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FIFTH INTERNATIONAL FUNGUS SPORE CONFERENCE

UNICOI STATE PARK AND CONFERENCE CENTER HELEN, GEORGIA

AUGUST 17-21, 1991

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SPONSORED BY THE UNIVERSITY OF GEORGIA AND THE CENTER FOR PLANT CELLULAR AND MOLECULAR BIOLOGY

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the University of Georgia in helping the organizers make this conference the success we hope it will be. University President Charles B. Knapp and Kevin Corbett of the Office of Development were helpful in the obtaining of industrial support. Vice presidents Joe L. Key, Research, and William F. Prokasy, Academic Affairs, provided moral and financial support during our preparation for this meeting. A special thanks to Ms. Kathy Vinson who organized both the organizers and the participants.

Conference support grants from the National Science Foundation and the Department of Energy are gratefully acknowledged.

We also express our thanks to the industrial sponsors whose support makes this conference a more pleasurable one for all participants

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SCHEDULE OF EVENTS

17-21 AUGUST, 1991

SATURDAY, AUGUST 17

1200 onwards — Registration. Basement of Building D.
Setup for Poster Session 1. Sporen Keller. Building D.

1400 - Sporen Keller open. Drinks and light snacks courtesy of our industrial sponsors.

2030 - Opening Dinner (Conference Rooms A-C):

Welcome and remarks - Bill Timberlake
Dinner
Remarks and Introduction of Speaker - Mel Fuller

KEYNOTE ADDRESS - DR. SALOMON BARTNICKI-GARCIA

"SPEAK SOFTLY, THINK DEEPLY, THE FUNGUS IS SLEEPING"

SUNDAY, AUGUST 18, MASTER'S HALL 3 & 4

INDUCTION OF SPORULATION

830	**Dr. Charles Mims**	The Fungal Spore — Form and Function
915	**Dr. Robert Ullrich**	Control of Fruiting by Mating Type
1000	**Coffee Break**	
1030	**Dr. Tom Adams**	Initiation of Conidiophore Development in *Aspergillus nidulans*
1115	**Dr. Lawrence Yager**	Light Control of Asexual & Sexual Sporulation in *Aspergillus nidulans*
1200	**Lunch. Unicoi restaurant.**	
1400 - 1800	**Research Discussions.** These will be *ad hoc* discussions organized by participants. See Charles Mims for use of available rooms and equipment.	
1400 - 1800	**Posters session I. Sporen Keller open.**	
1800 -1930	**Dinner. On your own. Unicoi restaurant or in Helen.**	

NUCLEAR DIVISION

2000	**Dr. Brent Heath**	The Cytoskeleton & Spore Formation

2045	**Dr. N. R. Morris**	Deletion of the Gene for One of the Two α-Tubulins of *Aspergillus nidulans* Blocks Ascosporogenesis
2130	**Dr. Berl Oakley**	Gamma Tubulin is Ubiquitous and is a Component of Micro tubule Organizing Centers

MONDAY, AUGUST 19. MASTER'S HALL ROOMS 3 & 4.

SPORE FORMATION

830	**Dr. Gordon Beakes**	Asexual and Sexual Spore Formation in Zoosporic Fungi
915	**Dr. John E. Hamer**	The SMO Locus of *Magnaporthe grisea*
1000	**Coffee Break. Please get coffee, etc. and return to room as we must keep on schedule.**	
1015	**Dr. Tommy Sewall**	Ultrastructural Analysis of Fungal Sporulation Mutants
1100	**Dr. Bruce Miller**	Genetic Control of Conidiophore Morphology
1145	**Load bus and vans for outing. Van for those taking the train ride must depart at 1145. Box lunch with chicken will be provided on board.**	

MOUNTAIN OUTING AND DINNER

Set up for Poster Session II. Sporen Keller.

TUESDAY, AUGUST 20. MASTER'S HALL ROOMS 3 & 4

SPORE RELEASE AND DISPERSAL

830	**Dr. John Taylor**	Spores and Molecular Evolution
915	**Dr. Jim Anderson**	Clonal Reproduction, Spore Dispersal & Mating in Basidiomycetes
1000	**Coffee Break**	
1030	**Dr. Nick Money**	Mechanisms of Spore Release in Zoosporic Fungi
1115	**Dr. Robert Hanau**	The Role of Fungal Spores in Plant Pathogenesis
1200	**Lunch. Unicoi Restaurant.**	
1400 - 1600	**Research Discussions**	
1400 - 1800	**Posters. Session II. Sporen Keller open.**	
1830 - 1930	**Dinner. On your own.**	

SPORE FORM AND FUNCTION

2000	**Dr. John Clutterbuck**	Molecular Genetics of Fungal Spores
2045	**Dr. Martha Powell**	Structure & Function of Motile Cells in Fungi
2130	**Dr. Adrienne Hardham**	The Use of Monoclonal Antibodies to Study Zoospore Form and Function

WEDNESDAY, AUGUST 21. MASTER'S HALL 3 & 4.

SPORE GERMINATION

830	**Dr. Kurt Mendgen**	Stage-Specific Production of Cell Wall-Degrading Enzymes by Uredosporelings of Rust Fungi
915	**Dr. P. E. Kolattukudy**	Molecular Communications between the Fungal Spore & its Host
1000	**Coffee Break**	
1030	**Dr. Richard Staples**	Germling Differentiation: an Environmentally Induced Process of Pathogen Ingress Adapated to the Host
1115	**Dr. Carmen Cano**	Differential Gene Expression During Spore Germination of *Mucor*. Role of DNA Methylation and Polyamines

ABSTRACTS OF INVITED SPEAKERS

(Arranged in Order of Talks)

Charles W. Mims

Department of Plant Pathology, University of Georgia, Athens, GA 30601.

The Fungal Spore — Form and Function

This is a very general presentation designed to provide an overview of various ultrastructural features of different types of fungal spores. Data obtained from both scanning and transmission electron microscopic studies will be presented. Examples of spore types to be considered include ascospores, conidia, basidiospores of rusts and various holobasidiomycetes, smut and rust teliospores, and aeciospores and urediniospores. Emphasis will be placed upon variations in the nature of spores surface markings and spore wall architecture. Attention will also focus upon cytoplasmic and nuclear components of spores and, when possible, results obtained from the study of freeze substituted samples will be compared to results from conventionally fixed samples. An attempt will be made to correlate certain morphological features of spores to various functions performed by spores.

--Notes--

Robert C. Ullrich[1] , Charles A. Specht[1], Mary M. Stankis[2], Huiling Yang[1] , Guang-Ping Shen[2] and Charles P. Novotny[2]

Departments of Botany[1] and Microbiology and Molecular Genetics[2], University of Vermont, Burlington, VT 05405.

Control of Fruiting by Mating Type

Mating type in *Schizophyllum commune* is typical of heterothallic basidiomycetes in the respect that it is the primary and critical regulator of sexual development leading to fruiting body formation and elaboration of meiotic products. Mating type in Schizophyllum is determined by four loci: *Aα*, *Aβ*, *Bα* and *Bβ*, each with naturally-occurring alternative forms. Regulation is an intracellular event exerted within the fusion cells formed between two paired haploids. Partial development results when the two haploids differ at either of the four loci; full development requires differences for at least one *A* locus and one *B* locus.

We have isolated three alternative forms (*Aα1*, *Aα3* and *Aα4*) of the *Aα* locus. The locus consists of 5-9 kb of heterogeneous DNA embedded within a common flanking sequence of the chromosome. Sequence and transcript analyses show the *Aα* locus to be a complex encoding a small cluster of genes. We have identified four *Aα*-specific transcripts and two transcripts from the common sequence flanking *Aα* in *Aα4* cells. *Aα1*, *Aα3* and *Aα4* contain different alleles for several of the *Aα*-specific genes. Transformation experiments demonstrate that at least part of the mating-type specificity associated with alternative forms of *Aα* is encoded within two genes, *ORF1* and *ORF2*. *ORF1* and *ORF2* are homeotic genes, and therefore are thought to be involved in controlling sexual development through DNA binding and the regulation of transcription from developmental genes. Alleles of *ORF1* show acidic domains common to transcription factors. A 2.4 kb transcript coded within the *Aα* locus is developmentally regulated with transcript about tenfold more abundant in dikaryons than haploids. The evidence presented above suggests the following working hypothesis. One or more mRNAs coded from the specific *Aα* present in each of the two haploid nuclei of the fusion cell are translated into polypeptides in the cytoplasm. These polypeptides are transported into each of the two haploid nuclei present in the fusion cell. These polypeptides form heteromultimeric complexes within each haploid nucleus, bind to regulatory sites on the DNA and cause the alteration of target gene transcription. This action of altered gene transcription (both activation and inhibition) is thought to be responsible for switching the cell to sexual development.

--Notes--

Thomas H. Adams

Department of Biology, Texas A&M University, College Station, TX 77843.

Initiation of Conidiophore Development in *Aspergillus nidulans*.

In contrast to many other cases of microbial development, *Aspergillus nidulans* conidiophore production initiates primarily as a programmed part of the life cycle rather than as a response to nutrient deprivation. The molecular genetic mechanisms controlling activation of the conidiation pathway are largely unknown but lead ultimately to the transcription of *brlA* which in turn results in activation of other genes required for conidiophore formation. Several developmental mutants have been described for *A. nidulans* that have morphological defects suggesting that they are altered prior to activation of *brlA* expression. One of the most commonly described phenotypes for such early developmental mutants is "fluffy". *Aspergillus nidulans* fluffy mutants characteristically produce an unrestricted proliferation of aerial hyphae and give rise to large cotton-like colonies. Fluffy mutants frequently produce relatively normal conidiophores from this hyphal mass but with a several day delay as compared to wild type. These mutants must therefore be capable of expressing the genetic machinery necessary for conidiophore development but are apparently altered in the ability to respond to the programmed signals that control timing. One typical fluffy mutant results from mutation of the *acoD* gene and is defined by a temperature sensitive mutation *acoD684*. I have obtained a plasmid clone (pAD4; L. N. Yager and W. Hide, unpublished) capable of complementing the *acoD684* mutation at high frequency, and have demonstrated genetically that this plasmid contains the wild type *acoD* gene. Strains containing a deletion of the *acoD* gene are completely aconidial and no *brlA* expression is detectable during growth on rich medium. However, this mutant phenotype can be partially remediated by conditions that limit growth suggesting that, unlike wild type strains, *acoD* null mutants develop in response to nutrient limitation. In addition, *acoD* mutant strains conidiate when grown in contact with either wild type colonies or with strains carrying different developmental mutations (e.g. *brlA*). Sequence analysis of the *acoD* coding region indicates that the gene encodes a protein that is greater than 750 amino acids in length and that the carboxy-terminus of the predicted polypeptide shares significant similarity with glutamine sythetase. I propose that *acoD* encodes an enzyme responsible for production of an extracellular factor that functions by directing growing cells to undergo development. In the absence of this factor, development can still occur in response to intracellular signals resulting from growth limitation.

--Notes--

Lawrence N. Yager

Department of Biology, Temple University, Philadelphia, PA 19122

Light Control of Asexual & Sexual Sporulation in *Aspergillus nidulans*

Light affects both asexual and sexual sporulation in *Aspergillus nidulans.* Light is required for the initiation of conidiophores and the formation of asexual spores (conidia). Although light is not required for sexual sporulation, it does delay the time of initiation of cleistothecial primordia. Light-dark shifting experiments indicate that two different, nonoverlapping critical periods of development exist for both asexual and sexual sporulation cycles. The ability of light to elicit conidiation or delay cleistotheciation is dependent upon the allelic state of the *velvet A* (*veA*) gene. Mutation of this gene allows conidiation to occur in the dark and delays cleistotheciation regardless of the presence or absence of light. Mutations in two new loci, designated *veB* and *veC*, have been isolated, which have similar phenotypes to the *veA1* mutation. The characteristics of these genes will be discussed. Lastly, it has been shown that both sporulation cycles respond to specific wavelengths of light. Irradiation by either blue light (440 nm) or red light (700 nm) elicits conidiation and delays sexual development. Mutants that affect the specific response to either wavelength have been isolated and characterized.

— Notes —

I. Brent Heath

Biology Department, York University, Toronto, Ontario, Canada M3J 1P3

The Cytoskeleton and Spore formation

Oomycetes produce two morphologically distinct types of biflagellate zoospores in their asexual life cycle. The development of these zoospores involves many highly reproducible and predictable forces requiring events such as cytoplasmic cleavage, organelle movements, germ tube production, shape generation, exocytosis and the synthesis and positioning of assorted organelles. Using a combination of electron microscopy, rhodamine-phalloidin based fluorescence microscopy and inhibitor studies, we can begin to understand the ways in which microtubules and actin filaments function singly, or in combination, to provide the mechanical basis for these various processes. I shall review previous work on microtubules and integrate it with our recent studies on actin to show the pivotal roles that these cytoskeletal elements play in all aspects of the zoosporic phases of the life cycle of *Saprolegnia ferax*.

— Notes —

N. Ronald Morris and Karen E. Kirk

Department of Pharmacology, University of Medicine and Dentistry - Robert Wood Johnson Medical School, Piscataway, N. J. 08854

Deletion of the Gene for One of the Two α-Tubulins of *Aspergillus nidulans* Blocks Ascosporogenesis

The filamentous fungus *Aspergillus nidulans* has two genes encoding α-tubulin, *tubA* and *tubB*. Mutations in *tubA* and molecular disruption of *tubA* have demonstrated that its gene product is essential for mitosis and nuclear migration during vegetative growth. We have now addressed the question of the function of *tubB* by deleting and replacing it with a selectable marker to produce a mutation that we term *tubBΔ*. Deletion of *tubB* has no demonstrable effect on vegetative growth and only a small effect on conidiation, but completely inhibits ascosporogenesis, i.e., *tubBΔ* strains produce no sexual spores when self-crossed. However, *tubBΔ* strains produce viable ascospores when outcrossed to *tubB*$^+$ strains, indicating that the *tubBΔ* mutation is recessive. We have studied the cytology of sexual development in wild-type strains and in the *tubBΔ* mutants to elucidate where ascosporogensis fails in the mutant. Both the wild type and *tubBΔ* are able to form a dikaryon, which in the wild type strain undergoes karyogamy, a meiotic division and two mitotic divisions to form an ascus with eight ascospores. However, *tubBΔ* strains, although they appear to be able to undergo karyogamy, are unable to proceed through meiosis. To determine whether the *tubB*$^+$ requirement for ascosporogenesis represents a specific requirement for *tubB* α-tubulin, we have introduced extra copies of the *tubA* α-tubulin gene under the control of various promoters into a *tubBΔ* strain. The effect of upregulating *tubA* α-tubulin is to ameliorate, but not completely reverse, the defect in ascosporogenesis caused by the *tubBΔ* mutation.

— Notes —

Berl R. Oakley, C. Elizabeth Oakley, Yixian Zheng, Yisang Yoon and M. Katherine Jung.

Department of Molecular Genetics, Ohio State University, Columbus, OH 43210

Gamma Tubulin is Ubiquitous and is a Component of Microtubule Organizing Centers

We have identified, in *Aspergillus nidulans*, a new member of the tubulin superfamily of proteins, gamma tubulin. By low-stringency hybridizations we have identified gamma-tubulin cDNAs from *Drosophila melanogaster* and *Homo sapiens*. Gamma tubulin is, thus, likely to be ubiquitous in eukaryotes. We have disrupted the gamma-tubulin gene of *A. nidulans* and have found that gamma tubulin is essential for viability, nuclear division and microtubule assembly. We have prepared gamma-tubulin-specific antibodies and have found that gamma tubulin is located at spindle-pole-bodies in *A. nidulans* and at centrosomes in *D. melanogaster* and mammalian cells. Gamma tubulin is thus a component of microtubule organizing centers of phylogenetically diverse organisms. Based on these findings, we propose that gamma tubulin nucleates the assembly of microtubules at microtubule organizing centers and establishes the polarity of these microtubules. Supported by a grant from the NIGMS.

— Notes —

Gordon Beakes

Department of Biology, the University, Newcastle upon Tyne, NE1 7RU, UK

Asexual and Sexual Spore Formation in Zoosporic fungi

In most aquatic fungi, three morphogenetic pathways/options are available to the organism upon the induction of sporulation viz.: 1) the formation of a sporangium to produce dispersive zoospores, 2) the formation of a resting spore, usually following gametangial contact and sexual reproduction and 3) the production of an *in situ* 'chlamydospore' derived from the delimitation of a segment of differentiated hyphal cytoplasm. However, the fundamental differences between these spore types and their biological significance is often not fully appreciated. Zoospore differentiation in the Saprolegniales (*Saprolegnia* and *Achlya*) and a number of chytrid parasites of planktonic algae (*Rhizophydium* and *Zygorhizidium* spp.) will be compared and contrasted. In particular the temporal and spatial differentiation of some of the specialized zoopore organelles will be traced and the structural aspects associated with the spore discharge apparatus described. Different patterns of operculum differentiation in the chytrids will be described and an evolutionary sequence suggested. The structural features of the corresponding resting spores produced by the same fungi will be described with emphasis on the nature and disposition of storage reserves and their wall structure. Changes in the resting spore wall may be the crucial event associated with the spore becoming quiescent, particularly in Oomycete fungi. Finally evidence will be presented to suggest that chlamydospores represent partially differentiated 'sporing structures'.

— Notes —

J. E. Hamer, S. Given, K. Dobinson and J. Stout

Department of Biological Sciences. Purdue University, West Lafayette, IN 47906

The *SMO* Locus of *Magnaporthe grisea*

Magnaporthe grisea is a filamentous Ascomycete, and is a pathogen of many cultivated and feral grasses. Isolates obtained from nature are usually infertile and thus reproduction is exclusively asexual and relies on the production and dispersal of conidia. Dispersed conidia attach immediately to leaf surfaces and germinate rapidly via the production of a germ tube. On the leaf surface or on artificial hydrophobic surfaces an infection structure called an appressorium differentiates from the hyphal tip. The appressorium becomes melanized and allows direct penetration of the plant epidermis. We have identified a genetic locus termed *SMO* (for spore morphology) that plays a role in directing cell shape and normal differentiation of conidia, appressoria, and asci. Wildtype strains produce pyriform shaped conidia containing two septa separating three nuclei. *Smo*$^-$ conidia contain between one to three cells, but usually three nuclei; however, the shape of the conidia becomes highly deformed and a wide variety of spore shapes can be observed. Appressorium formation is delayed in *Smo*$^-$ strains and abnormally shaped appressoria are frequently observed. In crosses between *Smo*$^-$ strains, asci are abnormally shaped, whereas crosses between *Smo*$^-$ and *Smo*$^+$ strains produce normal tapered shaped asci. The *SMO* locus also appears to be highly mutable and different genetic backgrounds of *M. grisea* produce different spontaneous mutation rates at the *SMO* locus. Dispersed repeated sequences in the *M. grisea* genome were used to map the *SMO* locus, and a chromosomal walk has been undertaken to clone the wildtype locus.

— Notes —

Tommy C. Sewall

Department of Biology, Texas A&M University, College Station, TX 77843

Ultrastructural Analysis of Fungal Sporulation Mutants

Conidiogenesis in *Aspergillus nidulans* consists of a series of developmental events that result in the formation of complex, multicellular conidiophores. The genetic regulation of conidiophore formation has been extensively studied using classical and molecular methods. Mutations in the *brlA*, *abaA*, and *wetA* genes block conidiogenesis during conidiophore vesicle formation, phialide differentiation, and conidium maturation, respectively. These three genes act sequentially in a regulatory pathway responsible for controlling numerous sporulation-specific genes. Mutant strains were compared to the wild-type strain using electron microscopy to determine the developmental event altered by each mutation. *brlA*$^-$ conidiophore stalks continued indeterminate apical growth rather than shift to radial expansion to form the conidiophore vesicle. Expression of *abaA* was required for conidium wall synthesis by the phialide apex and for the change in the nuclear division pattern required for conidium budding. Temperature-shift experiments demonstrated that *abaA* expression was required for both phialide differentiation and maintenance of conidium production. *wetA*$^-$ conidiophores were normal but produced conidia that were missing two distinct wall layers upon maturity. Progressively older conidia developed thinner walls and larger vacuoles until lysis occurred. Interactions between genes involved in the regulation of conidiogenesis were further characterized using mis-scheduled expression experiments. Precocious expression of *brlA* and *abaA* but not *wetA* in vegetative hyphae inhibited growth and caused over-production of septae and branches. *brlA* expression was sufficient to induce sporulation on a very limited conidiophore. Ultrastructural methods developed in these studies have been applied to more subtle phenotypes not easily seen at the morphological level.

— Notes —

Bruce L. Miller

Dept. of Bacteriology and Biochemistry, University of Idaho

Genetic Control of Conidiophore Morphology

Conidiation in *Aspergillus nidulans* is characterized by the orderly differentiation and spatial organization of well-defined cell types into an aerial reproductive apparatus, the conidiophore. Chains of haploid conidia are produced upon this structure. Loci that control conidiophore morphology and conidia formation fall into two major genetic regulatory networks: one (BRISTLE) directing the linear progression from vegetative hyphae to conidia, the second (STUNTED) directing conidiophore morphology by determining patterns of differentiation and spatial organization of cell-types.

We have isolated the developmental modifier genes, stunted and medusa. Two RNAs are transcribed from the stunted gene with the smaller transcript, *stuA* α being initiated 635 bp downstream of the larger *stuA* β transcript. α RNA start sites are located within a large intron of *stuA* β. This gene structure is novel in fungi, the bristle gene being the only other known example (Prado and Timberlake). Temporal expression of the stunted gene appears to be regulated transcriptionally in response to the establishment of developmental competence and translationally in response to the induction of conidiation. Structural elements within the long non-translated leaders of the stunted transcripts support the latter observation.. The induction-dependent increase in expression of a reporter fusion protein (STUA:β-galactosidase) is confined to the top of the conidiophore vesicle, metulae and phialides.

Genetic and molecular analysis have established that the genes bristle, abacus and wet-white define a linearly dependent regulatory pathway (*brlA* > *abaA* > *wetA*). Prior genetic analysis has also determined that correct morphological development requires the complex interaction of functions of the genes: abacus, bristle, medusa and stunted. We have begun a molecular analysis of these interactions and have found that they are also of a complex nature. The interaction of gene functions can be summarized as follows:

1. The induction-dependent increase in stunted expression requires a functional bristle gene product.
2. The loss of stunted gene function leads to incorrect spatial expression of the bristle and abacus gene products within the conidiophore.
3. As a corollary, increased dosage of the stunted gene results in the repression of *brlA* and *abaA*, but not *brlA* expression, in specific cell types of the conidiophore.
4. A functional medusa gene product apparently modulates the repressive effect of increased stunted gene dosage.
5. The medusa and bristle gene products may have some overlap in function during later stages of condiophore development. The medusa gene product is necessary for enhancement of *brlA* and *abaA* but not *brlA* expression. Increased bristle gene dosage can compensate, or suppress, deficiencies in *brlA* and *abaA* expression in a medusa mutant.

These results suggest that an important component of cell differentiation and pattern formation in *Aspergillus nidulans* requires tightly controlled temporal and spatial expression of the bristle gene.

— Notes —

John Taylor, M. Berbee, B. Bowman, A. Gargas, S. Lee, K. LoBuglio, G. Saenz, E. Swann and T. White

Department of Plant Biology, University of California, Berkeley, CA 94720 and Roche Diagnostics Research, Alameda, CA 94501

Spores and Molecular Evolution

Fungal classification is based upon spores and their associated structures, the key features being spore form. formation, discharge and dispersal. Phylogenetic analysis of fungal DNA sequences permits an independent investigation of the relationship of spores to classification. Some of our recent observations follow. There is a general agreement between classification based on spore morphology, nuclear ribosomal DNA internal trancribed spacer and mitochondrial small subunit ribosomal DNA in Sordariaceae. Mary Berbee has shown that higher taxa that have recently lost favor, such as Plectomycetes and Pyrenomycetes, are supported by comparison of 18S rDNA as well as their reproductive morphology. Barbara Bowman has shown that fungi with confusing, or missing, spore characters, such as *Neocallimastix* sp., *Coccidioides immitis* or *Trichophyton rubrum,* can be classified with 18S rDNA sequence. Mary Berbee has done the same for *Ophiostoma ulmi* and *Ascosphaera apis.* Clearly, molecular characters, which are universal and independent of expressed morphology, permit the classification in one system of fungi possessing and lacking the all important meiotic spores. Finally, Steve Lee, Eric Swann and Greg Saenz have shown that a few spores, or even one, provide enough nucleic acid for studies of their molecular evolution.

— Notes —

J. B. Anderson

Department of Botany, University of Toronto, Mississauga, Ontario, Canada L5L1C6

Clonal Reproduction, Spore Dispersal and Mating in Basidiomycetes

We are examining the extent to which reproduction is by vegetative growth or by dissemination of basidiospores followed by mating in a forest population of *Armillaria*, a genus of root-infecting basidiomycetes. Assay of mating-type alleles, nuclear RFLP and RAPD (Random Amplified Polymorphic DNA) markers, and mtDNA restriction fragment patterns in isolates collected in 1988, 89, and 90 uniquely identified several diploid, vegetative clonal genotypes on the site. The genetic evidence demonstrates that clonal distributions were the result of vegetative growth and not of mating among sib-related basidiospores. The larger clones occupied areas up to 1 km in diameter. Based on observed growth rates, we estimate (conservatively) that the larger clones have been growing on the site for at least 500 years. Up to 20 heterozygous nuclear loci, as well as mtDNA restriction fragment patterns, all indicated genetic stability during vegetative growth; no mutation or mitotic recombination was observed. In all but one case, each vegetative clone had only one mitochondrial type; in contrast, laboratory matings consistently resulted in diploid colonies mosaic for mitochondrial type. We are currently asking whether patterns of relatedness among vegetative clones, including "maternal" or "paternal" descent, can be inferred from the cytoplasmic and nuclear markers.

— Notes —

Nicholas P. Money

Department of Biochemistry, Colorado State University, Fort Collins, CO 80523

Mechanisms of Spore Release in Zoosporic Fungi

The zoosporic fungi have adopted a number of different strategies to expel their spores, in preparation for dispersal. However, these mechanisms appear to represent variations on a basic theme: release always involves the dissipation of hydrostatic pressure or turgor. Evidence for this conclusion comes from the inhibitory effect of small osmotic shocks on sporangial emptying in a variety of fungi. More is known about the mechanism of release in fungi like *Achlya* and *Saprolegnia* which liberate spores individually, through a single papilla. In *Achlya*, spore release appears to be coupled to the loss of a residual turgor pressure from the sporangial lumen, which remains after the completion of cleavage. This pressure may be as small as +0.01 bars, which compares with a sporangial turgor of more than +5.0 bars before cleavage. Turgor is also a key element in the mechanism of release of an uncleaved cytoplasmic mass, as we see in *Rhizidiomyces* and *Pythium*. However, in this case, water uptake and the development of a small pressure gradient within the cytoplasm may be responsible for its movement. Instruments such as the pressure probe offer exciting new approaches to the study of fungal reproductive physiology.

— Notes —

Daniel G. Panaccione, Zhenfan Yang, Lisa Vaillancourt, and **Robert M. Hanau.**

Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

The Role of Fungal Spores in Plant Pathogenesis

We are using *Collectotrichum graminicola* (teleomorph: *Glomerella graminicola*), which causes anthracnose disease of maize and numerous other cultivated and wild gramineous species, as a system to study the molecular basis for conidial development in phytopathogenic fungi. The fungus produces two types of conidia in culture as well as during host infections. One is falcateshaped and is produced from acervuli in lesions on the surface of infected leaves and by acervulus-like conidiomata on the surface of agar medium. Development of falcate conidia is blastic and involves morphologically distinct conidiogenous cells. The other conidium is ovalshaped and is produced below the surface in the host and during growth on agar medium. Oval conidia are variable in size and smaller than falcate conidia, and are produced blastically from hyphae that lack distinct conidiogenous cells. Oval conidia are the only type produced by cultures grown in the dark whereas production of falcate conidia is light dependent. *In vitro* translation of poly(A)-RNA from dark-grown and light-induced hyphae shows state-specific differences in mRNA at early stages in falcate conidial development. Current efforts are aimed at knowing: (i) how light regulates production of falcate conidia; (ii) the role of oval conidia in the disease cycle; (iii) if conidiation genes from other fungi share homology with the genome of *C. graminicola*.

— Notes —

John Clutterbuck

Genetics Department, University of Glasgow, Glasgow, Scotland

Molecular Genetics of Fungal Spores

It is striking that many of the *Aspergillus nidulans* conidiation genes so far analysed appear likely to encode regulators rather than enzymes or structural proteins. *brlA* mutants were isolated on account of their conspicuous phenotypes, and we now suspect that this is because BRLA protein concentration increases with development, controlling multiple responders at each stage. On the other hand, mutants in some putative responder genes are so inconspicuous as to be missed except in combination.

— Notes —

Martha J. Powell

Botany Department, Miami University, Oxford, OH 45056

Structure and Function of Motile Cells in Fungi

Although Chytridiomycetes and Oomycetes are not phylogenetically closely related, similarities in their habitats and in the role of their zoospores in dispersal are reasons for an integrated consideration of organellar function in their zoospores. Electron microscopy has revealed much about zoospore architecture, but only some functions of the bewildering assortment of single-membrane bounded organelles are known. Many of these organelles are newly synthesized in the transition from vegetative growth to asexual reproduction, and hence are specific to zoospore unique activities. In zoospores, we can now identify roles for single membrane-bounded organelles in five processes: (1) osmotic control, (2) storage, (3) metabolism, (4) ion regulation, and (5) cell surface modifications. Examples of mechanisms involved in these processes in zoospores of Oomycetes and Chytridiomycetes will be illustrated.

— Notes —

A. R. Hardham

Plant Cell Biology Group, Research School of Biological Sciences, The Australian National University, Canberra, ACT 2601, Australia

The Use of Monoclonal Antibodies to Study Zoospore Form and Function

Observations of cell structure have made a major contribution to our understanding of the function of cells and many of their organelles. There are, however, limitations to the interpretation of the function of cell components based solely on structural features. Chemical fixation can introduce structural artifacts and both chemical and freeze fixation arrest dynamic events. Immunological probes such as monoclonal antibodies allow the unambiguous identification of selected cell components and their use has greatly improved our ability to deduce function from structural images and to interpret changes in cell structure.

In our studies of *Phytophthora* zoospores, we have used monoclonal antibodies raised against proteins from other organisms, for example tubulin from pig brain, centrin from the alga *Tetraselmis* and calmodulin from pea, to study important highly conserved proteins. We have also raised monoclonal antibodies against antigens in *Phytophthora* spores. These latter antibodies target components associated with the flagella, a variety of zoospore vesicles and other zoospore surface and intracellular components. The antibodies have been used to follow the assembly and organization of these components during zoospore formation, their structure and distribution in mature zoospores and the changes that occur during zoospore encystment and the infection of host plants.

— Notes —

H. Deising and **K. Mendgen**

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Stage-Specific Production of Cell Wall-Degrading Enzymes by Uredosporelings of Rust Fungi

In order to elucidate fungal-plant interactions during early stages of infection by biotrophic fungi, infection structures of the broad bean rust fungus , *Uromyces viciae-fabae* , were produced on inductive membranes. These structures, comparable to those produced in the host plant, were tested for the presence of enzymes that may attack cuticles and cell walls of plants. Cutinase activity was detected on the surface of urediospores with the highest activities on the echinulations. This enzyme is not involved in penetration, but plays a role in the adhesion of the urediospores to the host cuticle. The formation of acidic cellulose isoforms begins with the differentiation of appressoria. Neutral isoforms which are endo-CMCases are produced during differentiation of infection hyphae and later. Pectinmethylesterase activity was found during differentiation of the substomatal vesicle, i.e. during the first contact of the fungus with the mesophyll cells. Polygalacturonate lyase is synthesized mainly during formation of the haustorial mother cells. In contrast to the enzymes mentioned above, polygalacturonate lyase is produced only in the presence of its substrate. Not only enzyme production occurs in accordance with infection structure formation. The wall layers of the hyphae change in composition during infection structure development and become resistant to chitinase and glucanase as soon as the fungus penetrates the host mesophyll. Also, the endomembrane system differs in germ tubes and infection hyphae. During growth of the fungus within the host plant, the endoplasmatic reticulum and the Golgi apparatus appear in new configurations. With monospecific antibodies against cell wall-degrading enzymes and *in situ* localization, secretion of the pectinolytic and cellulolytic enzymes will be studied in more detail.

— Notes —

P. E. Kolattukudy

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Molecular Communication Between the Fungal Spore and Its Host

Fungal spores that contact plant surfaces germinate, differentiate into infection structures and penetrate into the host. These events are regulated by signals received from the host. Germination and appressorium formation of *Colletotrichum gloeosporioides* is triggered by the surface components from its host, avocado. Surface components of other plants do not trigger this differentiation and avocado surface components do not trigger appressorium formation in other species of *Colletotrichum* that are pathogenic in other plants. The appressorium inducing component was chromatographically isolated and identified to be very long chain fatty alcohols. This conclusion was confirmed by bioassays with synthetic materials. Upon treatment of germinating spores with the appressorium-inducing agent, significant changes in gene expression occur. The next step is the formation of an infection peg that penetrates into the host with the aid of cutinase. This enzyme is secreted specifically at the site of penetration and secretion inhibitors disrupt this targeting. Cutinase gene expression is triggered by the host cuticle. The molecular mechanism by which the host component triggers the expression of the fungal gene appears to involve phosphorylation of a transcription factor that binds to a specific region of cutinase gene promoter only when phosphorylated. It might be possible to devise ways to intervene in the programmed differentiation of the germinating fungal spore induced by host components and thus protect plants against fungal attack.

—Notes —

R. C. Staples[1] and H. C. Hoch[2]

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Germling Differentiation: An Environmentally Induced Process of Pathogen Ingress Adapted to the Host

Propagules of a wide range of fungal pathogens of plants and insects penetrate their hosts via infection structures which develop when the germling attaches to a surface. While attachment alone appears to induce appressorium development in many pathogens, nutrients often suppress the process, and a nutrient-related signal may be required as well. For example, isolates of *Metarhizium anisopliae*, an aggressive entomopathogen, when isolated from cuticles of insects where nutrient levels are low, require low levels of complex nitrogenous compounds in order to develop the appressorium and induce secretion of proteases required for penetration. Small amounts of simple sugars or amino acids will suppress these processes. Such nutrient-related triggers are not needed by the obligately parasitic rust fungi; however, many rusts also require specific topographies as a signal in addition to attachment. Such topographies are used to ensure correct placement of the appressorium over the stomate, the point of entry. Apparently pathogens have developed attack strategies based on the environment of their host's surface, including the location of entry points and that colonization is unnecessary for survival. We will review some of the surface features which appear to regulate infection structure formation and the changes in metabolism and gene activities induced.

— Notes —

Jose Ruiz-Herrera[1] and **Carmen Cano**[2]

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Differential Gene Expression During Spore Germination of *Mucor*. Role of DNA Methylation and Polyamines

Spore germination in *Mucor* occurs by a process which can be divided into three steps. During the first step (Ia), spores which are originally ellipsoidal attain a spherical shape without increasing the major diameter. The second step (Ib) involves an increase in volume through isodiametric growth until a critical volume is reached. The spore enters then into a polarized phase of growth (II) with formation of germ tubes or buds according to the environmental conditions. Addition of cycloheximide or actinomycin D at different periods during germination, stops the process precisely at either one of those steps, suggesting that each one is under the control of a set of gene products. These steps are also regulated by polyamines, DNA replication and DNA methylation. Spore incubation in the presence of MGBG, and inhibitor of SAM decarboxylase which depresses the levels of spermidine, stops germination at stage Ia; whereas DAB an inhibitor of ornithine decarboxylase (ODC) which affects the contents of putrescine stops germination at phase Ib. Spores continue growing isodiametrically without formation of germ tubes or buds. Incubation with hydroxyurea (HU) brought about the same results as DAB. The effect of these two drugs was traced back to the stage of DNA methylation. By use of the isochizomers MspI and HpaII it was demonstrated that DNA from mature spores and stages Ia and Ib is highly methylated and becomes hypomethylated 5-azacytidine (AC) reverted the effect of DAB on both methylation and germination. mRNAs specific of stage II were purified and the corresponding cDNAs were prepared and used as probes to measure the effect of DAB and HU on their expression. Both drugs inhibited completely their formation. Finally, cloned *CUP* gene from *Mucor racemosus* was used as a specific probe of developmental alterations during germination. Through hybridization of the radioactive probe with DNA prepared from different stages and digested with Msp I or Hpa II. It was demonstrated the existence of a *CUP* multigene family. All of the members of this gene family were located in the methylated region of spore DNA and became demethylated in stage II. DAB inhibited hypomethylation of all *cup* genes, and their expression, which occurred only after stage II was initiated. It is suggested that spermidine is involved in the transcription of genes involved in phase Ia of germination, and that putrescine is required for hypomethylation of genes whose products are necessary for polarization of growth. This process of hypomethylation occurs during DNA replication, and permits the expression of genes which are maintained silent through their methylation state.

— Notes —

ABSTRACTS FOR POSTERS

SESSION I

(Arranged alphabetically)

On Display in Sporen Keller Building D

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The Movement of Actin in Growing Hyphae of *Saprolegnia ferax*

Filamentous actin is concentrated in the apices of emerging germ tubes and subsequently established hyphae. Thus it is thought to play a role in initiating and maintaining polarized apical growth. We have recently developed an electroporation technique that allows us to stain actin in live hyphae with rhodamine-phalloidin and thus observe the behavior of actin during growth. This technique allows us to visualize a population of actin that is distinct from the peripheral actin network which we have previously described in chemically fixed cells. In growing hyphae, actin is found throughout the cytoplasm and is most concentrated in the apex. However, the extreme tip contains little actin and there is an apical, central invagination where staining is low. The behavior of the invagination is analogous to that of the Spitzenkörper, an apical body consisting of a concentration of wall vesicles seen in the apices of septate fungi. *Saprolegnia* does not have a Spitzenkörper, however, it does have an apical concentration of wall vesicles. Thus, the location of the invagination and its behavior suggest that this actin-poor region is occupied by large numbers of wall vesicles. Fluorescence recovery after photobleaching of rhodamine-phalloidin stained F-actin has revealed three types of F-actin movement during growth. There is a population of actin that moves forward at the same rate as the tip extends such that it maintains its relative position during growth. Actin is also recruited from subapical regions and accumulates in the growing tip and some actin is left behind to populate the subapical cytoplasm as the tip grows forward. Thus F-actin moves in a cyclical fashion during tip extension.

— Notes —

Susan G. W. Kaminskyj and I. Brent Heath

Biology Department, York University, Toronto, Ontario, Canada

Independence of Turgor,Tip Growth and Hyphal Diameter in *Saprolegnia ferax*

Tip growth models assume a direct relationship between turgor and tip plasticity/hyphal extensibility resulting in growth. Increase in colony dry weight is affected by medium osmolarity, but the turgor/tip growth relationship has not been examined directly. We have done so for *Saprolegnia*.

If tip growth were correlated with turgor, interconnected coenocytic hyphae, which should have constant turgor, should also have similar growth rates. Instead, we found a three-fold variation, between tips and over time for any tip, although the average was constant. We sought to test this result another way: treatments which alter turgor should affect growth rate similarly.

We altered turgor with osmotica, sorbitol or polyethylene glycol-400 (PEG), or by selectively inhibiting putative osmoregulatory K+ channels with TPA. Increasing levels of osmotica reduced turgor linearly to a minimum plateau. More sorbitol than PEG was required to achieve similar turgor reductions. Lowered turgor initially correlated with reduced growth rate, but rate reduction continued in the constant minimum-turgor plateau. In contrast to osmotica, TPA did not reduce turgor despite dose-dependent growth rate inhibition; turgor increased with high TPA. Hyphal diameter increased with osmoticum, but was unaffected by TPA.

Hyphal growth rates varied widely and rapidly; there was no consistent relationship between growth rate and manipulated turgor. Hyphal diameter was not related to turgor. These results suggest that hyphal extensibility as measured by growth rate is predominantly mediated by factors other than turgor.

— Notes —

Jinliang Li and I. Brent Heath

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Zoospore Ultrastructure and Phylogeny of Chytridiomycetous Gut Fungi

Zoospores function to multiply and disperse the species in an aqueous environment but in spite of this singularity of function they show a remarkable diversity of form and organization. This diversity is considered to reflect differing evolutionary history and has therefore been used as a phylogenetic and taxonomic indicator. However, it is difficult to differentiate between diversity generated by adaptation to subtle functional specialization versus phylogenetic diversity. The gut fungi are a group of organisms which live in a very specialized, seemingly homogenous environment. Their origins are unknown, they may have originated by invasion from one or many free living ancestors, with subsequent specialization to the gut environment. Zoospores of gut fungi have basically similar ultrastructure. They all have a similar perikinetosomal apparatus and similar organelle distribution pattern. This gives us the opportunity to compare organisms in an environment likely to favour convergent evolution for zoospore form. We have compared relationships indicated by zoospore ultrastructure, and thallus morphology, with those indicated by an independent marker, rRNA base sequences using an internal transcribed spacer from the rRNA gene cluster. This sequence was chosen in order to reveal relationships between putatively closely related organisms. We shall discuss the results of our analysis by comparing phylogenetic trees generated from the different data in order to clarify both the phylogeny of the organisms and the value of zoospore ultrastructure as a phylogenetic indicator.

— Notes —

C. N. Rosendahl* & L. W. Olson

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In Vivo Screening for Antifungal Activity Against the Plant Pathogen *Pythium ultimum*

A new assay which can be used for *in vivo* screening of fungicides and microbiologically produced metabolites against *Pythium ultimum* Trow is described. The assay uses cress *(Lepidium sativum L.)* as a test plant and the assay can be performed quickly and does not require more space than many *in vitro* tests.

The assay uses *P. ultimum* oospores as the inoculum. For the production of oospores, Petri dishes (9 cm diam.) with potato carrot broth (20 g grated carrots and 20 g grated potatoes, boiled for 10 min in 250 ml distilled water, filtered through one layer of cheese cloth and diluted to 1 liter) are inoculated with agar blocks of *P. ultimum*. The cultures are incubated at room temperature and the myclium is harvested after 7 days and then dried on a stack of Whatman No. 1 filter papers. The oospores are harvested by hydrating the dried mycelium and then grinding it briefly in a mortar. After filtering the suspension through a 28 micrometer (pore diam) filter, the oospores are pelleted. The oospore concentration is determined with a haemocytometer and the desired dilutions are made in distilled water. The assay is performed in 6 well microtiter plates. Cotton wool (0.2g) is placed in each well and watered with 1.5 ml of a plant nutrient solution. One ml of the *P. ultimum* oospore suspension is added to each of the wells to be inoculated. Approximately 20 cress seeds are sown on the cotton. The microtiter plates are incubated at room temperature with artificial day light. Damping off is scored after 5 days. As few as 60 oospores/well will kill 100% of the cress plants.

— Notes —

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Synchronization of *Pythium ultimum* Oospore Germination

Pythium ultimum Trow was grown in potato-carrot broth for 1 week at room temperature in either light or dark and then dried. Oospores were collected from the dried mycelium and germinated on corn meal agar (Difco) in either light or dark. Rich nutrient agar inhibits oospore germination while oospores germinate well on nutrient poor agar.

Oospores produced in light and in darkness show very different rates of germination when germinated in light or dark. Oospores produced in light germinated better in light than in darkness. Oospores produced in darkness do germinate but the percentage of oospores which germinate is not nearly as great as when the oospores are produced in light. Oospores produced in darkness germinate somewhat better in light than in darkness.

For oospores produced in light, exposure to light before germination triggers oospore germination but light is not necessary during the subsequent germination. For oospores produced in darkness, exposure to light prior to the start of oospore germination inhibits germination.

These results suggest that oospores produced in light and dark are physiologically different and different environmental factors are required to trigger oospore germination.

— Notes —

Lyndon D. Waterman[1], Susan B. Persad-Chinnery[1], Louis E. Chinnery[1], Stan Michelini[2], Margaret A. Marshall[3], William E. Timberlake[3] and Richard B.G. Dales[1]

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Genetical Approaches to the Study of Vesicular-Arbuscular Mycorrhizal Fungal Spores of the Genus *Gigaspora*

The vesicular-arbuscular mycorrhizal (VAM) fungi are a group of about 150 species classified as six genera of the Zygomycetes. These filamentous fungi colonize the roots of most terrestrial plants forming mutualistic associations. The VAM fungal genera *Gigaspora* and *Scutellispora* form some of the largest known fungal spores, ranging from about 50 micrometers to over 500 micrometers in diameter. These spores are produced extra-radically and are usually globose to ovoid in shape with a characteristic bulbous suspensor cell between the spore and the single subtending hypha. The spore walls of *Gigaspora* species are multilayered with a composite width of 3-50 micrometers. On the inner surface of the spore wall there is a layer of densely-nucleated cytoplasm and, for one *Gigaspora* species, the number of nuclei per spore has been estimated at ~20,000. The interior of the spore is composed primarily of lipids. Spores of *Gigaspora* species can be germinated *in vitro*, though different spore samples show variation in germinability. The extent of development of the germ tube into a branching hyphal network is also variable but growth inevitably ceases unless a plant root system is available for colonization.

We have established methods for routine spore germination and are utilizing previously described procedures for the *in vitro* culture of mycorrhizae. We are now developing molecular genetic and cytogenetic approaches to the study of *Gigaspora* species. Information on the isolation and analysis of DNA samples from single spores and an investigation of nuclear division within germ tubes will be described.

— Notes —

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Pythium Zoospores Prefer Nori

The watermold *Pythium porphyrae* is a significant pathogen of natural and cultured *Porphyra* (Nori). Although maximally active at relatively high temperatures and low-salinities it, nevertheless, is well adapted to its role as a marine parasite. Comparative infection studies of algae from the Pacific N. W. USA indicate that the mycelium develops most rapidly in the haploid blades of *Porphyra nereocystis* as contrasted to *P. torta* or *P. san juanensis*. Both mycelium and zoospores, however, ignore the diploid, filamentous, conchocoelis phase of *Porphyra*.

The well known linkage between life-cycle stage and cell wall character in Rhodophyta points to a potential host-parasite recognition system working through zoospore identification of cell wall components. Assays of zoospore encystment, germination and appressorium formation in relation to natural and modified algae cell walls indicate that the zoospores of *Pythium porphyrae* quantitatively differentiate between different genera of red algae, but ignore green algae (*Ulva*), brown algae (*Laminaria*) and the leaves of *Zostera*.

Preliminary results tie encystment to the presence of sulfated-galactans, including those of commercial agar, with observed discrimination between lambda (-), iota (+) and kappa carrageenan (+). Sequential extraction of *Porphyra* cell walls suggest that separate signals control zoospore encystment and appressorium formation.

— Notes —

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Sexual Spore Formation in the Zygomycete *Absidia glauca*

Absidia glauca is a heterothallic zygomycete with two physiologically distinct mating types (+ and -). The mating types are morphologically indistinguishable, and they are very similar in growth, behavior and vegetative sporulation. Additionally, we could show by 2D gel analysis of *in vitro* translation products that mRNA populations are nearly identical in both mating types. On the genetic level, however, we could demonstrate that the complementary mating types differ in many respects.

Chromosome separations on pulsed field gels show that the (+) type contains an additional chromosome (Chr. 10), for which no electrophoretic equivalent exists. Also the rDNA repeats are differently organized in the mating types.

Only very few protein coding genes are specific for one mating type. We could identify a 15 kDa surface protein, which is specifically expressed in the (+) type ("APSP"). When the complementary mating types meet, a few mating type specific genes seem to be switched on. Hyphal branches grow towards each other, and the mating types cooperate in the synthesis of the pheromone trisporic acid. The hyphal branches form gametangia at their tips which fuse and finally differentiate into the zygospore.

Zygospore formation can occur as well in an artificial situation, obtained by protoplast fusion between auxotrophically labeled strains belonging to complementary mating types. In this homothallic situation, the regulation of genes responsible for mating seems to work properly. There are, however, negative regulatory effects exerted by the (-) type. We could show by means of a specific antibody that the expression of "APSP" in the (+) strain is completely suppressed in homothallic fusion derivatives.

— Notes —

ABSTRACTS FOR POSTERS

SESSION II

(Arranged alphabetically)

On Display in Sporen Keller Building D

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Image Analysis of Urediniospores Which Infect *Mentha*

Peppermint rust *(Puccinia menthae* Pers.) infects *Mentha piperita* L. and the same species, spearmint rust, infects common American spearmint, *M. spicata L.* The peppermint race will not infect the spearmint race and the spearmint rust will not infect the peppermint plant[1]. The urediniospores of both races of rust have a similar morphological appearance with SEM. The peppermint spores visually appear to be slightly more elongated. The Prism Image Analysis System software (Dapple Systems) was used with a Macintosh IIci to quantitate morphological differences. Thirty different morphological parameters of either size or shape were measured for sample populations of 47 native spearmint rust spores and 43 peppermint rust spores. Analysis of Variance tests were then performed using the Prism software to determine which parameters were significant between the two races. Separation was possible with 14 morphological parameters ranging from 92.25% to 99.99% probability of significant difference. The parameters were area, convex area, perimeter, convex perimeter, length, fiber length, equivalent diameter, form factor, roundness, extent, compactness, aspect ratio, elongation, and curl. Although separation of these urediniospores is not presently tied with economics and marketing, separation of spores of other fungi does have marketing implications[2,3].

References
1. M. J. Murray, Crop Science (1961), 1, 175.
2. J. S. Gardner and W. M. Hess, Proceedings EMSA (1990), 706.
3. W. M. Hess and E. J. Trione, Plant Disease (1986), 70, 548.

— Notes —

L. Epstein, M. J. Jones and T. C. Caesar-TonThat

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Mutants of the Plant Pathogenic Fungus *Nectria haematococca* with Adhesion-Reduced Macroconidia and Germ Tubes

Macroconidia of *Nectria haematococca* mating population I (anamorph, *Fusarium solani* f. sp. *cucurbitae*) become adhesive several hours before germ tube emergence. The germ tubes also are adherent. Proteinase K, but not the heat-denatured enzyme, removed both adherent spores and germ tubes, suggesting that the adhesin(s) may be proteinaceous. Two chemically-derived mutants with macroconidia with reduced capacity to adhere onto polystyrene and zucchini fruits were selected. The mutant germ tubes also were less adherent than the wild-type germ tubes, suggesting that the same gene(s) may be involved in spore and germ tube adhesion. Ultrastructurally, the outer portions of spore walls of the two mutant and wild type strains differed from each other; neither mutant had an extended matrix along the spore wall surface, in contrast to the wild type. When macroconidia were inoculated into wounded zucchini fruits, the adhesion-reduced mutants were as virulent as the wild-type strain. However, in disease assays in which macroconidia were deposited onto the surface of unwounded zucchini, the mutants were less virulent than the wild-type. Thus, adhesion of *N. haematococca* macroconidia to its host surface appears to be a virulence factor. Analysis of progeny of a cross of one of the mutants and the wild type indicated a 1:1 segregation, indicating that the mutation is segregating as a single gene.

— Notes —

David H. Griffin, Kathleen E. Quinn, Gregory S. Gilbert, C. J. K. Wang and Susan Rosemarin

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Conida or Spermatia of *Hypoxylon mammatum*?

Vegetative interactions between genetically different isolates of *Hypoxylon mammatum*, the cause of hypoxylon canker of *Populus tremuloides*, resulted in the formation of a conidial interface. We observed morphological variation in conidium formation during the vegetative interaction, and examined the germination properties of these conidia. The propensity to produce two forms of conidiophores, geniculate and nodulose, differed among ascospore isolates. Conidial sizes varied significantly among isolates and the conidia produced at the interface of opposed mycelia were usually significantly different from those of one or both of the parental isolates. Cultures formed a lawn of conidia *in vitro* when mycelial macerates of two different isolates were mixed and plated. These conidia germinated up to 80%, judged by germtube emergence. Filtering conidial preparations through glass wool removed most of the contaminating hyphal fragments. Colony formation by these preparations was only 5% of the estimated numbers of conidia plated, but 2 to 8-fold more than the estimated numbers of hyphal fragments contaminating the preparations. Germinating conidia produced a few short hyphal branches on the surface of the agar medium, and one long aerial hypha with branches at its tip. No further development occurred. Single ascospore isolates from 14 asci from six perithecia found on one canker were tested for interactions judged by the formation of a conidial interface between opposed mycelia. Each ascus contained four different interaction groups composed of identical twin ascospore isolates, indicating the heterothallic production of asci within all six perithecia and the utility of the vegetative interaction as a phenotypic marker of genetic identity. No isolates of the same group were found in interascus pairings. Mycelial isolations from cankers showed that each canker consisted of a single interaction type, suggesting its origin from a single spore. No isolates of the same type were found among cankers on adjacent trees, either in a wild clone or in a garden plantation, indicating that cankers caused by asexual propagation of the fungus were rare. We hypothesize that conidia of *H. mammatum* serve as spermatia rather than primarily as asexual propagates.

— Notes —

Gareth Griffith, Charles Birse and John Clutterbuck

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ivoA and *ivoB* Conidiophore Pigmentation Genes of *Aspergillus nidulans*

Genetic evidence suggests that *ivoA* and *ivoB* genes are closely regulated by *brlA*. Both genes have been cloned and are currently being sequenced. *ivoB* encodes an extracellular Cu^{2+}/Zn^{2+} - dependent phenol oxidase required for production of the grey-brown conidiophore pigment. The product of the action of *ivoA* is N-acetyl-6-hydroxytryptophan which is the substance of the *ivoB* enzyme.

Northern blots confirm that both genes are transcriptionally regulated by *brlA*, *ivoB*, being activated slightly earlier and in less leaky *brlA* mutants than *ivoA*. We therefore postulate that the two promoters will prove to differ in sensitivity to the BRLA protein.

— Notes—

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Composition of Telial Horn Gel in *Gymnosporangium nelsoni*

The protrusion of teliospores and the release of basidiospores in the life cycle of *Gymnosporangium nelsoni* (Juniper rust) is dependent upon the swelling of the telial horn gel. At the same time the gel acts as an indicator of favorable conditions since it will not swell until sufficient moisture has fallen. Microscopic examination of the gel revealed that the teliospores were located mainly on the surface of the gel. More mature teliospores were subtended by less mature teliospores and some teliospores were embedded in the gel. It was observed in TEM micrographs that the gelatinous materials supporting the teliospores consisted of concentric rings of electron dense material. The gelatinous material did not appear to be cellular in nature. Analysis of a number of SEM micrographs revealed that the gel material arose by the breakdown of the telial stalk or pedicel. There were no remnants of pedicels in the mature telial horns. The gel contained 82% carbohydrates and 18% fatty acids. No amino acids were detected. Chemical analysis by gas chromatography-mass spectrometry of acid hydrolyzed gel and gel hydrolyzed by selective enzymes indicated the following sugars: glucose, mannose, galactose, fructose, and arabinose. Nine fatty acids were detected after hydrolysis. Histochemical and additional enzyme tests gave positive tests for cellulose, chitin and pectin. However, ^{13}C NMR analysis indicated that the polymer structure was not similar to plant pectin. It appears that the get is a complex carbohydrate polymer with swelling properties similar to pectin.

— Notes —

Young H. Kwon, Harvey C. Hoch, James R. Aist, and Richard C. Staples

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Appressorium Development in *Uromyces appendiculatus*: Apical Vesicles and Cytoskeleton

The formation of appressoria of *Uromyces appendiculatus* urediospore germlings is triggered by external physical features of host stomata as well as artificial surfaces bearing inductive topographies. Germling apices sense inductive topographies, e.g., 0.5 micrometers high ridges, within 4 min after initial contact. The area in the germling that senses such topographies is located on the substrate side of the cell and within 6.0 micrometers of the apex.

The organization of the germling apex before and during initiation of appressorium formation was examined by both electron microscopy and laser scanning confocal microscopy. Most notable were changes in the distribution and organization of the apical vesicles. In germlings that contacted artificial inductive topographies in vitro, the distribution of apical vesicles in germlings did not change within the first 3-4 min. After 6-8 min, most apical vesicles became repositioned subapically in the enlarging apex. The apical cluster in developing appressoria was closely associated with the population of cytoplasmic microtubules that also became oriented parallel to the inductive ridge.

The microtubule and F-actin microfilament cytoskeletons were examined for their involvement in the process of appressorium formation in this fungus. Disruption of microtubules and microfilaments was visualized in the germling apex, only near the substrate, during stages of appressorium development. A significant population of microtubules and microfilaments appeared oriented parallel with the ridge near the substrate side of the cell, and reticulate in regions of the cell farther from the substrate.

— Notes —

D. E. Lusk and H. C. Aldrich

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Cytochemical Comparison of Ascospore, Ascus and Vegetative Walls of *Ascodesmis sphaerospora* Obrist (Ascomycete, Pezizales)

The majority of available chemical data on walls of Ascomycete fungi is derived from enzymatic and chemical degradation studies of hyphae. The function of hyphal walls is vastly different from that of ascus and ascospore walls. Therefore, structural and chemical differences may exist between these cell walls. Further support for such a hypothesis comes from differences in biosynthesis systems. Ascospore walls are formed totally *de novo* while ascus and vegetative cell walls are built on existing wall. Anti-ascospore wall polyclonal antibodies (in serum) label all walls suggesting some chemical similarities between these wall systems. Conversely, antibodies derived from hybridoma colonies, one uncloned and two monoclonals, label only various layers of the ascospore walls. ConA lectin labels all layers of the ascospore wall and the plasma membrane or the inner-most layer of the vegetative cells. WGA and GS II lectins label the ascus and vegetative walls, but not any part of the ascospore walls. LFA lectin labels cytoplasmic components of some cells, a glycocalyx-like layer of apparently dead vegetative cells, and what appears to have been cell-cell interaction sites between vegetative cells and an ascus. This cytochemical evidence suggests the existence of limited chemical similarity with distinct specific chemical differences between the various wall systems.

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The Effect of Specific Wavelengths of Light on Sporulation in *Aspergillus nidulans*

Light is an important environmental stimulus, which can induce conidiation in the filamentous ascomycete *Aspergillus nidulans*. Light also affects sexual sporulation in this organism. Although sexual sporulation occurs regardless of the presence or absence of light, light does affect time of initiation of cleistothecial primordia. Using the chromogenic staining assay for cleistothecial laccase described by Hermann et at. (1983), we show that sexual development initiates 15 hours earlier in the dark than in the light. Light-dark shifting experiments indicate that light can only elicit a delay in sexual sporulation if colonies are irradiated during a critical period of development. This period is different from that required for conidiation.

Previous work has shown that the ability of light to elicit conidiation is dependent on the allelic state of the *velvet A* (*veA*) gene. The *veA1* mutation abolishes light dependency allowing conidiation to occur in the absence of light. We show that this mutation abolishes the ability of colonies to initiate early sexual development in the dark; cleistothecial primordia initiate at the same time regardless of the presence or absence of light. Furthermore, the *veA1* mutation is temperature sensitive with respect to sexual development. Temperature shift experiments define a thermosensitive period for *veA* expression.

Lastly, Mooney and Yager (1990) have shown that conidiation is elicited by exposure to red light and suppressed by an immediate shift to far red light, reminiscent of phytochrome mediated responses observed in plants. We show that red light also causes the delay in initiation of sexual development. Moreover, we have found that the initiation of sexual sporulation in certain *A. nidulans* strains is delayed by blue light and that blue light can also induce conidiation in these same strains. This effect is due to a single Mendelian factor, which we have designated *bedA1* Preliminary results indicate that strains able to respond to blue light show a delay in the initiation of conidiation, but only in blue light. The kinetics of asexual development are normal at other wavelengths. Data is presented that compares the critical periods for both blue light and red light sensitivity.

Hermann, T.E., M.B. Kurtz and S.P. Champe (1983) *J. Bacteriol.* 154, 955-964.
Mooney, J.L. and L.N. Yager (1990) *Genes & Dev.* 4, 1473-1482.

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Hyperoxidant States at the Start of All Three Morphological Events During the *Neurospora crassa* Conidiation Process

Exponentially growing hyphae of *N. crassa*, when exposed directly to air, rapidly adhere to each other and synchronously form aerial hyphae and conidia. A rapid loss of the NAD(P)-reducing power and excretion of glutathione disulfide was detected during the first ten minutes of air exposure. These changes were followed by a peak of total protein oxidation, loss of glutamine synthetase activity and the presence of the oxidatively modified enzyme. During hyphae adhesion, reducingg power and glutamine synthetase activity recovered to initial values. An increased level of proteolysis reduced the amount of oxidized proteins. Two hours later, at the start of aerial hyphae formation, the NAD(P)-reducing power decreased a second time; glutathione was oxidized. A second peak of protein oxidation developed which coincided in time with the definitive loss of glutamine synthetase activity in the adhered mycelium. In the aerial hyphae at the start of conidia formation the NAD(P)-reducing power fell again and glutathione was oxidized. A third peak of protein oxidation developed which coincided in time with the loss of glutamine synthetase activity in the aerial hyphae. Oxidation of NAD(P), glutathione, proteins, and glutamine synthetase are thought to be due to an increased generation of reactive oxygen species (oxygen radicals). As a response to a hyperoxidant state, cells differentiate by activating diverse mechanisms that tend to avoid dioxygen.

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