation of transcripts is independent of light. Two initiation sites for transcription are detected in leaf cells (S1 and S2) whereas one initiation site only is functioning in root cells and in seeds (S2). Several constructions using promoter region with appropriate deletions were used for transient expression assays in Arabidopsis protoplasts. It is shown that the two start of transcription depends on the function of two separated promoter containing regions. Transcription starting at S1 is very efficient and tissue-specific. Transcription starting at S2 is constitutive and functions at a low level. The initiator element at S2 includes a pyrimidine stretch which probably have a general significance as it is present in the case of 4 other genes each one coding for a chloroplast ribosomal protein.

1920 REGULATION OF GENES ENCODING PLASTID PROTEINS DURING CHLOROPLAST BIOGENESIS IN EUGLENA. D. E. Buetow[1,2], L. S. H. Yi[1] and G. Erdös[1]. University of Illinois, Departments of Physiology and Biophysics[1] and of Plant Biology[2], Urbana, Illinois 61801.

The regulation of expression of eight chloroplast (ct) genes, i.e., atpB, atpE, atpH, psaA, psbA, psbB, psbC and rbcL, of Euglena gracilis was examined in cells grown continuously in the dark or first grown in the dark and then in the light for 72 h, during which ct biogenesis occurs. Dark-grown cells contain low levels of gene transcripts. rbcL, psaA, psbC, atpB and atpE transcript levels transiently increased per cell during ct biogenesis to a small extent (1.7 to 5.6X depending upon the transcript). Each transcript peaked in amount at 12 to 36 h and then decreased by 72 h to about the dark level. In contrast, psbB transcripts increased throughout ct biogenesis whereas psbA and atpH transcripts remained constant after 12 h. Most transcript levels correlate with their gene levels only during the first one-third to one-half of the ct-biogenesis period. Gene protein products were detected in dark-grown cells, and the steady-state level of each increased to different extents in cells grown continuously in the light, i.e., 6X (atpH) to 1000X (rbcL). During ct biogenesis, most of the proteins began to accumulate over the dark level by 3 to 12 h of light before any significant level of chlorophyll was present and continued to accumulate as the respective mRNA levels declined or became stable. Pulse-chase studies (^{3}H-phe) showed that cellular levels of plastid proteins are regulated at the posttranslational level of protein turnover with the proteins being stabilized as they accumulate during ct biogenesis. An ATP-dependent protease like the evolutionarily-conserved Clp protease of E. coli appears to be inactivated during ct biogenesis. Supported by NSF and McKnight Foundation.

1921 ISOLATION AND PURIFICATION OF PEA CHLOROPLAST SOLUBLE RNA POLYMERASE. Aigun S. Boyer and Richard B. Hallick. University of Arizona, Department of Biochemistry, Tucson AZ 85721

The soluble DNA-dependent RNA polymerase has been purified from *Pisum sativum* chloroplasts. This procedure involves a 100,000 x g centrifugation, ammonium sulfate precipitation, DEAE-CL6B and Affi-gel blue chromatography, followed by Sephacryl-300 gel filtration column. The most pure sample then is loaded to a 6% non-denaturing gel. Other than RUBISCO and RUBISCO activase, a band of about 620KD MW is detected. This band is cut out and run on SDS-PAGE to obtain the subunit composition. The enzyme activity is completely dependent on exogenous DNA for template. The native molecular weight of the enzyme was found to be around 620KD. There are six major polypeptide bands on the SDS-PAGE. The molecular weights are approximately 156, 138, 77, 60, 40 and 27KD. The gene for pea chloroplast rpoA which encode a subunit has been sequenced(1). The rpoA gene encodes a polypeptide of 39KD which is very similar to the 40KD subunit in this enzyme. (This work was supported by the U.S. Public Health Service, National Institues of Health.)

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POSTER ABSTRACTS

1922 GROUP III TWINTRON IN A CHLOROPLAST GENE IS EXCISED BY ALTERNATIVE SPLICING. Donald W.Copertino[1] and Richard B. Hallick[1,2]. University of Arizona, Departments of Molecular and Cellular Biology[1], and Biochemistry[2], Tucson, AZ 85721

The chloroplast genes of *Euglena gracilis* contain more than 100 group II and group III introns. Several chloroplast genes also contain introns within introns, or twintrons.[1] The structure of the gene encoding ribosomal protein L16, rpl16, is 66 nucleotides (nt) exon 1, 97 nt group III intron, 28 nt exon 2, 356 nt group II intron, 50 nt exon 3, 208 nt group III twintron, and 255 nt exon 4.[2] We have analyzed the excision of the group III twintron by polymerase chain reaction (PCR) amplification, cloning, and sequencing of cDNAs, and northern hybridization. We have determined that at least four alternative 5'- and five alternative 3'-splice sites are used during the excision of the twintron, including two cryptic 5'-splice sites in the 5'-exon. Three cDNAs representing splicing intermediates were generated by excision of internal group III introns from external group III introns (twintrons). Three other cDNAs representing "pseudo fully spliced" transcripts resulted from the use of the two 5'-splice sites in the 5'-exon. However, these transcripts do not accumulate and contain stop codons. Twintron formation by intron insertion may be a mechanism by which alternatively spliced genes are generated. (Supported by the U. S. Public Health Service, National Institutes of Health.)

[1] Copertino and Hallick, (1991) *EMBO J.*, 10:433-442.
[2]Christopher and Hallick, (1989) *Nucleic Acids Res.*, 17:7591-7608.

1923 EXPRESSION AND RNA PROCESSING OF THE *EUGLENA GRACILIS* CHLOROPLAST *rps2-atpI-atpH-atpF-atpA* OPERON. Robert G. Drager[1] and Richard B. Hallick[1,2]. University of Arizona, Depts. [1]Molecular & Cellular Biology & [2]Biochemistry, Tucson AZ 85721

Genes encoding the *Euglena gracilis* chloroplast ribosomal protein S2 and chloroplast ATPase subunits CF_0IV, CF_0III, CF_0I, and CF_1a, (*rps2*, *atpI*, *atpH*, *atpF* and *atpA*, respectively) have been cloned and sequenced. This operon is conserved in all known plant chloroplast genomes. The *Euglena* genes are interrupted by seven group II and eight group III introns. Based on comparison of genomic and cDNA sequence data. the group II introns range in size from 323 to 629 nt. These introns have typical group II intron 5' and 3' boundary sequences and secondary structure. Although group III introns are rare in photosynthesis related genes, there are five group III introns in *atpI*. The initiator codon of *atpF* is GUG rather than AUG. The second intron in *atpF* is in the same location as the only intron interrupting this operon in higher plant chloroplasts. The five *Euglena* genes are co-transcribed. Fully spliced polycistronic mRNAs are detected by northern hybridization. In addition, the polycistronic transcript is processed to monocistronic transcripts by intercistronic cleavage. There is no significant difference in transcript accumulation or processing in RNA from light grown and dark grown cells. Analysis of transcript accumulation and processing of RNA from cells grown anaerobically and mapping of transcript initiation, termination and intercistronic cleavage sites will be presented. (Supported by the U. S. Public Health Service, National Institute of Health.)

1924 PURIFICATION OF PEA CHLOROPLAST RNASE P - LIKE ACTIVITY. Catherine S. Hibbert[1]and Richard B. Hallick[1,2]. University of Arizona, Departments of Molecular and Cellular Biology[1] and Biochemistry[2], Tucson, AZ 85721

RNase P is an endoribonuclease that cleaves the terminal sequence from the 5'- ends of precursor tRNAs. It has been extensively studied in *E.coli* and *B.subtilus* and has also been found in the nucleus and organelles of eukaryotes. *In vitro* synthesized monomeric, dimeric and trimeric tRNA substrates have been used to investigate chloroplast tRNA maturation pathways and RNase P-like activity (1). RNase P-like activity has been purified >16,000 fold from isolated *Pisum sativum* by chromatography on DEAE Cellulose, heparin agarose and FPLC Mono S columns. The Mono S peak fraction contains two polypeptide bands of 36 Kd and 26 Kd apparent molecular weight as shown by 5%-15% gradient SDS PAGE analysis after either Coommassie Blue or Silver staining. The 37 Kd band was separated from the active fraction by FPLC Superose 6 gel filtration of the pooled Mono S active fractions. Under nondenaturing conditions the RNase P-like activity eluted off the Superose 6 column just prior to the 17,000 MW globular protein standard consistent with a native molecular weight of 22,000. Further characterization of this enzyme is in progress. This work has been supported by NIH grant GM 35625 to R.B.H.

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1925 ORF516: AN UNUSUAL 2.8KB GENE IN A CONSERVED CHLOROPLAST RIBOSOMAL PROTEIN OPERON OF *EUGLENA GRACILIS*. Kristin P. Jenkins[1], David A. Christopher* and Richard B. Hallick[1,2]. University of Arizona, Depts. [1]Molecular and Cellular Biology and [2]Biochemistry, Tucson AZ 85721; *Department of Biochemistry and Biophysics, Texas A&M University, College Station TX 77843

An operon coding for 11 ribosomal protein genes, a tRNA and a 2.8 kb region containing a large open reading frame was characterized. The 2.8 kb region is not found in the equivalent chloroplast ribosomal protein operons in tobacco, rice or liverwort; nor is it found in *E.coli* ribosomal protein operons. We have shown by northern analysis that orf516 is cotranscribed with the surrounding ribosomal genes in an 8.3 kb primary transcript and accumulates as a 1.4 kb mRNA. We have done primer extension studies of the 5' ends of orf516 and the downstream gene rpl16 to determine the orf516 transcript begins 8 nt from the upstream rps3 stop codon and terminates 7 nt after its own stop codon. No in frame AUG has been found, hence translation is believed to initiate at a TTG (Leu) codon (position 118). The orf516 contains 5 exons interrupted by one group III and 3 group II introns. Exon two is 3 nt, the smallest found in chloroplasts to date. Orf516 has a predicted MW of approximately 65, and a charge of +57. The protein is high in phenylalanine, leucine and lysine, and no homologous proteins have been found by computer analysis. Currently, expression studies of the putative protein are in progress to determine the properties or activity of this unusual orf. (Supported by the U. S. Public Health Service, National Institute of Health.)

1926 INTERCISTRONIC PROCESSING OF A CHLOROPLAST PRE-mRNA CODING FOR PROTEIN COMPONENTS OF PHTOSYSTEM I AND II REACTION CENTERS. Jennifer K. Stevenson*, Donald W. Copertino* and Richard B. Hallick*@. University of Arizona, Depts of Molecular and Cellular Biology*, and Biochemistry@ Tucson AZ 85721

The chloroplast genome of *Euglena gracilis* contains a transcription unit which codes for protein components of the photosytem I (PSI) and II (PSII) reaction centers. The pre-mRNA, containing the genes *psaA* and *psaB* (PSI), and *psbE, psbF, psbL,* and *orf42* (PSII),[2,3] undergoes two post-transcriptional intercistronic processing events. We have determined the precise intercistronic processing sites by northern hybridization, primer extension RNA sequencing and S1 nuclease analysis. One site maps between the *psaA* and *psaB* genes and the other between the *psaB* and *psbE* genes. Potential secondary structures are observed in these regions. The latter site effectively cleaves the tetracistronic PSII genes from the PSI genes. This cleavage appears to occur more frequently than the cleavage between *psaA* and *psaB*. The observed differential processing events may be related to the differential expression of the protein products during chloroplast development. (Supported by the U. S. Public Health Service, National Institute of Health.)

[1]Cushman JC, Hallick RB and Price CA (1988) *Curr Genet* 13:159-171; [2]Cushman JC, Christopher DA, Little MC, Hallick RB and Price CA (1988) *Curr Genet* 13:173-180; [3]Copertino DC and Hallick RB (1991) *EMBO J* 10:433-442

1927 LIGHT-INDUCED SWITCH IN PROMOTER UTILIZATION FOR A CHLOROPLAST *PSBD-PSBC* OPERON. David A. Christopher, Tineke Berends-Sexton and John E. Mullet. Texas A & M University, Department of Biochemistry & Biophysics, College Station, TX 77843-2128

Both the synthesis of reaction center protein D2 and the levels of a specific subset of mRNAs which encode this protein are maintained in mature barley chloroplasts. To understand the differential maintenance of *psbD* mRNA abundance, we have studied the transcription of, and the RNAs produced from, the *psbD-psbC* operon in light and dark-grown barley plastids. The operon consists of six loci, organized as *psbK-psbI-psbD-psbC-orf62-trnG*. At least 12 different *psbD-psbC* RNAs are produced from the operon in barley plastids, two of which accumulate in response to light. Each of the 12 RNAs has been mapped to their respective DNA sequences. We have shown that much of the RNA diversity is due to the action of at least three promoters which drive three overlapping transcription units in the operon. One of these promoters is relatively inactive in dark-grown plants. Upon illumination, this promoter is differentially activated and produces two RNAs which accumulate to become the predominant *psbD-psbC* RNA species in mature barley chloroplasts. Run-on transcription and RNA quantitation experiments provided evidence that both light-induced transcription and enhanced RNA stability play roles in the light response mechanism. The accumulation of the light-induced RNAs may be necessary to maintain D2 synthesis after photoinduced D2 breakdown, while the promoter switch itself appears to serve as a means to uncouple the expression of *psbK-psbI* from *psbD-psbC* during light-induced plastid development. The properties of the promoter and the factors involved in the light response mechanism are being studied.

POSTER ABSTRACTS

1928 cDNA SEQUENCE, mRNA ANALYSIS AND GENES CODING FOR TOBACCO CHLOROPLAST RIBOSOMAL PROTEINS L12 AND L24. Gasmalla Elhag, Betty Glinsmann-Gibson and Don P. Bourque, Department of Biochemistry, University of Arizona, Tucson AZ (USA) 85721

Chloroplast ribosomal proteins of *N. tabacum* are coded by both chloroplast and nuclear genes. Full length cDNAs have been characterized which code for proteins homologous to *E. coli* ribosomal proteins L12 and L24. Tobacco chloroplast L12 cDNA codes for a mature L12 protein of 133 amino acids preceded by an amino terminal transit peptide of 53 amino acids. The predicted N-terminal 12 amino acids of the mature protein were confirmed by protein sequencing. Protein L24 is 197 amino acids long, with a putative transit peptide of about 40 residues and a carboxyl-terminal extension, relative to homologous procaryotic ribosomal proteins. As judged by Northern blot analysis, the size of cytoplasmic mRNAs for these proteins corresponds to that of their full-length cDNAs. Analysis of multiple cDNAs and genomic Southern blots show that each of these proteins is coded by at least two expressed genes.

1929 AN ULTRASTRUCTURAL AND BIOCHEMICAL ANALYSIS OF THREE SOYBEAN VARIEGATED LEAF MUTANTS. Tai-Sheng Cheng and Joel M. Chandlee. Department of Plant Sciences, University of Rhode Island, Kingston, RI 02881

The y18 gene of soybean (Glycine max) contributes towards chloroplast development. A variant allele (y18-m) has been identified which normally gives rise to green leaf tissue, however, frequent somatic mutations produce yellow sectors on the leaves resulting in a variegated leaf pattern. The mutations can also occur germinally such that homozygous recessive yellow plants can be derived from a mutable plant. LDS-PAGE gel analysis of thylakoid membrane proteins from chloroplasts isolated from green or yellow tissue revealed the absence of four polypeptides (51, 35, 25, and 22 kD) in the mutant chloroplasts. Northern blots have been examined to determine the affect of the mutation on mRNA populations. An experiment involving in vitro translation coupled with an in vitro reconstitution system is being carried out in attempt to identify the primary lesion in the y18 mutant. A second variegated leaf mutant (E25-10), phenotypically similar to T225M, has been produced by exposure of seed to gamma radiation. EM studies of chloroplasts in yellow leaves revealed fewer grana stacks and a distortion of the membrane structure. Several polypeptides of the thylakoid membranes are missing, and many are reduced. A spontaneously occurring variegated leaf mutant of soybean (Ogemaw) has been recovered. This mutant shows a different variegated leaf pattern to those produced by either T225M or E25-10 in that it exhibits a green/white sectoring pattern. A genetic analysis is underway.

1930 PRESENCE OF ASPARTOKINASE GENE IN SPINACH CHLOROPLAST DNA.

S.Kochhar and V.K. Kochhar
National Botanical Research Institute, Lucknow, India

Aspartokinase (ATP: L aspartate 4-phospho transferase, EC 2.7.2.4) catalyzes the first step in the biosynthetic pathway that leads to the formation of lysine, threonine and methionine. Due to the complexity of this pathway plants have evolved diverse patterns of regulation of this enzyme. We have earlier isolated and purified the lysine and threonine sensitive isoenzymes of aspartate kinase and have studied their regulation. In order to understand the differential regulation of this enzyme we have probed the genomic and chloroplastic DNA from spinach leaves for aspartate kinase gene using the plasmid pNC 8641 containing the operon of the enzyme (kindly supplied by Prof. Henery Paulus). Besides nuclear DNA the chloroplastic DNA gave positive signals indicating the presence of the AK gene in the chloroplasts. This is the first report of AK gene from plant DNA.

1931 CLONING AND CHARACTERIZATION OF *SYNECHOCYSTIS* 6803, *ARABIDOPSIS*, AND PEA PROTEIN SYNTHESIS FACTOR EF-G GENES. Piri L. Welcsh, Mahinur S. Akkaya, and Caroline A. Breitenberger. Ohio State University, Departments of Molecular Genetics and Biochemistry, Columbus, Ohio 43210

Chloroplast protein synthesis factor EF-G (chlEF-G) activity is significantly higher in pea seedlings grown in red light compared to the dark. Since chlEF-G is encoded by a nuclear gene, light regulation of its activity may play an important role in coordinating nuclear and chloroplast gene expression in plants. EF-G specific oligonucleotides were generated which correspond to conserved regions in the amino acid sequences of *E. coli* and *M. luteus* EF-G and *M. vannielii* and hamster EF-2 and to the N-terminal amino acid sequence of pea chlEF-G. These oligonucleotides were used in various PCR reactions to amplify specific regions of the *Synechocystis* 6803, *Arabidopsis* and pea EF-G genes. PCR generated fragments have been cloned and sequenced. Both the *Arabidopsis* and pea chlEF-G fragments show a high degree of amino acid identity to each other as well as to *E. coli* and cyanobacterial EF-G's, although the sequences differ at the nucleotide level. A genomic clone encoding the *Synechocystis* 6803 EF-G has been isolated and sequenced. The PCR generated chlEF-G fragments are being used to screen an *Arabidopsis* genomic library, and to analyze chlEF-G gene transcription.

1932 TRANSCRIPTIONAL REGULATION OF CHLOROPLAST GENES RBCS, RBCL, AND PSBA IN THE CHROMOPHYTE O. LUTEUS. Ann E. Reynolds, Betty L. McConaughy, and Rose Ann Cattolico. University of Washington, Botany Department, Seattle, Wa. 98195

Autotrophic plants in oceanic systems process about 50% of the total carbon fixed globally. This extensive contribution to primary production places the algae in an indispensible position with respect to a balanced carbon budget. To study gene regulation in the chloroplasts of marine plants, a chloroplast run-on system was developed for the model chromophyte (chl a,c containing), *Olisthodiscus luteus*. Incorporation of ^{32}P into plastid RNA has been shown to be heparin independent, actinomycin sensitive, and DNA dependent. These data indicate that we are measuring elongation of pre-existing transcription complexes. We have assayed transcription initiation of Calvin cycle (Rubisco large and small subunit) and photosystem II (D1 protein) genes The *rbcL* and *rbcS* genes, which are both chloroplast encoded in this organism, are cotranscribed as a 2.4 kb mRNA; the *psbA* transcript is 1.3 kb. Analysis of transcript initiation during a diel 12hr light:12hr dark cycle has shown a 50 and 33 fold decrease for *rbcL,S* and *psbA* respectively when cells are harvested in the dark (D6) vs the light (L6). A very similar decrease was observed for the steady-state levels of mRNA for these genes. From these observations we conclude that initiation of mRNA transcription is the critical step for regulating *rbcL,S* and *psbA* genes during a diel cycle. This finding contrasts sharply with terrestrial plants in which chloroplast gene regulation is predominantly posttranscriptional. A profile of both steady-state RNA levels and transcript initiation throughout the diel cycle will be presented and the source of the signal(s) for the RNA fluctuation discussed.

1933 SEQUENCES AND FACTORS FOR THE EXPRESSION OF CHLOROPLAST GENES. Kai Tiller, Jorg Nickelsen and Gerhard Link. University of Bochum, Plant Cell Physiology and Molecular Biology, D-4630 Bochum 1, F.R.G.

Chloroplast gene expression appears to be regulated at various levels in a species-specific and developmental stage-specific way. In mustard (*Sinapis alba*), the *in vivo* transcripts of a number of chloroplast genes vary in their amounts throughout seedling development and in light-grown versus dark-grown plants. As part of our efforts to define the underlying control mechanisms at the level of transcription and RNA maturation, we have investigated proteins that interact with DNA and/or RNA sequence elements. We have purified three transcription factors that resemble bacterial sigma factors in conferring promoter binding and transcription specificity *in vitro*. They all show prefence for the *psbA* promoter as compared to the *rps16* and *trnQ* promoters, but each one at different assay conditions. Experiments using stage-specific extracts suggest a role of these proteins in chloroplast versus etioplast differentiation. Although they are all present in both plastid types, at least one of them shows plastid type-specific differences in its sequence requirements. In another kind of approach, we have isolated proteins that specifically interact with 3'-sequence elements of chloroplast RNA precursors. The *in vitro* binding site for one of these factors was shown to correlate with an RNA 3'-terminus *in vivo*, and the purified protein has associated endonucleolytic activity, suggesting an essential role in RNA 3'-end formation. For the *rps16* gene, the binding site reflects the only detectable *in vivo* end, whereas in the case of the *trnK* gene the situation is more complicated by the presence of more than one 3'-end. Here, the *in vitro* binding site is located downstream of the major 3'-end found *in vivo*, and a fraction of the *in vivo* transcripts was shown to extend even beyond the binding site, indicating cotranscription with downstream *psbA* sequences. Our current focus is on gene isolation and full functional characterization of these and other factors involved in chloroplast gene expression and its regulation.

POSTER ABSTRACTS

1934 PREFERENTIAL TRANSLATION OF CHLOROPLAST-ENCODED r-PROTEINS IN *CHLAMYDOMONAS*. C. R. Hauser[1], J. E. Boynton[1] and N. W. Gillham[2]. Duke Univ., Depts. of [1]Botany and [2]Zoology, Durham, NC 27706

Using the nuclear *cr-1* mutant deficient in the small subunit of the chloroplast ribosome, and the chloroplast *spr-u-1-27-3* mutant grown in the presence of spectinomycin (Liu et al., Plant Mol. Biol. 12:385-394,1989) we have shown previously that chloroplast encoded ribosomal (r-) proteins are synthesized preferentially to the photosynthetic proteins under conditions of reduced chloroplast protein synthesis. To identify the cis-acting sequences involved, 5' and 3' untranslated regions (UTRs) have been exchanged between the *rps12* gene encoding the S12 chloroplast r-protein and the *atpB* gene specifying the beta subunit of the CF_1/CF_0 ATP synthase. Additionally, chimeric constructs have been made in which the coding sequences for the respective genes have been exchanged leaving 5' and 3' UTR sequences intact. Plasmids with these chimeric constructs were introduced into an *spr-u-1-27-3* recipient strain by cotransformation with a second plasmid carrying the *psbA* gene containing a mutation to DCMU resistance. Transformants were selected on DCMU media and homoplasmic subclones containing the chimeric constructs isolated. They are being examined for accumulation of *atpB* and *rps12* chimeric mRNAs by Northern analysis and the respective levels of β and S12 proteins by pulse labelling and immunoprecipitation under conditions of reduced chloroplast protein synthesis. The 5' and 3' UTRs in the mRNAs for both *rps12* and *atpB* are predicted to contain a high degree of secondary structure. In the case of the *rps12* 5' UTR, the ability to form a stem-loop structure is evolutionarily conserved among chloroplast genomes in the absence of sequence homology. In contrast, the *atpB* 5'UTRs which are predicted to form stable stem-loop structures also contain a high degree of sequence homology between phylogenetically related organisms. Supported by an NIH-Postdoctoral Fellowship to C.R. Hauser and NIH Grant GM-19427.

1935 EXPRESSION OF CHLAMYDOMONAS REINHARDTII CHLOROPLAST psbB GENE DEPENDS ON NUCLEAR GENE PRODUCT. Caroline Monod, Michel Goldschmidt-Clermont, Jean-David Rochaix. University of Geneva, Department of Molecular Biology, Geneva, Switzerland.

We show that the expression of the chloroplast gene psbB, which encodes a chlorophyll a-binding polypeptide (P5) in Photosystem II, requires a nuclear gene product acting at either the level of transcription or mRNA accumulation. Mutant 222E was isolated on acetate-containing media after mutagenesis and suicide-selection, and was characterized as a Photosystem II mutant by its high fluorescence phenotype. Genetic crosses showed the mutation to be nuclear in origin. Pulse-labeling of total proteins with ^{14}C-acetate demonstrated the absence of the psbB protein product. Primer extension analysis showed that psbB transcription initiation occurs 35 bases upstream of the initiator codon. S1 analysis demonstrated that the 3' nontranscribed region consists of approximately 600 bases with two inverted repeats. Northern blot analysis not only failed to detect any psbB mRNA in the mutant, but also revealed downstream of psbB the absence of some other transcripts and the appearance of a novel one. Whereas in mutant cells permeabilized with toluene and pulse-labeled with ^{32}P-UTP psbB transcripts are made at wild-type levels, in mutant cells in vivo pulse-labeled with ^{32}P-phosphate (and not treated with toluene), psbB transcripts either are less efficiently transcribed or are more rapidly degraded as compared to wild-type.

1936 CHEMICAL MIMICRY OF DOMINANT/RECESSIVE ALLELES OF CAROTENE GENE: INDUCTION OF CIS/TRANS CAROTENOGENESIS BY SUBSTITUTED AMINES. S Madhavan[1] and C.R. Benedict[2]. [1] Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68583; [2]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Tx 77843

In normal tomato fruits the dominant gene TT, produces the *trans* carotenes, viz. 15-*cis*-phytoene, *trans*-phytofluene, *trans*-ζ-carotene, *trans*-neurosporene, *trans*-lycopene and ß-carotene. The homozygous recessive tt allele in the tangerine mutant of tomato produces the *cis*-carotenes viz. 15-*cis*-phytoene, di-*cis*-phytofluene, di-*cis*-ζ-carotene, tri-*cis*-neurosporene and tetra-*cis*-prolycopene Cotton cotyledonary pairs upon treatment with a substituted tertiary amine, MPTA [2-(4-methylphenoxy) triethylamine] in the dark for 69 h accumulate 15-*cis*-phytoene, *trans*-phytofluene, *trans*-ζ-carotene, and *trans*-lycopene. However, when treated with a substituted dibenzylamine (4-CH_3-$C_6H_4NHCH_2C_6H_5$) these dark grown cotton cotyledons synthesize only *cis*-carotenes viz. 15-cis-phytoene, di-cis-phytofluene, di-cis-ζ-carotene, tri-cis-proneurosporene, tetra-cis-prolycopene and poly cis-γ-carotene. The identity and distribution of these poly-*cis*-carotenes are similar to the individual poly-*cis*-carotenes synthesized in the homozygous (tt) tangerine mutant of tomato. Results from the simultaneous application of the antibiotics Act-D, α-amanitin, cordycepin, anisomycin or cycloheximide with the amines to these cotyledons support the conclusion that both these amines induce carotenogenesis in this tissue. Control cotyledons accumulate only negligible amounts of carotenes in the dark. Thus the induction of carotenogenesis by these amines involves gene expression, transcription and translation of the poly A$^+$ RNA on 80s ribosomes It is probable that MPTA mimics the expression of the dominant gene TT, producing *trans*-carotenes while the substituted dibenzylamine mimics the expression of the homozygous recessive tt allele by producing the *cis*-carotenes in cotton cotyledons

1937 TRANSCRIPTION OF COTTON CHLOROPLAST psbA GENE: EVIDENCE FOR 5'END PROCESSING. Tim Ulmasov, Eleonore Piruzian Institute of Molecular Genetics, Moscow 123182, USSR

Both mRNA levels and its translational efficiency of the chloroplast psbA gene encoding D1 component of photosystem II are regulated by light in higher plants though the mechanism of this regulation is not fully understood yet. We mapped 5' and 3' ends of psbA transcripts from cotton using S1-mapping and primer extension techniques and found their 5' ends to be heterogeneous starting at positions +1, +21-22 and +73-74 with refer to upstream canonical psbA promotor, while the 3' ends were localised after 3' stem-loop structure as usual. This pattern of 5' ends heterogeneity of psbA transcripts was not reported yet and has an unusual feature that trancripts starting at +73-74 lack S-D sequence and should not be translated efficiently. mRNA capping and T4 PNK phosphorylating experiments revealed that +21-22 and +73-74 transcripts occur by posttranscriptional processing and have 5'-OH groups at their 5' ends. Processing profile in young seedlings shifts from 50% of +73-74 transcripts relative to total psbA mRNA pool in normally grown plants to 80-90% in plants etiolated for 24 h, while no change was observed after 8 h of etiolation.

1938 STRUCTURE AND TISSUE SPECIFIC EXPRESSION OF MAIZE PLASTID GENOME ORF170. Jaakko Kangasjärvi[1] & Burle G. Gengenbach. Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN55108. [1]Permanent address:Botanical Garden, University of Kuopio, SF-70211 Kuopio, Finland.

Our previous studies of plastid genome transcription in maize leaves and developing endosperm revealed that a 2.4 kb transcript from plastid genome BamHI restriction fragment 14 accumulated preferentially in amyloplasts of developing endosperm. Sequence analysis of the fragment, which is located between *psaA* and *rps 4*, revealed two introns containing open reading frame (ORF170) encoding a 170 amino acid hydrophilic protein. 5' and 3' ends of both introns have splicing sequences conserved in plastids and sequence analysis of the PCR-amplified cDNA verified the splice junctions. Both exons I and II start with ATG and have an in-frame stop codon just within the following introns thus each is a separate shorter open reading frame. PCR analysis of leaf and endosperm RNA showed that the RNA populations contained transcripts continuous from ORF170 to *psaA* suggesting that ORF170 is expressed in maize chloroplasts and amyloplasts as a part of a *psaA-psaB-rps14* operon. Northern analysis with BamHI 14 supports this conclusion. Primer extension analysis of leaf and endosperm total and polysomal RNA using both ORF170 and *psaA*-specific primers revealed several transcript 5' ends, some of which were found only in chloroplasts. Capping of the 5' ends of the transcripts combined with RNase protection to identify the primary transcripts and processing sites is underway. The developmental processing of the transcript was different in amyloplasts and chloroplasts. During endosperm development the amount of unspliced transcript increased relative to the spliced transcript; in contrast, the fully spliced transcript remained most abundant in successively older leaves. The pattern of intron splicing differed in leaves and endosperm. In endosperm amyloplasts only one set of intermediately spliced (one intron removed) transcripts were amplified with PCR, while in leaves additional shorter transcripts were detected. Total polysomal RNA from various tissues contained transcripts from the BamHI 14 region. Only fully spliced transcripts were incorporated into polysomes in seedlings leaves and roots. In amyloplasts transcripts retaining both introns were also found in polysomal RNA We are currently making antibodies against the ORF170 protein expressed from cloned cDNA in E. coli to determine whether a corresponding protein is actually synthesized in plastids.

1939 CHARACTERIZATION OF mRNA-BINDING PROTEINS AND mRNPS IN MATURE AND DEVELOPING CHLOROPLASTS OF SPINACH. Martha Hamblin[1,2], Cynthia C. Adams[2], Hsu-Ching Chen[2], and David B. Stern[2]. [1]Cornell University and [2]Boyce Thompson Institute, Ithaca, NY 14853

The discovery that many chloroplast mRNAs are developmentally regulated at the level of transcript stability has led us to search for mRNA-binding proteins that mediate this regulation. In *petD* mRNA, which encodes subunit IV of the cytochrome b_6/f complex, the 3'-untranslated region (UTR) contains a hairpin structure shown to be important for transcript stability *in vitro*. Several proteins that interact with this 3'-UTR have been identified by *in vitro* UV-crosslinking studies. Binding sites that are sequence-specific and structure-specific have been identified for some of these proteins. We are developing purification strategies for isolating these proteins from chloroplasts. We have found that 57 kd and 100 kd *petD*-3'UTR-binding proteins can be enriched or purified by affinity chromatography on poly(U) and poly(C) agarose, respectively. Progress in the purification and functional analysis of these proteins will be reported. Evidence that mRNP complexes form in chloroplasts *in vivo* has been obtained by sedimentation studies. This analysis has included RNPs containing *petD* and *rbcL* (which encodes the large subunit of RUBISCO) mRNAs. We are currently looking in differentianng chloroplasts for changes in the composition of mRNP complexes that can be correlated with changes in the steady state levels of their respective mRNAs.

POSTER ABSTRACTS

1940 FUNCTIONAL ANALYSIS OF PROTEINS THAT BIND TO THE 3' INVERTED REPEAT REGION OF SPINACH CHLOROPLAST *petD* mRNA. Ouyun Chen, Hsu-Ching Chen, Cynthia Adams and David Stern. Boyce Thompson Institute, Tower Road, Ithaca, NY 14853

Two spinach chloroplast proteins have been identified that bind specifically to the 3' inverted repeat sequence (IR) region of *petD* mRNA, a transcript encoding subunit IV of the cytochrome b_6/f complex. A 33 kd protein binds to the stem of the IR hairpin structure, and a 57 kd protein binds to an AU-rich sequence present in two copies, one upstream and the other downstream of the hairpin structure. Partially purified 57 kd protein preparations contained an endonuclease activity that cleaved *petD* RNA about 25 nt 5' to the IR, a site close to the 57 kd protein binding site. To study the function of the 33 kd protein, a series of plasmids were tested in a chloroplast *in vitro* transcription system. These plasmids contained a $tRNA^{Ser}$ gene linked to a promoterless $tRNA^{Met}$ gene, and the 3' IR sequence of *petD* was inserted between the two genes. *In vitro* transcription resulted in the accumulation of a 93 bp and a 73 bp RNA, corresponding to the mature $tRNA^{Ser}$ and $tRNA^{Met}$, when a wild-type 3' IR was used. However, when a mutant 3' IR sequence was used, to which the 33 kd protein fails to bind, an additional 200 bp RNA accumulated to high levels, consistent with increased levels of transcription termination at the IR. Results concerning the role of 33 kd protein in transcription termination and mRNA metabolism in spinach chloroplasts will be presented.

1941 POST-TRANSCRIPTIONAL REGULATION OF CHLOROPLAST ELECTRON TRANSPORT GENE IN *CHLAMYDOMONAS REINHARDTII* STUDIED BY TRANSFORMATION-MEDIATED MUTAGENESIS. Wataru Sakamoto[1], Xuemei Chen[1], Karen L. Kindle[2] and David B. Stern[1]. [1]Boyce Thompson Institute and [2]Plant Science Center, Cornell University, Ithaca, NY 14853

In *Chlamydomonas*, photosynthetic mutants are able to grow on acetate, a reduced carbon source. Conversely, wild-type cells but not mutant cells are sensitive to a compound called metronidazole. We are taking advantage of these properties, to introduce specific mutations into photosynthetic electron transport genes of *C. reinhardtii* chloroplasts. Our initial experiments have focused on *petD*, which encodes subunit IV of the cytochrome b_6/f complex. DNA sequencing and transcript analysis revealed that *petD* mRNA contains a long 5' untranslated region (UTR) of 360 nucleotides, and a potential stem-loop structure in the 3' UTR. To determine how *petD* is regulated, we made a series of deletions in the 5' and 3' UTRs, and we are currently introducing these altered genes into chloroplasts. Several point mutations, including changes in the initiation codon, premature stop codons and the introduction of rare codons were also made. Transformants will be analyzed for transcription of *petD* gene, and the stability and translatability of the *petD* message.

1942 TRANSCRIPTION TERMINATION *IN VITRO* BY SPINACH CHLOROPLAST RNA POLYMERASE. L.-J. Chen[1], S.-T. Jeng[2], R. I. Gumport[3], J. F. Gardner[3] and E. M. Orozco, Jr.[4] [1]Agricultural Biolabs, National Chung-Hsing University, Taichung, Taiwan 40227, R.O.C.; [2]Dept. of Biochemistry and [3]Dept. of Microbiology, University of Illinois, Urbana, IL 61801, U.S.A.; [4]DEKALB Plant Genetics, Eastern Point Road, Groton CT 06340, U.S.A.

Spinach chloroplast RNA polymerase terminates transcription *in vitro* at the threonine attenuator terminator (*thrA*) from *E. coli*. We desired to quantitate the efficiency of termination at *thrA* by the chloroplast enzyme. The standard method of determining termination efficiency, by comparing the terminated to "run-off" transcripts, was not possible; the chloroplast RNA polymerase did not correctly initiate transcription on linear templates. An approach using S1 nuclease protection analysis of total and "read-through" transcripts was attempted, but spurious results were obtained. The successful approach involved cloning multiple copies of *thrA* downstream from a chloroplast promoter. These constructs were transcribed *in vitro* and the termination efficiency determined by quantitating the relative amounts of transcripts that ended at each successive terminator. Each *thrA* sequence terminated 63% of RNAs being made by the spinach chloroplast RNA polymerase. Twenty-one terminator variants were analyzed; for the chloroplast enzyme not only hairpin structure, but also sequence, influences termination.

1943 PHOTOAFFINITY LABELING OF CLONED RUBISCO SMALL SUBUNIT AND ITS PRECURSOR : SITE-DIRECTED MUTAGENESIS OF NUCLEOTIDE-BINDING SITES. Robert R. Klein and Michael E. Salvucci. USDA-ARS, University of Kentucky, Lexington, Ky 40546 USA.

Tobacco cDNA sequences encoding the RUBISCO small subunit (SSU) and small subunit precursor (pSSU) were cloned into peT plasmids and expressed in E. coli. Target genes in peT plasmids are under control of bacteriophage T7 promoter and T7 translation signals. Following induction with isopropyl-B-thiogalactoside (IPTG), the SSU or pSSU comprised nearly 50% of the total cell protein. In vitro protein import assays showed that the pSSU was transported into the chloroplast and was processed and assembled into the holoenzyme. The cloned SSU and pSSu were photoaffinity labeled with [^{32}P]8-azidoadenosine 5'-triphosphate (8-N_3ATP). An apparent K_D value of 13 uM N_3ATP was determined for both the SSU and pSSu, which was similar to the value determined for SSU in a holoenzyme complex isolated from tobacco leaves. The response of photoaffinity labeling of cloned SSU and pSSU to Mg^{2+} and pH was also similar to the response of SSU in the holoenzyme complex, indicating that the putative nucleotide binding site on the RUBISCO SSU is similar in the isolated and assembled state. Site-specific mutagenesis of specific residues in the nucleotide-binding domain was conducted to alter nucleotide binding. The effects of specific base substitutions on photoaffinity labeling of the SSU will be discussed in terms of the function of the SSU nucleotide binding site

1944 SEQUENCE ANALYSIS AND REGULATION OF THIOREDOXIN IN TWO RHODOPHYTES. A.E. Reynolds, J. Chesnick, J. Woolford, and R.A. Cattolico. University of Washington, Botany Department, Seattle, Washington 98195

Thioredoxins play a critical role in the regulation of enzyme activity both in oxygenic and anoxygenic organisms. This small polypeptide of approximately 12,000 molecular weight is viewed as a general protein disulfide reductase. In photosynthetic cells, photosystem I modulation occurs when electrons originating from chlorophyll are transferred to ferredoxin and then to thioredoxin by the enzyme ferredoxin thioredoxin reductase. In turn, activation of enzymes needed to catalyze metabolic and biosynthetic pathways (e.g. Calvin cycle enzymes, CF_1 ATPase function) is mediated by the reduced thioredoxin produced in the light. In terrestrial plants (Chlorophyta), all thioredoxin (trx) genes are nuclear encoded. We have identified the first chloroplast encoded trx genes in two red algae (Rhodophyta), *Porphyra yezoensis* and *Griffithsia pacifica*. Both genes are 324 bp in size and are 64% similar to each other. The genes show greatest similarity (68%) to that of *Anabaena 7119* but share only 38% and 50% similarity with *Anabaena 7120* and spinach (m form) respectively. The trx genes of both rhodophytes are located immediately 5' to the rbcL/rbcS operon. Northern analysis of *Porphyra* RNA indicates the presence of two transcripts, 0.7 and 1.0 kb in size. Primer extension has shown that the chloroplast trx transcription start site in this alga is 210 bp, 5' from the initiating methionine. The *Griffithsia* trx gene is unique in that it lacks a methionine initiating codon. Whether this chloroplast encoded trx is transcriptionally active is presently unknown. The *Griffithsia* trx sequence may represent a pseudogene, for the alga also encodes trx within the nuclear genome. Ongoing studies in our laboratory will address questions concerning transcriptional regulation of these genes.

1945 NUCLEOTIDE SEQUENCE AND CHARACTERIZATION OF A CHLOROPLAST ENVELOPE PROTEIN. David W. Budd and Kenton Ko Queen's University, Department of Biology, Kingston, ON K7L 3N6

The chloroplast envelope plays a major role in a number of important biochemical processes such as lipid and carotenoid biosynthesis, metabolite transport, and protein import. In comparison to other chloroplast compartments, very little is known about envelope membrane proteins. In this study we report the isolation and characterization of a tomato cDNA clone encoding a chloroplast envelope membrane protein from tomato. The nucleotide and deduced amino acid sequence do not exhibit homology to any sequence in the databanks. Northern RNA blot analysis indicates that although steady state transcript levels are very low, the mRNA is most abundant in flower tissue and mature fruit. Southern genomic DNA analysis shows cross reactivity to 2000 and 5700 bp fragments. The gene does not appear to be part of the complex families observed with other plastid genes. We are currently examining the expression characteristics of this gene during plant development.

POSTER ABSTRACTS

1946 LONG DIVERGENT OVERLAPPING TRANSCRIPTION IN THE CODING REGION OF TOBACCO PLASTID ndhF GENE: USE OF ALTERNATIVE PROMOTERS ON THE OPPOSITE STRAND. Antonio Vera, Tohru Matsubayashi and Masahiro Sugiura. Nagoya University, Center for Gene Research, Nagoya 464-01, Japan.

A novel transcriptional unit has been detected in the SSC region of Nicotiana tabacum chloroplast DNA. Transcript mapping revealed several outstanding features. A precursor transcript (> 1.5 kb) divergently overlaps with the messenger from ndhF in a great extent (> 400 nt), being the coding region of this gene largely involved (> 350 nt). This precursor transcript includes no other ORFs than ORF55 located more than 1 kb downstream from the start site, and recently identified as the gene for ribosomal protein L32 (2). Up to date this is the first example of such an extensive overlapping phenomenon observed in plastid genomes, being a promoter region and a coding sequence affected.

By in vitro capping experiments we also discovered that a small (215-220 b) and highly stable RNA is independently transcribed in the 3' distal region on the same transcriptional unit. This small RNA displays a secondary structure which does not resemble any other RNAs so far identified nor includes ORFs. It is expressed both in chloroplasts and non-photosynthetic plastids.

The elucidation of its biological function, and possible regulatory implications regarding overlapping transcripts are currently under investigation.

(1) Matsubayashi et al., Mol. Gen. Genet. (1987) 210:385-393
(2) Yokoi et al., FEBS Lett.(1990) 276:88-90

1947 EXPRESSION OF CHLOROPLAST tRNA GENES : THE TRANSCRIPTION AND PROCESSING OF PRECURSOR tRNAs. Tatsuya Wakasugi and Masahiro Sugiura. Nagoya University, Center for Gene Research, Nagoya 464-01, Japan

All chloroplast tRNAs are likely encoded in the chloroplast genome. The tRNA genes are transcribed as precursor RNAs, which are converted into functional tRNAs by processing, splicing, CCA addition and base modification. Northern hybridization assay revealed that eighteen tRNA genes are transcribed monocistronically while twelve other tRNA genes are cotranscribed with adjacent genes. We have analyzed processing reaction of monocistronic and polycistronic tRNA precursors by using an in vitro processing system. 5'endonuclease(ctRNaseP), 3'endonuclease and 3'exonuclease are involved in the processing reaction in both monocistronic and polycistronic precursors. These enzymes show different processing activity depending on tRNA substrates. We have then attempted to purify these enzymes. ctRNaseP has been purified 1800-fold from tobacco chloroplasts. It has a 30 kd component but no large RNA components as present in E. coli RNaseP. 3'exonuclease has been partially purified and characterized.

1948 EXPRESSION OF THE TOBACCO CHLOROPLAST GENES FOR THE PHOTOSYNTHESIS SYSTEM B.-Y. Meng, T. Mastubayashi, T. Wakasugi and M. Sugiura
Nagoya University, Center for Gene Research, Japan

Tobacco chloroplast DNAs contains the genes for at least 28 components of th photosynthetic apparatus. In this work, 15 transcription units covering these 28 genes have been mapped by northern blot hybridization, in vitro capping, S1 mapping and primer extension assays. Some of the genes are transcribed polycistronically (e.g. psaA-psaB-rps14, psbK-psbI-trnG, psaI-ORF229-ORF184-petA) and others are transcribed monocistronically (e.g.rbcL, psbA, psbN). Interestingly, we found a transcription start site for psbC within the coding region of psbD, two promoter within the psbK-psbI-trnG cluster and that a psbN transcript complementary to a part of the primary transcript from the psbB operon. The upstream DNA sequence of identified initiation site are similar to the prokaryotic consensus promoter. Beside that "ta" sequences at "-25 region" were found in several promoter regions and the number of the "ta" units seems be related to the amount of transcripts. The distinctive feature of the 3'end of these 15 transcription units will be discussed.

1949 TWO NUCLEAR GENES CODING FOR CHLOROPLAST EF-Tu OF TOBACCO. Yuhko Murayama, Tohru Matsubayashi, Mamoru Sugita and Masahiro Sugiura. Nagoya University, Center for Gene Research, Nagoya 464-01, JAPAN

We have purified an elongation factor EF-Tu from a tobacco chloroplast soluble fraction by GDP-affinity chromatography. The N-terminal amino acid sequence of this protein was determined in order to isolate and characterize the chloroplast EF-Tu cDNAs and their genes. Two distinct cDNAs were isolated from a N. sylvestris leaf cDNA library and found to encode the N-terminal sequences of the purified EF-Tu. By using the cDNA probe the corresponding genes were isolated from a genomic library. DNA sequence analysis and genomic Southern analysis revealed that the EF-Tu is encoded by two genes, tufA and tufB, on the N. sylvestris nuclear genome. Both genes have no intron. The tufA gene encodes a polypeptide of 478 amino acid residues, including a putative transit peptide of 70 residues. The tufB encodes a polypeptide of 485 residues, including a transit peptide of 77 residues. The mature proteins (408 residues) encoded by both genes differ only in 19 positions while the transit peptides are highly divergea. The tobacco mature EF-TuA and EF-TuB have 84% and 81% amino acid sequence homologies with Arabidopsis EF-Tu. RNase protection assay and northern analysis using gene specific oligonucleotide probes revealed that relative amounts of tufA and tufB mRNAs vary in tobacco leaves and roots. This suggests that the expression of two tobacco tuf genes are regulated by organ-specific manner.

1950 RIBONUCLEOPROTEIN FAMILY IN TOBACCO CHLOROPLASTS
Lizhen Ye, Yuqing Li, Satsuki Nagayoshi, Mamoru Sugita and Masahiro Sugiura, Nagoya Univ., Center for Gene Research, Nagoya 464-01, Japan

Three chloroplast ribonucleoproteins(RNPs) cp28, cp31 and cp33 have been characterized from tobacco chloroplasts(1). Here we report the identification and sequences of two additional chloroplast RNPs (cp29A and cp29B) isolated from a different protein fraction eluted from an ssDNA affinity column. These two chloroplast RNPs have high global homology to cp28, cp31 and cp33 and contain a transit peptide, an acidic amino-terminal domain and two highly conserved RNA-binding domains. Genomic Southern analysis showed that each of the five RNPs is encoded by a single nuclear gene. Sequence analysis of the genes for cp29B, cp31 and cp33 revealed that their genes contain three introns inserted at the identical sites. The expression of the cp31 gene was studied in detail. No alternatively spliced transcript was detected in both leaf and root. Taken together, we suggest that there is a sizable RNP family in chloroplasts and the diversity of these chloroplast RNPs is achieved through gene duplication rather than through alternative splicing as in the case of the human hnRNP proteins.

(1) Li,Y. and Sugiura,M. (1990) EMBO J

1951 THE CHLOROPLAST GENES FOR RIBOSOMAL PROTEINS GL23 AND CL32 ARE FUNCTIONAL IN TOBACCO. Fumiaki Yokoi, Tatsuya Wakasugi and Masahiro Sugiura. Nagoya University, Center for Gene Research, Nagoya, Japan

The 50S subunit of chloroplast ribosomes was prepared from tobacco leaves. The ribosomal proteins were fractionated and the N-terminal amino acid sequences of 13 kDa and 14 kDa proteins were determined. The N-terminal sequence of the 13 kDa protein matches that deduced from rpl23 and therefore we have called it as CL23. This demonstrates that rpl23 is a functional gene in tobacco (1). The N-terminal sequence of the 14 kDa protein matches the deduced protein of ORF55. The protein predicted from ORF55 shows homology to E. coli L32 protein and hence it has been named as CL32 and ORF55 as rpl32 (2). The tobacco chloroplast genome therefore contains 21 different genes for ribosomal proteins. The remaining ribosomal proteins will be presented.

(1)Yokoi,F. et.al : FEBS Lett. 281, 64-66, 1991
(2)Yokoi,F. et.al : FEBS Lett. 276, 88-90, 1990

POSTER ABSTRACTS

1952 THE TRANSCRIPTION PATTERN OF TOBACCO CHLOROPLAST ndh GENES. Tohru Matsubayashi, Toshiaki Kato, Masahiro Sugiura. Nagoya University, Center for Gene Research, Nagoya 464-01, Japan

Chloroplast ndh genes seem to encode the subunits of NADH dehydrogenase, which raises the possibility of the existense of mitochondoria-like respiratory chain in chloroplasts of higher plants. We have previously reported[1] that 6 tobacco ndh genes (ndhA-F) are actively expressed[1]. In this study we have determined a transcription unit of 9 tobacco ndh genes, including additional 3 ndh genes (G, H and I) by northern analysis, S1 mapping and primer extension. ndhA-ndhI-ndhG-ndhE on the small single copy region are co-transcribed to produce a 3.9 kb transcript, which is then processed into each mature mRNA. ndhD is co-transcribed with the upstreem gene (psaC) as a 2.2 kb transcript. ndhF is transcribed as a 2.6 kb mRNA. The transcript of ndhH has been also detected. ndhB on the inverted repeat sequence is transcribed as a 2.5 kb precursor, which is then spliced into a mature mRNA. ndhC on the large single copy region is co-transcribed with the downstreem gene (psbG) and ORF158 as a 2.3 kb transcript, which is then processed into each mature mRNA. We have determined the transcription initiation sites of ndhA and psaC by an in vitro capping method. Prokaryotic promoter-like sequences (-10 and -35 regions) have been found upstreem of these transcription initiation sites.

(1)Matsubayashi et al., Mol. Gen. Genet.(1987) 210:385-393

1953 THE TRANSCRIPTION PATTERNS OF RIBOSOMAL PROTEIN AND RNA POLYMERASE GENES IN TOBACCO CHLOROPLAST GENOME Keita Torazawa, Tatsuya Wakasugi & Masahiro Sugiura. Nagoya University, Center for Gene Research, Japan

The entire sequence of the tobacco chloroplast genome (N. tabacum BY4) has been determined. Genes coding for 21 ribosomal proteins and 4 subunits of RNA polymerase were found based on homologies to those of E. coli. We have analyzed the transcription patterns of these genes and mapped their transcription units. rps4, rps16 and rpl32 genes are transcribed as a monocistronic mRNA and the remaining genes are co-transcribed with other ribosomal protein or photosynthetic protein genes. For instance, rps12, which has 74% homology with the E. coli counterpart, is "devided" into a single copy of exon 1(5'-rps12) and two copies of exon2-intron-exon3(3'-rps12). 5'-rps12 is co-transcribed with rpl20 and clpP(putative, ATP dependent protease, catalytic subunit gene). 3'-rps12 is co-transcribed with rps7.

We present transcription patterns of these ribosomal protein and RNA polymerase genes.

1954 IDENTIFICATION OF IN VIVO PROCESSING INTERMEDIATES AND OF SPLICE JUNCTIONS OF tRNAs FROM MAIZE CHLOROPLASTS BY PCR AMPLIFICATION. Gaby Delp, Gabor Igloi and Hans Kössel. Institut für Biologie III, Universität Freiburg, Schänzlestr. 1, D-7800 Freiburg, Germany

Total RNA from chloroplasts of maize seedlings was used for polymerase chain reaction (PCR) mediated amplification of tRNA precursors and of mature tRNAs encoded by the two split tRNA genes of the ribosomal spacer ($tRNA^{Ile}GAU$ and $tRNA^{Ala}UGC$) and the single intron-containing $tRNA^{Gly}UCC$ gene. Sequence analysis of DNAs amplified from the mature tRNAs by combinations of exon specific primers allows unambiguous identification of the respective splice junctions. Primer combinations in which 5'- or 3'-flanking precursor tRNA sequences are included, leads to the amplification of processing intermediates in which 5'-terminal extensions are still present, whereas no PCR products corresponding to 3'-terminal extensions could be detected. From this it is concluded that in chloroplasts the 5'-terminal endonucleolytic cleavage by RNase P occurs as the final step in the tRNA processing pathway of which the endonucleolytic cleavage at the 3' side probably occurs prior to the splicing of the intron sequences.

1955 DYNAMIC REGULATION OF CHLOROPLAST GENE EXPRESSION DURING BARLEY LEAF DEVELOPMENT. Jeffrey C. Rapp, Brian J. Baumgartner and John E. Mullet. Texas A & M University, Department of Biochemistry and Biophysics, College Station, Texas 77843

Using a lysed plastid "run-on " transcription assay, we previously determined that barley chloroplast transcription increases 10-fold early in chloroplast development and declines during chloroplast maturation. We have expanded our analysis of chloroplast gene transcription by determining the transcription rates and *in vivo* RNA levels of fourteen chloroplast genes at four stages of chloroplast development. The fourteen chloroplast genes assayed are representative of genes involved in protein synthesis, transcription and photosynthesis. Run-on assays were performed on plastids isolated from the four developmental stages, and the radiolabeled transcripts produced during the five minute assay were hybridized to dot blots containing *in vitro* generated antisense RNAs from the fourteen genes. After estimating the efficiency of hybridization of radiolabeled plastid transcripts and normalizing for the length (Kb) of each antisense RNA probe, greater than 50-fold differences in relative transcription rates among genes were observed. The ratio of transcription activities of specific genes also changed during development. Utilizing Northerns and quantitative RNA dot blots, transcript populations and RNA levels corresponding to each of the fourteen genes were also determined. These results indicate that transcription is a primary determinant of chloroplast gene expression during barley chloroplast development. In addition, the expression of several chloroplast genes is modulated through changes in RNA stability.

1956 THE STRUCTURE AND EXPRESSION OF THE *EUGLENA GRACILIS* CHLOROPLAST PETB-ATPB-ATPE OPERON. Ling Hong and Richard B Hallick, University of Arizona, Department of Biochemistry, Tucson AZ 85721.

The genes encoding the chloroplast cytochrome b6 (petB), the ATP synthase b-subunit (atpB) and ATP synthase e-subunit (atpE) have been located on the EcoD fragment of the chloroplast genome of *Euglena gracilis*. The complete nucleotide sequence for this region has been determined. The gene order of this operon is petB-atpB-atpE. Unlike higher plants, the petD gene is not located distal to petB. Based on northern hybridization analysis, the atpE, atpB and petB genes are co-transcribed. The petB gene is not co-transcribed with the psbB gene in *Euglena gracilis*, whereas, in the higher plants, petB and psbB are in the same operon. All of the intron boundaries have been characterized by sequence analysis of PCR amplified cDNAs. The *Euglena gracilis* chloroplast atpB and atpE genes are conserved in gene order compared to the analogous genes from the *E.coli* unc operon and the similar atpB-atpE operons in higher plants. The gene products of petB and atpB share very high similarity between *Euglena gracilis* and higher plants, whereas, the gene product of atpE does not. The entire nucleotide sequence, the gene expression pattern and intron boundaries of this operon will be presented. (Supported by the U. S. Public Health Service, National Institutes of Health)

1957 ISOLATION AND FUNCTION OF RNA-BINDING PROTEINS IN CHLOROPLASTS OF SPINACH AND *ARABIDOPSIS THALIANA* Rüdiger Hell, Gadi Schuster and Wilhelm Gruissem. University of California, Dept. Plant Biology, Berkeley, California 94720, USA.

Processing and stability of chloroplast mRNAs is accompanied by the binding of proteins to 3' inverted repeat sequences downstream of the coding regions that are conserved between the individual genes of different species. A 28 kD RNA binding-protein has been identified in spinach chloroplast extracts which is required for specific RNA 3' end processing. The amino acid sequence of the protein reveals two conserved RNA binding domains and a novel acidic and glycine-rich amino terminal domain. To investigate the *in vivo* function of this and other RNA-binding proteins in processing and stability of chloroplast mRNAs, *Arabidopsis thaliana* will be transformed with sense and antisense DNA constructs of the corresponding genes. Experiments are currently under way to isolate *Arabidopsis* genes for RNA-binding proteins from a leaf cDNA expression library using two different strategies. The gene for the *Arabidopsis* homologue to the spinach 28 kD RNA binding protein is being isolated by PCR with degenerated oligonucleotides against the two RNA binding consensus sequences as primers. For the isolation of other RNA-binding protein genes, a North-Western screen is being developed to identify positive clones by the binding of proteins to a chloroplast mRNA 3' probe.

POSTER ABSTRACTS

1958 CHLOROPLAST mRNA 3' END PROCESSING REQUIRES A NUCLEAR-ENCODED RNA-BINDING PROTEIN. Gadi Schuster and Wilhelm Gruissem. Department of Plant Biology, University of California Berkeley, CA 94720.

To dissect the molecular components involved in chloroplast mRNA 3' end processing and stability, a spinach chloroplast protein extract containing mRNA 3' end processing activity was fractionated by FPLC and RNA-affinity chromatography. The purified fraction consisted of several proteins and was capable of processing the 3' ends of the *psbA*, *rbcL*, *petD* and *rps14* mRNAs. This protein fraction was enriched for a 28kD RNA binding protein (28RNP) which interacts with both the precursor and mature 3' ends of the four mRNAs. Using specific antibodies to this protein the poly(A) RNA-derived cDNA for the 28RNP was cloned and sequenced. The predicted amino acid sequence for the 28RNP reveals two conserved RNA-binding domains, including the consensus sequences RNP-CS1 and CS2, a novel acidic and glycine-rich amino terminal domain and a putative transit peptide. The accumulation of the nuclear-encoded 28RNP mRNA and protein are developmentally regulated in spinach cotyledons, leaves, root and stem, and are enhanced during light-dependent chloroplast development. The general correlation between accumulation of the 28RNP and plastid mRNA during development, together with the result that depletion of the 28RNP from the chloroplast protein extract interferes with the correct 3' end processing of several chloroplast mRNAs, suggests that the 28RNP is required for plastid mRNA 3' end processing and/or stability.

1959 CHARACTERIZATION OF A PROTEIN BINDING SEQUENCE IN THE PROMOTER REGION OF THE 16S rRNA GENE OF THE SPINACH CHLOROPLAST GENOME. Silva Lerbs-Mache, Laurence Baeza, Lab. Biol. Molec. Végétale, U of Grenoble-CNRS, Grenoble, France.

We have determined a 14 basepairs sequence located in front of the 16S rRNA initiation start site which is protected from exonuclease III digestion. The sequence does not include neither of the two E. coli-like promoter elements of the gene which are well recognized by E. coli RNA polymerase in vitro. The specificity of the protein-DNA interaction in this region was further verified by competition and oligonucleotide mobility shift assays. The corresponding trans-acting factor(s) were characterized by UV cross linking experiments. Analysis of several spinach chloroplast protein extracts revealed that the presence of the binding protein(s) is very variable and depends on the physiological status of the plant material. Since the sequence is not found upstream of any of the chloroplast genes it might be specific for rRNA genes and thus might be implicated in the specific regulation of rRNA gene expression in chloroplast.

1960 STRUCTURE OF THE CHLOROPLAST S1 RIBOSOMAL PROTEIN AND FUNCTION IN THE INITIATION OF TRANSLATION. Bruno Franzetti, Pierre Carol and Régis Mache. Biologie Moléculaire Végétale, U. Grenoble-CNRS, Grenoble, France.

The largest ribosomal protein of *E. coli* ribosomes, S1, plays a major function in protein synthesis. This 63kDa-protein is conserved in Gram-negative bacteria but not in Gram-positive bacteria. It contains a ribosome binding domain and a mRNA binding domain. We have isolated and characterized a cDNA clone coding for a 43kDa-chloroplast S1 r-protein in spinach (*Soc* S1). The *Soc* S1 is homologous to the mRNA binding domain of the*E. coli* S1 only and contains 3 internal sub-domains. An element of secondary structure present in the *E. coli* S1 is highly conserved in *Soc* S1 as shown by hydrophobic cluster analysis. The *Soc* S1 contain polypeptide extensions typical of most of the nuclear encoded chloroplast r-proteins.

Experiments are presented showing that the *Soc* S1 r-protein is needed for a specific recognition of chloroplast mRNAs by 30S ribosomal subunit in the presence of fMet-tRNA and of Mg^{2+}. Transcripts from the chloroplast encoded *rpl22* gene are used for these experiments. It is shown that the *Soc* S1 r-protein is in closed contact with the mRNA in the translation initiation complex. Other 30S r-proteins do not have this property. Assays with reverse transcriptase determine the site of binding of the initiation complex on the mRNA. Condition of formation of the initiation complex are studied. Results are presented using mutated *rpl22* transcripts and mutated rpl22 transcripts.

1961 A SINGLE BASE PAIR DELETION IN THE *psaB* GENE ALTERS mRNA ACCUMULATION IN A PHOTOSYSTEM I MUTANT OF CHLAMYDOMONAS REINHARDTII. Ruohui Xu, Scott P. Bingham & Andrew N. Webber, Department of Botany and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, Arizona 85287-1601.

A chloroplast photosystem I mutant of C. reinhardtii, ac-u-g-2.3, has been characterized and shown to contain a single A-T base pair deletion in the psaB gene. The reading frame-shift results in a premature stop codon that terminates polypeptide chain elongation 150 codons early. Northern blot analysis of RNA from mutant cells shows that the steady state level of transcripts from psaB are 3-fold higher than from wild type cells. Transformation of chloroplasts, using the biolistics technique, with DNA encoding the 3' half of the psaB gene restores the steady state level of psaB transcripts to that of wild type cells. This demonstrates that the altered mRNA accumulation is a direct result of the single A-T base pair deletion. RNA isolated from darkgrown cells show a similar pattern of psaB transcript accumulation in mutant and transformed cells indicating that neither light or the lack of a functional photosynthetic electron transfer chain are having any effect on psaB gene expression in the mutant cells. Expression of the psaA gene is also unaffected by the mutation. Results of RNA pulse labeling experiments, to determine if the mutation causes an increase in transcription or mRNA stability, will be presented. We are now investigating the impact of protein translation on the accumulation of the psaB transcripts in mutant and transformed cells.

1962 INTRON-ENCODED PROTEINS FROM ALGAE AND FILAMENTOUS FUNGI: EXPRESSION IN YEAST AND IN E. COLI. Stefanie Faßbender, Karl-Heinz Brühl, Michael Ciriacy and Ulrich Kück.
Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, D-4630 Bochum 1, Germany; Institut für Mikrobiologie, Universität Düsseldorf, D-4000 Düsseldorf 1, Germany

The genomes of eukaryotic organelles contain a variable number of mosaic genes. They are interrupted by intronic sequences which in some cases contain open reading frames capable of encoding polypeptides of different functions. Beside 'maturases' and 'endonucleases', some introns encode 'reverse transcriptase' like enzymes. Sequence comparison indicate a close relationship of the intron-encoded polypeptides with RNA-dependent DNA-polymerases from retroviruses or retrotransposons. But so far, enzymatic activity of intron-encoded reverse transcriptase has not been demonstrated. Here we reported the expression in S. cerevisiae and E. coli of putative reverse transcriptases. The enzymes are encoded by introns of both, the plastid petD gene from the green algae Scenedesmus obliquus (1) and the mitochondrial coxI gene from the filamentous fungus Podospora anserina (2). Attempts are presented to measure the enzymatic activities of the intron encoded proteins after synthesis in S. cerevisiae and E. coli.

(1) Kück U, Mol Gen Genet 218: 257-266
(2) Osiewacz HD, Esser K, Curr Genet 8: 299-305

Supported by the 'Deutsche Forschungsgemeinschaft'

1963 TWO DISTINCT TRANSCRIPTIONAL ACTIVITES OF PEA CHLOROPLASTS SHARE IMMUNOLOGICALLY RELATED FUNCTIONAL POLYPEPTIDES. Navin C.Khanna, Sujata Lakhani & Krishna K. Tewari, Plant Molecular Biology, International Centre for Genetic Engineering & Biotechnology, New Delhi, India.

A RNA polymerase was isolated from pea chloroplasts according to a procedure previously described by Sun et al (1986, Pl. Mol.Biol. 6, 429-439) and used for the *in vitro* transcription of recombinant plasmids containing the 16S rRNA and *psbA* promoters. This enzyme (rb-RNA pol) was found to preferentially transcribe the ribosomal gene. Subsequently, we developed an alternate method for the isolation of a pea ct RNA polymerase (ms-RNA pol) which showed a highly selective transcriptional preference for a ct mRNA gene promoter (*psbA* gene which codes for a 32 kDa polypeptide component of Photosystem II). The two transcriptional activities were distinguishable by their distinct promoter specificities and metal ion requirements. Therefore, in order to assess whether the transcriptional activities were associated with a single RNA polymerase or variants of the enzyme, we developed monoclonal antibodies (mabs) against the rb-RNA pol to address the question of common functional polypeptides in the two RNA polymerase preparations . The mabs were screened on the basis of (i) ELISA, (ii) Western blots, and (iii) their ability to inhibit 16S rRNA gene transcription *in vitro*. Three clones were selected and were checked for interference in the transcription of the *psbA* gene by ms-RNA pol. These mabs effectively inhibited *in vitro* transcription of the *psbA* gene. The mabs cross-reacted with polypeptides of identical mol. wt. (27, 75, 90 and 95 kda) in both rb- and ms-RNA pol preparations on Western blots.

POSTER ABSTRACTS

1964 REGULATION OF PHOTOSYNTHETIC ACTIVITY IN FLOWER COROLLAS. A. Vainstein, A. Halevy, I. Smirra and R. Sharon. The Hebrew University of Jerusalem, Department of Horticulture, Rehovot, Israel.

The presence of chloroplasts in mature corollas has been largely overlooked in the past. However, the colored corollas of petunia and carnation have been found to contain active chloroplasts that may play a role in the regulation of flower development and pigmentation. These chloroplasts carry out electron transport at ca. 25% the efficiency of those from green leaves. This low chloroplast efficiency can be attributed to a decreased amount of specific photosynthetic components. The level (transcriptional/translational) at which this regulation occurs will be described. Similar data will be presented for corollas in which a transition from chloroplast to chromoplast occurs during flower development. In the latter tissue EM immunolocalization, site of synthesis and accumulation of the chromoplast specific protein will also be shown.

1965 cpDNA-BINDING PROTEINS IN THE CHLOROPLAST ENVELOPE MEMBRANE IN PISUM SATIVUM. N. Sato, C. Albrieux[1], J. Joyard[1], R. Douce[1,2] and T. Kuroiwa, Department of Biology, University of Tokyo and [1]DBMS-Physiologie Vegetale Cellulaire, CENG, [2]Universite Joseph Fourier, Grenoble

The envelope membranes of chloroplasts of young pea leaves contain two proteins (175 and 130 kDa) that bind specifically chloroplast DNA. The 175 kDa protein is also present in a large quantity in the stroma, while the 130 kDa protein was localized in the inner envelope membrane. The DNA region that binds to the 130 kDa protein was found to be situated within a 414 bp sequence located in the 5.7 kbp PstI fragment of pea cpDNA. The DNA-binding proteins are present in young leaves in which chloroplast nucleoids are located in the periphery of the chloroplasts, whereas no DNA-binding protein was detected in the chloroplast envelope membranes from aged leaves in which chloroplast nucleoids are dispersed in the interthylakoidal stroma. These findings suggest that the chloroplast DNA is anchored on the internal surface of envelope membrane through the DNA-binding proteins when it is rapidly replicated.

1966 CHLOROPLAST DNA POLYMERASE FIDELITY OF AN ARABIDOPSIS THALIANA (L.) CHLOROPLAST MUTATOR STRAIN CHM^{-1}. J. Clinton Bailey, II, Sabine Heinhorst and Gordon Cannon. University of Southern Mississippi, Department of Chemistry and Biochemistry, Hattiesburg, Mississippi 39406

An Arabidopsis thaliana (L.) nuclear encoded chloroplast mutator strain, CHM^{-1}, is characterized by white, yellow, green variegation of two leaves. (Mutational Research 18 (1973) 149-162). The altered phenotype is transmitted through non Mendelian inheritance, which is consistent with Redei's interpretation that a nuclear encoded gene is inducing genetically stable alterations in the plastome. We are testing the hypothesis that the resulting CHM^{-1} mutations are products of chloroplast DNA replication generated by high error rates of a mutant chloroplast DNA polymerase. The fidelity of partially purified DNA polymerases from the chloroplasts of the CHM^{-1} mutant and of wild type Columbia plantlets was assessed using an assay detecting forward mutational frequencies (Journal of Biological Chemistry 263 (1988) 4450-4459). In addition, frequencies of nucleotide misincorporation into deoxyribo-homopolymers will be presented. The observed differences in fidelity between CHM^{-1} and wild type chloroplast DNA polymerases will be discussed.

POSTER ABSTRACTS

1967 ANALYSIS OF SITE SPECIFIC DNA REPLICATION WITH AN IN VITRO SOYBEAN CHLOROPLAST EXTRACT. Lisa Hedrick, Sabine Heinhorst and Gordon Cannon. University of Southern Mississippi, Department of Chemistry and Biochemistry, Hattiesburg, Mississippi 39406

An in vitro chloroplast DNA replication system has been derived from partially purified chloroplast extracts of the photoautotrophic soybean cell line SB-1. The extract contains at least 18 proteins, as evidenced by SDS-PAGE. Enzymatic activities observed in the extract and likely to be involved in DNA replication include DNA polymerase, helicase, topoisomerases I and II, primase and single stranded DNA binding activities. The DNA polymerase activity present in the extract represents form II soybean chloroplast polymerase (Heinhorst, et al. Plant Phys. 92: 939-945). A 1.5 Kb SacI/BamHI subclone of the SacI 14.5 Kb soybean chloroplast DNA fragment was preferentially used as a template for replication. This 1.5 Kb SacI/BamHI subclone is utilized by the extract 10 times more efficiently than most other cloned soybean chloroplast DNA fragments. Required enzymatic activities for site specific initiation are being studied by component depletion and complementation assays. We are further examining the role of the sequences contained within the 1.5 Kb SacI/BamHI fragment as an in vivo DNA replication origin by two-dimensional Fangman-Brewer electrophoresis of DNA replication intermediates.

1968 A GENE CO-INDUCED WITH ACCELERATED CAROTENOGENESIS IN *Dunaliella bardawil* IS HOMOLOGOUS TO EARLY LIGHT INDUCED GENES IN HIGHER PLANTS Amnon Lers, Haim Levy, Irena Gokhman and Ada Zamir, Biochemistry Dept. Weizmann Institute, Rehovot 76100, Israel

Dunaliella bardawil is a halotolerant, unicellular green alga potentially capable of accumulating massive amounts of β-carotene in response to intense illumination and other evironmental factors. This capability makes this organism particularly suitable for studies of carotenogenesis regulation and its links to chloroplast development and adaptive processes. Differential screening of cDNA libraries from light-induced *D. bardawil* have led to the isolation of a clone corresponding to a transcript whose accumulation paralleled the rate of carotenogenesis under a variety of conditions. The cloning and sequence determination of the gene (*cbr* for *c*arotene *b*iosynthesis *r*elated) indicated the deduced gene product was closely related to Elips, major early light induced proteins of still unknown function in pea and barley (Green *et al.*, TIBS 16, 181 (1991)). Similarity also exists between an internally repeated oligopeptide motif spanning hydrophobic domains I and III in Cbr/Elip and in Cab proteins. Upstream of the *cbr* transcription start site, 3 tandemly repeated 21 bp include an octamer and hexamer identical to sterol regulatory elements controlling mammalian genes catalyzing early reactions common to the sterol and carotenoid biosynthetic pathways. Based on these results we propose that Cbr/Elip might belong to a family of pigment binding proteins, acting during developmental stages of the photosynthetic apparatus whose synthesis is coordinately regulated with carotene biosynthetic genes. Aspects of this proposal are currently being investigated.

1969 CHLOROPLAST GENE EXPRESSION IN NUCLEAR, PHOTOSYNTHETIC MUTANTS OF ARABIDOPSIS THALIANA. R.D. Dinkins and B.R. Green. Department of Botany, University of British Columbia, Vancouver, B.C. Canada.

Six photosynthetic mutants of Arabidopsis thaliana which are high chlorophyll fluorescent have been characterized with respect to their chloroplast proteins, chlorophyll-protein complexes and RNAs. Each mutant is deficient in one or more of the thylakoid complexes involved in chloroplast electron transport. Mutant RD1 is severely reduced and mutant RD4 is slightly reduced in photosystem I complex, but other thylakoid complexes appear to be normal. Chloroplast and nuclear RNAs for thylakoid membrane proteins are normal and suggest that the block in the photosystem I assembly or stability is post transcriptional. Mutants RD2, RD3, have reduced levels of both photosystem I and II, as well as a reduction in the cytochrome b6/f complex. Chloroplast RNA levels were also slightly reduced, except for the petA transcript encoding the cytochrome f in mutant line RD2 which appears to be significantly overexpressed. The reason for this is unknown at the present. Both mutants RD5 and RD6 are severely depleted in all thylakoid membrane complexes involved in electron transport. The latter two mutants were also observed to display alterations in the abundance of many chloroplast transcripts. Transcripts of nuclear-encoded chloroplast proteins were normal. This suggests that the wild-type gene product for mutant RD5 and RD6 is involved in chloroplast RNA processing and/or stability or translation.

1970 NUCLEAR MAIZE TIMING MUTANT HAS UNIQUE CHLOROPLAST TRANSLATIONAL DEFICIENCY. Mary Polacco, Scott Harding. University of Missouri, Department of Biochemistry, Columbia, Missouri, 65211.

Mutations at nuclear maize locus, v24 (v: virescent; delayed greening), have a striking effect on the assembly timing of the major chlorophyll a/b light harvesting complex, LHCII. Normally, in developing mesophyll thylakoids, LHCII assembly is a late event, occurring largely after reaction centers become functional. In v24 seedlings, LHCII assembles prematurely and results in a developmentally conditional, high yield of leaf chlorophyll fluorescence. Mature, v24 thylakoids have a normal stoichiometry of LHCII and other protein complexes. We have previously reported that transcript levels of the chloroplast gene, psbA, and the nuclear gene family, cab, are unaffected by v24. We find that this is true of other plastid and nuclear photosynthesis genes (psaA, rbcL, rbcS and individual members of the cab family) and that cab polysome profiles are normal. The v24 timing perturbation does not alter the Circadian rhythm of cab transcripts. Expression of v24 severely reduces thylakoid-bound translation complexes during chloroplast morphogenesis. There is no apparent reduction in soluble, cytoplasmic polysomes. Perturbation of LHCII assembly timing may be unique to the translational lesion of v24: (1) cold sensitive, maize v mutations, blocked in chloroplast ribosome assembly at restrictive temperatures, do not alter LHCII assembly timing; (2) Several v mutants, independently isolated in Mutator backgrounds, were screened for premature LHCII assembly. All mutants, with this v24 trait, are allelic to v24.

POSTER ABSTRACTS

1971 THE LHC POLYPEPTIDES OF GYMNOSPERMS AND ANGIOSPERMS AND A NEW NOMENCLATURE FOR THEIR CORRESPONDING GENES (FORMERLY THE *cab* GENES).

Stefan Jansson and Petter Gustafsson, Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden.

The genes encoding the major apoprotein of LHC II has been extensively studied whereas very little work has focused on the genes encoding the other chlorophyll *a/b*-binding proteins of PS I and PS II. They have with very few exceptions only been characterized from tomato, and very little is known about the presence of these genes in other species and their evolutionary conservation. We have sequenced cDNAs encoding a number of different chlorophyll a/b-binding polypeptides (Type I and II LHC II, Type I, II, III and IV LHC I and Type I CP29) from the gymnosperm Scots pine and we here present a comparative analysis of the angiosperm and gymnosperm LHC polypeptides. All angiosperm genes have their highly homologous counterparts in Scots pine, and thus all flowering plants have probably the same set of LHC polypeptides. Based on these findings and protein sequencing data which correlates the different LHC proteins to their corresponding genes, we present a nomenclature for the genes encoding LHC polypeptides that should be valid for all flowering plants The genes encoding LHC polypeptides associated with PS I and PS II are given the group names *lha* and *lhb*, respectively, analogous to the nomenclature for the genes encoding other photosynthetic thylakoid polypeptides.

1972 THE GENETIC CONTROL OF POLLEN PLASTID DNA LEVELS.

Neil O. Polans[1], Joseph L. Corriveau[2] and Annette W. Coleman[2]
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We used DNA-fluorochrome/epifluorescence microscopy to examine germinated pollen from different pea inbred lines and found substantial variability among accessions with respect to both the proportion of pollen grains containing plastid DNA and the average number of nucleoids per generative cell. These observations suggested genetic variability for plastid DNA levels in pea and the possibility that P. sativum might exhibit biparentalism in some instances. At issue was whether paternal plastid transmission was correlated with the availability of plastids in the pollen. RFLP markers were then used to follow the transmission of DNAs from parents to their F_1 offspring. Results from these initial inheritance studies indicated the presence of only the maternal plastid markers in the F_1 progeny, irrespective of the pollen plastid DNA levels of the paternal parent. The same result was obtained when offspring were sampled at early developmental stages. Preliminary studies also indicated that variability in pollen plastid DNA levels appears to be under nuclear control. Once we establish the degree to which pollen plastid DNA levels are regulated by the nuclear genome, we intend to use our nuclear genetic marker systems (including RFLPs and RAPDs) to dissect and genetically characterize the components of this trait.

1973 ASSEMBLY OF THE CYTOCHROME b-f COMPLEX IN CHLOROPLAST THYLAKOID MEMBRANES.

F. Madueño , J.A. Napier and J.C. Gray. Botany School, University of Cambridge, U.K.

The cytochrome *b-f* complex of chloroplast thylakoid membranes mediates electron transfer between PSI and PSII, being also involved in cyclic electron flow around PSI (1). The cytochrome *b-f* complex is the simplest of the multisubunit complexes that catalyse the light reactions of photosynthesis. The complex is essentially composed of five polypeptide subunits, four of which, cytochrome *f*, cytochrome *b*-563 and subunits IV and V, are encoded by chloroplast DNA and synthesised within the chloroplast, and the fifth, the Rieske FeS protein, is encoded by nuclear DNA and synthesised in the cytoplasm (2,3). The simple composition of cytochrome *b-f* complex and the feature that it contains only one nuclear-encoded polypeptide, make this particular complex an ideal model for the study of the assembly of protein complexes containing subunits encoded by both nuclear and chloroplast genomes. The goal of our work is to understand the role of the nuclear-encoded Rieske FeS protein in the assembly of the cytochrome *b-f* complex. With this aim we have isolated and characterized tobacco cDNA clones encoding the Rieske FeS protein. A full-length cDNA clone has been inserted in both sense and antisense orientation under the control of either *rbcS* or 35S CaMV promoters into pBin19-derived vectors and the constructs produced in this way have been used to generate transgenic tobacco plants. The analysis of these plants is currently underway and the effect of altered levels of the Rieske FeS protein on the assembly of cytochrome *b-f* complex will be discussed.

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3. Haley J. and Bogorad L. (1989) *Proc. Nat. Acad.* Sci. USA 86, 1534-1538.

1974 ACTION SPECTRUM OF LHC-II APOPROTEIN TRANSCRIPT ACCUMULATION AND COORDINATED TRANSLATION.

Kostas Triantaphylopoulos, Thalia Bei-Paraskevopoulou and Joan H. Argyroudi-Akoyunoglou.
Institute of Biology, NRCPS "Demokritos", Athens, Greece.

To ascertain that the phytochrome protein is indeed the photoreceptor involved in the synchronization of the endogenous circadian clock and the LHC-II apoprotein mRNA accumulation (Tavladoraki, Kloppstech, Argyroudi-Akoyunoglou, Plant Physiol 90, 665-672, 1989), we studied the action spectrum of the LHC-II gene transcription in etiolated bean leaves. Transcript accumulation was detected by dot-hybridization experiments or immunoprecipitation of in vitro translated products of isolated poly(A)mRNAs. We found that the accumulation of the LHC-II-precursor-apoprotein (32 kDa)-mRNA depends on wavelength of pre-exposure light, the action spectrum of its accumulation being closely correlated with the absorption spectrum of phytochrome (Pr). In addition, Western-blot analyses of total leaf LiDS-extracts showed that the accumulation of the mature apoprotein in etiolated leaves preexposed to a light pulse of equal dose but various wavelengths, follows closely the pattern of its transcript accumulation. Thus, translation of the apoprotein in vivo is coordinated with the accumulation of its transcript. The immunodetected translation product in vivo (25 kDa) accumulates also in a circadian rhythm, following a Red-light pulse, similar to that of its transcript. Furthermore, the regeneration of PChlide in the dark following the Red light pulse follows a similar rhythm. The results indicate that the small amount of Chla formed after the Red light pulse (from preexisting PChlide) is sufficient to stabilize the LHC-II apoprotein in thylakoids, suggesting that the thylakoid protease, beleived to regulate LHC-II complex assembly, may be inactive under these conditions.

1975 PLASTID RIBOSOME DEFICIENCY RESULTS IN A MAJOR CONVERSION IN NUCLEAR GENE EXPRESSION.

Thomas Börner and Wolfgang R. Hess. Humboldt-Universität Berlin, O-1040 Germany, Invalidenstrasse 43.

The barley mutant *albostrians* lacks plastid ribosomes. The plastid ribosome deficiency blocks chloroplast development at an early stage and causes a drastic change in the accumulation of several nuclear genes transcribed by RNA polymerase II. The transcript level of the genes encoding chloroplast proteines like rbcS, cab, gapA and the cytoplasmic enzyme nitrate reductase is reduced whereas the mRNA level of the genes encoding gapC and chalcone synthase is distinctly increased.
We suppose that the reduction of mRNA of the genes studied indicates the effect of (a) plastid-derived factor(s) triggering gene expression in correspondence with the developmental status of the chloroplast. Probably another regulatory principle causes the increase in expression of certain other genes.

1976 REPLICATION OF BARLEY CHLOROPLAST GENOME: POSSIBLE ROLE OF HAIRPIN STRUCTURES.

Sylvie Collin and T. H. Noel Ellis, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH, England.

Aberrant linear plastid genomes have been described in barley albino plants (1), (2). These studies led to the conclusion that hairpin structures might be involved in the mechanism of replication of chloroplast DNA. We describe, in this poster, the consistent presence, in green barley plants, of chloroplast DNA molecules which have the following properties: their apparent size doubles after denaturation; they cannot be detected on Southern blots if they are not depurinated first; these structures are also sensitive to S1 nuclease and insensitive to Topoisomerase I (3). These characteristics are the expected properties of hairpin DNAs. The data collected so far show that the ends of these molecules map all around the chloroplast genome and represent a maximum of 2% of the chloroplast DNA. We are currently investigating whether they form at preferential sites.

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(2) Ellis, T.H.N. and Day, A. (1986) The EMBO Journal 5:2769-2774
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POSTER ABSTRACTS

1977 ChrA AND ChrB, CHROMOPLAST-SPECIFIC PROTEINS IN RIPENING FRUITS OF *Capsicum annuum*. Lee A. Newman, Miguel Cervantes-Cervantes, Noureddine Hadjeb, Michal Oren-Shamir, and Carl A. Price. Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759, USA

Chromoplasts are a differentiation state of plastids characterized by the accumulation of carotenoids. Our studies have been conducted on the sweet pepper, *Capsicum annuum* L., which displays a rich variety of genes affecting fruit color. A 58-kDa protein named ChrA and a 35-kDa protein named ChrB occur in chromoplast membranes and represent approx. 50% and 10%, respectively, of the plastid membrane protein. Western blots of total membrane proteins probed with antibodies raised against ChrA and ChrB showed no detectable protein in green immature fruit, leaves, stems, or roots, but striking increases early in fruit ripening in the case of ChrB, and late in fruit ripening in the case of ChrA. ChrA occurs in association with xanthophylls as the principal protein component of a carotenoid-protein complex isolated from chromoplast membranes. We also examined the protein composition of chromoplasts isolated from different genotypes *C. annuum* in which the timing or pigmentation of chromoplasts is affected. ChrB occurs in chromoplasts of all genotypes examined. The nuclear genes c_1, c_2, *cl*, and g_1 each affect the nature or timing of pigmentation in plastids of *Capsicum* fruits, but we detected no proteins specific to these genes. The occurrence of ChrA, however, correlates strictly with the nuclear gene y^+. Deciding whether ChrA is encoded at the y^+ locus or y^+ is pleiotropic on *chrA* will require more direct evidence, such as complementation or sequencing.

1978 ISOLATION OF *chrA*, A CHROMOPLAST-SPECIFIC GENE FROM *Capsicum annuum*. Noureddine Hadjeb, Lee A. Newman, and Carl A. Price. Waksman Institute, Rutgers University, Piscataway, NJ 08855-0759, USA.

ChrA and ChrB are chromoplast-specific proteins in the sweet pepper *Capsicum annuum* L. (cf. abstract of L. A. Newman et al.). Information on the regulation of expression of *chrA* and *chrB*, the genes encoding these proteins, could have wide significance for understanding the differentiation of proplastids, leucoplasts, and chloroplasts into chromoplasts. We describe here the isolation of *chrA*, the first gene encoding a chromoplast-specific protein to be isolated. From a partial amino acid sequence of the N-terminus of ChrA, we synthesized an oligonucleotide designed to minimize degeneracy and to correspond to codon usage in tomato. A *Sau* 3A genomic library of seedling DNA from *C. annuum* var. Emerald Giant was prepared in λ FIXII and probed with the synthetic oligonucleotide. An insert from a positive plaque was isolated and subcloned into pTZ18 for sequencing. The sequence hybridizing with the oligo was located about 1000 bp from one end of the insert. The sequence of bases around this site corresponds to a predicted amino acid sequence which includes the six amino acids of the oligo preceded by the following 12 amino acids of the N-terminus of ChrA determined by microsequencing. Upstream from the site found by the probe are 22 amino acids, which may correspond to the transit peptide of ChrA. We infer that an open reading frame coding for 130 amino acids corresponds to the first exon of *chrA*. On the basis of Southern blots, it appears that *chrA* is represented by a single copy. Comparison of some 6000 base pairs within or near *chrA* with sequences in GenBank turned up no similarities.

1979 DIFFERENTIATION OF PLASTIDS IN DEVELOPING PEA LEAVES. Yasuko Kaneko and Kenneth Keegstra. Department of Botany, University of Wisconsin-Madison, Madison, Wisconsin 53706

In developing plant tissue, plastids exhibit very dramatic structural changes during their differentiation into specialized plastids, such as chloroplasts. Although the functions of the various plastids in developing plant tissue is not well defined, ultrastructural observation suggests several specific changes during each step of plant development. Since most of the plastid proteins are synthesized in cytoplasm, the coordination between the expression of nuclear encoded plastid proteins and the developmental sequence of the plant tissue is expected. In order to characterize the differentiation of plastids at a cellular and molecular level, ultrastructural differentiation of the plastids was examined in young developing pea leaves and correlated with the expression of some nuclear-encoded plastid proteins using molecular and immunochemical techniques. In young developing pea leaves following germination, plastids were observed in the amoeboid stage, starch accumulating stage, and prolamellar body stage successively before they fully differentiate into chloroplasts. Results from molecular and immunochemical analysis on the appearance and localization of some of the nuclear-encoded plastid proteins in the developing pea leaves will also be presented.

1980 CHARACTERIZATION OF NONSEDIMENTABLE MICROVESICLES DERIVED FROM THYLAKOIDS. Sibdas Ghosh, K. Yao, G. Paliyath, E.B. Dumbroff and J E. Thompson, University of Waterloo, Department of Biology, Waterloo, Ontario, N2L 3G1, Canada.

Nonsedimentable lipid-protein microvesicles (termed deteriosomes) have been isolated from the stroma of intact chloroplasts obtained from primary leaves of bean (Phaseolus vulgaris L. cv. Kinghorn) and were also generated in vitro from isolated thylakoids. The deteriosomes are bilayered in nature, range from 100 to 400 nm in diameter, and are enriched in thylakoid lipid and protein catabolites. Western blot analyses have indicated that catabolites of photosynthetic proteins are among the protein degradation products found in deteriosomes. The data are consistent with the hypothesis that deteriosomes serve as a vehicle for removing partially degraded lipids and proteins from thylakoid membranes.

1981 ASSEMBLY OF THYLAKOID MACROMOLECULAR COMPLEXES IN DEVELOPING PLASTIDS. Beth A. Welty, Shivanthi Anandan, Daryl T. Morishige and J.Philip Thornber. Dept. of Biology, University of California, Los Angeles, CA 90024-1606.

In fully developed thylakoids, each photosystem is composed of a core component (CC) and a light-harvesting component (LHC), containing several different pigment-proteins within each multiprotein complex. Plastids from seedlings grown under intermittent light (IML) contain the functional CC I and CC II, but there is very little accumulation of chl a/b proteins of either LHC I or LHC II. These protochloroplasts can be followed to maturity during exposure to continuous illumination. After various periods of light the assembly of PS I and PS II multiprotein complexes in isolated thylakoids was examined for their spectral characteristics and protein composition by a 2-D electrophoresis system. 1) Synthesis of the major LHC II pigment-protein occurred via addition of pigments to the monomeric polypeptide prior to assembly of the pigment-protein monomer into a trimer. Distinct absorption changes occur in the pigment-protein during this process. 2) Formation of a complete PS I occurred by addition of LHC I as a multiprotein unit to the P700 containing CC I, as evidenced by a distinct size increase and shifts in 77°K fluorescence emissions. 3) A novel group of low molecular weight polypeptides (13-20kDa) whose abundance correlated inversely with the increase in chl b content and LHC II accumulation were identified. 4) The smallest low MW polypeptide was purified and internal amino acid sequence determined. B A. Welty was supported by a USDA Fellowship

1982 ABERRANT CHLOROPLAST PHENOTYPE IN TOMATO GENOTYPES CARRYING *DIVERGENS* (DI), A NUCLEAR RECESSIVE MUTATION. A. Zelcer[1], M. Koornneef[2], S. Gepstein[3]

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During a routine examination of tomato protoplasts from multimarker tester lines, an aberrant chloroplast phenotype was detected in genotype LA 1166. This line carries four monogenic recessive mutations. Linkage studies indicated cosegregation of the locus divergens (*di*, chromosome IV, one of the markers in LA 1166) with the aberrant phenotype. Moreover, the genotype LA 599, monogenic for *di*, exhibits identical aberrant chloroplasts.

The *di* mutation is manifested as slightly reduced plant vigor, white stems and petioles with occasional green streaks, and delayed fruit ripening. Microscopic observations on isolated mesophyll protoplasts or leaf sections, as well as EM examination of thin sections indicated that individual cells contain an extremely reduced number (as low as 1-3) of mature plastids. Plastids are unusually big and irregularly shaped. This phenotype can be easily determined in leaf squashes.

Immunodetection analyses of chloroplast proteins in wt and *di* genotypes are currently being performed and will be summarized. These studies represent the first report regarding the effect of a genetically defined nuclear locus on chloroplast morphology in higher plants.

POSTER ABSTRACTS

1983 EXPRESSION OF THE GENE FOR HYDROXYMETHYLBILANE SYNTHASE IN PEA AND ARABIDOPSIS. Huguette Albrecht, Michael Witty and Alison G. Smith. Department of Botany, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

Hydroxymethylbilane synthase (HMBS, also known as porphobilinogen deaminase) is one of the early enzymes in the porphyrin biosynthesis pathway. It is responsible for the synthesis of HMB, a linear tetrapyrrole which is cyclised by uroporphyrinogen III synthase to form uroporphyrinogen III. This is the precursor to all biological porphyrins, including haem and chlorophyll. Although all the enzymic steps, at least for the formation of haem and chlorophyll, are well established and characterised, the controls operating on the pathway remain largely unknown. Genes and/or cDNA clones for HMBS from E. coli, Euglena gracilis and mammalian sources have been isolated and characterised, but to date no clones for HMBS of plant origin have been studied. One of our main interests is to investigate the possible regulation of the HMBS gene in photosynthetic and nonphotosynthetic tissue, which have very different requirements in terms of the amount and type of porphyrins they require. In order to address this question, clones for HMBS from cDNA libraries of pea and Arabidopsis have been isolated, and in addition a number of genomic clones. Data on the structure of the gene and its control sequences will be presented, together with preliminary studies on the expression of the gene in different tissues and stages of greening.

1984 VARIATION IN MECHANISMS PRODUCING UNIPARENTAL INHERITANCE AMONG ANGIOSPERMS. A.W. Coleman and J.L. Corriveau, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912.

Among angiosperms, plastids (in 80% of species) and mitochondria show maternal inheritance. Cytological examination, using DAPI to look for mitochondrial nucleoids fails to reveal mtDNA in the nucleoid conformation from shortly after first pollen mitosis in either generative or tube cells. However we have found mtDNA in total DNA from germinated pollen. Thus mtDNA must be eliminated subsequently, or else by anthesis only tube cell mitochondria contain DNA. The situation is more complex for plastid DNA during pollen development. In the tube cell, ptDNA in the nucleoid conformation is not seen in maturing pollen. In a genetically biparental species, *Medicago sativa*, ptDNA nucloids are visible in generative cell plastids of growing pollen tubes, and ptDNA is present in total DNA of germinated pollen. In the uniparental species, *Antirrhinum majus*, pt DNA nucloids are absent in mature pollen and no ptDNA was detected in total DNA of germinated pollen. In the uniparental species, *Nicotiana tabacum*, pt DNA nucleoids were absent from germinated pollen, but ptDNA was detectable in total DNA from germinated pollen. The source of this ptDNA, whether tube or generative cell, was not determined. Recently, others have detected trace biparentalism of ptDNA in this species. Two further genera of interest are *Pelargonium* and *Oenothera*, both known to be capable of biparental plastid inheritance. Cytological examination shows that both contain ptDNA nucleoids in generative cells of mature pollen; those of *Pelargonium* remain unchanged during 6 hrs of pollen germination and growth; those of *Oenothera* have essentially disappeared after 6 hr of pollen tube growth. Taken together, these observations suggest that the mechanism of mtDNA elimination may be uniform among angiosperms, while those for ptDNA elimination vary with the species, at least in timing.

1985 CHARACTERIZATION OF THE *nv*-MUTATION IN *L. esculentum*. Eva Eicher and Artur. J P.Pfitzner. University of Munich, Botanical Institute, D-8000 München, FRG

The recessive *nv*-mutation was introgressed into *L. esculentum* from *L. peruvianum* during a breeding program for resistance against tobacco mosaic virus (TMV) and the gene was mapped to chromosome 9. Plants homozygous for the *nv*-gene typically exhibit yellow leaves and are stunted as compared to the wildtype plants. By applying biochemical, electron microscopical and molecular biological methods we have analysed the physiological defects underlying the *nv*-phenotype. The mutant plastids display a significant reduction in chlorophyll content . The number and hight of grana stacks are also decreased by a factor of about 2 in the mutant as compared to the wildtype plants.On the other hand, analysis of the chloroplast proteins on 2D gels revealed that the mutant shows the same overall protein pattern as the wildtype, disregarding a reduction in the total amount of proteins which is paralleled by the decrease in chlorophyll content. This result is, however, not caused by a reduction of transcript levels for chloroplast proteins, because *in vitro* translation of poly A^+ RNA isolated from mutant as well as from wild type plants yielded comparable levels of radiolabelled proteins. Pulse chase experiments strongly suggest that the lower accumulation of various chloroplast proteins is due to an increased protein turnover in mutant plants. Thus, in contrast to the *Su-* mutation in tobacco, the *nv*-mutation in tomato does not seem to affect the accumulation of transcripts for photosynthetic proteins, but rather results in a reduced stability of chloroplast proteins.

1986 CHLOROPLAST DEVELOPMENT IN SPIRODELA OLIGORRHIZA. D.J. McCormac, D. Bruce* and B.M. Greenberg. Dept of Biol, U of Waterloo, Ontario, Canada. N2L 3G1. *Dept. of Biol. Sci., Brock U., St. Catharines, Ontario, Canada. L2S 3A1.

We are studying chloroplast development in the higher plant *Spirodela oligorrhiza* because of its ability to grow indefinitely as a heterotroph in the dark. With this system we are able to follow chloroplast development from proplastids. The dark grown plants are devoid of chlorophyll, but upon illumination synchronously green over a 120 h period. Ultrastructural, physiological and molecular techniques were used to monitor proplastid to chloroplast transitions. Synthesis of chloroplast proteins that have roles in photosynthesis correlates with thylakoid assembly, chlorophyll accumulation and appearance of carbon fixation activity. The developmental sequence in *Spirodela* was slow enough to reveal the biosynthesis of the D1 photosystem II reaction center protein occurred prior to that of the major light-harvesting antenna proteins. This, coupled with the high chlorophyll a/b ratio observed early in development, indicates that reaction center assembly precedes accumulation of the light-gathering complexes. Currently, we are monitoring mRNA levels for the D1 and LHCII proteins to determine if differential synthesis of the reaction centers and antenna is regulated at the level of transcription or translation. We have also found that chloroplast development can be sensitively monitored by fluorescence at 77K. With this technique, correctly assembled PSI and PSII centers can be detected shortly after light exposure. This method is being used to study the photoreceptors involved in proplastid to chloroplast transitions.

1987 CHARACTERIZATION OF NUCLEAR-ENCODED PLASTID RIBOSOMAL PROTEIN GENES *rps17, rpl9, rpl15*, AND THE GENE ENCODING PLASTID SPECIFIC CL25. Michael Thompson, Colleen Jacks, Todd Lenvik, and Steve Gantt. University of Minnesota, Department of Plant Biology, St.Paul, MN 55108

We have isolated cDNA and genomic clones that encode *Arabidopsis thaliana* plastid ribosomal proteins CS17, CL15, CL9 and CL25 (not homologous to *Escherichia coli* L25). The genes for these proteins are encoded in the nuclear genome. The nucleotide sequence of all four cDNAs and genes was determined. Primer extension and RNase protection experiments were used to map the 5'-ends of transcripts from *rpl15*, *rpl9* and *rps17*. The deduced amino acid sequence suggests that each cDNA contains a complete coding region. Southern blot analysis suggests that CS17, CL9, CL15, and CL25 are each encoded by a single gene. Northern blot analysis reveals the levels of mRNAs encoding CS17, CL9, and CL15 to be higher in leaves and stems than in roots, while the level of mRNA encoding the cytosolic ribosomal protein S11 appears to be the same in these tissues. Analysis of the 5'-flanking regions of each gene reveals direct and inverted repeats which could play a role in the expression of these genes. Furthermore, three sequence motifs are present in the 5'-flanking regions of all four genes. One of which contains the sequence ATGATAAGG which is present in virtually all *rbcS* and *cab* genes and has been identified by computer search within the promoters of numerous phytochrome regulated genes. The functional significance of these sequences is currently being defined.

1988 *OENOTHERA* CHLOROPLAST DNA POLYMORPHISMS ASSOCIATED WITH PLASTOME MUTATOR ACTIVITY. Barbara B. Sears, Wan-Ling Chiu, Ellen Johnson, S.A. Kaplan, and Ruth T. Wolfson, Michigan State University, Department of Botany and Plant Pathology, East Lansing, Michigan 48824.

Oenothera plants homozygous for a recessive allele at the plastome mutator (pm) locus show non-Mendelian mutation frequencies that are 200-fold higher than spontaneous levels. Chloroplast DNA (cpDNA) was isolated from nine mutants and two green isolates of the plastome mutator line. When compared to cpDNA from a representative of the progenitor Johansen strain, cpDNAs from all eleven plastome mutator lines show changes of fragment mobility due to deletion events at five discrete regions of the plastome. At least three of the deletions target regions of cpDNA where multiple direct repeats are found. In one case, a 15-bp duplication has occurred. In contrast to the plastome mutator line, cpDNA from several populations of a closely related wild-type *Oenothera* species have few restriction fragment length polymorphisms. This suggests that both mutation frequencies and site-specific cpDNA deletions are elevated in the plastome mutator line, and implicates a defect in the cpDNA repair or replication machinery.

POSTER ABSTRACTS

1989 REPLICATION SLIPPAGE NEAR AN *OENOTHERA* CHLOROPLAST DNA REPLICATION ORIGIN. Wan-Ling Chiu and Barbara B. Sears, Michigan State University, Department of Botany and Plant Pathology, East Lansing, Michigan 48824.

The origins of *Oenothera* chloroplast DNA replication were mapped to determine whether variation at this site could account for the different transmission abilities associated with the various plastome types. Two pairs of displacement loop (D-loop) initiation sites were observed on intact, circular chloroplast DNA molecules by electron microscopy. Analysis of restriction fragments allowed each pair of D-loops to be mapped to the inverted repeat of the *Oenothera* cpDNA molecule, where they bracket the 16S rRNA gene. A hypervariable region of cpDNA was found to lie in the spacer between the 16S rRNA and tRNA-ile genes, near one of the D-loop initiation sites. The highly variable region contains a number of direct repeats, ranging in size from 6-bp to 142-bp. DNA sequence data from a number of different plastome types indicate that the fragment size variability is due to duplications and deletions of a subset of these direct repeats. Strand slippage during replication is a likely cause of these saltatory changes.

1990 IMPACT OF PLASTOME-GENOME COMPATIBILITY ON BIPARENTAL INHERITANCE OF CHLOROPLASTS IN *OENOTHERA*. Wan-Ling Chiu and Barbara B. Sears, Michigan State University, Department of Botany and Plant Pathology, East Lansing, MI 48840.

In crosses of *Oenothera*, the evening primrose, plastids can be transmitted from both parents to the progeny, but, the frequency of biparental plastid inheritance is strongly influenced by the plastome types involved in a given cross. In testing plastid transmission in a number of nuclear backgrounds, we have found that the compatibility of the plastome and genome has a strong influence on plastid inheritance. In general, the transmission efficiency of each plastome correlated strongly with its compatibility with the nuclear genome of the progeny, suggesting that plastome-genome interactions can influence plastid transmission by affecting the efficiency of plastid multiplication after fertilization.

Other specific features of pollination and fertilization that are controlled by nuclear genes can impact plastid transmission. Lower frequencies of plastid transmission from the paternal side were observed when the pollen had poor vigor due to an incompatible plastome-genome combination, indicating that plastome-genome interactions may also affect the input of plastids during fertilization. Crosses using maternal parents with long styles or pollen with relatively low growth capacity resulted in reduced frequencies of paternal plastid transmission. These observations suggest that degeneration of pollen plastids may occur as the time interval between pollination and fertilization is lengthened.

1991 VECTORS CONTAINING DOMINANT SELECTABLE MARKER GENES NPT-II & SPT-I FOR THE TRANSFORMATION OF CHLOROPLAST. D.K. Srivastava*, T. Ulmasov and E.S. Piruzian. Institute of Molecular Genetics, USSR Academy of Sciences, Moscow-123182,USSR, *Department of Biotechnology, Dr.Y.S.Parmar University of Horticulture and Forestry,SOLAN-173230, INDIA.

Chloroplast transformation and subsequent identification of the transformants require a selectable marker gene. We have constructed the chimeric plasmid expression vectors pTD21(with SPT-I gene) and pTD22(with NPT-II gene) from pTZ18/19 plasmids, which can be used for the transformation of chloroplast by liposome-mediated gene transfer method. These vectors contain promoter DNA sequences from spinach chloroplast rbcL gene and coding sequences of SPT-I and NPT-II from the bacterial transposon Tn5, Oricp from tobacco chloroplast, and terminator sequences from psbA gene of tobacco chloroplast. In addition, plasmids pGSΔ6 and pGSΔ2 were constructed which contained the chimeric gene encoding transit peptide-NPT-II fusion protein (transit peptideof rbcS of the pea chloroplast). The distance between the Shine-Dalgarno site and the first coding sequence was maintained to six nucleotide base pairs by treating the restricted plasmid with Bal31 to have an appropriate expression of the fused protein. The fusec protein isolated by affinity chromatography followed by coating on the liposome containing suitable plasmids will facilitate the entry of liposome into the chloroplast. These plasmids after transformation can replicate autonomously and express their genes.

1992 STABLE FOREIGN GENE EXPRESSION IN CHLOROPLASTS OF HIGHER PLANTS H.Daniell Dept of Biological Sciences, University of Idaho, Moscow ID 83843, USA

A workable system for foreign gene incorporation or replacement of native genes in chloroplasts or mitochondria has great merit as a tool for the study of their functions and regulated gene expression. We have recently demonstrated transient expression of chloramphenicol acetyl transferase (cat) in chloroplasts of cultured tobacco cells, after biolistic delivery of chloroplast vectors (Daniell et al., Proc.Natl.Acad.Sci.USA, 1990, 87, 88-92) and established conditions for optimal DNA delivery into cultured plant cells, using an improved biolistic device (Ye et al, Plant Mol.Biol.,1990, 15, 809-820). We have also reported transient expression of b-glucuronidase (GUS) in different cellular compartments following biolistic delivery of chloroplast or nuclear vectors into wheat leaves and calli, derived from anther culture or immature embryos (Daniell et al.,1991, Plant Cell Rep.9, 615-619). All these observations demonstrate a reproducible foreign DNA delivery system into higher plant chloroplasts (Daniell 1991 Methods Enzymol. in press).

Despite several successful reports of transient foreign gene expression in chlorplasts of higher plants, stable integration and expression of a foreign gene has not yet been accomplished. A mutant EPSPS gene conferring a high level of resistance to glyphosate (obtained from MONSANTO), driven by a chloroplast promoter (psbA) and flanked by chloroplast DNA border sequences, has been introduced into chlorplasts of cultured plant cells, using the GENE GUN. Results on stable integration of EPSPS gene into the chloroplast genome and expression of EPSPS in the chloroplast compartment will be presented.

1993 GENETICALLY ENGINEERED ALTERATION OF FATTY-ACID SATURATION OF CHLOROPLASTIC PHOSPHATIDYLGLYCEROL IN TOBACCO PLANTS

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The substrate selectivity of glycerol-3-phosphate acyltransferase in chloroplasts determines the level of saturated species of phosphatidylglycerol (PG), which is correlated to the low-temperature sensitivity of plants. A tobacco plant, containing a high level of saturated species of PG, was transformed with a cDNA for glycerol-3-phosphate acyltransferase of Arabidopsis, which contains a low level of saturated species of PG and is a chilling-resistant plant. The level of saturated species of PG of the transgenic tobacco plant was intermediate between the levels of wild-type tobacco and Arabidopsis. These results suggest that the level of saturated species of PG, and very probably the chilling sensitivity, can be altered by gene manipulation of glycerol-3-phosphate acyltransferase.

1994 VECTOR FOR INTEGRATION OF FOREIGN DNA INTO THE INVERTED REPEAT OF *NICOTIANA TABACUM* PLASTID DNA. Jeffrey M. Staub and Pal Maliga. Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, NJ 08855

Vector pJS75 has been constructed to facilitate insertion of foreign DNA into the inverted repeat region of the tabacco plastid genome via linkage to flanking selectable antibiotic resistance markers. The flanking markers in pJS75 (10 kb) include spectinomycin resistance encoded in the 16s rDNA (SPC1, A to C position 1138) and streptomycin resistance encoded by rps12 (C to T, codon 90). These selectable markers are separated by 3 kb, between which novel restriction enzyme site markers have been created. Linkage of these sites to the selectable markers and thus their suitability as insertion sites for gene size foreign DNA is examined. Additional restriction site markers within intergenic regions of the rRNA operon are also examined for linkage. It is found that insertion of oligonucleotides encoding the restriction site markers cause no observable phenotypic effect. Most notably, insertion of a frameshift mutation into ORF131, and insertion between the tRNA-Val and rRNA promoter shows no interference. Likewise, insertion into intergenic regions of the rRNA operon have no effect. All sites show strong linkage to the selectable markers and are recovered in homoplasmic form in subclones of the original transformant. Nearly complete replacement of the 6.2 kb region of homology in pJS75 is the most common event. Stable integration of chimeric reporter gene constructions into plastids of *N tabacum* is being tested by selection for the flanking antibiotic resistance markers.

POSTER ABSTRACTS

1995 EVIDENCE FOR A RecA-LIKE PROTEIN IN CHLOROPLASTS OF *Pisum sativum* L. AND *Arabidopsis thaliana* L. Heriberto Cerutti and Andre T. Jagendorf. Cornell University, Section of Plant Biology, Ithaca, New York 14853.

Several lines of evidence suggest that recombination and related processes, such as gene conversion, occur in the chloroplast genome of higher plants. However, our understanding of the biochemical mechanism(s) involved is still extremely limited. A relationship between the chloroplast recombination system(s) and its eubacterial (particularly cyanobacterial) counterpart would be expected, considering the endosymbiotic origin of plastids. We found a DNA strand transfer activity in Triton X-100 permeabilized chloroplasts and in crude stromal extracts from pea. This activity was similar to that of the purified RecA protein from *E. coli*. Fractionation of pea and Arabidopsis protoplasts showed the presence of four proteins in the chloroplast fraction crossreacting with 2 antibodies raised against *E. coli* K12 RecA protein. The most intense band (apparent MW 36.5 KDa) is slightly smaller than RecA and is uniquely recognized by an affinity purified antibody. Its steady state level is enhanced by DNA damaging agents like mitomycin C, nalidixic acid or UV irradiation. In addition, it binds ssDNA and it comigrates with the recombinant molecules in band shift assays. This protein has been partially purified and shown to be in a multimeric state in its native conformation. All the evidence is consistent with it being a RecA analogue presumably involved in chloroplast DNA recombination and repair. By Southern hybridization, sequences homologous to the *Synechococcus* recA gene were identified in the nuclear genome of pea.

1996 CHLOROPLAST GENE TRANSFER VIA RECOMBINATION AND TRANSFORMATION IN HIGHER PLANTS. Gábor V. Horváth, Carmel O'Neill, Philip J. Dix and Péter Medgyesy. Biological Research Center, H-6701 Szeged, Hungary

Green cybrid plants with a new nucleus-chloroplast combination have not been selectable after protoplast fusion between Nicotiana and Solanum species (from different subfamilies in the Solanaceae). A limited chloroplast gene transfer via recombination, however, overcame this incompatibility as was demonstrated by the production of normal N. tabacum plants with a plastome of a stable mosaic of N. tabacum and S. tuberosum chloroplast genomes. Physical mapping of the entire recombinant plastid genome revealed the formation of 18 recombinations scattered along the plastome. The inverted repeat regions showed identical recombination patterns. Detailed analysis of the recombination sites indicated both homologous (predominantly in the inverted repeat regions) and illegitimate (predominantly in the large single copy region) crossovers. This "potacco" plastome proved to be stable in two successive backfusion experiments. Spontaneous plastid fusion and subsequent particular integration of potato genes resulted in this recombinant plastome fully compatible with the tobacco nuclear genome while containing numerous plastid genes from a somatically/sexually incompatible species. In an analogous manner direct transfer of a defined plastid gene has also been attempted. A cloned 16S rRNA gene containing mutations coding for a double antibiotic resistance was introduced by simple polyethylene glycol mediated DNA uptake into N. plumbaginifolia protoplasts. Putative chloroplast DNA transformants were selected by an expression of spectinomycin resistance, and identified with a test for the unselected streptomycin resistance. A comparative molecular analysis of the integration sites after recombination or transformation mediated plastid gene transfer is in progress.

1997 A SELECTABLE MARKER FOR CHLOROPLAST REVERSE GENETICS IN CHLAMYDOMONAS : TRANS-SPLICING (tscA) AND PHOTOSYSTEM I (psaC) MUTANTS.

M.Goldschmidt-Clermont, Y. Takahashi and J.-D. Rochaix. Departements of Molecular Biology and of Plant Biology, University of Geneva, Sciences II, 1211 Geneva 4, Switzerland.

The E.coli *aadA* gene (amynoglycoside adenyl transferase) was inserted in a chloroplast expresssion cassette and introduced into *Chlamydomonas reinhardtii* by particle gun bombardment. The chimeric *aadA* gene is stably expressed in the transgenic chloroplast and confers spectinomycin resistance to the transformed cells. It can be used as a portable selectable marker for transformation and also as a reporter gene. Gene disruption mutants of photosynthetic genes can thus be directly selected. Disruption mutants of the *psaC* gene, coding for the 9 kD subunit of PS I with the F_A and F_B iron sulfur centers, show a complete loss of photosynthetic activity. Other subunits of PS I are subject to post-translational degradation so that they also fail to accumulate in these mutants. The 9kD subunit is thus required for the stable assembly of PS I. The *tscA* gene encodes a small chloroplast RNA required *in trans* for the *trans*-splicing of *psaA* exons 1 and 2 (1,2). Disruptions of *tscA* can lead to a complete block of *psaA* mRNA maturation depending on the site of insertion and the orientation of the *aadA* cassette. Experiments are in progress to introduce site-specific mutations in the genes to explore structure-function relationships.

(1) Choquet et al. (1988) Cell 52, 903-913.
(2) Goldschmidt-Clermont et al. (1991) Cell 65, in press.

1998 CHLOROPLAST TRANSFORMATION OF rbcL IN *CHLAMYDOMONAS REINHARDII*. Günter F. Wildner, Genhai Zhu, Richard G. Jensen and Richard B. Hallick. University of Arizona, Department of Biochemistry, Tucson AZ 85721

An rbcL gene with a nonsense mutation in *Chlamydomonas reinhardii* chloroplast DNA was transformed to a functional gene by bombardment with tungsten microprojectiles coated with plasmid DNA carrying the rbcL gene. This plasmid originated from the temperature-sensitive missense mutant 68-4PP (R. J. Spreitzer et al, 1988, Plant Physiology 86:773-777), which requires acetate for growth at 35°. All transformants showed both a full-sized Rubisco large subunit in SDS gel electrophoresis, and temperature sensitivity at the non-permissive growth temperature. Randomly selected clones of transformants were analyzed by DNA sequencing. All clones showed the same change in amino acid residue Leu-290 (CTT) to Phe (TTT) as the parental plasmid 68-4PP. The RuBP carboxylase/oxygenase specificity factor was 42 for the transformants as well as for the mutant, 68-4PP. The wild-type specificity factor was 64. A new method was developed for the determination of the specificity factor, based on the specific radioactivity of PGA formed in the reaction. An additional reaction product was detected and identified by HPLC as xylulose-1,5-bisphosphate, which accumulated significantly during the reaction with the purified RuBP carboxylase isolated from the transformants. We propose that the change of the amino acid residue Leu-290 to Phe causes a steric strain on the large subunit and this distortion results in the improper protonation of the activated enediol form of RuBP forming subsequently xylulose-1,5-bisphosphate.

1999 CHLOROPLAST ENCODED CHLOROPHYLL BIOSYNTHESIS GENE(S) OF *Chlamydomonas rheinhardtii*. Jon Y. Suzuki and Carl E. Bauer. Department of Biology, Indiana University, Bloomington, IN 47405

In angiosperms, the production of chlorophyll involves the enzyme PCR which utilizes light energy to catalyze the reduction of protochlorophyllide to chlorophyllide. In contrast, "dark greening" organisms such as gymnosperms and algae, as well as photosynthetic bactena have an additional light-independent enzyme which catalyzes a similar reaction. We have recently identified the *bchL* gene in the bacterium *Rhodobacter capsulatus* that encodes one of the subunits of the dark-driven protochlorophyllide reductase. *bchL* has striking homology to *frxC*, a *Marchantia polymorpha* chloroplast gene of previously unknown function and which is conspicuously absent from a number of angiosperms which have been examined. In this study, a site-directed mutational analysis of *frxC* was undertaken to determine whether it also encodes a subunit of the dark-driven protochlorophyllide reductase of plants. Toward this end, we have isolated a homolog of *frxC* in the chloroplast genome of *C. rheinhardtii* which has up to 80% sequence identity to the *M. polymorpha frxC* gene. A plasmid containing selectable markers as well as a plasmid containing a mutated copy of *frxC* were cotransformed into *C. rheinhardtii* using the particle gun transformation technique. RFLP analysis has revealed that 90% of the transformants were cotransformed for the mutated *frxC* and that approximately 50% are homoplastic. The transformants which are homoplastic for the mutant copy of *frxC* are yellow in the dark, but are green in the light. We are currently analyzing the expression of *frxC* in a number of nuclear mutants of *C. rheinhardtii* which exhibit the same yellow in the dark phenotype to check whether or not nuclear genes are involved in *frxC* expression. In addition, sequence information at a second locus *bchB*, which is required for dark-driven protochlorophyllide reduction in *R capsulatus* indicates the existence of at least one other subunit, and this subunit has homology to ORF 465 of *M. polymorpha* chloroplast.

2000 SELF-SPLICING AND MOBILITY OF A GROUP I INTRON IN THE CHLAMYDOMONAS REINHARDTII CHLOROPLAST. F. Dürrenberger and J.-D. Rochaix. University of Geneva, Departments of Molecular Biology and Plant Biology, Geneva, Switzerland

The chloroplastic 23S ribosomal RNA gene of the green unicellular alga Chlamydomonas reinhardtii harbours a group I intron which contains an internal open reading frame (ORF). A precursor encompassing the intron with its 5' and 3' flanking sequences was shown to undergo self-splicing during in vitro transcription resulting in an excised intron and joined exons. Isolated precursor, corresponding to the full-length in vitro transcript, is capable of self-splicing in vitro also in the absence of protein factors.

When the internal ORF was expressed in E. coli in the presence of a second plasmid which contained a cDNA corresponding to the intronless form of the 23S rRNA gene, specific cleavage close to the exon-exon-junction was observed.

Chloroplasts of C. reinhardtii were transformed with the same cDNA as a model for an intronless copy of the 23S rRNA gene. All the transformants which had the cDNA integrated at the expected site in the chloroplast genome had also the ribosomal intron precisely inserted into this cDNA.

These experiments show clearly that the intron of the chloroplastic 23S rRNA gene of C. reinhardtii behaves as a ribozyme at the RNA level and as a mobile genetic element at the DNA level.

POSTER ABSTRACTS

2001 STUDIES ON TRANSFORMATION AT THE CHLOROPLAST psaB LOCUS OF CHLAMYDOMONAS REINHARDTII. Scott E. Bingham & Andrew N. Webber, Department of Botany and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, AZ 85287.

A chloroplast photosystem I mutant of Chlamydomonas reinhardtii ac-u-g-2.3, has been transformed with a wild type psaB gene to restore photosynthetic competence. The mutation in psaB was localized in the coding sequence by complementation using subcloned restriction fragments of psaB. Transformation frequencies in the range of $1x10^{-5}$ to $1x10^{-6}$ were obtained using psaB cloned in pUC19. However, these frequencies were reduced by approximately one half when psaB was introduced in single or double stranded M13mp19. In order to test the feasibility of inserting site directed mutations into psaB, we have complemented the mutant strain with a psaB gene carrying a silent mutation creating a new StuI restriction site in transformed cells. Analysis of transformants using PCR and Southern hybridizations indicates that the site directed mutant gene has stably inserted into all copies of chloroplast DNA. Co-transformation of this gene with a plasmid carrying ribosomal DNA resistance markers against streptomycin and spectinomycin (Newman et al.1990 Genetics 126: 875-888.) has also been achieved, suggesting that mutations in psaB which confer a non-photosynthetic phenotype to transformed cells can be selected by this method.

2002 DIATOM PLASMIDS HAVE CHARACTERISTICS OF BACTERIAL TRANSPOSONS. Mark Hildebrand, Patricia Hasegawa, Robin W. Ord, and Benjamin E. Volcani. Scripps Institution of Oceanography, and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093-0202. In collaboration with V. Samantha Thorpe and Charles Glass (Scripps Clinic and Research Foundation, La Jolla, CA 92037), we have sequenced two small circular plasmids, pCf1 (4.27 kbp) and pCf2 (4.08 kbp), found in the diatom Cylindrotheca fusiformis. Available evidence suggests that the plasmids are located in the chloroplast. pCf1 encodes 5 open reading frames (ORFs) and pCf2 encodes 6 ORFs. Two ORFs are similar in the two plasmids; ORF218 (pCf1) and ORF217 (pCf2) share 80% amino acid identity and ORF482 (pCf1) and ORF484 (pCf2) share 54% amino acid identity. ORF218/217 share 29% amino acid identity with the resolvase protein encoded by Tn3-family bacterial transposons. Resolvases have only been identified in bacterial transposons and are responsible for the resolution of a transposon genomic co-integrate. The majority of amino acid residues conserved in all resolvases are found in ORF218/217, including the active site serine. Both plasmids pCf1 and pCf2 hybridize with several restriction fragments of chloroplast DNA, and pCf2 also hybridizes with nuclear DNA. These results suggest that the plasmids contain transposons which have integrated into the chloroplast genome, and in the case of pCf2, the nuclear genome as well.

2003 PRELIMINARY *IN SITU* LOCALIZATION STUDIES ON THE PLASMID GC2 IN THE RED ALGA *GRACILARIA CHILENSIS*. Rama K. Singh, Michael Reith and Lawrence Fritz. NRC-Institute for Marine Biosciences, Halifax, NS., Canada. A 3.8Kb cryptic plasmid (GC2) from the red alga *G. chilensis* has been previously isolated, cloned and sequenced (Villemur (1990) Plant Mol Biol 15: 237-243). This plasmid was shown to contain a large open reading frame (ORF411) and to be transcriptionally active. However, the cellular location of this plasmid (nuclear or organellar) remains undefined. As we are interested in using this plasmid as a possible transformation vector, *in situ* hybridization experiments were carried out to address these questions. ^{35}S-radioactive transcripts of each strand of the entire plasmid were generated *in vitro* utilizing the T3 and T7 promotors of Bluescript SK$^+$. The transcripts were hydrolized to produce fragments 150-200 bp in length and were hybridized to 5µm paraffin sections of *G. chilensis*. Preliminary results confirm that the plasmid is transcriptionally active and indicate that *in vitro* transcripts antisense to ORF411 hybridize more strongly to *G. chilensis* cells than sense transcripts. In addition, these results indicate that the GC2 plasmid is of red-algal origin and not of associated bacteria or green algae. Studies at the EM level are underway to determine the cellular location of this plasmid in *G. chilensis*.

2004 STABLE MAINTENANCE OF EPISOMAL DNA IN THE CHLOROPLAST OF *Chlamydomonas reinhardtii*. Karen L. Kindle* and David B. Stern†, *Plant Science Center and †Boyce Thompson Institute, Cornell University, Ithaca, New York 14853.

Mutations that remove the 3' untranslated region of the *Chlamydomonas* chloroplast *atp*B gene, including a region that has the potential of forming a stable stem-loop structure, prevent the accumulation of a discrete size class of *atp*B mRNA This truncated gene functions well enough to permit phototrophic growth, but *Chlamydomonas* chloroplast transformants carrying such mutations do not grow well phototrophically, accumulating only about 30% of wild-type levels of *atp*B mRNA. The *atp*B mRNA is heterogeneous in size, and accumulation of the β subunit of chloroplast ATPase is only half of wild-type levels (Stern, Radwanski, and Kindle, *Plant Cell* 3: 285-297). Some transformants that had been bombarded with the deleted stem-loop *atp*B gene grew better than the majority class under selective conditions, and analysis of these strains indicates that they transcribe the gene at a high rate, ranging from 2-5 fold higher than wild-type. Although they still do not accumulate a discrete size mRNA, the accumulation of RNA and protein are at or above normal levels. The increased transcription appears to be due to the high copy number of the transforming plasmid in the chloroplast compartment. Quantitative DNA blot analysis indicates that in some variants there are >500 copies of the plasmid per chloroplast. This poster will describe the characterization of these transformants, including the form of the plasmid DNA copies and additional recombination events in the chloroplast genome.

2005 REGULATION OF CHLOROPLAST MATURATION AND SENESCENCE IN PRIMARY FOLIAGE LEAVES OF BARLEY. Karin Krupinska and Angela Thomsen, Institut fur Allgemeine Botanik der Universitat Hamburg, Ohnhorststraße 18, D-2000 Hamburg 52, FRG.

Leaves of monocots like barley are particularly well suited to study chloroplast differentiation during maturation and senescence. Cell division occurring only in a basal meristem results in a developmental gradient of cells and homogeneous plastid populations with increasing age from the base to the tip. Taking advantage of this arrangement we used leaf sections cut at various distances from the leaf base to study chloroplast maturation and senescence.

By this approach we could show that maturation of chloroplasts in expanding primary foliage leaves of barley seedlings grown under a daily light-dark regime depends on the age of the corresponding cells and is characterized by an increase in photosynthetic capacity and specific changes in the transcription of the plastid genome. After leaf expansion is complete the photosynthetic capacity decreases and senescence specific processes get going.

In order to test whether senescence like maturation depends on the age of the cells and is sequentially induced within a leaf or instead is affecting the entire leaf, the decline in photosynthesis, chlorophyll and protein content, and in the expression of photosynthesis specific genes was studied in various leaf segments during aging of fully expanded leaves. The results suggest that senescence affects cells of different age simultaneously and thus refers to the entire leaf. Experiments with the aim at identifying factors involved in senescence induction are discussed.

2006 PROTEIN TARGETING IN CYANOBACTERIA. Dawn Smith, Derek.S.Bendall and Christopher.J.Howe. Department of Biochemistry,University of Cambridge,Tennis Court Road, Cambridge, CB2 1QW, UK.

Cyanobacteria are prokaryotes capable of oxygenic photosynthesis. They contain two distinct membrane systems;the thylakoid membranes which are analogous to the thylakoid membranes of higher plants,and the plasma membrane. These two membrane systems have clearly distinct polypeptide and pigment compositions. It is possible to separate them by sucrose density gradient centrifugation. The distribution of a number of polypeptides (cytochrome aa_3 oxidase and components of photosystem II) is being studied with the aim of elucidating the mechanism of polypeptide targeting.

POSTER ABSTRACTS

2007 MUTATIONAL ANALYSIS OF THE PHOTOSYSTEM II MANGANESE-STABILIZING POLYPEPTIDE I. B. Philbrick, K. S. Century and B. A. Zilinskas. Dept Biochem & Microbiol, Rutgers University, New Brunswick, NJ, 08903.

Photosynthetic water oxidation is catalyzed by a complex of proteins known as Photosystem II (PS II), located in the thylakoid membrane. It requires the inorganic cofactors Mn^{2+}, Ca^{2+} and Cl^-. We are investigating the role of the manganese-stabilizing polypeptide (MSP) in PS II function by the specific mutation of *psbO*, the gene which encodes it, in the cyanobacterium *Synechocystis* sp. PCC 6803. Several types of mutants have been constructed. Mutants lacking the polypeptide were obtained separately by both deletion and insertional inactivation of *psbO*. In these MSP$^-$ mutants, the PS II polypeptides D1, D2 and CP-43 accumulate to wild type levels, as determined by immunoblot analysis. These mutants are capable of photoautotrophic growth and light-induced water oxidation, but at a rate only 60-70% that of wild type. They exhibit a pronounced sensitivity to inhibition by high light intensity or Ca^{2+} depletion, but not by Cl^- depletion. To identify functionally significant amino acid residues and domains of MSP, we have used oligonucleotide-directed site-specific mutagenesis to engineer amino acid substitutions and deletions in the protein. Specific residues of MSP were selected based on their conservation in the seven reported MSP sequences and on their potential for interacting with the other PS II polypeptides, the Mn cluster, and Ca^{2+}. Results from structural and functional analyses of the various mutants will be presented.

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2008 ROLE OF OSMOTIC STRESS INDUCED PROTEINS IN TOLERANCE OF CYANOBACTERIA TO OSMOTIC STRESS. Shree K. Apte, T.A. Fernandes and V.Iyer. MB & AD, B.A.R.C., Bombay-400085

Effect of hyperosmotic stress on modification of protein syntheses and its correlation with observed tolerance/sensitivity have been examined using a cyanobacterial model system. Anabaena L-31, a nitrogen fixing, fresh-water strain exhibits a unique combination of high osmotolerance with high salt sensitivity. Exposure of this strain to osmotic stress(sucrose,0.35M) results in rapid and selective synthesis of certain peptides, called osmotic stress proteins or OSPs, in the 12-50kDa molecular mass range. Exposure to salt(NaCl,0.125M) elicits all the OSPs and 2 more peptides(salinity stress proteins, or SSPs). Expression of OSPs and SSPs is facilitated by transcriptional activation of certain genes. Presence of low levels of nitrate(10 mM)or ammonium(3 mM) confer significant protection from salt stress. Presence of nitrate, but not ammonium, also enhances the osmotolerance. Both nitrate and ammonium rapidly and efficiently inhibit Na^+ influx during salt stress but are not themselves accumulated as internal osmoregulators. Nitrate, per se, induces the synthesis of almost all the OSPs. Ammonium fails to evoke such a response. Previous exposure of cultures to permissive(sub-lethal) levels of salt or sucrose results in higher tolerance to subsequent exposure to non-permissive levels of these stresses. Permissive levels allow synthesis of OSPs, do not inhibit general protein synthesis and do not affect growth. OSP induction in nitrate cross-protected and pre-adapted cultures suggest their role in osmotolerance.

2009 CLONING OF SALINITY INDUCED GENES FROM A DIAZOTROPHIC HALOTOLERANT CYANOBACTERIUM. Shree K.Apte* and Robert Haselkorn**. *BB&AD,BARC,Bombay; **MGCB,U.Chicago,Chicago

Salt (NaCl) tolerance of a heterocystous, nitrogen fixing, salt tolerant cyanobacterium Anabaena torulosa has been characterized. Na^+ exclusion, achieved by reduced influx and active efflux of Na^+, forms the basis of salt tolerance of this strain. The inherent ability to tolerate salt stress can be further modified by environmental factors which modulate transmembrane Na^+ fluxes. High salinity inhibits nitrogen fixation due to diversion of energy from nitrogenase to osmoregulation, especially Na^+ efflux. Factors which prevent such diversion of energy enhance salinity tolerance and protect nitrogen fixation during salinity stress. Exposure to salt stress induces rapid and selective expression of salinity stress proteins (SSPs) by transcriptional activation of certain genes. A subtractive RNA hybridization technique has been developed to detect genes whose transcription is induced by salt stress. Screening of 4,500 clones from a genomic library of Anabaena torulosa has identified 11 cosmids which harbor >90% of the salinity stress induced genes in the chromosome.Estimates show that nearly 100 kb DNA (100 genes or 1.5% of the genome) of this strain may be expressed during exposure to salt stress. Further characterization of these cosmids in terms of detection of structural and functional homologs of Escherichia coli salt tolerance genes, identification of genes encoding Anabaena torulosa salinity stress proteins, and isolation of tentative salt stress induced Anabaena promoters will be described.

2010 THE OPERON rbc AND ITS GENOMIC REGION ARE INVOLVED IN THE ABILITY OF CYANOBACTERIA TO GROW UNDER LOW-CO_2. Schwarz R., M. Hassidim, J. Lieman-Hurwitz, M. Ronen, L. Reinhold, A. Kaplan. The Hebrew University, 91904 Jerusalem, Israel.

Modification of rbcS (encoding the small subunit of rubisco, SSU) in Synechococcus PCC7942 resulted in a high-CO_2-requiring (HCR) mutant, EK6, with a 17 kDa rather than a 14 kDa SSU. The in situ level of activation of the modified rubisco in mutant cells exposed to low (but not to high) inorganic carbon (Ci) was significantly lower, presumably accounting for the 100-fold lower apparent photosynthetic affinity for Ci. The photosynthetic characteristics of the HCR mutants D4 and R14, on the other hand, were similar to those of high-CO_2 grown wild type cells. These mutants were raised by modification of an open reading frame (ORF) immediately downstream of rbc. Sequence analysis of this ORF indicated high homology with purK from Bacillus subtilis (encoding phosphoribosylaminoimidazole carboxylase subunit II, involved in the synthesis of inosine monophosphate, IMP). When D4 and R14 were supplied with IMP they were able to grow in the presence of low CO_2. The mutations in four other HCR mutants were also mapped in the region of rbc. Northern analysis indicated that the abundance of the transcript from rbc increased following the exposure of the cells to low-CO_2. Our data suggest the presence of a cluster of genes near and including rbc, involved in the ability of Synechococcus to grow under low ambient Ci.

2011 CYTOKININ PRODUCTION BY THE CYANOBACTERIUM AGMENELLUM QUADRUPLICATUM PR6. W. A. Mulroy, B. J. Taller and S. E. Stevens. Memphis State Univ., Biology Dept. Memphis, Tennessee 38152.

Cytokinins are N^6-substituted adenine derivatives which function as hormones in plants. They occur both as free molecules and as constituents of tRNA. As the production of free cytokinins is thought to be limited to plant-associated microbes, it was of interest to examine cytokinin synthesis by the marine cyanobacterium Agmenellum quadruplicatum PR6. Cytokinins were isolated from the culture medium of an early stationary phase culture by chromatography on XAD-2, Sephadex LH-20 and phenylboronate agarose. Cytokinins were analyzed with the tobacco callus bioassay, HPLC and enzyme immunoassay. Two cytokinin-active compounds were detected and tentatively identified as isopentenyladenosine (IPR) and methylthioribosylzeatin (msZR) on the basis of chromatographic properties, UV scan and immunoassay. The total cytokinin activity was equivalent to 3.8 ug/L kinetin. To our knowledge this is the first report of cytokinin production by a cyanobacterium. Investigation of the biosynthetic origin of these compounds is in progress. As there is evidence for the subcellular localization of msZR and IPR in plant chloroplast tRNA, the cytokinin content of Agmenellum tRNA will be presented. The relationship of these results to the endosymbiotic origin of chloroplasts will be discussed.

2012 DNA SYNTHESIS IN THE CYANOBACTERIUM *SYNECHOCYSTIS* 6803. X. Yang, H. Daniell, and B. A. McFadden. Washington State University, Department of Biochemistry and Biophysics, Pullman, WA 99164-4660. Studies of DNA replication in cyanobacteria has been limited, although this phenomenon has been well investigated for chloroplasts in higher plants, with which cyanobacteria share many common characteristics. We have characterized DNA replication in *Synechocystis* 6803 by permeabilizing the cells with either L-α-lysophosphatidylcholine (LPC) or lysozyme/EDTA. Permeability of the treated cells was evidenced by the incorporation of added ^{32}P-TTP into DNA. The ^{32}P-TTP incorporation in cells permeabilized by either method was appreciably higher than that in untreated cells, and increased linearly with time for about 4 hrs. In addition, treated cells became permeable to proteins such as DNase I and micrococcal nuclease. More significantly, however, lysozyme/EDTA treated cells (permeaplasts) not only incorporated TTP more efficiently than LPC-treated cells, but also were capable of uptake and replication of exogenously-supplied cyanobacterial plasmids or pXY 205, one of several recombinant plasmids with a *synechocystis* plasmid fragment, which has been also shown to replicate actively in an *in vitro* replication system that we have developed (unpublished). This capacity of *Synechocystis* permeaplasts to replicate exogenous DNA should enable us to identify DNA replication origins and their related regulatory sequences in cyanobacteria. Supported in part by NIH grant GM-19, 972.

POSTER ABSTRACTS

2013 LIGHT REGULATION OF *psbA* GENE EXPRESSION IN THE CYANOBACTERIUM *SYNECHOCYSTIS* 6803.

Mohamed Abdalla, Jan Eriksson and Christer Jansson. Department of Biochemistry, The Arrhenius Laboratories, University of Stockholm, S-106 91 Stockholm, Sweden.

We are trying to identify and characterize the mechanisms involved in light regulation of *psbA* gene expression in the cyanobacterium *Synechosystis* 6803. The *psbA* gene family in *S.* 6803 contains three members, *psbA*-1, *psbA*-2 & *psbA*-3 [1]. Construction of mutants with only one active *psbA* gene showed that either of *psbA*-2 or *psbA*-3 has the capacity to produce wildtype levels of transcripts and that transcription of both genes is light-stimulated [2]. No transcripts could be detected from the *psbA*-1 gene. Primer extension analyses revealed that ~90% of the steady state *psbA* transcript levels in the wildtype originated from *psbA*-2. Inactivation of *psbA*-2 resulted in an 8-fold increase in *psbA*-3 transcript levels whereas inactivation of *psbA*-3 resulted in a 2.5-fold increase in *psbA*-2 transcript levels.

The light-stimulated accumulation of *psbA* transcripts was independent of the photosynthetic electron transport [3]. In comparison, the accumulation of *rbcL-S* transcripts, which encode the large and small subunits of the Calvin-Benson cycle enzyme ribulose 1,5-bisphosphate carboxylase, required either an active photosynthetic electron transport or glucose.

The *psbA* transcripts were remarkably stable in the dark, with a $t_{1/2}$ of ~7 hours [3]. This should be compared to some minutes for a typical prokaryotic transcript. The half-life under illuminated conditions was ~15 minutes. When inhibitors were used to shut down the photosynthetic electron transport in the light the *psbA* transcript stability was comparable to that in darkness [3]. Degradation of the *psbA* transcripts in darkness proceeded through the accumulation of a degradation product not observed under normal illuminated conditions.

We are now furher studying the role of transcript production and transcript stabilty in the regulation of the *psbA* gene family

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2014 MOLECULAR ANALYSIS OF AN EXTRACELLULAR PROTEIN FROM A CYANOBACTERIUM.

Janet P A Varley, Paul Nicholson and Christopher J Howe. Biochemistry Department, University of Cambridge, Tennis Court Rd., Cambridge CB2 1QW, UK.

A polypeptide of molecular weight 37kDa is found in the extracellular medium of *Phormidium laminosum* grown in liquid culture. This polypeptide has been purified by gel electrophoresis, digested using trypsin and a partial amino acid sequence determined. The amino acid data have been used to derive two degenerate oligodeoxynucleotides. These have been used to probe a *P. laminosum* genomic library for the gene(s) encoding the polypeptide. Several independent clones have been identified and DNA sequence analysis is in progress. Additional work has been carried out to establish that the polypeptide is genuinely extracellular, rather than being associated with cellular debris. The polypeptide has also been partially purified by gel filtration, and analysed for possible protease or nuclease activity.

2015 COMPONENTS FOR HIGH-AFFINITY Fe UPTAKE IN CYANOBACTERIA.

Malcolm Potts and Kerthanne Mockaitis.
Virginia Tech., Department of Biochemistry, Blacksburg, Virginia 24061

A 3414-bp fragment of DNA from *Nostoc commune* UTEX 584 carries a gene (*iphP*) encoding a secreted cell-surface phosphatase of Mr = 33,000 and with an apparent pH dependence of pH 7.4. *iphP* can be expressed in *E. coli* where the gene product accumulates in the periplasmic space. The phosphatase shows no correspondence with known prokaryotic phosphatases, however, marked sequence similarity to a highly-conserved region of eukaryotic protein tyrosine phosphatases is evident. Structural studies of *iphP* and its flanking regions, including deletion analysis and *in-vitro* translation of sense and antisense transcripts, confirmed the boundaries of the gene and revealed a number of unusual features:

1. The DNA strand opposing the *iph* coding sequence represents an ORF (*iphK*) of virtually identical size to *iphP*. The derived amino acid sequence of *iphK* shows similarity to known eukaryotic kinases.
2. Repeated sequences, with the potential to form very stable secondary structures, effectively bracket the *iphP* and *iphK* coding regions.
3. Two ORF's - for a membrane-bound Fe-receptor (IphA) and a Fe-transporter (IphB) occur upstream and downstream, respectively, of *iphP* and are both orientated (5' to 3') in the same direction as *iphP*.

A discussion of the relationships between, and functions of, these proteins will be presented for critical analysis.

2016 RAPD MARKERS IN PLANT GENETICS

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The use of DNA markers in genetic mapping, genetic diagnostics, molecular taxonomy, and evolutionary studies has been well established. The most commonly used DNA marker is the restriction fragment length polymorphism (RFLP). Detection of RFLPs by Southern blot hybridization is laborious and incompatible with applications requiring high analytical throughput. Genetic tests based on the polymerase chain reaction are simple to perform, but require target DNA sequence information in order to design specific primers. We have recently described [Nucleic Acids Res. 18, 6531-6535(1990)] a novel class of genetic marker which is based on the DNA amplification reaction, but requires no knowledge of target DNA sequence. These markers, called RAPD™ markers (for Random Amplified Polymorphic DNA), are generated by amplification of random target DNA segments with single primers of arbitrary nucleotide sequence. RAPD™ markers can be used for genetic mapping applications, population studies, fingerprinting and for genetic diagnostics. These markers can easily be converted to classical RFLP markers and can be thought of as sequence tagged sites. The non-radioactive assay requires nanogram quantities of DNA and is applicable to a broad range of species. In this communication we describe several techniques that enhance the value of RAPD™ markers. Design of RAPD™ experiments, including optimization of reaction conditions and choice of mapping populations will also be discussed. Examples will be drawn from corn and soybean mapping.

2017 PRIMARY STRUCTURE OF PHYCOCYANIN FROM A THERMOPHILIC CYANOBACTERIUM.

T. Shimazu, K. Miura, M. Soga, M. Hirano and *S. Katoh.
Toray Res. Center Inc. Tebiro, Kamakura 248, *Univ. of Tokyo, Hongo, Bunkyo-ku 113, Japan

Phycocyanin is a light harvesting biliprotein which captures radiant solar energy and transfers it to photosystem II reaction center in cyanobacteria and red algae. Here we report the primary structures of α- and β-subunits of phycocyanin(PC) from the thermophilic cyanobacterium, *Synechococcus elongatus*. The amino acid sequences were determined by automated Edman degradation of protease digested peptides. The nucleotide sequence for β-subunit was also determined with PCR amplified fragments which were obtained using its amino and carboxyl terminal oligonucleotide primers. Both sequential results are completely coincided. The α- and β-subunits consist of 162 and 172 amino acid residues, respectively. One chromophore binds to Cys-84 in PC-α and two chromophores bind to Cys-82 and Cys-153 in PC-β. A post-translationally modified residue γ-*N*-methylasparagine was found in the β-subunit at position 72. Comparison of the sequences of two subunits from *S. elongatus* phycocyanin with those of the corresponding subunits from other 7 organisms shows homologies ranging from 67 to 84%. The sequence homology between *Synechococcus* phycocyanin and those from two other thermophilic organisms is relatively high (79-84%). On the other hand, several unique variations of amino acids were found in the primary structures of the *Synechococcus* proteins, which show a significant thermostability. This work was supported by New Energy & Industrial Technology Development Organization (NEDO).

2018 ANALYSIS OF *ANABAENA* PCC 7120 PHOTOSYNTHESIS GENE PROMOTERS.

James A. Martin, Richard A. Whitaker and Stephanie E. Curtis, NCSU, Department of Genetics, Raleigh, NC 27695

We are interested in the identification gene promoters from the cyanobacterium *Anabaena* sp. strain PCC 7120 as tools to study the regulation of gene transcription during heterocyst development. Examination of sequences 5' to the transcription initiation sites of a collection of photosynthesis genes revealed a conserved region at -10 nucleotides, but no consensus sequence further upstream. To define the sequences required for transcription in vegetative cells, we performed a series of experiments designed to functionally identify *Anabaena* vegetative cell promoters. These experiments made use of a shuttle vector in which transcriptional fusions can be made to a promoterless reporter gene encoding chloramphenicol acetyl transferase (CAT). A series of fragments of varying lengths which include the transcription start sites and 5' proximal sequences from the *rbc*L, *atp*1, *atp*2, *pet*F1 and *psb*AI photosynthesis gene operons were cloned in front of the CAT gene. Following mobilization of these constructs into *Anabaena*, expression of CAT was measured by both activity and antibody assays. For each operon, fragments containing less than 200 base pairs upstream of the transcription start sites were able to support accurate transcription initiation. Fragments containing only 39 base pairs upstream of the *psb*AI transcription start site and 100 base pairs upstream of the *rbc*L start site were able to support accurate transcription initiation and expression of CAT. Expression of CAT was much greater from fusions with the *rbc*L 5' proximal sequences than from fusions with sequences 5' to the other operons.

POSTER ABSTRACTS

2019 ISOLATION AND CHARACTERIZATION OF A DNA TOPOISOMERASE I FROM ANACYSTIC NIDULANS AND PRELIMINARY CHARACTERIZATION OF ITS GENE. Patricia McCormick, Bernice Smith, Gordon Cannon and Sabine Heinhorst. University of Southern Mississippi, Department of Chemistry and Biochemistry, Hattiesburg, Mississippi 39406

DNA topoisomerases are enzymes that play a vital role in DNA replication, transcription, recombination and gene expression. We have isolated and characterized a topoisomerase from the cyanobacterium Anacystis nidulans R2. The enzyme relaxes negatively, but not positively supercoiled DNA in the presence of a divalent cation. Taken together with its inability to introduce supercoils into a relaxed template and its insensitivity to the topoisomerase II inhibitors nalidixic acid and novobiocin, these features suggest that the cyanobacterial enzyme is a topoisomerase I. Preliminary studies have identified the putative gene encoding the cyanobacterial topoisomerase I by heterologous hybridization with the cloned E. coli top A probe. Further studies will involve cloning and characterization of the gene

2020 A DNA POLYMERASE FROM THE CYANOBACTERIUM ANACYSTIC NIDULANS R2. Hsiu-Jin Lin, Gordon Cannon and Sabine Heinhorst. University of Southern Mississippi, Department of Chemistry and Biochemistry, Hattiesburg, Mississippi 39406

Cyanobacteria are the only known prokaryotes capable of oxygen evolving photosynthesis and they represent the most likely ancestors to modern photosynthetic organelles of eukaryotic algae and higher plants. A DNA polymerase has been highly purified and characterized from the cyanobacterium Anacystis nidulans R2. The enzyme has demonstrated several common features with E. coli DNA polymerase I, among them being its use of activated DNA as a preferred template and its resistance to N = ethylmaleimide, dideoxynucleotides, arabinosyl-CTP and aphidicolin. Like its E. coli counterpart, the cyanobacterial enzyme possesses both 3'→5' and 5'→3' exonuclease activities. Our current approaches to identify the gene encoding the enzyme will be discussed.

2021 Mu AS A GENETIC TOOL IN THE ANALYSIS OF THE GENE CLUSTERS AROUND THE rbc OPERON IN THE CYANOBACTERIUM Synechococcus PCC7942. Devorah Friedberg, Martin Kessel* and Nava Folkman. The Hebrew University, Department of Applied Microbiology and *Department of Membrane and Ultrastructure Research Jerusalem 91010, Israel.

The isolation and characterization of high-CO_2-requiring mutants of the cyanobacterium Synechococcus PCC7942 have led to the demonstration of open reading frames (orfs) around the rbc operon. We have recently used Mu derivatives for genetic analysis of the gene cluster around rbc. Mutagenesis using the Mu elements resulted in new high-CO_2 requiring mutants which do not contain any apparent carboxysome structures and exhibit a low photosynthetic affinity to CO_2. The generation of "carboxysome-less" mutants as a result of insertion mutagenesis will be further discussed in view of their possible role in photosynthesis. Transcriptional fusions of the Mu elements with the cyanobacterial sequences enabled analysis of mutative promoter sequences in the orfs around rbc. Sequence analyses revealed that the insertion of Mu elements into the cyanobacterial genome follow mechanisms similar to those described in E. coli.

This research was supported by a grant from the GSF/NCRD.

2022 PUTATIVE GYYCOGEN METABOLISM GENE ADJACENT TO NIFH* IN ANABAENA 7120 Barbara A. Cannell and Steven J. Robinson. University of Massachusetts, Department of Botany, Amherst, MA 01003

Adjacent to the second copy of the nitrogenase reductase gene *nifH* in the filamentous cyanobacterium *Anabaena* 7120 are three open reading frames, possibly transcribed as an operon, reading in the opposite direction of *nifH**. A database search of protein sequences detected homology between the amino acid sequence of one of the open reading frames (552 amino acids) and a group of proteins involved in the catabolism or synthesis of α-glucosidic bonds in starches, bacterial glycogen or other α-linked glucose polymers. These include bacterial glycogen branching and debranching enzymes, pullanases, α-amylases, cyclodextrin glucanotransferase, and an exodextranase, as well as two genes expressed in the salivary glands of *Drosophila* and mosquito. Each of the twelve genes chosen from among these for sequence analysis have approximately 25% amino acid identity with ORF1. Although these genes represent diverse species, range in size from 494 to 1444 amino acids and may be intracellular or secreted, it was possible to describe 11 regions that are conserved, each gene having at least 8 of these regions. Three of these sites have previously been identified in a wide range of α-amylases (as well as in some of those genes listed above) and implicated in substrate binding and/or enzyme activity. It is not evident from patterns of similarity or difference among these genes that any of these conserved regions are indicative of a particular function or substrate conformation. The common denominator among these genes appears to be the presence of α-linked glucose units in the polymers upon which they act. Experiments under way to determine timing and conditions of expression of ORF1 may help determine whether its function is synthetic or catabolic and whether the pattern of expression is related to that of nitrogen fixation genes.

2023 TRANSCRIPTION OF A CYTOCHROME P-450 ω-HYDROXYLASE AND OTHER OPEN READING FRAMES WITHIN THE *ANABAENA* 7120 *nifD* EXCISION ELEMENT. Sherry McLaughlin and Peter Lammers. Dept. of Chemistry, New Mexico State University, Las Cruces, N.M. 88003

An 11 Kb DNA element interrupts the *nifD* gene in most filamentous cyanobacteria and is excised from the chromosome during the differentiation of nitrogen-fixing heterocysts. The element is not necessary for normal growth or nitrogen fixation, yet is quite persistent during laboratory culture. Sequence analysis of 5,922 nucleotides within the *Anabaena nifD* excision element revealed three open reading frames (Lammers et al., [1990] J. Bacteriol. *172*, 6981). One of the open reading frames, ORF3, is highly homologous with members of the cytochrome P-450 family which encode steroid and fatty acid ω-hydroxylases. This finding suggests that the substrate for the *Anabaena* P-450 enzyme could be a lipid component. ORF2 is the most highly conserved of the three open reading frames in *Nostoc* and *Calothrix* genomes based on Southern hybridization. Recent experiments have demonstrated the three element-encoded ORF's are transcribed at low levels. Dot blot analysis of total RNA isolated from filaments at 3, 6, 9, 12, 15, 20, 24, 30 and 36 hours after removal of combined nitrogen indicated that all three ORFs are expressed constitutively at very low rates under laboratory growth conditions irrespective of nitrogen status. Steady-state levels of ORF 2 transcripts are the most abundant; 2 and 4 fold higher than ORF's 3 and 1, respectively, and approximately 1/50 of that of the *nifD* signal 36 hours after induction. The construction of orf-specific insertional mutants will be documented and discussed with respect to variations in lipid content. The most closely related bacterial P-450 ω-hydroxylase is induced at the transcriptional level by treatment with barbiturates (Wen et al., [1989] J. Biol. Chem. 264, 10996). The activity of a microsomal laurate hydroxylase from Jerusalem artichoke is also enhanced by phenobarbitol (Salaun et al., [1981] Eur. J. Biochem. 119, 651). The inducibility of the *Anabaena* P-450 is under investigation.

2024 STUDY OF A GENE INDUCED AT LOW TEMPERATURE IN A CYANOBACTERIUM, *ANABAENA* PCC7120.

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Mutant TLT2, obtained by insertion of a transposon with luciferase as a reporter, increases in luminescent intensity when transferred from 30 to 20°C (1). The addition of different types of antibiotics to the culture medium has shown that some of those which are known to have the ribosome as target lead to an increase of luminescence of TLT2 at 30°C, in accord with the idea that ribosomes could be sensors for cold shock (2). Rifampicin reduces luminescence at 20°C and decreases the rate of loss of luminescence upon transfer from 20 to 30°C. To find out what controls the mutated gene *tlt2*, we have replaced the transposon in the cells by a construction containing *lux* as a promoter probe but lacking most of the transposon. We are now proceeding to mutagenize the resulting strain with a new transposon to find genes whose products regulate the response of *tlt2* to temperature.

(1) Wolk C.P., Y. Cai and J.-M. Panoff (1991). Proc. Natl. Acad. Sci., in press.
(2) VanBogelen R.A. and F.C. Neidhardt (1990). Proc. Natl. Acad. Sci., 87:5589-93.

POSTER ABSTRACTS

2025 THE MITOCHONDRIAL COMPLEX I-HOMOLOGOUS NADH-DEHYDROGENASE OF THE CYANOBACTERIUM SYNECHOCYSTIS 6803. Klaus Steinmüller, Ulrike Ellerstek and Susanne Berger. Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Heinrich-Heine-Universität Düsseldorf, Universitätsstraße 1, D-4000 Düsseldorf 1

Ten genes (ndhA-J) of the plastid DNA of higher plants are homologous to subunits of the mitochondrial NADH-ubiquinone oxidoreductase (complex I of the respiratory chain). We have isolated and sequenced the corresponding genes from the cyanobacterium Synechocystis 6803 using gene-specific plastid-DNA fragments as hybridization probes. The cyanobacterial genes form the same transcription units as in plastid-DNA: 1.ndhH, 2.ndhA-I-G-E, 3.psaC-ndhD, 4.ndhF and 5.ndhC-psbG1-ndhJ. The amino acid sequence similarity to the plastidial ndh-genes ranges between 50-75%). Fusion proteins of psbG1 (probably also a ndh-gene) and ndhJ with the gene 10-product of bacteriophage T7 were expressed in E.coli and used to generate antibodies. Both antibodies identified their respective antigens in cytoplasma and thylakoid membrane preparations of Synechocystis 6803 demonstrating the presence of the NADH-dehydrogenase on both membrane systems. Since recently the location of a cytochrome c oxidase and the cytochrome b_6/f complex on the thylakoid and the cytoplasma membrane has been reported, the results indicate, that each membrane contains a complete respiratory chain.

2026 CYANOBACTERIAL METALLOTHIONEIN GENE: *SMTA*. James W. Huckle, Amit Gupta, William P. Lindsay, Jennifer Turner, Brian A Whitton, Andrew Morby and Nigel J.Robinson, Department of Biological Sciences, University of Durham, Durham DH1 3LE, U.K.

Part of the coding region of a prokaryotic metallothionein gene was amplified in the polymerase chain reaction (Robinson et al. *Proc. R. Soc Lond. B* 242, 241, 1990) using template DNA from *Synechococcus* PCC6301 (and also *Synechococcus* PCC7942) and inosine containing degenerate oligonucleotide primers designed from the known amino acid sequence (Olafson et al. *Biochem. J.* 251, 691, 1988). The nucleotide and encoded amino acid sequence of the amplified fragment corresponded to part of the known sequence of the cyanobacterial metallothionein but with the substitution of Ser for Cys_{32}. Anchored polymerase chain reactions were used to chromosome crawl and thereby characterise gene flanking sequences. This included associated translational signal sequences (ATG and Shine/Dalgarno) and a further c a. 750 bp of 5' flanking sequence. The prokaryotic metallothionein gene was also isolated from a *Synechococcus* PCC7942 size fractionated *Sal I/ Hind III* genomic library prepared in the vector pGEM4z and the gene has been designated *smtA*. The abundance of *smtA* transcripts increases following exposure to 2.5 μM Cd. An increase in transcript abundance was detected 5 min after exposure to 2.5 μM Cd and further increases in transcript abundance were detected up to 2 h. Increases in transcript abundance were also observed following exposure to elevated concentrations (1 μM, 2.5 μM, 5 μM, 10 μM) of Zn, Cu, Hg, Cr, Co, Cd and Ni but there was no detectable change in transcript abundance in response to conditions known to induce the synthesis of heat shock proteins in this organism. Fusions of the 5'flanking region of *smtA* and reporter-genes have been introduced into transgenic *Synechococcus* PCC7942 (PIM8) to identify putative metal regulatory sequences. Cd-resistant cultures of *Synechococcus* PCC6301 have been selected by step-wise adaptation. Cultures resistant to 0.8 μM, 1.2 μM and 1.75 μM Cd were obtained and these cultures have been maintained in media containing the respective concentrations of Cd for longer than six months. DNA was isolated from these cultures at different times over a six month period. Southern blots revealed transient amplification and rearrangement of *smtA* in these resistant cultures. As a control, Southern blots were also probed with a second gene *psaE*. There was no evidence of amplification or re-arrangement of *psaE* in the Cd-resistant cultures.

2027 A HOTSPOT FOR CHLOROPLAST GENE RECOMBINATION AND TRANSFORMATION IN *CHLAMYDOMONAS*. S.M. Newman[1], J. E. Boynton[1], E. H. Harris[1] & N. W. Gillham[2]. Duke Univ., Depts. of [1]Botany and [2]Zoology, Durham, NC 27706

Chloroplast gene recombination in *C. reinhardtii* can be analyzed in sexual crosses and following biolistic transformation. The number, position and orientation of small dispersed repeat sequences (often marked by KpnI and AatII restriction sites) present in intergenic regions are variable and account for most of the restriction fragment length polymorphisms (RFLPs) between the colinear chloroplast genomes of the interfertile strains *C. reinhardtii* and *C. smithii*. The pattern and position of specific exchange events within the 22 kb inverted repeat of the chloroplast genome have been examined in recombinant progeny from several two- and three-point crosses between *C. reinhardtii* and *C. smithii* which contain herbicide (*dr*) and antibiotic resistance (*er* and *spr*) point mutations in the *psbA*, 23S and 16S ribosomal RNA genes, respectively. Exchange events were not randomly distributed but were found to occur preferentially in a 0.7 kb region near the 3' end of the *psbA* gene. These data are corroborated by measurements showing that the *psbA dr* mutation was genetically unlinked to the 23S and 16S rRNA mutations, which are linked and show about 1% recombination per kb. This is significant since the *dr-er* and *er-spr* intervals are about the same length (ca. 7 kb). The 0.7 kb recombination hotspot also functions as a preferential site for exchange events leading to the integration of donor sequences during transformation of the chloroplast *psbA* and 23S rRNA genes. Sequence analysis of the 0.7 kb region at the 3' end of the *psbA* gene has identified four classes of 18-37 bp direct repeats but no open reading frame. Using deletion constructs in a chloroplast transformation assay, the hotspot was further localized to a 325 bp region that lacks most of these repeats. This suggests that the repeats themselves are not responsible for the increased recombination frequency. Studies are underway to define further the recombinogenic sequences and determine their interactions with specific proteins. Supported by NIH Postdoctoral Fellowship GM-12934 to S.M.N. and by NIH Research Grant GM-19427.

2028 CONSTRUCTING AND INTRODUCING SITE-SPECIFIC MUTATIONS WITHIN THE PSBA GENE OF *CHLAMYDOMONAS REINHARDTII*

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We are utilizing *Chlamydomonas reinhardtii* as a model for higher plant systems to study the D1 protein subunit of photosystem II encoded by the *psbA* gene. We are currently constructing site-specific mutations at the nucleic acid level that lead to single residue changes at the amino acid level on the acceptor side of the Q_b-binding site. Our work involves amplifying portions of the *psbA* gene in *C.reinhardtii*, a chloroplast-encoded gene known to have 5 exons and 4 introns, and introducing mutations within the region corresponding to the Q_b-binding site and adjacent areas that span more than one exon and intron. We have explored several methods to generate a mutated template suitable for introduction into *C.reinhardtii* via biolistic transformation. The results of these comparative studies will be presented, employing several types of starting template, and several methods of site-specific mutagenesis to determine which starting templates provide acceptable chloroplast transformation results within *C.reinhardtii*.

2029 COPPER REGULATION OF CYTOCHROME C553 AND PLASTOCYANIN IN *SYNECHOCYSTIS* PCC 6803.

Lili Zhang[1], Himadri B. Pakrasi[4] and John Whitmarsh[1,2,3]. [1]Department of Plant Biology, [2]Department of Physiology and Biophysics, University of Illinois, [3]Photosynthesis Research Unit, USDA/ARS, Urbana, IL 61801. [4]Department of Biology, Washington University, St Louis, MO 63130.

In certain cyanobacteria electron transport between cytochrome bf and photosystem I can be mediated by either plastocyanin or cytochrome c553. The availability of copper in the growth medium regulates which protein is present. We are investigating the transformable cyanobacterium *Synechocystis* sp. PCC 6803 to understand the molecular mechanism controlling the expression of cytochrome c553 and plastocyanin. Based on Western blot analysis and spectroscopic measurements of *in vivo* photosynthetic activities, we have determined the copper concentration dependence of the switch between cytochrome c553 and plastocyanin. We have cloned the gene for cytochrome c553 (*cytA* gene) from this species. Combining the DNA and protein sequence data, we show that the 120 amino acid open reading frame of the *cytA* gene encodes an 85 amino acid mature protein and 35 amino acids in the transit sequence. It is noteworthy that, although both cytochrome c553 and plastocyanin are destined to the lumen of thylakoids and have the same function in photosynthetic electron transport, the signal sequences that direct them across the membrane bear little homology between the two proteins. Perhaps the difference in the transit sequence may reflect the fact that cytochrome c553 is targeted to the periplasmic compartment as well. We are currently creating an insertion inactivation mutant of the *cytA* gene to determine the impact of cytochrome c553 on photosynthetic and respiratory electron transport.

2030 CMS-ASSOCIATED mtDNA SEQUENCES FROM SUGAR BEET.

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We have isolated CMS-specific clones from mtDNA libraries of sugar beet. A 5 kb *EcoRI* clone found only in the male sterile cytoplasm carries a potential open reading frame (ORF) of 651 bp unique to the CMS cytoplasm. Sequence analysis revealed extended homology of this ORF with two conserved domains of the nuclear encoded mitochondrial RNA polymerase from *Saccharomyces cerevisiae* (RPO4) and ORF1 of mitochondrial plasmid S2 from *Zea mays* as well as with phage T3 and T7 RNA polymerases. We are currently investigating the possible functional role of this sequence in mitochondria of male sterile sugar beet and its involvement with expression of CMS. Mapping and sequence data and results of transcriptional analyses will be presented. We are also investigating *in organello* polypeptide synthesis of isolated mitochondria to analyze CMS-specific effects on the protein level. Utilizing CMS-specific mtDNA sequences we have developed a simple dot blot miniassay for rapid discrimination between N (fertile) and CMS cytoplasms of sugar beet based on hybridization with alkaline-SDS plant extracts as target, enabling large-scale screening of breeding populations. Another part of our studies is aimed at identifying the progenitor cytoplasm of the CMS in sugar beet. For this purpose, we have started investigations on the presence of the unique CMS-associated ORF of *Beta vulgaris* in wild *Beta* beets.

POSTER ABSTRACTS

2031 THE MITOCHONDRIAL GROUP IIB INTRON FROM THE GREEN ALGA SCENEDESMUS OBLIQUUS. Ulrich Kück, Michael Winkler and Francoise Herdenberger. Allgemeine Botanik, Ruhr-University, P.O.Box 102148, D-4630 Bochum, Germany.

We recently have discovered a group IIB intron in the mitochondrial gene for the small ribosomal RNA from the eukaryotic green alga Scenedesmus obliquus (Kück et al., 1990). In the presence of high molar salt concentrations, the mitochondrial intron (rI1) is capable of splicing in vitro. After establishing the optimal conditions for RNA processing the in vitro splicing products were unequivocally identified in self-splicing experiments by northern hybridization analysis employing 3'end-labelled RNAs or exon and/or intron specific probes. Finally two transesterification products were identified by sequencing of the spliced RNA. From our data we conclude that the processing of groupII introns from both algal and yeast mitochondria are preceed by identical consecutive transesterification steps. The predicted secondary and tertiary structure of intron rI1 of Scenedesmus obliquus contains all motifs necessary for optimal self-splicing and which are characteristic of other group IIB introns from different species.

2032 THE MITOCHONDRIAL ATPA GENE ENCODING THE ALPHA SUBUNIT OF ATP SYNTHASE IN SUGARBEET. Tetsuo Mikami, Mineo Senda and Toshiro Kinoshita. Hokkaido University, Faculty of Agriculture, Sapporo 060 Japan

We have isolated atpA from the male fertile (N) cytoplasm (cv TK81-0) of sugarbeet by heterologous hybridization using atpA probe from pea, and the DNA sequences were determined. The gene has a length of 1518 bp and encodes a 506 amino acid polypeptide with a molecular weight of 54937 dalton. The amino acid sequence shared 93.9% homology with the ATPase alpha subunit of maize. In order to examine the genome organization of the atpA in CMS lines of sugarbeet, atpA probe DNA was hybridized to HindIII digests of mtDNAs from the S (TK81-MS) and different type of CMS line (I-12CMS(2)). The analysis revealed the polymorphic HindIII fragments containing atpA sequences; 3.7 kbp fragment from TK81-0, 2.7 kbp from TK81-MS and 6.0 kbp from I-12CMS(2). These polymorphic fragments were cloned into pUC119 for fine mapping and nucleotide sequencing. The atpA from S cytoplasm showed a remarkable divergence from N atpA around 50 bp upstream from the ATG start codon. On the other hand, only downstream of atpA indicated rearrangement between TK81-0 and I-12CMS(2) mtDNAs.

2033 PHYLOGENY OF THE MITOCHONDRIAL GENOMES OF FUNGI AND ALGAE. Bruno Paquin, Lise Forget, Isabelle Plante, Yun Zhu, Gabi Wolff[1], Gertraud Burger and Franz B. Lang. Université de Montréal, Département de Biochimie, Montréal, Canada. [1]Ruhr Universität, Lehrstuhl Botanik, Bochum, Germany.

Our aim is to reconstruct the phylogeny of the mitochondrial (mt) genomes within the fungal and algal branches of eukaryotes, by comparative sequence analysis of entire mtDNAs. We have determined (i) almost completely the mtDNAs from the oomycete *Phytophthora infestans* (an algal "fungus"; genome size: 36 kbp) and from the chytridiomycete *Allomyces macrogynus* (a primitive fungus; genome size 56 kbp); (ii) about 50% of the mtDNA from the "colourless" green alga *Prototheca wickerhamii* (genome size 52 kbp), and (iii) we started sequencing of the mtDNA from the chrysophyte alga *Ochromonas danica*[2] (genome size 41 kbp), the red alga *Plocamiocolax pulvinata*[3] (genome size 28 kbp), and of a close relative of *P. infestans*, namely *P. megasperma* (genome size 41 kbp).

Our results position (i) *A. macrogynus* at the root of the fungal branch, both in the nuclear and in the mitochondrial lineages, thus pointing to a single common ancestor of the fungi, (ii) the oomycete *Phytophthora* and the chrysophyte alga *Ochromonas danica* in very close relationship. We detected numerous mt introns in *A. macrogynus* (group I and II introns), and in *Prototheca wickerhamii* (sofar group I introns, only). Some of these mt introns reveal an unusually high similarity of the intronic open reading frames (ORFs) as well as of the sequences forming the core secondary structure to those of other fungi and algae. We suggest that these introns stem from an ancestral precursor of mitochondria or from the bacterial ancestor itself.

Collaboration with [2] Dr. A.Coleman, Brown University; [3] Dr. L. Goff, Santa Cruz

2034 TRANSFORMATION OF THE CHLAMYDOMONAS MITOCHONDRIAL GENOME. B. Randolph-Anderson, J. Boynton, N. Gillham, E. Harris, and A. Johnson, Duke University, Durham NC and R. Matagne, Universite de Liege, Liege Belgium.

The respiratory deficient dum-1 mutant of C. reinhardtii, which is incapable of growth in the dark because of a 1.5 kb deletion including most or all of the mitochondrial apocytochrome b gene, can be restored to respiratory competence following bombardment with high velocity microprojectiles coated with wild type mitochondrial DNA. After four to eight weeks dark incubation, a total of 22 respiratory competent colonies were isolated from plates of dum-1 cells bombarded with C. reinhardtii mitochondrial DNA (frequency 7.3 x 10^{-7}) and a single colony was isolated from plates bombarded with C. smithii mitochondrial DNA (frequency 7.7 x 10^{-8}). No colonies were seen on the control plates (frequency <0.96 x 10^{-9}). All transformants grew normally in the dark on acetate media and were homoplasmic for the wild type mitochondrial genomes typical of the C. reinhardtii or C. smithii donor, which differ by several characteristic restriction fragment length polymorphisms. No copies of the diagnostic deletion fragments of the dum-1 recipient were detected in any of the transformants. Mitochondrial genomes of the dum-1 mutant were also found to be unstable, with the region affected by the deletion varying in size between single cell clones from the mutant population. Furthermore, in contrast to wild type which has only 15.8 kb linear molecules, the mutant genomes were present as a mixture of 14.3 kb monomer and 28.6 kb dimer molecules. The mutant dimers appeared to result from head to head fusions of two deleted molecules. If the deletion in the dum-1 mutation includes loss of a telomeric structure necessary to maintain the integrity of one end of the wild type mitochondrial genome, such head to head fusions of the deleted ends may produce dimer molecules with telomeres at each end which may be inherently more stable than the deleted monomer molecules. Supported by NIH Grant GM-19427.

2035 ANALYSIS OF CHLOROPLAST AND MITOCHONDRIAL DNAs IN SOMATIC HYBRIDS OF Solanum brevidens AND S.tuberosum. Y-S. Xu, M.G.K. Jones*, A. Karp** and E. Pehu Dept. of Crop Husbandry, University of Helsinki, SF-00710 Helsinki, Finland *School of Biological and Environmental Sciences, Murdoch University, Perth, Western Australia, 6510 **Long Ashton Research Station, Bristol BS18 9AF, U.K.

The organellar DNAs of somatic hybrids obtained by electro- and chemical fusion of mesophyll protoplasts of S. brevidens and S. tuberosum PDH 40 have been analyzed. cpDNA was examined by restriction enzyme fragment patterns, and mtDNA by Southern hybridization using digoxigenin labeled mtDNA sequences nad5 or orf25 (kindly supplied by Dr. S. Levings). Fifty-eight hybrids were analyzed: patterns showed that the chloroplasts originated either from one parent or the other in equal frequency, with only one chloroplast type present in each hybrid. In the Southern analysis of the hybrid mtDNA, 9 of the 15 analyzed had an RFLP pattern similar but not identical to the PDH40 parent. Similarly, two of the hybrids had most of the S. brevidens fragments. One of the hybrids had an identical RFLP patterns to that of the potato and another two hybrids had a completely novel RFLP pattern. These results clearly show that mtDNA rearrangements had occurred at a high frequency. There were no differences in the frequencies of the rearrangements observed between the hybrids regenerated from chemical or electrofusions. The relationships between the organellar populations and the nuclear genome dosage in the hybrids will be discussed.

2036 LOCATION AND NUCLEOTIDE SEQUENCE OF A CT-LIKE $tRNA^{Val}$ GENE IN THE SUNFLOWER MITOCHONDRIAL DNA. A. Salardi and C. Quagliariello, Università della Calabria, Dipartimento di Biologia Cellulare, 87030 Arcavacata di Rende, Italy.

We report the complete nucleotide sequence of a region transferred from the chloroplast DNA (ctDNA) and subsequently integrated into the mitochondrial DNA (mtDNA) of sunflower. This region (415 nt) from the large inverted repeat of the ctDNA contains the 3'- 20 nt of the 16S rRNA, the intergenic spacer region and the entire tRNAV (GAC) gene. The tRNAV gene is 552 nt apart from the coxIII gene but in opposite orientation. Southern hybridization experiments suggest that the tRNAV (GAC) is a single copy gene in the sunflower mtDNA. This is the first tRNAV(GAC) gene sequenced in plant mtDNAs to our knowledge. The high degree of homology between the deduced tRNAV sequence from sunflower mitochondria and the respective plastid sequences in other species allows the classification of the potentially encoded tRNA gene as a mitochondrial ct-like tRNAV. The high conservation of this tRNA and its flanking regions in sunflower mitochondria suggests that this gene may be functional in these organelles.

POSTER ABSTRACTS

2037 GENETIC ORIGIN OF POTATO MITOCHONDRIAL TRANSFER RNAs. Laurence Maréchal-Drouard, Frédérique Weber, Thierry Desprez, Omaïma Khamis, Jacques-Henry Weil and André Dietrich. Institut de Biologie Moléculaire des Plantes du CNRS, Université Louis Pasteur, 12, rue du Général Zimmer, F-67084 Strasbourg Cedex France.

It has become evident that plant mitochondria (mt) contain transfer RNAs (tRNAs) of at lesat three different genetic origins. As expected, some tRNA genes ("native") are part of the genome of the endosymbiont which was progenitor of plant mitochondria. Other mt tRNA genes ("chloroplast-like") are part of promiscuous chloroplast (cp) DNA sequences which have been inserted into the mt genome during evolution. Finally, some mt tRNAs are coded for by the nuclear genome and must therefore be imported from the cytosol into the mitochondria. In an extensive study, we have identified 31 potato mt tRNAs. This tRNA population comprises 15 "native", 5 "chloroplast-like" and 11 nuclear-encoded species.

The import of tRNAs from the cytosol and the presence of "chloroplast-like" tRNAs seem to be two general features of plant mitochondria, but differences in the imported and "chloroplast-like" tRNA species appear between the dicotyledon and monocotyledon plants studied so far. For instance, potato mt $tRNAs^{Val}$, in contrast to wheat and maize mt $tRNAs^{Val}$, are coded for by the mt genome. Results will be presented concerning the potato mt $tRNA^{Val}$ genes. On the other hand, the fact that chloroplast tRNA genes have been recruited to function in plant mitochondria raises the problem of the relative distribution and expression of the "chloroplast-like" and "native" mt tRNA genes in different plants. This question has been addressed by comparing the distribution and expression of "chloroplast-like" tRNA genes in potato mitochondria and in mitochondria of other plants. Sequence comparisons between the regions of the potato mt and cp genomes containing the $tRNA^{Trp}$ and the $tRNA^{Asn}$ genes, respectively, will also be presented.

2038 *IN VIVO* HETEROLOGOUS tRNA IMPORT INTO THE MITOCHONDRIA OF TRANSGENIC PLANTS. Ian Small[1], Laurence Maréchal-Drouard[2], Jean Masson[1], Georges Pelletier[1], Jacques-Henry Weil[2] and André Dietrich[2].

[1]Laboratoire de Biologie Cellulaire INRA, Route de St-Cyr, F-78026 Versailles Cedex, France ; [2]Institut de Biologie Moléculaire des Plantes du CNRS, Université Louis Pasteur, 12 rue du Général Zimmer, F-67084 Strasbourg Cedex, France.

Some plant mitochondrial tRNAs, including the mitochondrial leucine tRNAs from bean and potato, are nuclear-encoded and therefore imported from the cytosol (1). The elucidation of the mechanisms involved in this import process may suggest ways of targetting other nucleic acids to mitochondria. As a first step, we are studying the feasibility of using naturally imported tRNAs as vectors to carry short extra RNA sequences into plant mitochondria.

After introduction of the bean leucine tRNA(NAA) gene into potato protoplasts by electroporation, we were able to demonstrate the presence of this foreign bean tRNA in the mitochondria of the transformed potato plants. Oligonucleotide-directed mutagenesis has been used to generate alterations and insertions in the bean leucine tRNA gene in order to examine the specificity determinants of the import process, and to see whether extra sequences can be added without compromising the expression of the gene or the mitochondrial import of the transcripts. In this context, we were able to show that the bean leucine tRNA(NAA) gene is still expressed in transgenic potato plants after introduction of a four base-pair insertion into the anticodon loop and that the corresponding product is imported into the mitochondria. The expression of other modified bean leucine tRNA(NAA) genes in transgenic potato plants is currently being examined.

(1) Maréchal-Drouard L, Guillemaut P, Cosset A, Arbogast M, Weber F, Weil JH and Dietrich A (1990), Nucleic Acids Res. 18, 3689-3696.

2039 GENE STRUCTURE AND TRANSCRIPT EDITING OF SORGHUM MITOCHONDRIAL ATP6. F. Kemken, J. A. Hullen, D. R. Pring, and H. V. Tang, Department of Plant Pathology and USDA-ARS, University of Florida, Gainesville, FL 32611 USA and Department of Botany, Ruhr-Universitat, P.O. Box 102148, D-4630 Bochum, Germany. Mitochondrial atp6 genes from two sorghum lines were isolated and sequenced. The line Tx398 carried one copy of the gene on a 5 kb BamHI fragment, while the line IS1112C carried the 5 kb copy and an additional copy on a 6.3 kb fragment. Genomic and cDNA sequencing of the two copies in IS1112C revealed a repeated sequence with a 5' breakpoint within the open reading frames. The 5 kb copy in Tx398 and IS1112C has a 381 bp amino extension to a conserved 756 bp core gene, while the 6.3 kb copy has a 246 bp extension. The two genes are 1137 and 1002 bp and encode polypeptides of 379 and 334 residues, respectively. Both copies are transcribed. PCR amplification, cloning and cDNA sequencing revealed extensive transcript editing in the 756 bp core gene. Three edits were silent, but 16 resulted in changing 15 amino acids. Editing of a CAA codon to TAA truncates the carboxy terminus to a position identical to that of yeast atp6. This codon is conserved in all higher plants examined to date, suggesting that plant atp6 genes may be edited to a common carboxy terminus. Interestingly the 381 bp amino extension exhibited no amino acid changes due to editing, while the 246 bp extension had one amino acid change. While editing frequency was very high for sites which changed amino acids, frequency of silent editing varied between the two sorghum lines.

2040 CHARACTERIZATION OF A PUTATIVE SOYBEAN MITOCHONDRIAL DNA REPLICATION ORIGIN. Stephanie L. Fico[1], Henry Daniell[2] and Brent L. Nielsen[1]. [1]Auburn Univ., Dept. of Botany & Microbiology, Auburn, Alabama 36849 and [2]Univ. of Idaho, Dept. of Biol. Sci., Moscow, Idaho 83843.

Mitochondrial DNA polymerase has been partially purified from etiolated soybean seedlings by chromatography through DEAE-cellulose and heparin-Sepharose columns. Glycerol gradient analysis of the enzyme revealed a single peak of DNA polymerase activity, with an estimated native molecular mass of between 150,000 and 200,000 daltons. The soybean enzyme shows preference for either activated calf thymus DNA or a poly($dAdT_{12}$) template, but does not support replication using poly($rAdT_{12}$) in the presence of either $MgCl_2$ or $MnCl_2$. The characteristics of the soybean enzyme are similar to those of wheat embryo and yeast mitochondrial enzymes, and different from those reported for animal and cultured soybean cell mitochondrial DNA polymerase. Approximately 25 PstI fragments of soybean mitochondrial DNA have been cloned into pUC19, and tested for in vitro DNA replication activity using the partially purified soybean mitochondrial DNA polymerase fraction. Most clones support only a background level of activity, similar to pUC19 without an insert, while one clone, pMP3.0, supports a 7- to 10-fold higher level of activity, as determined by incorporation of [^{3}H]-TTP in in vitro DNA replication assays. The activity with pMP3.0 is stimulated by the addition of rNTPs to the reaction. Gel analysis of [^{32}P]-TTP labelled replication reaction products showed specific labelling of pMP3.0 but not pUC19 or other mitochondrial clones. pMP3.0 was used for biolistic transformation of sugarbeet cells, and after transformation, the cells were allowed to recover for 24 or 44 hours before isolation of total DNA from the mitochondrial fraction. The presence of pMP3.0 in transformed organelle fractions from both the 24 and 44 hr samples was visualized by Southern blot hybridization, while DNA from cells similarly transformed with pUC19 alone showed no hybridization with a pUC19 probe. The DNA sequence of pMP3.0 is currently being determined, and an analysis of the sequence for this clone will be reported.

2041 IDENTIFICATION OF SUBUNIT 6 OF NADH-OXIDOREDUCTASE IN MITOCHONDRIAL DNA FROM WHEAT. N. Haouazine, M.-F. Jubier, E. Delcher, D. Lancelin*, G. Pelletier* and B. Lejeune.

Biologie moléculaire végétale, Université Paris-Sud, 91405 Orsay, *Biologie Cellulaire, CNRA, 78036 Versailles, France.

In order to characterize new coding sequences in the mitochondrial (mt) genome of wheat (*Triticum æstivum*), we have analyzed a SalI fragment that was selected among those displaying strong homology with mt DNA from other Angiosperms, thus indicating a high degree of sequence conservation.

Northern hybridization experiments with RNAs from etiolated wheat seedlings revealed an unique 1.3 kb transcript.

Southern cross-hybridization experiments between this Sal I fragment and *Brassica oleracea* mt DNA allowed us to localize more precisely the homologous region on a 1.5 kb SacII-Xho I fragment.

This fragment was entirely sequenced, and shown to include a 705 bp ORF . The predicted sequence of 235 amino acids was compared with the Swiss-Prot protein sequence database. Homologies with several subunits 6 of NADH-ubiquinone oxidoreductase were detected, the highest similarities being found with the fungal subunits.

Comparison with the corresponding cDNA is under way in order to detect possible RNA editing as already evidenced in other mitochondrial gene products.

2042 EXPRESSION OF THE WHEAT MITOCHONDRIAL *NAD4* GENE. EVIDENCE FOR A ROLE OF RNA EDITING IN INTRON SPLICING. Lorenzo Lamattina and Jean-Michel Grienenberger. Institut de Biologie Moleculaire des Plantes, CNRS, Université Louis Pasteur, Strasbourg, FRANCE

The wheat mitochondrial (mt) NADH dehydrogenase subunit 4 gene (*nad*4) has been localized and sequenced. This gene, about 8 kb long, is composed of four exons separated by three class II introns. The *nad*4 gene exists as a single copy in the wheat mitochondrial genome and it is transcribed into one abundant mRNA of 1.8 kb, whose extremities have been mapped. The complete cDNA sequence corresponding to the *nad*4 transcript has been determined by combining direct sequencing of uncloned cDNAs and a method involving cDNA synthesis and PCR amplification using specific oligonucleotides as primers, followed by cloning and sequencing of the amplification products. Comparison of the genomic sequence with that of the cDNA shows that all *nad*4 transcripts are fully edited at 23 positions, with an uneven distribution of the editing sites between the different exons: While exon 1 and exon 4 are extensively edited (with a change of 11% of the amino acid sequence), exon 2 is not edited at all and exon 3 is 0.5% edited. The nucleotide at the 5'-end of the exon 3, which would be involved in the recognition of the "guide pair" located on exon binding site 1 (EBS1) of the D3 terminal loop of the intron 2, must be edited for the correct splicing of the intron. The relevance of these data to the function and mechanism of RNA editing in plant mitochondria are discussed.

POSTER ABSTRACTS

2043 A *TRANS*-SPLICING MODEL FOR *nad1* GENE EXPRESSION IN WHEAT MITOCHONDRIA. Yvan Chapdelaine and Linda Bonen. University of Ottawa, Department of Biology, Ottawa, Canada K1N 6N5

The *nad1* gene encoding subunit I of the respiratory chain NADH dehydrogenase is fragmented into five unique-copy coding segments that are scattered over at least 55 kb and interspersed with other genes in the wheat mitochondrial genome. The *nad1* segments are flanked by sequences with group II intron features and direct sequencing of *nad1* cDNAs as well as cloned PCR-amplified reverse transcripts demonstrate the presence of correctly-spliced mRNAs. Northern hybridization analysis indicates that all *nad1* pieces and flanking regions are transcribed with a complex pattern. RNA editing occurs at sites asymmetrically distributed along the wheat *nad1* coding region and the initiation codon is created by RNA editing of an ACG codon to AUG. The unusual organization of the wheat *nad1* gene is attributed to mitochondrial DNA rearrangements within introns and a *trans*-splicing model involving secondary structural interactions between group II-like intron pieces is proposed for its expression. Our findings illustrate the remarkable plasticity of the structure of this gene and raise questions about splicing mechanisms in plant mitochondria. Supported by NSERC Canada and le Fonds FCAR.

2044 TRANSCRIPTION IN RICE MITOCHONDRIA. Edward Kaleikau, Charles André and Virginia Walbot. Stanford University, Department of Biological Sciences, Stanford, California 94305-5020

Transcript patterns observed on Northern blots of rice mitochondrial RNA are simple compared to the multiple RNA species found in mitochondria for other plant species. We utilized this finding to help elucidate the nucleotide signals regulating transcription initiation and termination in rice mitochondria. Towards this understanding, we cloned and sequenced three rice mitochondrial genes; *cob*, *atp9*, and *coxJ*. Our transcriptional analysis of these genes by RNase protection, primer extension, G-capping and sequencing has: [1] identified an 11 base motif at the transcription start site that closely matches the consensus promoter motif proposed for maize mitochondrial genes and [2] provides support for the importance of a 3' structural element in mitochondrial transcript stabilization.

2045 IN VIVO AND IN VITRO STUDIES OF mRNA EDITING IN WHEAT MITOCHONDRIA. Alejandro ARAYA, Dominique BEGU, Pierre V.Graves, Armand Mouras, Michel Hernould, Patricia Gobin and Simon Litvak. **Laboratory of Plant Molecular Biology. IBCN-CNRS. 1 rue Camille Saint Saëns. 33077 Bordeaux cedex. France**

A post-transcriptional mRNA modification process, called RNA editing, which converts Cs into Us was recently described in plant mitochondria. Wheat mtDNA carries two copies of ATP synthase subunit 9 gene (*atp9*) closely associated with two copies of ATP synthase subunit alpha gene (*atpA*). The *atp9* and *atpA* genes are cotranscribed. Isoplasmic fertile, alloplasmic cytoplasmic male sterile (CMS) and alloplasmic fertile-restored wheat lines share identical transcription pattern of these genes. Sequence analyses of *atp9* genes and cDNAs from different lines showed that the mRNA coding region is modified by eight C to U conversions leading to five amino acid changes and the creation of a stop codon; two of the conversions are silent considering the protein primary sequence. Direct sequencing and amino acid analysis of the ATP synthase subunit 9 from fertile wheat confirmed for the first time that RNA editing process has consequences at the translational level. Partially edited molecules in 5' end and/or in 3'end in the RNA which could be intermediates of RNA editing were isolated in CMS wheat mitochondria. Similar differences in the editing pattern of apocytochrome b (cob) transcripts from fertile and CMS wheat lines were observed and will be presented and discussed. Transgenic plants containing the *atp9* gene and the peptide signal for mitochondrial import were constructed with the edited and non-edited genes. The effect of this approach on the emergence of the CMS phenotype after plant regeneration will be shown. The incubation of non edited *atp9* RNAs with a wheat mitochondrial protein extract showed the emergence of correctly edited molecules identical to those observed *in vivo* . The RNA editing activity detected *in vitro* is heat and protease sensitive. Micrococcal nuclease treatment inhibits this activity suggesting the requirement of nucleic acid factor(s).

2046 A TOMATO MITOCHONDRIAL MUTATION ALTERS PLANT GROWTH. Mary O'Connell, Augusta Bonnema and Susan Fender. New Mexico State University, Plant Genetic Engineering Lab, Department of Agronomy and Horticulture, Las Cruces, NM 88003

Plant growth and development are a result of the coordinated expression of genes located primarily in the nucleus, but also of genes located in cytoplasmic organelles. Mutants in organellar gene expression are being used to study the interaction in gene expression between the nucleus and the chloroplast and mitochondria. We have recovered a collection of tomato (*Lycopersicon esculentum*) mutants following cybridization. The cybrids were generated by fusing leaf mesophyll protoplasts of tomato cv. UC82 with irradiated protoplasts isolated from a suspension culture of *L. pennellii*. The mutants display a five-fold reduction in reproductive growth and a two-fold reduction in vegetative growth. The mutation is heritable, and the mutant phenotype is correlated with the presence of mitochondrial DNA from the wild species used in the protoplast fusion, *L. pennellii*. All of the cybrids have the chloroplast genome of cultivated tomato. The altered organizations of the mitochondrial DNA associated with the mutant phenotype are stably inherited. We propose that the mutation is the result of a recombination event between the two mitochondrial genomes. The growth of the mutants versus control regenerants and non-mutant cybrids will be described. A detailed description of the of the organization of the mitochondrial DNA in these mutant and non-mutant cybrids will be presented. The organization of the mitochondrial DNA has been characterized using cloned heterologous probes for several mitochondrial genes as well as a cosmid clone bank of tomato mitochondrial sequences.

2047 SEQUENCE OF A cDNA ENCODING A DIFFERENTIALLY EXPRESSED MITOCHONDRIAL POLYPEPTIDE. C. Colas des Francs-Small, F. Ambard-Bretteville, I.D. Small*, F. Vedel & R. Rémy. Génétique Moléculaire des Plantes, URA 115, UPS, Bât. 360, F-91405 Orsay cedex ; *Biologie Cellulaire, INRA, Route de St Cyr, F-78026 Versailles cedex

A 40 kDa polypeptide, which is abundant in non green potato tissue mitochondria but scarce in mitochondria from photosynthetic tissues was characterized by 2-D PAGE. It was also found to have a similar tissue-specific distribution in other plant species, and in quantities which suggest an important role in plant mitochondria. A λgt11 cDNA expression library was screened with an antibody raised against this polypeptide. The sequence of a positive (but not full length) cDNA clone was determined. Using an oligonucleotide deduced from the N-terminal sequence of the polypeptide and another one from the cDNA sequence, the cDNA encoding the mature protein was reconstituted by PCR and sequenced. The amino acid sequence deduced from the cDNA was used to search the NBRF and Swissprot protein data bases, and found to have significant homology (28%) with *E. coli* D-3-phosphoglycerate dehydrogenase and certain other NAD-specific 2-hydroxy acid dehydrogenases of the same type.

2048 THE MITOCHONDRIAL GENOME OF THE CLAVAROID FUNGUS CLAVICORONA PYXIDATA. Carol Contolini, Karen Hughes and Qiuxin Wu. University of Tennessee, Department of Botany, Knoxville, Tennessee 37996-1100

Mitochondrial DNA from the clavarioid fungal species, Clavicorona pyxidata was obtained and a restriction site map prepared for three enzymes, Hind III, Hpa II and Xba I. Hybridization with probes from Suillus and yeast elucidated the location of LrDNA, SrDNA, cytochrome c oxidase complex (subunits I, II and III) and cytochrome b genes. From restriction fragment digests, the genome size was estimated to be approximately 54kb. MtDNA of C. pyxidata isolates collected from oak logs within 300 meters of each other differed by a single deletion. Isolates from Wisconsin differed from a Tennessee isolate by loss of a restriction site. These preliminary results confirm the within-species variability associated with fungal mtDNA and may allow analysis of fungal population structure.

POSTER ABSTRACTS

2049 TRANS-SPLICING IS A COMMON CHARACTER IN PLANT MITOCHONDRIA. B. Wissinger, V. Knoop, S. Binder, A. Marchfelder, W. Schuster and A. Brennicke. Institut für Genbiologische Forschung GmbH, Ihnestr.33, D-1000 Berlin 33, FRG.

Until recently only a few introns had been identified in higher plant mitochondria. All those are cis-splicing and belong to the group II intron type. We have now identified and analyzed the complete open reading frames coding for subunits 1, 2 and 5 of the NADH dehydrogenase in Oenothera mitochondria. Each of these genes is composed of five coding exons. Coupling of the exons and locations of exon/intron borders were shown in cDNA clones or by specific PCR experiments. Linkage analysis shows that these genes are split to two (nad2), respectivly three (nad1, nad5), genomic locations. Transcription data indicate independent precursor molecules for these locations and shows that mRNA maturation depends on trans-splicing reactions. All five trans-splicing introns show typical characteristics of group II introns with the discontinuity located in structural domain IV. Base pairings may participate in the recognition between the two parts of each trans-splicing intron. Comparative analysis suggests that trans-splicing of these genes is conserved in other plant mitochondria. A trans-splicing intron of the nad1 gene is edited (C to U transitions) at relevant structural positions which may be a prerequisite for splicing. cDNA analysis and transcript data suggests differences in splicing efficiencies for the introns within each gene.

2050 SEQUENCE ANALYSIS OF A MITOCHONDRIAL GENOME OF *ARABIDOPSIS THALIANA* - A PROGRESSREPORT. P. Gronger, M. Unseld, U. Eckert and A. Brennicke. Institut für Genbiologische Forschung Berlin GmbH, Germany.

We isolated from a total DNA cosmid library from *Arabidopsis thaliana* cosmids containing mitochondrial DNA fragments by heterologous hybridization with known mitochondrial genes. So far, 350 kb of unique sequence of the mitochondrial genome of *Arabidopsis* have been identified. Cosmids, representing these sequences could be arranged in several linkage groups. The definite structure however is still unclear. A number of cosmids were selected to start sequence analysis. We used different cloning strategies to prepare templates for automated fluorescent sequencing. Up to now, around 160 kb of unique sequence have been established in numerous small linkage groups. Among these we were able to identify the tRNA genes $tRNA^{Tyr}$ and $tRNA^{Ser}$, the coding regions (or parts of them) for *nad1*, *nad5*, *cox*III, *atp6*, *atpα*, *cob* and *rps14* and several short sequence repeats. The latter may play a role in recombination of mitochondrial DNA or possibly in RNA editing. All coding regions show a high degree of similarity with corresponding genes from other organisms. The protein coding genes from *Arabidopsis* appear to be preedited to a high degree.

2051 TRANSFER AND STABLE MAINTENANCE OF ISOLATED MITOCHONDRIA IN BRASSICA NAPUS CALLI AFTER MICROINJECTION OF PROTOPLASTS H.A. Verhoeven, J.W. van Eck, J. Blaas, P Dijkhuis, C.M. Colijn- Hoogmans. Dept. of Cell Biology, Centre for Plant Breeding and Reproduction Research, CPRO, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Cytoplasmic male sterility (CMS) in plants is caused by alterations in the mitochondrial genome, resulting in impaired interaction with the nuclear genome. Cytoplasmic male sterility is applied in plant breeding, especiallv in the production of F1 hybrid seed. We report here on the isolation of viable mitochondria from a cytoplasmic male sterile line of the "Ogura" type of Raphanus sativus. Furthermore we describe the transfer and stable transmission of those mitochondria to a male fertile line of Brassica napus through microinjection without the use of selective markers. Injected protoplasts were identified and followed individuallv throughout their development. Restriction fragment length analysis with mitochondrial probes indicated the presence of donor mitochondria in 3 out of 17 calli tested, whereas no donor chloroplasts could be detected with a chloroplast specific probe indicating that no chlorosis will occur Karyotype analysis showed that all calli contained only B.napus chromosomes. This procedure is a valuable alternative for introduction of CMS and for the study on the interaction between nucleus and mitochondria. Furthermore this method opens new perspectives for the transformation of mitochondria and their transfer to protoplasts

2052 MESSENGER RNAs, BUT NOT rRNAs ARE EDITED IN *OENOTHERA* MITOCHONDRIA. Rainer Ternes, Rudolf Hiesel, Volker Knoop, Bernd Wissinger, Axel Brennicke, and Wolfgang Schuster. Institut für Genbiologische Forschung, Ihnestraße 63, D-1000 Berlin 33, Germany.

The mRNAs of protein coding genes in plant mitochondria are frequently modified by RNA editing. To investigate whether RNA editing can be a criterion for determining translated orfs or whether structural RNAs are edited as well, we compared the distribution of RNA editing sites in mRNAs and rRNAs in *Oenothera* mitochondria. In addition to the sequences analysed so far we compared cloned cDNAs with genomic sequences of the following genes and the respective leader, trailer and spacer sequences: the alpha-subunit of ATPase (atpA), cytochrome b (cytb), orfB, and the 5S, 18S and 26S rRNAs. While all open reading frames in *Oenothera* mitochondria are edited, no editing was found in the 5S rRNA and the 1.820 nucleotides analysed of the 18S rRNA. In the 1.833 nucleotides compared for the 26S rRNA, two altered nucleotides were found. The least edited mRNA is the atpA transcript with four editing sites in the 1.533 nucleotides open reading frame. While RNA editing is not stringently restricted to orfs, we observe clustering of RNA editing sites in translated open reading frames. From this analysis we conclude that frequent RNA editing is indicative for functional protein coding regions in plant mitochondria. The extensive editing in orfB, for example, thus further supports the contention that this orf encodes a mitochondrial protein.

2053 IDENTIFICATION AND COMPARISON OF SUNFLOWER MITOCHONDRIAL AND CHLOROPLAST tRNA GENES. L.R. Ceci, M.Ambrosini, S.Fiorella and R.Gallerani* Sezione di Trani del C.S.M.M.E.-C.N.R. e Dipartimento di Biochimica e Biologia Molecolare*, Università di Bari, Italia.

Total sunflower mitochondrial tRNAs, specifically labelled at their 3' termini,have been used to detect mtDNA fragments carrying tRNA genes. The sequencing analysis of some of them led to the identification of genes belonging both to the "genuine" and to the "cp-like" class. Among the former, trnM(i) and trnE were identified and among the latter, those specific for methionine (e), histidine, triptophane and asparagine.

In order to have more insight in to the origin of the "cp-like" mt tRNA genes we began the identification of their counterparts on the sunflower chloroplast genome. trnH, trnN and trnM chloroplast genes lie in restriction fragments of different sizes compared to those carrying mitochondrial genes. Furthermore, sequencing analysis of trnH genes, both in chloroplast and in mitochondria, reveals that they are located in highly conserved regions among higher plants. However, while the coding regions show 98% similarity, the regions flanking the genes differ completely. These observations, together with other results reported in the literature, suggest a new classification of "cp-like" tRNA mitochondrial genes.

2054 CHARACTERIZATION OF A MAIZE MITOCHONDRIAL PROMOTER USING A HOMOLOGOUS *IN VITRO* TRANSCRIPTION SYSTEM. William D. Rapp and David B. Stern. Boyce Thompson Institute, Ithaca, NY 14853

The mitochondrial genome of plants encodes several components of the mitochondrial electron transport chain and ATPase. It also encodes the rRNAs, many of the tRNAs, and a few of the ribosomal proteins required for translation of mitochondrial mRNAs. Currently, little is known about how the expression of plant mitochondrial genes is regulated. Promoter consensus sequences have been proposed based on comparisons of sequences surrounding transcription initiation sites, but the lack of mitochondrial transformation or *in vitro* transcription systems has precluded the conclusive identification of these or other sequences as being essential for the control of transcription. We report the purification of a maize mitochondrial protein fraction that accurately initiates transcription of the cloned maize mitochondrial *atp1* gene *in vitro*. Primer extension analysis confirmed that *in vitro* transcription initiation of the *atp1* gene occurred at the same site as *in vivo*. Deletion of 13 base pairs surrounding the initiation site, including the 11 base pair maize promoter consensus sequence, completely abolished transcription. The *atp1* promoter was extensively characterized by using a series of 5' and 3' deletions and mutants with single base pair alterations as templates in *in vitro* transcription reactions.

POSTER ABSTRACTS

2055 MITOCHONDRIAL GENOME ANALYSIS IN THE CONIFER, *LARIX*.
L. DeVerno, P.J. Charest and L. Bonen*. Petawawa National Forestry Institute, Chalk River, Ontario, Canada K0J 1J0. *University of Ottawa, Department of Biology, 30 George Glinski, Ottawa, Ontario, Canada K1N 6N5.

Despite their ecological and commercial importance the basic information available on conifer mitochondrial genome organization, complexity, and mode of inheritance is limited. As descendants of some of the most primitive land plants, conifers have unique physiology and genetics. Thus, only a limited amount of the molecular genetic information gained from the study of agricultural crops can be applied directly to conifers. We have isolated both mitochondrial and total genomic DNA from *Larix* species to examine the physical complexity of mitochondrial genes and to determine mitochondrial inheritance patterns in *Larix* hybrid crosses, using restriction endonuclease and Southern hybridization analyses with specific wheat mitochondrial gene probes. Some conifers appear to have evolved mechanisms to exclude the maternal transmission of plastids and the paternal transmission of mitochondria, leading to uniparental inheritance of organelles. Our results indicate that the mitochondrial genome is maternally inherited in *Larix* hybrid crosses. Specific wheat mitochondrial gene probes are also being used in heterologous hybridization experiments to study the complexity of *Larix* mitochondrial genes and to evaluate the effect of *in vitro* embryogenic cell culture on individual mitochondrial gene structure and organization.

2056 DIFFERENCES IN AMINO ACID TRANSPORT IN ISONUCLEAR LINES OF CMS AND FERTILE PETUNIA CELLS. M. Perl, Dvora Swartzberg and S. Izhar. The VOLCANI Center ARO, Bet Dagan,50250, ISRAEL.

The uptake of ten amino acids (aa) by cultured cells which originated from CMS (CMScells) or from fertile (Fcells) petunia, was examined. The transport of some aa is relatively poor and their accumulation in the , acid - soluble fraction does not exceed 25% of the external concentration. Transport of those aa seems to be by a simple diffusion process.

Some aa accumulate in the cells in their acid-soluble form, to a much higher concentration than the aa concentration added to the growth media, indicating an active uptake system.

The presence of KCN (2 mM) partially inhibited the uptake of most amino acids. The incorporation of the aa into the acid-insoluble fraction was diminished to a lesser extent than the accumulation of the aa in the acid soluble fraction. This may lead to the conclusion that the active transport system is not entirely energy-dependent.

A comparison between CMScells and Fcells revealed that the Fcells have a higher rate of uptake (RU) for most of the examined aa , but the ratio $RU_{CMScells}/RU_{Fcells}$ differs among the various amino acids.

A study of the translocation of amino acids from leaves to various parts of the plant in CMS and fertile plants revealed more rapid transport in the latter.

2057 ALTERED MITOCHONDRIAL GENE EXPRESSION ASSOCIATED WITH THE "POLIMA" CMS OF BRASSICA NAPUS , Mahipal Singh and Gregory G. Brown, Department of Biology, McGill University, Montreal, Quebec, Canada H3A 1B1

The Polima or pol system of cytoplasmic male-sterility in Brassica napus has received widespread attention with respect to its potential for hybrid rapeseed production. To identify potential CMS-associated regions of the pol mitochondrial genome, fourteen mitochondrial gene regions were used to probe Northern blots of floral mtRNA from normal or nap cytoplasm plants, pol male-sterile and fertility-restored pol cytoplasm plants. Differences in expression were detected only with the region carrying the atp6 gene. Sequence analysis of this region reveals a novel upstream 224 codon open reading frame (ORF) in pol mtDNA that is co-transcribed with atp6. The ORF consists of the first 58 codons of the orfB gene joined to a region of unknown function. Nuclear fertility restoration is specifically associated with the appearance of 5' transcript termini mapping within the ORF as well as with an increased level of the smallest atp6 transcript. Levels of this smallest transcript, which corresponds to the single atp6 transcript of fertile nap cytoplasm plants, are also specifically increased when male-sterility is suppressed by elevated temperature.

2058 SITE-DIRECTED AND RANDOM SATURATION MUTAGENESIS OF THE T-*urf13* GENE OF *cms*-T MAIZE. Mark E. Williams, Gerty C. Ward and Charles S. Levings III. North Carolina State University, Department of Genetics, Raleigh, NC 27695.

The Texas (T) cytoplasm of maize is characterized by two traits, male sterility and sensitivity to pathotoxins produced by the fungal pathogens *Bipolaris maydis* Race T and *Phyllosticta maydis*. This sensitivity has eliminated the use of T-cytoplasm in the production of F_1 hybrid seed. The T-*urf*13 gene is unique to T-cytoplasm and encodes a 13-kd polypeptide, URF13. Expression of URF13 in *Escherichia coli* has shown that URF13 is responsible for pathotoxin sensitivity; correlative evidence suggests that URF13 is also the cause of male sterility. Site-directed and random saturation mutagenesis is being used to determine the structure of URF13, define which regions of URF13 are important in conferring toxin-sensitivity and how the URF13-toxin interaction causes toxin sensitivity. Putative mutants were transformed into *E. coli* and plated on medium containing methomyl{S-methyl-N-[(methylcarbamoyl)-oxy]thioacetimidate}, a carbamate insecticide which causes equivalent toxic effects on cms-T mitochondria. Colonies which grew in the presence of methoyml were tested by Western blot analyses to determine if they were expressing full-length versions of the URF13 polypeptide. T-*urf13* DNA from these colonies were sequenced. Numerous toxin-insensitive mutations were found throughout the regions of the T-*urf13* gene tested. These mutants were tested and classified according to their stability and toxin-binding capacity.

2059 TOPOGRAPHY OF A MAIZE MITOCHONDRIAL MEMBRANE PROTEIN ASSOCIATED WITH FUNGAL TOXIN SUSCEPTIBILITY.
Kenneth L. Korth, Cyril I. Kaspi*, J. N. Siedow* and C.S. Levings III. North Carolina State University, Dept. of Genetics, Raleigh, NC 27695 and *Duke University, Dept. of Botany, Durham, NC 27706.

Acute susceptibility of Texas cytoplasmic male-sterile (cms-T) maize plants to *Bipolaris maydis* race T is due to the unique presence of a 13 kD mitochondrial encoded protein, URF13. Exposure of isolated cms-T mitochondria or *E. coli* expressing URF13 to pathotoxins produced by *B maydis* leads to rapid permeabilization of URF13 containing membranes. Results obtained through cross-linking studies indicate that URF13 exists in membranes as a multimeric form. SDS gel electrophoresis of membrane proteins from either cms-T mitochondria or URF13-expressing *E coli* cells treated with bifunctional cross-linking reagents reveals the presence of monomers, dimers, and higher-order aggregates of URF13. Protease accessibility studies of spheroplasts and inside-out membrane vesicles have been used with C- and N- terminal antigenic markers to examine the topological properties of URF13 in the membrane. The results show that the C- and N- termini of URF13 are localized on opposite sides of the membrane, which accomodates a model of URF13 containing three membrane-spanning helices. In addition, URF13 was found to be inserted into the *E. coli* membrane in both possible orientations. URF13 was expressed in invertebrate cell cultures using a baculovirus expression system to test for insect cell membrane permeabilization in the presence of *B maydis* toxin.

2060 MANIPULATION OF CYTOPLASMIC MALE STERILITY BY PROTOPLAST FUSION
Tina L. Barsby, Sarah Bowden, Jane Coventry, Elizabeth Davies and Peter G. Isaac. Nickerson International Seed Company, Cambridge Science Park, Milton Road, Cambridge, England.

Donor-recipient protoplast fusion is being used to convert breeding lines to cms for evaluation in commercial hybrid breeding programmes. We present up to date information concerning the efficiency of these methods in Brassica spp. The results of RFLP analysis of mitochondrial DNA following protoplast fusion are also discussed.

POSTER ABSTRACTS

2061 S_1 MAPPING ANALYSIS OF atpA GENE TRANSCRIPTS IN FERTILE AND MALE STERILE SUNFLOWER LINES. L. D'Ambrosio, L. Siculella*, A.D. De Tuglie and R. Gallerani. Dipartimenti di Biochimica e Biologia Molecolare e Biologia*, Universita di Bari e Lecce*, Italia.

We have sequenced the regions of mtDNAs coding for atpA genes in sunflower fertile and male sterile lines,for approximately 4,000 and 3,000 bp respectively. The coding regions of both genes are almost identical as well as those of 5' flanking non-coding regions for approximately 1,500 bp. In the 3' flanking non coding regions three relevant differences have so far been found between the two lines. The first concerns the presence, only in the fertile line,of two almost perfect inverted repeats located 317 bp downstream of the the stop codon, the second a snarp divergence point located 196 bp downstream the same points, and the third, an open reading frame of at least 204 nucleotides present only in the male sterile line and co-transcribed with the atpA gene. This last conclusion has been drawn from the observation that in male sterile line at least two transcripts with different 3' termini can be detected. However in the fertile line a single transcript, very similar to the smaller one in male sterile line has been found. S_1 mapping experiments, carried out using probes containing 5' coding and their adjacent non coding regions, seem to demonstrate the existence of very similar multiple transcription initiation points in both lines. The eventual expression of the open reading frame would suggest a new model to explain the biogenesis of cytoplasm male sterility in higher plants.

2062 ENERGY AND ACTIVATED OXYGEN METABOLISMS IN ANTHERS OF HUBEI PHOTOPERIOD-SENSITIVE GENIC MALE-STERILE RICE. Liang Chengye and Chen Xianfeng. South China Institute of Botany, Academia Sinica, Guangzhou 510650, P.R.China.

The energy and activated oxygen metabolisms in male-sterile and fertile anthers of Hubei Photoperiod-sensitive Genic Male-sterile Rice(HPGMR) have been comparetively studied. The results indicate that the male-sterile anthers all show lower total activities of cytochrome oxidase(COD), ATPase, peroxidase(POD), catalase and superoxide dismutase(SOD) in six anthers of a spikelet which are equivalent to about 2-64%, 20-76%, 26-60%, 6-77%, 29-55%, respectively, of those of their fertile counterparts during different development stages of pollens. The abortive anthers lack 1-5, 1, 1-2 isozyme bands of COD, POD and SOD, respectively, and also display lower content of ATP, higher content of H_2O_2 and malondialdehyde and higher efficiency of O_2^- which are equivalent to about 14-77%, 152-424%, 153-238%, 230-340%, respectively, of those of their corresponding fertile ones. We conclude that the male-sterile anthers show the physiological features of lower efficiency of oxidative phosphorylation, higher efficiency of O_2^- and H_2O_2 generation, weaker activity of scavenger system of activated oxygen and higher level of lipid peroxidation, which may be the physiological mechanism of pollen abortion of HPGMR.

2063 RICE MITOCHONDRIA AND CYTOPLASMIC MALE STERILITY B.Wang, B.T.Guo, J.Z.Wang, D.D.Li and L.Hu. Institute of Genetics, Academia Sinica, Beijing 100101, China

Mitochondria and chloroplast are two types of organelles with genetic functions in cytoplasm of higher plants. In some plants, it was reported that cytoplasmic male sterility (CMS) was related to mitochondria. In some other plants, CMS was reported to be related to choiroplast. however, it is not clear in rice until now. CMS rice has been widely used in hybrid rice production and great economic benefits have been achieved in China. In order to know if CMS is related to mitochondria and / or choiroplast, we did some comparative study between CMS rice and the corresponding normal fertile rice. Through electron microscopic analysis, electrophoretic analysis and RFLP(restriction fragment length polymorphism) analysis. We found the mitochondria from CMS rice and normal fertile rice are different in the following four aspects: (1)Ultramicroscopic structure in the inner membrance and cristae. (2)Low molecular weight mitochondrial nucleic acid (mtNA) including DNA and RNA.(3)Electrophoretic pattern of mtDNA digested with restriction enzyme. (4)Hybridization pattern of restricted mtDNA with mtDNA probe. However, regarding to these four aspects, there is no defference between chloroplast DNAs from CMS rice and its normal fertile rice. Therefore, we speculate that CMS is mainly related to mitochondria in rice.

2064 CMS IS ASSOCIATED WITH LARGE mtDNA DELETIONS IN TWO *N. SYLVESTRIS* PROTOCLONES. Philippe Chétrit, Raoul Rios, Véronique Vitart, Xiu-Qin Li, Rosine De Paepe and Fernand Vedel.
Génétique Moléculaire des Plantes, URA115, UPS, Orsay F-91405

Two CMS diploid plants (CMSI and CMSII) were regenerated from the same callus after two cycles of protoplast culture from a fertile botanical line. Both plants showed altered morphological traits, identical *in organello* mt protein synthesis variations and large deletions in their mtDNA. Analysis of the reorganised regions indicated that the deletions occured via two homologous recombination events (Rec1 and Rec2) in the parental mt genome. Rec1 event, common to both CMS, lies in a transcribed region which does not share homology with any of the mt gene probes available so far. Rec2 event, specific to CMSI was found to contain a sequence homologous to the *nad5* mt gene. Rec1 was responsible for a deletion of about 49 kb in CMSII, i.e., the largest mt deletion described so far in higher plants. Rec2 reduced this deletion to 16 kb in the CMSI plant. The target site for the sterility trait may therefore lie within a common deleted region spanning 14.5 kb in both plants. A model for the timing of Rec1 and Rec2 in the initial callus is proposed. This CMS trait results from a different mechanism compared to CMS-T maize and CMS Petunia.

2065 AMPLIFIED mt DNA SEQUENCES IN A NUCLEAR MALE STERILE PROTOCLONE OF *N. SYLVESTRIS*. Véronique Vitart, Rosine De Paepe, Philippe Chétrit and Fernand Vedel.
Génétique Moléculaire des Plantes, URA115, UPS, Orsay F-91405

Late flowering male sterile plants appeared in the progeny of a fertile plant obtained after protoplast culture from a fertile *Nicotiana sylvestris* line. Genetic studies indicated that both traits were controlled by the same nuclear recessive gene, *ms4*. Fertile and sterile progenies from the regenerated plant used as female in sexual crosses were characterized by identical changes in their mitochondrial genome. Two SacI mitochondrial DNA fragments found at low levels in the parent plant were amplified in the mutant plants. Cosmid cloning and mapping demonstrated that these fragments resulted from the same homologous recombination event, Rec3. This event was shown to involve a pair of repeated elements, several hundred bp in length. Rec3 site was located close to Rec1 site common to both CMS mutants (see accompanying poster). The region of the genome implicated in Rec3 event is transcribed. *In organello* mt protein synthesis variations found in both CMS were not observed in this nuclear male sterile mutant.

2066 DIFFERENT ORGANIZATION AND ALTERED TRANSCRIPTION OF THE MITOCHONDRIAL *atp6* GENE IN THE MALE-STERILE CYTOPLASM OF RAPESEED (*Brassica napus* L.). Hirokazu Handa and Kousuke Nakajima
National Institute of Agrobiological Resources, Department of Cell Biology, Tsukuba, 305, JAPAN

The F_0-ATPase subunit 6 gene (*atp6*) of rapeseed mitochondria has been isolated from both *pol* male-sterile and normal (fertile) cytoplasms in order to determine whether the rearrangements around the *atp6* locus in *pol* male-sterile cytoplasm play a role in cytoplasmic male-sterility (cms). The *pol* cms and normal *atp6* genes are completely identical, encoding a 261-amino acid polypeptide exhibiting approximately 85% sequence homology with other plant ATP6 proteins. The *pol* cms and normal *atp6* loci share a common 1569-base pair region consisting of the *atp6* coding region (786bp), 211bp 5'-flanking, and 572bp 3'-flanking regions. As a result of extensive rearrangement, a novel reading frame (*pol-urf*) was generated upstream of the *atp6* gene in *pol* cms mitochondria, which encoded 105-amino acids and was co-transcribed with *atp6*. A 5'-portion of this ORF shows sequence homology to the *Oenothera* ORFB gene associated with *coxIII*. A 5'-flanking region of the *pol-urf* also shows homology to that of ORF105 in Ogura cms radish mitochondria. These rearrangements forming the *pol-urf* at the *atp6* locus may be responsible for cms in rapeseed.

POSTER ABSTRACTS

2067 CHARACTERIZATION OF THE UNIDENTIFIED OPEN READING FRAME ASSOCIATED WITH CMS IN PETUNIA (URF-S)
N. Firon, O. Soferman, D. Gidoni, Y. Tabib, V. Yesodi, D. Nachshoni, and S. Izhar. Department of Plant Genetics, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.
In petunia, a mitochondrial (mt) sequence comprised of rearranged mt DNA sequences (S-Pcf locus) was found to be strongly associated with CMS. The S-Pcf locus consists of three open reading frames (ORF). The first ORF consists of the sequences of the gene for ATP synthase subunit 9 (atp9), cytochrome oxidase subunit II (coxII) and an unidentified reading frame (urf-s). The other two ORFs are the NADH dehydrogenase subunit 3 (nad3) and ribosomal protein S12 (rps12) gene. So far, urf-s was found to be unique to sterile lines and sterile somatic hybrids. Further study is needed to establish the direct involvement of this locus in the disruption of normal pollen development. In our efforts to better characterize the mt S-Pcf locus of the CMS cytoplasm, we identified an urf-s related sequence in a fertile petunia line (F5) which contains otherwise a normal cytoplasm and no nuclear fertility restorer genes. The urf-s related sequence which was identified in the fertile petunia line was termed urf-f. The 5' region of urf-f was found to be different from that of urf-s, since atp9 and coxII seqences were not detected 5' to urf-f. These results suggest that the genome organization around urf-f is different from that around urf-s. Isolation and characterization of the urf-f sequence from the mt genome of the fertile F5 plant is now in progress.

2068 ASSOCIATION OF THE *S-pcf* LOCUS AND A PROTEIN PRODUCT WITH ALTERNATIVE OXIDASE IN *PETUNIA*.
M.B. Connett[1], W.E. Peiffer[2], H.T. Nivison[3], S. Ferguson-Miller[2], S. Izhar[4], and M.R. Hanson[3]. [1]*Dept. of Biol. Sci, University of Waikato, Hamilton, New Zealand.* [2]*Dept. of Biochemistry, Michigan State University, E. Lansing, Michigan USA.* [3]*Dept. of Genetics and Development, Cornell University, Ithaca, N.Y. USA.* [4]*Volcani Center, ARO, Bet Dagan, Israel.*
Analysis of 17 CMS and male fertile somatic hybrids of *Petunia* was found to strengthen a correlation previously shown in a number of isonuclear pairs of genotypes between low alternative oxidase activity in culture, the presence of the *Petunia* cytoplasmic male sterility (CMS)-associated locus (*S-pcf*), and cytoplasmic male sterility.

An originally CMS *Petunia* line that has given rise to revertants to male fertility was also shown to have both anomalous respiratory partitioning and an altered *S-pcf* locus. The rearranged locus is not part of a general pattern of rearrangement as detected by probes for normal *atp9* genes. Although the genotype carrying the rearrangements is a non-regenerable callus line, so the relationship of the altered *S-pcf* locus to male sterility cannot be tested, the data concerning this rearranged genotype argue for association between *S-pcf* and low alternative oxidase activity in culture.

A 25 kD protein encoded in the *urfS* region of the *pcf* gene in a CMS line was partially purified in a native state by Fast Protein Liquid Chromatography (FPLC). Mitochondrial protein fractions identified as containing NADH dehydrogenase activity, cytochrome oxidase activity and antigens, ATP synthase antigens, or cytochrome *bc1* were not found to contain detectable 25 kD protein. However, the 25 kD protein was found in the same fractions as the alternative oxidase constitutive antigen in FPLC fractionation of CMS mitochondrial protein by both anion exchange and cation exchange. The co-fractionation of the CMS-associated antigen and the alternative oxidase antigen in two different fractionation schemes may indicate an association between the two proteins *in vivo*.

2069 NEW NUCLEUS-ORGANELLE COMBINATIONS OBTAINED BY CYTOPLASM TRANSFER THROUGH ASYMMETRIC SOMATIC HYBRIDIZATION IN ECONOMICALLY IMPORTANT CROPS.
F.A. Krens, F.H.M. Derks, P.F. Fransz, J. Creemers-Molenaar, R.D. Hall. Dept. of Cell Biology, Centre for Plant Breeding and Reproduction Research, CPRO, P.O.Box 16, 6700 AA Wageningen, The Netherlands.
The applicability of cell fusion as a technique in breeding programs aimed at cytoplasm transfer,or rather,at making new nucleus-organelle combinations has been assessed in our laboratory. This study concerned several research projects using a number of economically important crops, such as tomato, Brassica spp., perennial ryegrass and sugarbeet. In tomato, the project was focussed on chloroplast transfer in order to reduce energy requirements. In the other crops transfer of mitochondria (cytoplasmic male sterility) was the goal. A survey will be presented of the state of the art in the separate projects including aspects of plant regeneration, cell fusion conditions, genome inactivation, cybrid selection and cybrid characterization, e.g. at the molecular level. This last technique involved analysis of the nuclear and the organellar genome organization. The results obtained have led to the initiation of new programs on biochemical/ physiological performance and on mitochondrial genome organization and recombination.

2070 IMPORT OF THE MAIZE T-URF13 PROTEIN INTO TOBACCO MITOCHONDRIA CONFERS MALE STERILITY.
François Chaumont, Mark Williams*, Charles S. Levings, III* and Marc Boutry, Unité de Biochimie Physiologique, Université Catholique de Louvain, Place Croix du Sud 2-20, 1348 Louvain-la-Neuve, Belgium and *Genetics Dept., North Carolina State University, Raleigh, North Carolina 27695-7614 USA.
Maize carrying a Texas cytoplasm is male-sterile and susceptible to fungal toxins and the insecticide methomyl. Both traits are correlated with the presence in mitochondria of a 13 kd protein, T-URF13, encoded by the mitochondrial T-*urf13* gene specific to the Texas cytoplasm. We have investigated whether the T-*urf13* gene expressed in another plant species would confer male-sterility and susceptibility to methomyl. We introduced into the nuclear genome of tobacco, the T-*urf13* gene linked to a mitochondrial targeting presequence. Transgenic plants obtained with the control T-*urf13* without presequence were sensitive to methomyl but were male-fertile. Fewer transgenic plants were obtained with T-*urf13* linked to the mitochondrial presequence. They grew slowlier and their susceptibility to methomyl was less severe than that of the plants transformed with the T-*urf13* gene without presequence. However, transgenic plants were partly or completely male-sterile according to the transformant. These plants were fertilized by heterologous pollen. The sterile trait was inherited in the F1 progeny.

2071 A CHIMERIC GENE IN THE coxI REGION OF CYTOPLASMIC MALE STERILE LINES OF WHEAT.
Harold B. Rathburn, Jiasheng Song and Charles Hedgcoth. Kansas State University, Department of Biochemistry, Manhattan, KS 66506-3702.
Crosses yielding hybrids with Triticum timopheevi mitochondria (mt) and T. aestivum nuclei yield cytoplasmic male steriles (CMS). We observed restriction fragment length polymorphisms for coxI-specific fragments from T. aestivum and CMS mtDNAs and sequenced a 3.1 kb Hind3 fragment from a CMS line. There is an insertion encoding a gene (orf256) for a 30 kd protein (ORF256) between the presumptive transcriptional promoter and the translational start site of coxI as compared with coxI of T. aestivum. The gene must have originated from a partial duplication of the coxI region as 228 bp of 5' coxI flanking region provide the 5' flanking region of orf256. The first 11 amino acids encoded in orf256 are the same as the first 11 amino acids of COXI. There is an intergenic region of 117 bp separating the termination codon of orf256 from the ATG of the following intact coxI gene. About half of this region is unique to orf256 and half is like the aestivum flanking region of coxI. The DNA sequence of orf256 following the encoded 11 amino acids of coxI is not found in aestivum mtDNA. Southern analyses with probes specific for orf256, coxI, and the 3' flanking region of CMS coxI indicate that a very similar or identical arrangement exists in mtDNA of T. timopheevi and fertility restored lines. (USDA Grant 89-37262-4912)

2072 mRNA VARIATIONS FOR coxI IN FERTILE, CMS, AND FERTILITY-RESTORED LINES.
Jiasheng Song, Harold B. Rathburn, and Charles Hedgcoth. Kansas State University, Department of Biochemistry, Manhattan, KS 66506-3702.
coxI of Triticum aestivum (Ta) gives a transcript of about 2 kb. coxI of a CMS line (Ta nucleus; T. timopheevi (Tt) mitochondria) is 3' to a chimeric gene (orf256) with a 5' flanking region like that of Ta coxI and which encodes the first 11 amino acids of COXI in a 256 amino acid reading frame. Probes for orf256, coxI, and the 3' flanking region of the CMS coxI showed a similar arrangement for Tt, CMS, and fertility-restored mtDNAs and served to characterize mRNAs for the genes. The orf256 region does not occur in Ta mtDNA. The major coxI RNA in Tt mitochondria is 2.2 kb with a 5' end in the orf256 3' coding region. A minor 2.5 kb Tt RNA has a 5' end within orf256. CMS and fertility-restored lines have major 2.5 kb RNAs with that same 5' end. CMS and restored lines contain major RNAs of 2.9 kb, covering both orf256 and coxI. The RNA 5' ends map about 86 bases 5' to the orf256 translation start site. All RNAs hybridize with the probe for the 3' flanking region of CMS coxI. The 2.9 kb RNAs of CMS and fertility-restored lines could serve to produce both ORF256 and COXI, but RNAs in Tt and Ta mitochondria could only yield COXI. The Ta nuclear background in CMS and fertility-restored lines appears to influence some aspect of the transcription process resulting in an RNA spanning both orf256 and coxI. (USDA Grant 89-37262-4912)

POSTER ABSTRACTS

2073 STUDIES ON GENETIC EFFECT AND IN POPULATION IMPROVEMENT OF DOMINANT STERILE GENE IN WHEAT. Qin Taichen, Li Zenglu, Deng Dezang, Bian Yunlong, Laboratory of Application of Hybrid Vigour J.A.C. Yangzhou, 225001, Cheng Shunhe, The Research Institute of Yangzhou Agricultural Science

The genetic effect on population of Taigu nucleus sterile were studied with NC II design of Taigu nucleus sterile wheat and normal varieties. The results showed that there were nonsignificant difference between morphyological characters of sterile and fertile populations. Yield characters of sterile population was lower than that of fertile population, but 1000 kernels weight was higher. General combining ability and special combining ability variation of parents were about same between sterile and fertile populations. The genetic parameters of two populations were different, but the heritability of characters were about same. Nucleus sterile lines Taxuan t, Tazhong No.2 and normal variety Yanshi No.9 were better breeding materials. The results of population improvement showed that recurrent selection population C1 composed by Taigu nucleus sterile lines gained greater genetic advance of yield and the genetic advance of most characters were also greater. Variation coefficient of C1 population was higher that of base population C0. The genetic advance of improvement population A1 composed by artificial selecton was smaller than that of C1 and the variation coefficient was lower than that of base population C0.

2074 ACCOMODATION AND STABILIZATION OF THE MITOCHONDRIAL GENOME BY DNA RECOMBINATION. Peter Meggyesy, Nguyen Duc Thanh, Gábor V. Horváth and Lada Rasochová. Biological Research Center, Szeged, Hungary

We have performed experiments aimed at partial transfer of the mitochondrial genome (chondriome) in isonuclear-isoplastidic tobacco combinations where cytoplasmic male sterility (CMS) marked the presence of alien chondriome in the donor (from another Nicotiana species). After a fusion transfer a recombinant chondriome intermediate between the fertile and CMS types was formed. After a subsequent fusion transfer (backfusion to the fertile type) the mitochondrial genome demonstrated further recombinations resulting in a chondriome much more similar to the fertile type although the plants remained fully male sterile. Interestingly, this newly recombinant chondriome remained unchanged after an additional backfusion to fertile tobacco. Very similar results were obtained in a taxonomically distant combination (tobacco and potato from different subfamilies). After two rounds of mitochondrial DNA recombination (mediated by two successive cytoplasm transfers by protoplast fusion) a stable recombinant chondriome was obtained which remained unchanged after additional backfusions. The stable mitochondrial recombinant tobacco plants showed a completely normal appearance, in contrast to the reduced growth ability of the CMS tobacco line containing the entire alien chondriome also indicating that an accomodated chondriome was formed. It was notable that these chondriome recombinant plants were fully male sterile. Subsequent mitochondrial DNA recombinations, therefore, formed a stable, accomodated, new chondriome, by which process one form of nucleus-mitochondrion incompatibility, the reduced growth ability (which was expressed and, therefore, counterselected in cell culture) became separated from the developmentally late expressed CMS in the flowering regenerawes.

2075 ORGANIZATION AND TRANSCRIPTION OF ATPA CODING AND 3' FLANKING SEQUENCES IN CMS *P. VULGARIS*. C.D. Chase, V.M. Ortega and S.F. Fogg. University of Florida, Vegetable Crops Department, Gainesville, FL 32611.

A region of the mitochondrial genome associated with cytoplasmic male sterility (CMS) in *Phaseolus vulgaris* (*P. vulgaris*) was flanked by two different repeated sequences, designated x and y. The DNA sequence of the CMS-unique region and a portion of each flanking repeat was determined. Repeat x contained a complete coding copy of the *atpA* gene, as well as an open reading frame (orf) predicting a protein of 209 amino acids. The TGA termination codon of *atpA* and the ATG initiation codon of *orf209* were overlapping. The junction between repeat x and CMS-unique sequences was located 14 nucleotides 3' to the *orf209* termination codon. The CMS-unique region of 3735 nucleotides contained two orfs. These predicted proteins of 151 and 98 amino acids. The region of repeat y immediately adjacent to the CMS-unique region shared sequence similarity with an internal coding region of the *cob* gene. The *cob* sequences adjacent to the CMS-unique sequences were not fused to an in-frame initiation codon. Northern blot hybridization experiments demonstrated cotranscription of *atpA* and *orf209* in CMS and fertile revertant seedlings, and cotranscription of *atpA*, *orf209*, *orf98* and *orf151* in CMS seedlings. No CMS-unique transcripts were detected by northern blot hybridization of mitochondrial RNAs isolated from seedlings carrying the sterility-inducing cytoplasm in combination with fertility restoration genes from the line XR235-1-1. The molecular basis of this observation is under investigation.

This work was supported by USDA Competitive Research Grant 89-37262-4827.

2076 GENETIC ANALYSIS OF *Rf3* FUNCTION IN CMS-S MAIZE. T.L. Kamps, D.R. McCarty, and C.D.Chase. University of Florida, Vegetable Crops Department, Gainesville, FL 32611.

The S-type cytoplasmic male sterility (cms-S) of maize is gametophytically restored to fertility by the nuclear gene *Rf3*. Dominance and recessive descriptions cannot be assigned to alleles expressed in haploid tissue. We have tested the dominance-recessive relationship of the restoring (designated *Rf3*) and non-restoring (designated *rf3*) alleles in diploid pollen. The meiotic mutant *elongate* (*el*) was utilized to generate cms-S tetraploids (4N). The S cytoplasm and restoring allele were contributed by Ky21/S. The 2N pollen of *Rf3Rf3rf3rf3* plants was examined microscopically and scored for phenotype. A ratio of 5 filled grains : 1 shrunken grain was observed, indicating dominance of the restoring allele (*Rf3*). These results also argue against the possibility of the *rf3* allele functioning to induce sterility. Transmission of the non-restoring *rf3* allele through the diploid pollen will provide conclusive evidence of *Rf3* dominance. Linked restriction fragment length polymorphisms (RFLPs) will be used to verify transmission of *rf3*.

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2077 TRANSLOCATION AND TEMPLATE ACTIVITY OF FOREIGN GENETIC MATERIAL IN ISOLATED MAIZE MITOCHONDRIA. Yury L.Konstantinov, Vladimir A.Pogosonny, Galina L.Lutsenko, Elena L.Tauson. Siberian Institute of Plant Physiology and Biochemistry, Irkutsk 664033, USSR

The translocation of DNA of bacterial and plant origin has been studied in intact mitochondria of maize hybrid (Krasnodarsky 303 ATV) seedlings. The transport of DNA of bacterial pBR322 vector plasmid into maize mitochondria was shown by radioautographic analysis of preparations of the purified mitochondrial DNA. Using the system of in organello DNA synthesis, the incorporation of the labelled precursor of the synthesis in bacterial vector plasmid DNA was registered. Only covalently closed form of plasmid DNA was found in the inner space of the organelles. Altogether, the study of the transport into isolated mitochondria of the DNA of bacterial pBR327 plasmid vector and recombinant pZmS2.5 plasmid carrying Pst1-fragment of linear S2 plasmid-like DNA of maize mitochondria produced similar results. The translocation and template activity of linear S1 and S2 plasmid-like mitochondrial DNA to synthesize DNA were particularly studied. In this case, no template activity was registered in this type of DNA which could be accounted for by the loss of terminal proteins connected with 5'-ends of these DNA molecules during purification procedure. The data obtained suggest that the reported experimental system may be promising in development of the genetic transformation system of cereal mitochondria.

2078 STUDY ON ISOZYMES OF DIFFERENT CYTOPLASMIC TYPES IN MAIZE. Qin Taichen, Wen Xianmong, Laboratory of Application of Hybrid Vigour, Jiangsu Agricultural College, J.C.A. Yangzhou 225001, Huang Bingsheng, The Research institute of Agricutural Science of Huaiyin 223000

The peroxidase esterase and α-amylase isozymes were analysed with three groups of isonuclear alloplasmic lines in maize by using vertical slab polyacry lamide gel electrophoresis. Samples for analysis were taken from different tissues in various developmental stages: embryo of germinating seeds, leaves of seedling, third leaves countered from up to down and roots in various stages, anthers of mother pollen stage and two or three nuclei pollen stage, pistil, the endosperm in milk stage after pollination. It was found that the isozymograms of three kinds of isozymes showed significant differences in anthers among cytoplasmic lines with the same backgrounds. It showed that the numbers of esterase isozyme bands of all cytoplasmic lines reduced from mother pollen stage to two nuclei or three nuclei stage, and the reducing degree of band numbers in male-sterile line was less than in their respective normal lines. About α-amylas isozyme in anthers, males-terile lines showed either more in number of bands or higher in activity of certain bands than their respective maintainers. The peroxidase isozymograms in counter-third leaves of various cytoplasmic lines showed a developing regularity with the advance of growth. In the developing process, the differences in the peroxidase isozymogram among various cytoplasmic lines appeared gradually, and the stage and degree of the differences varied with different nuclear backgrounds. From these results, it was suggested that the isozymes were mainly controlled by nuclear genes, and the differences of zymograms in certain tissues among different lines were due to the interaction between nuclear genes and cytoplasms. No significant differences in three kinds of isozymogram among various cytoplasmic lines were observed in embryo, seedling, root, pistil and endosperm in milk stage.

POSTER ABSTRACTS

2079 MITOCHONDRIAL GENETICS OF *CHLAMYDOMONAS REINHARDTII*

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The first point mutation in the mitochondrial genome of *C. reinhardtii* was reported recently (Bennoun, Delosme and Kück, Genetics, 1991) . In order to improve the isolation procedure of such mutants, new methods were required. We report here on the efficiency of manganese to induce mitochondrial mutations in his alga and on the use of cytoduction to transfer mitochondrial markers in appropriate nuclear and chloroplast genetical backgrounds. Both methods were used to isolate new mitochondrial mutants subjected to genetic and molecular analysis.

2100 ANALYSIS OF CIS ELEMENTS AND TRANS FACTORS CONFERRING XYLEM- AND TUBER-SPECIFIC EXPRESSION

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The genes encoding enzymes of phenylpropanoid metabolism are regulated in a complex tissue-specific and temporal manner in response to developmental and environmental cues. The first enzyme in the pathway, PAL, is encoded by a small gene family which show differential tissue specificities of expression. In addition, each gene shows a complex pattern of expression. This pattern was shown to be due to discrete *cis* elements in the first 250bp of the promoter. Sequences conferring expression in xylem tissues are located at two positions in the promoter, and mutations that specifically abolish binding of a DNA-binding factor isolated from developing xylem abolish expression in that tissue. Progress is being made towards cloning the gene encoding this binding factor. The expression of some genes that are abundantly expressed in potato tubers is dependent in part on levels of sucrose. We have identified *cis* sequences that confer sucrose-inducibility and other sequences adjacent that act as non-specific enhancers, and the binding activity of nuclear factors follows the activity of the promoter closely during the early stages of tuber development. Progress is being made in cloning the cognate transcription factors involved. Once cloned, the factor(s) will enable us to understand and manipulate sucrose partitioning and production in crop plants.

2101 CYTOCHROME P-450 IN BRASSICA NAPUS SEEDLINGS.

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Microsomal preparations from the roots of 4-day-old Brassica napus cv Bienvenu seedlings contained cytochrome P-450 monooxygenase protein at a specific activity of over 100 pmol/mg protein, as determined by CO difference spectra. Significant quantities of cytochrome P-420 were also observed. Hypocotyl and cotyledon microsomal preparations did not contain either cytochrome P-450 or cytochrome P-420. Immunoblot analysis of root, cotyledon and hypocotyl microsomal proteins with polyclonal antibodies raised against bacterial cytochrome P-450 (camphor hydroxylase) from Pseudomonas putida) showed a distinct cross-reaction with a 62.5 kD polypeptide present only in root microsomes. Anti-avocado fruit cytochrome P-450 (ARP-1) polyclonal antibodies cross-reacted with this 62.5 kD root microsomal polypeptide and polypeptides of greater M_r present in all microsomal preparations. A cDNA library has been constructed with mRNA extracted from 4-day-old root tissue. An initial gene expression study with a C-terminal 383 bp Bgl II fragment from avocado P-450 cDNA (Bozak et al., 1990) containing conserved domains was unsuccessful. Synthetic oligonucleotides based on highly conserved regions of known P-450 sequences have been prepared and results of gene expression studies will be presented.

2102 TRANSGENIC TOBACCO CONTAINING pTiBo542 T-DNA FRAGMENT EXHIBIT ANCREASED RHIZOGENIC CAPACITY.

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pTiBo542 BamHI fragment 5 (Hood E.E. et al, 1984, 1986) from supervirulent *Agrobacterium tumefaciens* strain A281 was cloned in a binary vector BinI9. The fragment contains tms1 and tms2 genes involved in auxin biosynthesis (Komari et a., 1986). This construct was used to transform *Nicotiana tabacum* SR1 leaf disks with a helper strain LBA4404. Kanamycin resistant regenerants were fertile. Their progeny was tested for Km-resistance, and leaf disks from resistant plants were incubated on MS media with kanamycin and different phytohormone content. The progeny of one of the regenerants showed capacity to form roots on leaf disks on medium without phytonormones, while leaf disks from control plants (nontransformed or transformed with pBI121) did not form roots. Root formation was not observed on cytokinin containing media.

2103 EFFICIENT GREEN PLANT REGENERATION FROM ANTHER CALLI OF INDICA RICE. N. Lenka and G.M. Reddy, Department of Genetics, Osmania University, Hyderabad - 500 007, India.

Anthers from four cultivars of indica rice, CR 666-36-4, SR 26-B, Basmati mutant semidwarf 10 and Ptb-33 were inoculated onto N6, Chaleff's R-2 and MS media with varying concentrations of sucrose and hormonal combinations. Among media, N6 and Chaleff's R-2 with 2mg/l NAA and 0.5mg/l kinetin were superior for callus induction. With 6% sucrose, the callusing frequency was 1.2-1.5 times more compared to 3% sucrose. Cultivar Ptb-33 exhibited maximum callusing (42%) in N6 medium with 2mg/l NAA, 0.5mg/l kinetin and 6% sucrose. Plant regeneration in the four cultivars was maximum on MS medium containing 1mg/l NAA or IAA, 2mg/l BAP and 0.6mg/l kinetin. High frequency regeneration (75%) was found in this particular medium with 6% sucrose in Ptb-33. However, the proportion of green and albino plant regeneration varied with the type of media and sucrose concentration among the cultivars. SR 26-B and Basmati semidwarf 10 gave only albinos with 18-28% and 15-60% frequency respectively. While the anther callus of CR 666-36-4 gave 5.0-18.5% green and 7.0-21.5% albinos, Ptb-33 exhibited 10.0-45.0% green and 18.0-30.0% albinos in different media. Higher concentration of sucrose (6%) enhanced the regeneration frequency by 1.2-2.0 times compared to lower level of sucrose (3%) in all the media used. These studies clearly suggest that, specific cultivar, suitable hormonal combinations and sucrose concentration can be exploited successfully in green plant regeneration from anther callus of indica rice.

2104 CHARACTERIZATION OF THE ASSIMILATORY NITRATE REDUCTASE OF THREE HETEROCYSTOUS CYANOBACTERIA

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Assimilatory nitrate reductase (NR) plays a key role in nitrate-utilizing organisms, for it catalyzes the first step of nitrate metabolism. Plant NRs have been extensively studied in several instances at a molecular and genetic level and, consequently, are already well characterized. In contrast, the characterization of cyanobacterial NRs is still incipient, specially of those of heterocystous strains, since the scarce reports refer mainly to unicellular (*Anacystis nidulans*) and filamentous, nitrogen non-fixing (*Plectonema boryanum*) cyanobacteria. In these cases NR consists of a single polypeptide of an apparent Mr of about 85 kDa. We present here recent data on the NRs of the heterocystous strains *Anabaena* sp 7119, and *Nodularia* spp M1 and M2. These two *Nodularia* strains, isolated and purified to axenity in our laboratory, exhibit an anomalous regulation of nitrogen-fixation, for nitrate (even 20 mM) fails to impair nitrogenase activity. NR from the three strains show similar characteristics. It seems to be an oligomer of more than 200 kDa, made up of a single polypeptide type, with an apparent Mr of about 90 kDa. Cell concentration of the popypeptide is greatly increased by nitrate, and decreased by ammonium in the culture medium. In all instances the polypeptide is about 10 times less abundant in the *Nodularia* strains than in the *Anabaena* one. This fact suggests that the basis of the failure for nitrate to regulate nitrogenase activity in the *Nodularia* strains is a reduced level of the NR apoenzyme, caused by an altered activity of the structural NR gene.

POSTER ABSTRACTS

2105 MODIFICATIONS OF MITOCHONDRIAL DNA CAUSE CHANGES IN FLORAL DEVELOPMENT IN HOMEOTIC-LIKE MUTANTS OF TOBACCO.

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To investigate the influence of mitochondrial genes on stamen development of higher plants, protoplasts were fused from 3 male-sterile cultivars of *Nicotiana* with different floral morphologies. With these fusion experiments it was demonstrated that fusion of protoplasts from male-sterile tobacco plants with different mitochondria can lead to male-fertile cybrid plants and also to male-sterile plants with stamen morphologies combining stamen features from both parents in a novel arrangement. The male-sterile morphologies of these cybrids and their parents support the hypothesis that nuclear mitochondrial interaction occurs at several stages in tobacco floral development and that several mitochondrial genes are necessary for normal stamen and corolla development. Homeotic mutants described in other organisms exhibit phenotypes similar to those found in cytoplasmic male-sterile cultivars of *N. tabacum* and the cybrids obtained in our study. Stigmatoids, ovules and petals were found in conjunction with, or in place of stamens. The finding that nuclear and cytoplasmic mutations result in similar developmental defects is consistant with the hypothesis that floral development requires the cooperative interaction of both genomes. Close morphological resemblances between stamens of more primitive plant species and the stamens of male-sterile tobacco cultivars and male-sterile cybrids indicate that mitochondrial-nuclear coevolution was important in defining the transition of primitive stamens into modern stamens which are characterized by non-pigmented filaments and petaloid-free anthers.

Mitochondrial DNA studies involving comparisons between restriction patterns from cybrids and male-sterile parents showed that mtDNA changes always accompanied changes in floral phenotype and changes in floral phenotype were always predictive of changes in mtDNA. Analysis of mtDNA isolated from male-fertile cybrids led to the identification of a few restriction fragments that always appeared in conjunction with normal stamen development. Southern hybridization analyses and transcription studies using probes for mitochondrial genes revealed specific differences between male-sterile and male-fertile plants that involved both the coding and the 3 flanking regions of the alpha-subunit of the ATPase complex.

2106 Organization and Sequence Analysis of 5S rDNA in Brassica campestris and Eruca sativa

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5S rDNA sequences have been cloned and characterized from two important crucifers, namely Brassica campestris and Eruca sativa. Our hybridization data indicate that 5S genes from both the species and other crucifers are tandem repeats of 500bp and clustered in the genome. These sequences are also methylated as demonstrated with other plant 5S genes. The B. campestris 5S rDNA has been cloned as 496bp BamHI fragment whereas the E. sativa 5S rDNA has been cloned as 980bp BamHI fragment. The coding region of B. campestris 5S rDNA is 120bp in length and is highly conserved. It is nearly identical to that of Pisum sativum and Matthiola incana except for one or two nucleotide differences. Whereas sequence analysis of the intergenic spacer (non-coding region) of B. campestris shows no homology to any other reported plant 5S rDNA sequences. In contrast to B. campestris, we have identified two variants of 5S rDNA in E. sativa. One is 487bp in length (Variant I) and the other is 480bp in length (Variant II). These two variants are tandemly arranged and exist as 1kb fragment in the genome. At least two clones exhibiting 1kb BamHI fragments have been sequenced in both directions. Analysis reveals that there is at least 15-20% variation even in the coding region when compared to B. campestris and other plant 5S rDNA sequences. There is no homology between the non-coding spacer of E. sativa and other reported plant 5S rDNA sequences. Variant nature of 5S gene was confirmed on the basis of differences in the coding region. Sequences analysis of B. campestris and E. sativa 5S rDNA and its relationship to other crucifers will be discussed.

2107 A MOLECULAR APPROACH TO STUDY THE PERCEPTION OF THE AUXIN SIGNAL. K. Palme, B. Brzobohaty, Feldwisch, J., Garbers, C., Hesse,F., Hesse, T., I. Moore, R. Walden, Zettl, R., and J.Schell
Max-Planck-Institut fur Züchtungsforschung, Carl-von-Linné-Weg 10, D-5000 Köln 30, F.R.G.

Plant development is influenced by phytohormones, which elicit a remarkably wide variety of responses. Of particular interest are auxins which modify elongation growth, selective transcription of genes, control of cell division and vascular tissue differentiation. Receptor like proteins have been assumed to recognize and transmit the auxin signal. The search for such proteins led to the identification of auxin binding sites to the endoplasmic reticulum, the tonoplast and the plasma membrane. We have cloned from maize and A. thaliana members a gene family encoding auxin binding proteins located to the endoplasmic reticulum. We have expressed these genes in transgenic plants and will present the analysis.
Using photoactivatable probes we were successful in isolating from Zea mays different auxin binding proteins which are located either in the cytosol or in the plasma membrane. We will present recent results on identification and characterization of a NPA binding protein that is located in the plasma membrane. We assume that this protein is a component of the auxin efflux carrier.

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