international conference on plant vascular biology 2010

Hosted by The Ohio State University and Iowa State University

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Plant Vascular Biology 2010

July 24-28, 2010

The Fawcett Center, The Ohio State University

Sponsors

The Ohio State University
Department of Plant Cellular & Molecular Biology
Plant Molecular Biology & Biotechnology Program

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Integrative Organismal Systems

Plant Vascular Biology 2010

Program Schedule

(The Fawcett Center, The Ohio State University)

FRIDAY, JULY 23

Participant arrival; Shuttle bus transports participants from Port Columbus International Airport to campus dorms and hotels.

SATURDAY, JULY 24

8:30-12:30 Shuttle transports participants from campus dorms and hotels to The Fawcett Center. The shuttle runs continuous loops between these destinations during these hours. Continental breakfast provided at The Fawcett Center.

8:30-1:00 Registration; Poster set-up (all posters will be on display throughout the conference)

11:30-12:45 Lunch

1:00-1:30 Welcome and Introduction

1:30-2:30 **Keynote address**: **William Lucas** (University of California at Davis, USA)
"The Plant Vascular System: Its Secrets Hold Great Promise for Sustainable Food, Fiber and Energy Initiatives"

Session I. Development of Vascular Tissues I
Co-Chairs: Ji-Young Lee & Yka Helariutta

2:30-3:00 **Thomas Berleth** (University of Toronto, Canada)
"Control of Leaf Vascular Patterning"
3:00-3:30 **Karl J. Oparka** (University of Edinburgh, UK)
"Super-resolution Imaging of the Phloem"

3:30-4:00 Coffee break

4:00-4:30 **Hiroo Fukuda** (University of Tokyo, Japan)
"An Arabidopsis Cell Culture System Harboring Inducible VND6 - Cellular and Transcriptome Analyses of Tracheary Element Formation"
4:30-5:00 **Zheng-Hua Ye** (University of Georgia, USA)
"Molecular Dissection of Secondary Cell Wall Biosynthesis"
5:00-5:20 Short talk: **William W. Adams III** (University of Colorado, USA)
"Phloem Anatomy and Photosynthetic Capacity"
5:20-5:40 Short talk: **Makoto Shirakawa** (Kyoto University, Japan)
"Vacuolar SNAREs Function in the Formation of the Leaf Vascular Network by Regulating Auxin Distribution"
5:40-6:00 Short talk: **Rebecca S. Lamb** (The Ohio State University, USA)
"*The Paralogous Poly(ADP-ribose) Polymerases RCD1 and SRO1 Control Vascular Patterning and Differentiation"*

6:00-6:30 Group Photo

6:30-9:30 Reception and Social (The Fawcett Center)

8:00-10:00 Shuttle runs loops to transport participants back to campus dorms and hotels

SUNDAY, JULY 25

Session II. Development of Vascular Tissues II
Co-Chairs: Andrew Groover & Hiroo Fukuda

7:30-8:15 Shuttle transports participants from campus dorms and hotels to The Fawcett Center. Continental breakfast provided at The Fawcett Center.

8:30-9:00 **Ji-Young Lee** (Boyce Thompson Institute for Plant Research, USA)
"Root Vascular Tissue Patterning Directed by Cell-to-Cell Communication"

9:00-9:30 **Yka Helariutta** (University of Helsinki, Finland)
"Cell Signaling during Root Procambial Development in Arabidopsis"

9:30-9:50 Short talk: **Thomas Greb** (Gregor Mendel Institute of Molecular Plant Biology, Austria)
"Microdissecting the Cambium: New Insights into Secondary Growth Regulation in Arabidopsis thaliana"

9:50-10:10 Short talk: **Hans E. Waldenmaier** (Miami University, USA)
"Gene Expression Patterns of Programmed Cell Death During Vascular Aerenchyma Formation in Seedling Roots of Glycine max cv. 'Yukihomare'"

10:10 – 10:40 Coffee break

Session III. Secondary Growth in Woody Species
Co-Chairs: Mechthild Tegeder & David Granot

10:40-11:10 **Andrew Groover** (Institute of Forest Genetics, US Forest Service, USA)
"Evolution and Development of Vascular Cambia and Secondary Growth"

11:10-11:40 **Vincent Chiang** (North Carolina State University, USA)
"Regulation and Modeling of Lignin Biosynthesis: A Systems Biology Approach"

11:40-12:00 Short talk: **Xin-Qiang He** (Peking University, China)
"Polycomb Group Proteins Are Involved in Regulation of Plant Secondary Vascular Tissue Development"

12:00-12:20 Short talk: **Rachel Spicer** (Harvard University, USA)
"Auxin Transport in Populus during the Shift from Primary to Secondary Growth"

12:20 - 1:10 Lunch break

Session IV. Mechanisms of Vascular Transport I
Co-Chairs: John Patrick & Katarzyna Sokołowska

1:10-1:40 **N. Michele Holbrook** (Harvard University, USA)
"Physical and Physiological Constraints on Xylem Transport"

1:40-2:10 **Norbert Sauer (**FAU Erlangen-Nürnberg, Germany)
"Phloem and Nerve Cells - Common Proteins, Common Functions?"

2:10-2:30 Short talk: **Barbara Demmig-Adams** (University of Colorado, USA)
"Adjustment of Leaf Structure and Function to Cold Temperature"

2:30-2:50 Short talk: **Michael R. Thorpe** (Forschungszentrum Jülich, Germany)
"Briefly Arresting: The Mechanism for Transient Cessation of Phloem Transport after Abrupt Stimuli Remains Mysterious, Other Than in Legumes Where Calcium Triggered Dispersion of Forisomes Is Responsible"

2:50-3:10 Short talk: **Hui Tian** (University of Minnesota, USA)
"AtNaKR1 Is a Phloem Mobile Protein Necessary for Phloem Function and Root Meristem Maintenance"

3:10-3:30 Short talk: **Ziv Spiegelman** (The Hebrew University of Jerusalem, Israel)
"LeCyp1 Is Involved in Long-distance Signalling in Tomato (Solanum lycopersicum) Plants"

3:30-4:00 Coffee break

Session V. Mechanisms of Vascular Transport II
Co-Chairs: Thomas Greb & Norbert Sauer

4:00-4:30 **Robert Turgeon** (Cornell University, USA)
"Regulation of Sucrose Transporter Activity in Tobacco Leaves"
4:30-5:00 **Rainer Hedrich** (Universität Würzburg, Germany)
" Sucrose- and H^+- dependent Gating of the Power Horse of Phloem Loading SUC2"
5:00-5:30 **John Patrick** (University of Newcastle, Australia)
"Phloem Unloading of Resources - A Push-me Pull-me Phenomenon"
5:30-5:50 Short talk: **Qiushi Fu** (Cornell University, USA)
"Leaf Polar Metabolite Concentrations and Starch Levels in Relation to Phloem Loading Type"
5:50-6:10 Short talk: **Mechthild Tegeder** (Washington State University, USA)
"Phloem Loading of Amino Acids Affects Plant Metabolism and Productivity"
6:10-6:30 Short talk: **Katarzyna Sokołowska** (University of Wroclaw, Poland)
"The Dynamics of the Symplasmic Transport in the Cambial Region"

6:30-10:00 Dinner, Refreshments and Poster Viewing

8:00-10:00 Shuttle runs loops to transport participants back to campus dorms and hotels

MONDAY, JULY 26

Session VI. Mechanisms of Vascular Transport III
Co-Chairs: Julia Kehr & Brian Ayre

7:30-8:15 Shuttle transports participants from campus dorms and hotels to The Fawcett Center.
Continental breakfast provided at The Fawcett Center.
8:30-9:00 **David Braun** (University of Missouri, USA)
"Genetic Regulation of Sucrose Transport into the Phloem"
9:00-9:30 **Michael Grusak** (USDA-ARS Children's Nutrition Research Center, USA)
"Microelement Trafficking in Plants: A Multi-compartmental Journey from the Rhizosphere to Seeds"
9:30-9:50 Short talk: **David Granot** (The Volcani Center, Israel)
"Role of Hexose Phosphorylating Enzymes in Vascular Development and Function"
9:50-10:10 Short talk: **Ralf Metzner** (Forschungszentrum Jülich, Germany)
"A Snapshot of Xylem Vessel - Stem Tissue Interactions: New Perspectives on Mineral Nutrient Transport"

10:10-10:40 Coffee break

Session VII. Vascular Trafficking and Development I
Co-Chairs: David Braun & Susanne Hoffmann-Benning

10:40-11:10 **Julia Kehr** (Centro de Biotecnología y Genómica de Plantas**,** Spain)
"Phloem RNAs, Nutrient Stress, and Systemic Mobility"
11:10-11:40 **David Hannapel** (Iowa State University, USA)
"The Long-distance Transport of a Full-length mRNA Mediates Development"
11:40-12:10 **Paula Suarez-Lopez (**Centre for Research in Agricultural Genomics, Spain)
"Involvement of a miRNA in the Long-distance Regulation of Potato Tuber Induction"
12:10-12:30 Short talk: **Brian G. Ayre** (University of North Texas, USA)
"Geminivirus-mediated Delivery of Florigen to Ancestral Cotton Uncouples Flowering from Photoperiod and Promotes Determinate Growth"
12:30-12:50 Short talk: **Shweta Shah** (Iowa State University)
"Photoperiodic Effect on Protein Profiles of Potato Petiole and Phloem"

1:00 Box lunch for free time and social

For participants choosing tours of Easton Mall and Short North, the shuttle bus starts to pick up at 1:00 pm from The Fawcett Center and sends to these destinations. The shuttle runs hourly loops among Fawcett Center – Hotels – Dorms – Short North – Easton Mall. The last return shuttle pick up from Easton Mall is 9:00 pm.

TUESDAY, JULY 27

Session VIII. Vascular Trafficking and Development II
Co-Chairs: Paula Suarez-Lopez & Jae-Yean Kim

7:30-8:15 Shuttle transports participants from campus dorms and hotels to The Fawcett Center. Continental breakfast provided at The Fawcett Center.
8:30-9:00 **Leslie Sieburth** (University of Utah, USA)
"*Long Distance Root-to-Shoot Signaling: Intersection between the bps1 Mobile Signal and Auxin*"
9:00-9:30 **David Jackson** (Cold Spring Harbor Laboratory, USA)
"The Role of Protein Folding in Cell-Cell Protein Trafficking"
9:30-9:50 Short talk: **Susanne Hoffmann-Benning** (Michigan State University, USA)
"Identification of Putative Lipid Binding Proteins in the Phloem and Their Role in Plant Development and Stress Response"
9:50-10:10 Short talk: **Sylvie Dinant** (INRA-AgroParisTech Versailles, France)
"P-proteins in Sieve Elements: A Role in Macromolecular Trafficking?"

10:10-10:40 Coffee break

Session IX. Vascular Trafficking and Development III
Co-Chairs: Sylvie Dinant & Michael Thorpe

10:40-11:10 **Andy Maule** (John Innes Centre, UK)
"Omics Approaches to Defining the Plasmodesmal Proteome"
11:10-11:40 **Patricia Zambryski** (University of California at Berkeley, USA)
"Three Mutations, ise1,ise2, dse1, Affect Arabidopsis Embryo Development and Increase or Decrease Plasmodesmata Function"
11:40-12:00 Short talk: **Byung-Kook (Brian) Ham** (University of California at Davis, USA)
"PLASMODESMAL GERMIN-LIKE PROTEIN 1 (PDGLP1) Functions in Selective Cell-to-Cell Trafficking of Non-cell-autonomous Proteins (NCAPs)"
12:00-12:20 Short talk: **Ikuo Nishida** (Saitama University, Japan)
"Roles of RESTRICTED SUCROSE EXPORT1 Pectate Lyase in Sucrose Translocation and Secondary Plasmodesmal Biogenesis in the Leaf Vein of Arabidopsis Source Leaves"
12:20-12:40 Short talk: **Jae-Yean Kim** (Gyeongsang National University, Korea)
"Callose Synthase, A Fine Tuner of Intercellular Movement of Signaling Molecules through Plasmodesmata"

12:40-1:30 Lunch break

1:30- 4:30 Coffee and Poster viewing

4:30-6:30 Poster Presentations by Authors I: **Poster Numbers P1-P33** (Authors Need to Be at Posters)

6:30-7:30 Dinner

7:30-9:30 Refreshments and Poster Presentations by Authors II: **Poster Numbers P34-P67** (Authors Need to Be at Posters)

8:00-10:00 Shuttle runs loops to transport participants back to campus dorms and hotels

WEDNESDAY, JULY 28

Session X. Biotic Plant Interactions I
Co-Chairs: Na-Sheng Lin & Aardra Kachroo

7:30-8:15 Shuttle transports participants from campus dorms and hotels to The Fawcett Center. Continental breakfast provided at The Fawcett Center.
8:30-9:00 **Peter M. Gresshoff (**The University of Queensland, Australia)
"Long-distance Chemical Signaling Facilitates Autoregulation of Nodulation in Legumes"
9:00-9:30 **Ulrich Hammes** (University of Regensburg, Germany)
"Differential Vascularization of Nematode-induced Feeding Sites"
9:30-10:00 **Linda Walling** (University of California at Riverside, USA)
"Phloem-feeding Whiteflies and the Evasion of Plant Defense"
10:00-10:20 Short talk: **Fiona L. Goggin** (University of Arkansas, USA)
"Influence of a Fatty Acid Desaturase in Tomato on Plant Defenses Against a Phloem-feeding Herbivore"

10:20 – 10:50 Coffee break

Session XI. Biotic Plant Interactions II
Co-Chairs: Linda Walling & Fiona Goggin

10:50-11:20 **Daniel Klessig (**Boyce Thompson Institute for Plant Research, USA)
"SA, Methyl Salicylate, Lipids, and Systemic Acquired Resistance – the Plot Thickens!"
11:20-11:50 **Pradeep Kachroo** (University of Kentucky, USA)
"Photoreceptors and Resistance Protein-mediated Signaling against Turnip Crinkle Virus in Arabidopsis"
11:50-12:10 Short talk: **Ratnesh Chaturvedi** (University of North Texas, USA)
"Identification of a Diterpenoid as a Vasculature Translocated Signal Associated with the Activation of Systemic Acquired Resistance"
12:10-12:30 Short talk: **Aardra Kachroo** (University of Kentucky, USA)
"RPG1-B Derived Resistance to AvrB Expressing Pseudomonas syringae Requires RIN4-like Proteins in Soybean"

12:30-1:30 Lunch break

Session XII. Biotic Plant Interactions III
Co-Chairs: Shmulik Wolf & Ulrich Hammes

1:30-2:00 **Richard S. Nelson** (The Samuel Roberts Noble Foundation, Inc., USA)
"Virus Transport: Diversity in Motion"
2:00-2:30 **Michael E. Taliansky (**Scottish Crop Research Institute, UK)
"Role of the Plant Nucleolus in Virus Movement through the Phloem"
2:30-2:50 Short talk: **Na-Sheng Lin** (Institute of Plant and Microbial Biology, Academia Sinica, Taiwan)
"Multiple Factors Coordinate the Movement of Satellite RNA Associated with Bamboo mosaic virus in Infected Plants"
2:50-3:10 Short talk: **Byoung-Eun Min** (University of Kentucky, USA)
"Cytoplasm-tethered Transcription Activators Are Implicated in the Cell-to-Cell Movement of Sonchus yellow net virus"
3:10-3:30 Short talk: **Alison Roberts** (Scottish Crop Research Institute, Scotland)
"An N-terminal Domain of Potato mop-top virus TGB1 Protein Mediates Nucleolar Targeting and Is Essential for Long-distance Movement of Viral RNAs"
3:30-3:50 Short talk: **Lidor Gil** (The Hebrew University of Jerusalem, Israel)
"Sucrose Transporters Play a Role in Phloem Loading of CMV-Infected Melon Plants that Are Defined as Symplastic Loaders"

3:50-4:20 Coffee break

Session XIII. Integrative Plant Vascular Biology
Co-Chairs: Gary Thompson & Michael Grusak

4:20-4:40 Short talk: **Carel W. Windt** (Forschungszentrum Jülich, Germany)
"How Fast Can Phloem Sap Flow?"
4:40-5:00 Short talk: **Denis Renard** (INRA, UR 1268 Biopolymères, Interactions, Assemblages, F-44300 Nantes, France)
"Towards An Elucidation of the Three-Dimensional Structures of Complex and Flexible Biological Materials"
5:00-5:20 Short talk: **Shu Fujimaki** (Japan Atomic Energy Agency, Japan)
"Tracing Cadmium from Culture to Spikelet: Noninvasive Imaging of Long-distance Transport of Cadmium in Rice Plant"
5:20-5:40 Short talk: **Johannes Liesche** (University of Copenhagen, Denmark)
"Phloem Loading in Gymnosperms and the Functional Analysis of Plasmodesmata"

5:40-6:00 Closing remarks

6:00-6:30 Shuttle picks up participants from The Fawcett Center and transports to The Blackwell Inn

7:00-9:00 Conference Banquet and Best Short Talk and Poster Awards (at The Blackwell Inn)

9:00-10:00 Shuttle picks up and transports participants back to campus dorms and hotels

THURSDAY, JULY 28

Participant departure; Shuttle bus transport from campus dorms and hotel to Port Columbus International Airport at designated times.

Invited Lectures & Short Talks

Control of Leaf Vascular Patterning

Thomas Berleth*,[1], Enrico Scarpella[2] and Danielle Marcos[1]

[1]Dept. of Cell and Systems Biology, University of Toronto and [2]Department of Biological Sciences, University of Alberta, Canada
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Feedback-regulated auxin flows have been implicated in an amazing number of plant patterning processes and have become subject to mathematical modeling. To this end, improved genetic dissection, experimental interference and novel visualization tools have to be integrated with computer simulations towards increasingly precise theoretical predictions and experimental quantifications. Auxin Response Factors have critical, partially overlapping functions in controlling the expression of AtPIN auxin-efflux associated proteins and can be used as genetic tools to locally manipulate auxin signal transduction and auxin transport. We have used genetic and experimental interference tools as well as live visualization to dissect the formation of *Arabidopsis* leaf venation patterns. During leaf development networks of procambial cells, the precursors of all mature vascular cell types, emerge from homogeneous subepidermal tissue. A crucial member of the AtPIN family of auxin efflux proteins, AtPIN1, is expressed prior to preprocambial and procambial cell fate markers in domains that become restricted toward sites of procambium formation. Subcellular AtPIN1 polarity indicates that auxin is directed to distinct "convergence points" in the epidermis, from where it defines the positions of major veins. Integrated polarities in all emerging veins indicate auxin drainage toward pre-existing veins, but veins display split polarities as they become connected at both ends. Auxin application and transport inhibition reveal that convergence point positioning and AtPIN1 expression domain dynamics are self-organizing, auxin transport-dependent processes. Our results suggest that epidermal "convergence points" are part of a more general developmental module defining not only the positions of major leaf veins, but also the positioning of lateral shoot organs. Live visualization of individual pre-procambial domains demonstrates the dynamic nature of the process selecting procambium cells.

Super-resolution Imaging of the Phloem

Karl Oparka*, Jessica Fitzgibbon, Jens Tilsner and Karen Bell

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Unravelling the substructure of the phloem remains an elusive goal in plant-cell biology. To date, we have depended largely on traditional transmission electron microscopy (TEM) to obtain details of the substructure of phloem cells. In our lab, we have been exploring new approaches to bridging the resolution gap between TEM and fluorescence imaging. In this talk, I will describe the use of so-called 'super-resolution' approaches for studying phloem tissues at previously unobtainable resolution. Conventional fluorescence microscopy is limited by the diffraction of light to approximately 200 nm in the lateral (*x*-*y*) plane and to about 500 nm in the axial (*z*) plane. This is because light travelling through a lens cannot be focussed to a point, only to an Airy disk with a diameter of about half the wavelength of the visible emitted light. Confocal laser scanning microscopy has produced improvements in axial resolution due to the removal of out-of-focus flare, but is also limited by diffraction We have been exploring new imaging techniques for imaging phloem tissues at resolutions significantly greater than offered by conventional optical methods. In 3D-Structured Illumination Microscopy (3D-SIM) objects beyond the diffraction limit are illuminated with multiple interfering beams of light transmitted through a series of diffraction gratings, producing a resolution of 100 nm in *x*-*y* and 200 nm in *z*. Individual plasmodesmata (PD) of the phloem are now coming within the range of such subdiffraction imaging protocols. I will describe our recent uses of 3D-SIM and field-emission scanning electron microscopy (FESEM) with respect to imaging the location of different PD within the phloem and will discuss the advantages and pitfalls of imaging PD at subdiffraction resolution. I will present new images of phloem PD that show clearly that proteins can now be localized to PD at resolutions significantly greater than previously possible.

Cellular and Transcriptome Analyses of Xylem Formation with Arabidopsis Culture Systems Harboring Inducible *VND6* and *SND1*

Hiroo Fukuda*, Kyoko Ito-Ohashi, Yoshihisa Oda

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Wood composed of xylem tissues occupies the major part of biomass. To understand machinery underlying xylem formation, we need to search key factors involved in secondary wall formation. For this purpose, we have established novel Arabidopsis culture systems in which master transcription factors, VND6 and SND1, which belong to the similar NAC subfamily, are induced by a steroid hormone. In these systems, approx. 80% of cells differentiate to tracheary elements (TEs) and xylem fiber cells within 3 days. Using these cultures, we performed transcriptome analysis and compared the expression patterns of downstream genes between VND6 and SND1. This analysis revealed that each transcription factor induced distinct gene sets in addition to common genes such as secondary wall formation-related genes. This culture system is also useful to follow the cellular process of differentiation after induction. Therefore, using the *VND6*-inducive culture, we analyzed cytoskeleton dynamics and its regulation during pitted secondary cell wall formation. As a result, we revealed that microtubules are instabilized in future pit area and discovered a novel microtubule associated protein, MDD1, which depolymerizes microtubules in the future pit area.

Molecular Dissection of Secondary Wall Biosynthesis

Zheng-Hua Ye

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Secondary walls are the major constituent of wood (secondary xylem), which is the most abundant biomass produced by plants. Secondary walls are composed mainly of cellulose, lignin and hemicelluloses (xylan and glucomannan). To make secondary walls, genes involved in the biosynthetic pathways of cellulose, lignin and hemicellulose need to be coordinately switched on. Understanding the molecular switches controlling secondary wall biosynthesis in wood is of importance in basic plant biology as well as for potential genetic engineering of wood quality and quantity in tree species. We have been using Arabidopsis and poplar as model plants to characterize genes involved in transcriptional regulation of secondary wall biosynthesis and genes involved in xylan biosynthesis. We have uncovered key roles of several transcription factors in regulating secondary wall biosynthesis. One of these transcription factors, SND1, together with its homologs were found to be master switches activating the biosynthetic pathways of the secondary wall components. In addition, we have discovered a number of additional players in the SND1-mediated transcriptional regulation of secondary wall biosynthesis. We hypothesize that a transcriptional network is involved in the activation of secondary wall biosynthetic genes during wood formation. We have also dissected functional roles of a number of glycosyltransferases involved in xylan biosynthesis during wood formation. Further studies of the transcriptional network regulating secondary wall biosynthesis will likely enable us to genetically alter the biosynthetic pathways of individual secondary wall components, and knowledge gained from such studies promises to lead to better strategies for genetic manipulation of wood quality and quantity.

Phloem Anatomy and Photosynthetic Capacity

William W. Adams III*,[1], Véronique Amiard[2], Robert Turgeon[3] and Barbara Demmig-Adams[1]

[1]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309-0334 USA, [2]Centro de Genómica Nutricional Agro acuícola, Unidad de Biotecnología de Plantas, INIA-Carillanca, Casilla 58-D, Temuco, Chile, and [3]Department of Plant Biology, Cornell University, Ithaca, NY 14853 USA
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Foliar minor loading vein density, the number of phloem loading cells, and phloem cell ultrastructure were characterized and evaluated in relationship to photosynthetic capacity (the maximal rate of light- and CO_2-saturated photosynthetic oxygen evolution) among different species and within species for individuals acclimated to different levels of light. In some species (two symplastic loaders and one apoplastic loader), minor loading vein density was significantly greater in leaves that developed under high light (HL) compared to leaves that developed under low light (LL). For those species able to adjust vein density, transfer of already fully expanded LL-grown leaves (with relatively low vein density) to HL led to an incomplete upregulation of photosynthetic capacity, i.e. capacity failed to reach the level exhibited by HL-grown leaves with high vein densities. In contrast, transfer to HL of LL-grown leaves of apoplastic loaders that exhibited constant vein density resulted in a complete upregulation of photosynthetic capacity – to the level observed in HL-grown leaves. In apoplastic loaders with transfer cells, cell wall ingrowths were greater in HL- versus LL-grown leaves and in leaves transferred from LL to HL for one week. Increased wall ingrowth magnifies the plasma membrane area available for proteins involved in sucrose transport (sucrose/H^+ symporters and ATPases). Moreover, there was a very strong (r^2 = 0.999), and highly significant ($p<0.0001$), correlation between the mathematical product of foliar vein density X loading cells per sieve element when plotted against photosynthetic capacity in leaves of five apoplastically-loading species grown under HL conditions favoring growth. Minor loading vein density is a measure of the capacity of the physical pathway through which sugars are exported from leaves, and the number of loading cells per sieve element represents the number of cells providing the driving force for, and presumably contributing to, phloem loading of the sugars into those veins. The results presented here suggest that these two parameters in combination are able to predict what is apparently the maximally possible photosynthesis rate for a species. A better understanding of the mechanisms (i) underlying the development of phloem cells within the minor loading veins and (ii) those controlling the pattern and density of veins within a leaf may thus be essential to further increase the yields of important food, fiber, and biofuel crops.

Vacuolar SNAREs Function in the Formation of the Leaf Vascular Network by Regulating Auxin Distribution

Makoto Shirakawa*, Haruko Ueda, Tomoo Shimada and Ikuko Hara-Nishimura

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In normal leaf development, a two-dimensional pattern of leaf veins is known to form by differentiation of vascular cells from ground meristem cells in a manner that is regulated by the polar flow of auxin. However, the mechanisms regulating the distribution of auxin in the leaf primordium are largely unknown. Here we show that vacuolar SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors), VAM3 and VTI11, are required for the formation of the leaf vascular network in a dosage-dependent manner. This is the first report to show that the pre-vacuolar compartment (PVC)–vacuole traffic pathway is required for the formation of the leaf vascular network. *vam3-4*, a VAM3-defective mutant, was found to have an immature vascular network. An analysis of the DR5 reporter in *vam3-4* indicated that VAM3 is involved in the proper pattern formation of auxin maxima in the leaf primordium. This suggests that the immature vascular network in *vam3-4* was mainly determined at the stage of procambium formation in the leaf primordium. The abnormal distribution of auxin maxima was caused by the non-polarized localization of the auxin efflux carrier PIN1 (PIN-FORMED 1) in leaf primordium cells. VAM3 is the first key protein which is required for the proper localization of PIN1 in leaf cells. Finally, we found that PIN1 proteins were constitutively transported to vacuoles in leaf and roots cells. Our findings demonstrate that the PVC–vacuole pathway is required for the formation of auxin maxima, which regulates the polar localization of PIN1, which, in turn, is required for the formation of the leaf vascular network (Plant and Cell Physiology, 2009, 50: 1319-1328). Recently, we found that *vam3-4* mutants developed a large number of myrosin cells, which play an important role in plant defense and were distributed only along with the veins in the wild-type. Interestingly, the network of myrosin cells was found throughout the leaves of the *vam3* mutants and was compensating the immature network of vascular cells. Our findings suggest that VAM3 plays a role in development of both vascular cells and myrosin cells.

The Paralogous Poly(ADP-ribose) Polymerases RCD1 and SRO1 Control Vascular Patterning and Differentiation

Rebecca S. Lamb*, Zachary Skidmore and Sachin Teotia

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Poly(ADP-ribosyl)ation is an important post-translational modification of proteins that is only beginning to be understood in plants. The model plant species Arabidopsis thaliana encodes nine putative poly(ADP-ribose) polymerases (PARPs), of which six belong to a land plant specific group. Two members of this group, *RADICAL-INDUCED CELL DEATH 1 (RCD1)* and *SIMILAR TO RCD1 ONE (SRO1)*, encode proteins with WWE domains and RST domains in addition to their putative catalytic core. *RCD1* and *SRO1* are partially redundant with one another and have pleiotropic roles in abiotic stress response and development. *rcd1-3; sro1-1* mutants have extensive vascular defects that include a reduction in the amount of vascular tissue, unevenly spaced vascular bundles in the stem and a loss of vascular cambium. In addition, these plants have abnormally differentiated xylem vessels and lack phloem fibers. We will present work characterizing the vascular defects conditioned by loss of *RCD1* and *SRO1* and our progress toward understanding the molecular mechanisms underlying these defects.

Root Vascular Tissue Patterning Directed by Cell-to-Cell Communication

Jing Zhou, Jose Sebastian and Ji-Young Lee*

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A key question in developmental biology is how cells exchange positional information for proper patterning during organ development. In plant roots, tissue organization including a central vascular cylinder is highly conserved, suggesting the presence of evolutionarily conserved molecular regulation that directs tissue organization. In the vascular cylinder, xylem and phloem develop multilaterally. These tissue types are determined in the procambium of the root apical meristem. Protoxylem with lignified cell walls in a spiral form develops in the periphery of vascular cylinder and metaxylem with lignified cell walls in a pitted form develops in the center. In mutants of SHORT-ROOT (SHR), a GRAS gene family transcription factor (TF), proliferation and patterning of xylem and phloem are significantly perturbed. We found that SHR is required for xylem patterning as a mediator of crosstalk between the vascular cylinder and the surrounding endodermis. SHR, produced in the vascular cylinder, moves into the endodermis and activates SCARECROW. Together these transcription factors activate *MIR165a* and *166b* in the endodermis. Our investigation showed that these miR165/6 travel to neighbouring cells and degrade its target mRNAs encoding class III homeodomain-leucine zipper TFs (HD-ZIP III) in the endodermis and the periphery of vascular cylinder. Resulting differential distribution of HD-ZIP III TFs in xylem precursors is critical for xylem patterning since a high dosage of HD-ZIP III TFs specifies metaxylem and a low dosage does protoxylem. To understand where SHR affects the phloem development, we expressed non-mobile SHR under vascular cell type specific promoters. Unlike xylem patterning, SHR seems to promote phloem formation in a cell autonomous manner. Based on our developmental studies, we are further dissecting the SHR regulation in the vascular tissue patterning at a genome-wide level.

Analysis of Cell Signalling during Vascular Morphogenesis in Arabidopsis

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Vascular plants have a long-distance transport system consisting of two tissue types, phloem and xylem. During root primary development, xylem is specified as an axis of two vessel element cell files, centrally located metaxylem and peripherally located protoxylem. We have recently identified AHP6, an inhibitory pseudophosphotransfer protein for cytokinin signaling as a spatially specific regulator facilitating protoxylem specification (Mähönen et al. Science 311, 94). Subsequently, we have identified two regulatory interactions that regulate AHP6 and the xylem pattern. First, we have shown that cytokinin and auxin interact in a spatially specific manner during procambial development to specify the AHP6 pattern. Furthermore, in collaboration with the laboratories of Philip Benfey, Ji-Young Lee and Annelie Carlsbecker, we have shown that the miR165/6 species act non-cell autonomously to regulate the differential gene dosage of the class III HD-ZIP genes, and thus the AHP6 pattern during protoxylem and metaxylem development (Carlsbecker, Lee et al. Nature, in press). Finally, through identification of dominant mutations affecting callose biosynthesis, we have engineered a temporally and spatially controlled system to control plasmodesmatal trafficking during root procambial development. The mobility of the various signals is discussed based on the analysis with this system.

Microdissecting the Cambium: New Insights into Secondary Growth Regulation in *Arabidopsis thaliana*

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In addition to longitudinal growth, lateral expansion of growth axes is essential for land plants for creating extended shoot and root systems. Lateral or secondary growth is mediated by the activity of the cambium, a two-dimensional meristematic tissue, which is organised as a cylinder enclosing the centre of growth axes. During the onset of secondary growth in the shoot, cambium activity is established *de novo* between primary vascular bundles, in the interfascicular regions. Attempts to study the molecular regulation of cambium initiation have been hampered by limitations in performing genetic analyses in trees as well as by the difficulty in accessing the tissue in *Arabidopsis thaliana*. Hence, in spite of its essential role for many aspects of plant growth, our knowledge about cambium regulation is scarce. Here, we describe an *in vitro* system by which we can successfully induce secondary growth in isolated stem segments of *Arabidopsis*. Analyses of molecular markers show that the system largely reflects the situation in intact plants and suggest that it represents a valuable tool for dissecting the process under highly controlled conditions. Taking advantage of these benefits, we performed Laser Capture Microdissection (LCM)-based transcriptional profilings at different time points during the establishment of the interfascicular cambium in *in vitro*-incubated segments. Expression analyses in intact plants revealed that identified genes are expressed specifically in the cambium demonstrating the relevance of the system. Receptor-like kinases belong to the group of identified genes and reverse genetics confirmed that they represent novel regulators of cambium activity. Our findings open up the possibility of deciphering the nature of signalling events essential for early stages of cambium initiation.

Gene Expression Patterns of Programmed Cell Death During Vascular Aerenchyma Formation in Seedling Roots of Glycine max cv. 'Yukihomare'

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Each year flooding greatly reduces the yield of many crops. The flooding of crop lands results in the depletion of soil oxygen levels, which causes a physiological stress in the plants. Understanding the morphological and the molecular adaptations to hypoxic conditions is essential for the development of flood-tolerant crops. Some crops develop lysigenous aerenchyma in their roots in response to hypoxic soil conditions, usually in the cortex. The gas-filled aerenchyma can potentially provide new conduits for gas diffusion between the root tip and aerial portions of the plant. Evidence shows that lysigenous aerenchyma form by programmed cell death. Like some other legumes, when flooded *Glycine max* cv. 'Yukihomare' develops a longitudinal cavity in the vascular cylinder beginning 1.0 cm behind the root tip and extending for varying distances toward the root base. Studying vascular cavity formation in *G. max* has advantages, large amounts of molecular and genetic data are now available, including the completed genome sequence and DNA microarrays. Currently we are investigating vascular cavity formation in order to identify genes involved. This study is focused around a 24 hour time course DNA microarray analysis of total RNA of the *Glycine max* primary root during vascular cavity formation. Preliminary results indicate that about 3500 genes are temporally up- or down-regulated two-fold or more in response to flooding.

Evolution and Development of Vascular Cambia and Secondary Growth

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Vascular cambia were a major innovation in vascular plant development. By supporting the radial growth of woody stems, vascular cambia enable plant forms ranging from tropical lianas to massive forest trees. Our understanding of the developmental mechanisms regulating vascular cambia and secondary growth has been greatly accelerated by genetic and genomic studies in the model trees of the genus *Populus*. Results are presented demonstrating fundamental roles for Class I KNOX and Class III HD ZIP transcription factors in regulating cambial functions, daughter cell differentiation, and patterning of secondary vascular tissues in *Populus*. A next stage of cambial research will likely involve comparative genomic and evolution of development studies. In that light, an overview of cambial evolution and taxonomic relationships of cambial variants is presented.

Regulation and Modeling of Lignin Biosynthesis: A Systems Biology Approach

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Lignin is a complex phenolic structural component of the secondary cell walls of all vascular plants. It is an irreversible end point of a major metabolic pathway in plant secondary metabolism. Lignin is fundamental to the adaptation of plants to land, the evolution of vascular transport and the resistance of plants to pests and pathogens. Lignin is a major barrier to the utilization of biomass for energy, for papermaking, and for forage digestibility due to the interaction of lignin with cellulosics in the plant cell secondary wall. The past research on lignin biosynthesis is substantial, creating one of a few opportunities in higher plants to integrate genomics, proteomics, biochemistry, chemistry and modeling to develop a comprehensive understanding of biosynthesis and structure of a major component of morphology and adaptation. We are conducting a systems biology study on regulation of lignin biosynthesis in wood formation. We seek to build models to quantitatively illustrate how the entire pathway is organized and regulated and to reveal regulatory and metabolic flux control mechanisms, leading to lignin quantity and structures. We use the model woody plant, *Populus trichocarpa* (Nisqually-1), and the systems approach including advanced quantitative methods of genomics, proteomics, metabolomics, biochemistry and structural chemistry, to provide a comprehensive analysis of the regulation of lignin biosynthesis. A perturbation strategy is used to systematically knock down the expression of all pathway and regulatory genes known to be involved in lignin biosynthesis during wood formation, and the effects on lignin biosynthesis (gene transcripts, proteins, metabolites, quantity and structures) analyzed using advanced genomic methods available. This information forms the foundation of statistics-based mechanistic modeling and lignin quantity/structural predictions for a quantitative model of lignin biosynthesis. Details of systems data generation, data analyses and model development will be presented. Substantial data have been generated for gene-specific (amiRNA & RNAi) transgenic *P. trichocarpa*, enzyme kinetics, and stable-isotope-dilution based absolute quantitation of proteins and of metabolites. These data will be discussed, with the focus on functional redundancy-mediated feedback and feed-forward loop regulations of lignin biosynthesis at the transcript and protein levels. Our long term goal is a predictive model of lignin biosynthesis and quantity/structure for greater understanding of the plant response to environmental stress and for more precise strategies to improve plant productivity and the production of energy, biomaterials and food.

Polycomb Group Proteins Are Involved in Regulation of Plant Secondary Vascular Tissue Development

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Polycomb group (PcG) proteins are highly conserved transcriptional regulatory factors that are required to maintain normal developmental programs by suppressing key regulators of differentiation pathway. In plants, PcG are reported to be involved in stem cell maintenance in shoot apical meristem, floral induction, gametogenesis and seed development. Our previous studies on transcriptional and protein profiling of xylem cell transdifferentiation and dedifferentiation during bark regeneration after girdling in *Populus tomentosa* showed that some PcG maybe involved in the secondary vascular tissue regeneration process. In order to study the role of PcG in plant secondary vascular tissue development, three polycomb repressive complex 2 (PRC2) genes, *NbFIE*, *NbMSI1* and *NbCLF,* were silenced by virus-induced gene silencing (VIGS) in *Nicotiana Benthamiana*. The results showed that the silencing of plant PRC2 component genes generally lead to irregular phenotypes in leaf shape, shoot branching, shoot apical meristem determinacy and impaired secondary growth in stems. Silencing of *NbFIE1.1* and *NbFIE1.2* genes resulted in lack of differentiation to tracheary elements and fibers in the secondary xylem, while downregulation of *NbMSI1* gene leads to disorganization of xylem-phloem radial pattern in newly-formed stem. Full genome microarray analysis revealed the dramatically differentially expressed genes categorized to diverse genetic pathways, including meristem specific genes, plant hormone biosynthesis and signaling, flowering time control, programmed cell death and plant defense responses. Our results provided the direct evidence that Polycomb group proteins are involved in regulation of plant secondary vascular tissue development. Based upon the phenotype and microarray data analyses, the role of PcG in plant secondary vascular tissue development will be discussed. This work is supported by grants from the National Natural Science Foundation of China (30872001).

Auxin Transport in *Populus* during the Shift from Primary to Secondary Growth

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Auxin is known as a master regulator of plant morphology and has been studied extensively in herbaceous plants. However, despite the prominent role of auxin in apical control, phyllotactic patterning, gravitropism, and vascularization, little is known about auxin in woody plant development. We have produced separate transgenic lines of *Populus* that (1) express GUS under the control of the synthetic auxin-responsive promoter DR5, and (2) show reduced expression of the genes encoding auxin efflux proteins PtaPIN1 and PtaPIN7. pDR5-GUS expression patterns in *Populus* stems coupled with radiolabeled auxin transport assays suggest that there are two separate routes for basipetal auxin transport in young woody plants and that these routes are linked via the rays. The primary xylem parenchyma of leaf traces form a basipetal route of auxin transport that links leaves and stem beginning just below the shoot apex. As the vascular cambium is established it forms a second basipetal route, and dominates free auxin transport down the stem once developing leaves have ceased to export auxin. Radiolabeled auxin is freely exchanged between these two compartments via the rays and moves about ten times faster than the diffusion control benzoic acid, but its movement is unaffected by the polar auxin transport inhibitor NPA. Ray and axial parenchyma in mature *Populus* wood were also found to express several members of the PIN, AUX/LAX, and ABCB gene families. RNAi lines containing a hairpin construct designed to target a region of PtaPIN1 show reduction of *PtaPIN1* and *PtaPIN7* expression by up to 80%. Despite this substantial reduction, *in vitro* grown plantlets show no obvious phenotype and appear to have rates of auxin transport similar to wild-type plants. These lines will be re-evaluated once plants are established in soil and develop woody stems. Current work in the lab is focused on determining whether radial transport is an important route of exchange between leaf-derived auxin and the vascular cambium, and if so, whether rays transport auxin by moving it in conjugated form.

Physical and Physiological Constraints on Xylem Transport

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The transport of water at negative pressures places the xylem at risk for cavitation, with the structure and functioning of the xylem reflecting strategies to prevent or contain the spread of embolism. Once cavitation occurs, however, plants must replace or repair cavitated conduits to maintain hydraulic capacity. How embolized vessels are restored to their functional state represents perhaps the major challenge to our understanding of xylem transport. Here I review recent work, both empirical and theoretical, on embolism repair, relating the physical challenges of restoring xylem continuity to the structure and physiology of this vascular tissue.

Phloem and Nerve Cells - Common Proteins, Common Functions?

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Phloem companion cells, pollen tubes and plant neurons express genes encoding plasma membrane-localized transporters for the cyclic polyol *myo*-inositol. The respective proteins [INT2 and INT4 (inositol transporter1 and 2) in *Arabidopsis thaliana*; HMIT (H^+/*myo*-inositol transporter) in animals] are closely related, and the animal HMIT proteins are members of the animal GLUT family (13 GLUT proteins found in man) that typically represent hexose transport facilitators (GLUT1 to GLUT12 in man). In all animals, however, a single GLUT protein is responsible for the transport of inositol (GLUT13 in man; usually named HMIT) and this very protein is not a facilitator like all other GLUTs, but rather a H^+/inositol symporter. As all other energy-dependent carbohydrate transporters of animals depend on Na^+ gradients [e.g. SGLT proteins (sodium-dependent glucose transporters) and SMIT proteins (sodium-dependent *myo*-inositol transporters)] the evolutionary conservation of a single H^+/symporter was a surprising observation. Sequence comparisons of plant and animal H^+/inositol symporters revealed another unexpected conservation also on the structural level. All of these proteins contain a large extracellular loop (about 100 aa) between the predicted transmembrane helices IX and X. In contrast to the rest of the protein there is hardly any sequence conservation between the loop sequences of different plant or animal inositol transporters. The only conserved amino acids are 8 cysteine residues, with 4 of these residues forming 2 CXXC motifs. Blast searches, phylogenetic and structural analyses of these loop sequences revealed that these H^+/inositol IX/X loops represent extracellular PSI-domains that are frequently found in type-I receptors of animal plasma membranes, such as semaphorins, integrins, plexins or attractins. In these proteins, the PSI domains were shown to represent sites of extracellular protein/protein interaction, to be involved in the functional regulation of the respective proteins, and to be abused by viruses as extracellular attachment sites.

Adjustment of Leaf Structure and Function to Cold Temperature

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Plant species that load sugars into the phloem apoplastically (via membrane transporters) tend to be tolerant of cool temperatures, while symplastic loaders (moving sugars through plasmodesmatal passageways and preventing backflow by sugar polymer trapping) typically/frequently occur in warmer climates. While upregulation of photosynthetic capacity (the maximal rate of light- and CO_2-saturated photosynthetic oxygen evolution) under growth at cool temperatures has been reported for many apoplastic loaders, no corresponding information is available for symplastic loaders. We here report that the symplastic loader *Verbascum phoeniceum* also exhibited a higher photosynthetic capacity, which was associated with a greater density of minor foliar loading veins, under low versus moderate growth temperatures (in climate-controlled growth chambers). In contrast, another symplastically loading species, *Cucurbita pepo*, showed neither a higher photosynthetic capacity nor a greater minor loading vein density when grown at low temperature compared to moderate temperature. We also characterized two pairs of congeneric species (lowland *Mimulus cardinalis* and upland *Mimulus lewisii*, both symplastic loaders) or ecotypes (ecotypes 'Italy' versus ecotype 'Sweden' of the apoplastic loader *Arabidopsis thaliana*) with adaptations to contrasting temperature regimes. Both the upland *Mimulus* species and the *Arabidopsis* 'Sweden' ecotype exhibited a higher photosynthetic capacity when grown under low versus moderate temperatures. In both cases, the greater photosynthetic capacity at low temperatures was associated with specific leaf anatomical characteristics.

Briefly Arresting: The Mechanism for Transient Cessation of Phloem Transport after Abrupt Stimuli Remains Mysterious, Other Than in Legumes Where Calcium Triggered Dispersion of Forisomes Is Responsible

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Transient cessation of phloem transport in dicot stems can occur after various stimuli such as rapid cooling, osmotic shock or scorching a nearby leaf tip, but remains unexplained. For *Vicia faba,* the effects of both pathway chilling and leaf-tip burning were measured for three different aspects of the phloem (the conformation of forisome, a P-protein; the transport of carbon-11 labelled photoassimilate; and the membrane electrophysiology of sieve elements: Thorpe *et al.* 2010). Forisomes dispersed after rapid cooling, with a delay that was longer for slower cooling rates. Phloem transport stopped about 20 s after forisome dispersed. At the lower temperature, transport resumed and forisomes re-condensed in a similar time-frame. Chilling induced both fast and slow depolarization of SE membranes, the electrical signature indicating that forisome dispersion was due to a transient promotion of SE free calcium. The role of other P-proteins was therefore studied. In wheat, free of P-protein, the transport response to tip-burning was the same as in *Vicia*–a brief cessation of flow; but with rapid chilling there was merely a sustained reduction of transport, an effect of viscosity. In dicots, PP2 proteins are thought to be a component of P-proteins. Yet in *PP2*-knock-down Arabidopsis plants, the transient cessation of transport still occurred after rapid chilling. It seems therefore that these proteins play no major role in the shock-response, and its cause continues to be a mystery for species other than legumes.

Thorpe MR, Furch ACU, Minchin PEH, Föller J, van Bel AJE, Hafke JB (2010) *Plant, Cell & Environment* 33: 259-271.

AtNaKR1 Is a Phloem Mobile Protein Necessary for Phloem Function and Root Meristem Maintenance

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SODIUM POTASSIUM ROOT DEFECTIVE 1 (*NaKR1*) encodes a soluble metal binding protein that is specifically expressed in companion cells of the phloem. The *nakr1-1* mutant displayed high Na, K, and Rb accumulation in leaves, short roots, and late flowering. Starch accumulation in the leaves of the mutant was also consistent with defective function on the phloem. Using traditional and DNA microarray-based mapping, a 7 bp deletion was found in an exon of *NaKR1* that caused a premature stop. The mutant phenotypes were complemented by the native gene and by GFP and GUS fusions driven by the native promoter. NAKR1-GFP was mobile in the phloem, it moved into sieve elements and into a novel symplasmic domain of the root meristem. Grafting experiments revealed that the high Na accumulation phenotypes were due to loss of NaKR1 function in the leaves. This supports a role for the phloem in recirculating Na to the roots to provide Na tolerance. The onset of root phenotypes coincided with NaKR1 expression after germination. Short root length was primarily due to a decrease in cell division rate in the root meristem indicating a role for NaKR1 expression in the phloem in root meristem maintenance.

LeCyp1 Is Involved in Long-distance Signalling in Tomato (*Solanum lycopersicum*) Plants

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It is now evident that apart from photoassimilates, the phloem translocation stream contains a variety of proteins and mRNAs. One of the more abundant proteins present in phloem sap is Cyp1, a member of the cyclophilin family. Interestingly, we identified melon *CmCyp* transcripts in the phloem sap. Moreover, using a pumpkin-melon heterograft system, we demonstrated long-distance movement of *CmCyp* transcripts from melon rootstocks to pumpkin scions. The tomato *diageotropica* (*dgt*) mutation has been mapped to the *LeCyp1* gene. Tomato plants containing the *dgt* mutation are auxin-insensitive and exhibit a pleiotropic phenotype: altered gravitropism, limited secondary growth, lack of lateral roots and an aberrant xylem. Although the phenotype of the *dgt* tomato mutant has been well characterized, involvement of the *LeCyp1* gene product in the molecular mechanism causing the *dgt* phenotype is unclear. In the present study, we explored the involvement of *LeCyp1* product in long-distance signalling. Reciprocal grafting experiments including *dgt* mutants and VFN8 control tomato plants indicated that long-distance trafficking of the *LeCyp1* gene product can restore lateral root formation in *dgt* mutant rootstocks, as well as vascular development. To further explore the molecular mechanism by which long-distance movement of the *LeCyp1* product affects developmental processes, expression of auxin-responsive genes was analysed in *dgt* mutant and control plant tissues. This set of experiments demonstrated that long-distance movement of the *LeCyp1* gene product causes significant elevation in the expression of *Aux/IAA* transcripts in *dgt* mutant rootstocks grafted under control tomato scions. Collectively, our study establishes that auxin-response-mediated developmental processes can be controlled by long-distance trafficking of the *LeCyp1* gene product. A model for the mode by which *LeCyp1* mediates long-distance signalling in tomato plants will be presented.

Regulation of Sucrose Transporter Activity in Tobacco Leaves

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Feedback regulation of photosynthesis occurs when the source-sink balance of the plant is altered. For example, when source leaves are removed, suites of responses occur to compensate for the loss of carbohydrates, including upregulation of photosynthesis in the remaining leaves. There is an emerging consensus that feedback regulation involves sugar signaling, but little is known about where or how these signals are identified and transduced. Phloem loading, the process in which sucrose and other photoassimilates are transferred from the mesophyll to the phloem, seems ideally placed to play a regulatory role in carbon partitioning because it is poised between the photosynthetic and long-distance transport systems. It is possible that loading is regulated by signals from sink tissues, thus raising or lowering sucrose concentration and photosynthetic activity in mesophyll cells. In plants that load from the apoplast, including most crops, loading is driven by sucrose-proton co-transporters (SUTs or SUCs). Several lines of evidence support the view that SUTs are dynamically regulated at the transcript and protein levels over periods of hours. Transcript levels also change markedly over the diurnal cycle. While many of these findings suggest dynamic control, few measurements have been made of SUT1-mediated sucrose uptake in vivo. Here the results are mixed, some indicating that raising sucrose levels, either by heat girdling or providing exogenous sugar, increases or reduces sucrose uptake, or has no effect, depending on the system. One of the reasons for disparate results in SUT studies could be that time frames differ. Some physiological responses to modifying the source-sink balance are rapid while others require longer times. In these studies we assayed *SUT1* transcripts and sucrose transporter activity under physiological conditions associated with leaf ageing, shifts in photon flux density, the diurnal cycle, and reduction of source strength. Transporter activity was assayed by measuring [^{14}C]sucrose uptake in tobacco leaf discs. Our results indicate that alteration of sucrose uptake does not necessarily match changes in *NtSUT1* mRNA levels. Transporter activity is positively correlated with changes in export rate over a period of days, but is relatively constant throughout the diurnal cycle. Therefore, the sucrose transporter appears to be involved in long-term, but not short-term, acclimatory responses to photoassimilate flux.

Sucrose- and H^+- dependent Gating of the Power Horse of Phloem Loading SUC2

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In plants sucrose is loaded in the phloem for long distance transport from source leaves to sink tissues. Phloem loading of sucrose synthesized in mesophyll cells takes place in sieve tube adjacent companion cells. These transport-active cells appear interconnected via plasmodesmata to the sieve tubes. The flux and direction of sucrose is catalyzed by AtSUC2 in *Arabidopsis* and ZmSUT1 in maize. Plant and animal sugar carriers shuttle their substrates in cotransport with protons or sodium ions respectively. Our previous biophysical studies of ZmSUT1 revealed that this carrier is working like a perfect thermodynamic machine by which the proton gradient drives sucrose transport and vice versa on the basis of a 1:1 H^+:sucrose stoichiometry. As a matter of fact ZmSUT1 is capable to mediate sucrose loading and unloading of the phloem. Since the knowledge about the transport cycle of plant sucrose transporters is still very limited, we here focused on Sucrose- and H^+- dependent gating of ZmSUT1. When expressed in *Xenopus* oocytes application of sucrose in the presence of acidic pH results in sustained inward proton currents. Sucrose-dependent H^+ transport is associated with a decrease in membrane capacitance C_m. In the absence of sucrose, C_m of ZmSUT1 injected oocytes was voltage dependent. Addition of sucrose, however, induced a dose-dependent, reversible decrease of C_m. In addition to sucrose C_m was modulated by external protons. In order to explore the molecular mechanism underlying the C_m change presteady-state currents (I_{pre}) of ZmSUT1 transport were measured. Our results indicate that in the absence of sucrose `trapped´ protons move back and forth between an outer and an inner site within the transmembrane domains of ZmSUT1. This movement of protons in the electrical field of the membrane gives rise to the presteady-state currents and in turn to C_m changes. Upon application of external sucrose, protons can pass the membrane generating transport currents and thereby abolishing presteady-state currents. At the meeting data gained with mutants and complementary VCF method will be presented.

Phloem Unloading – A Push-me, Pull-me System

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Münch pressure flow predicts that resource partitioning within plants is primarily regulated by abilities of competing sinks to remove phloem sap from their importing sieve elements (SEs). This places phloem unloading (a process conveying phloem sap from SEs to sink cells by a cohort of uni-directional transport events) at the regulatory hub of resource partitioning and hence crop productivity. Exit from SEs ± companion cells commonly follows symplasmic routes that thereafter, in certain sink types, may be disrupted by an apoplasmic step. This step can be reversed to re-instate an uninterrupted symplasmic route (transport phloem located along axial pathways), be developmentally programmed or be permanent (release phloem located in sinks). Each scenario depends upon sink type and physiological state. Large differences in solute concentrations (C) and accompanying hydrostatic pressures (P) between SEs and sink cells drive symplasmic unloading by diffusion and bulk flow respectively. Their contributions to overall unloading rates depend upon the relative magnitudes of C and P differences **– push-me's.** Unloading by bulk flow neatly integrates with that of longitudinal delivery through importing SEs and avoids decoupling of water and solute exit from SEs. Large P differences and P maintenance of elongating sink cells points to bulk flow rates being regulated by plasmodesmal hydraulic conductances (L_o) rather than by sink cell P. Plasmodesmal L_os are modulated by phytohormones that, in some physiological contexts, could function to signal sink demand – **pull-me's**. Osmolyte accumulation by sink cells axiomatically requires their hydraulic isolation from the phloem or alternatively unloading must be mediated by diffusion alone. Hydraulic isolation is secured by compartmenting phloem and sink cell apoplasms or by insertion of an intervening apoplasmic step (e.g. fleshy fruit). In the latter case, resource transfer between juxtaposed cells either side of the symplasmic discontinuity is mediated by transporters operating in efflux and influx modes. For sinks containing high cell wall invertase (CWINV) activities, facilitated diffusion accounts for sucrose efflux to the sink apoplasm. In the absence of CWINV, a component of sucrose efflux is likely to be energy coupled. Sink demand is linked with phloem import by a regulatory complex. Complex components include transporter regulation by substrate repression (influx) and turgor homeostasis (efflux) arranged in series with modulating plasmodesmal L_os. Key remaining questions to progress a mechanistic understanding of phloem unloading include identifying transporter(s) mediating energy-coupled membrane efflux of sucrose and discovering underlying molecular events regulating transporter activities and particularly plasmodesmal L_os.

Leaf Polar Metabolite Concentrations and Starch Levels in Relation to Phloem Loading Type

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The objective of this study was to determine leaf polar metabolite concentrations and starch levels in relation to phloem loading type. Over 40 dicotyledonous species, including both herbaceous and tree species, were selected based on their phloem loading type as determined by ^{14}C-autoradiography (Rennie and Turgeon, PNAS, 106:14162-14167). Recently fully expanded leaves were taken between 3:30 to 5:00PM on clear days to measure polar metabolites (via GC/MS), starch levels, and leaf sap osmolality. We found that concentrations of leaf transport sugars correlated with phloem loading type, with passive, symplastic loading species having higher concentrations than apoplastic loading and polymer-trapping species. For species that do not transport sugar alcohols, a positive correlation was found between concentrations of transport sugars and other polar metabolites, which suggests that other polar metabolites accumulate in the vacuole to balance the preferential compartmentalization of transport sugars in the cytosol. Sugar alcohol-transporting species had higher total concentrations of polar metabolites primarily due to the presence of sugar alcohols and the lack of corresponding increase in other polar metabolites in these species suggests a significant proportion of the sugar alcohols resides in the vacuole. Leaf starch level was significantly lower in passive, symplastic loading species than in apoplastic loading and polymer-trapping species, suggesting a larger proportion of the carbon resources is allocated to soluble metabolites in these species. Of the herbaceous species examined, passive, symplastic loading species had higher leaf osmolality than apoplastic loading and polymer-trapping species. However, this trend was not apparent in tree species and all of them had high osmolality, especially in sugar alcohol-transporting tree species. The results are consistent with the hypothesis that both apoplastic loading and polymer trapping have evolved in response to selective pressure for rapid growth, particularly in herbaceous plants, which can afford to have low leaf solute levels because of high hydraulic conductance. In contrast, passive, symplastic loading helps to maintain leaf turgor particularly in tree species, which have low hydraulic conductance. Presence of sugar alcohols enhances the ability of plants to tolerate low water potential, regardless of phloem loading type.

Phloem Loading of Amino Acids Affects Plant Metabolism and Productivity

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Nitrogen (N) is an essential macronutrient for plant growth and development. It is important for plant biomass production, seed yield, and seed protein quality. To study the importance of source-sink translocation of N for plant development, metabolism and productivity, the function of *Arabidopsis* amino acid transporter AAP2 that localizes to the phloem throughout the plant, was analyzed. *AAP2* T-DNA insertion lines showed a decrease in the amount of seed total N and storage proteins, supporting a role of AAP2 in phloem loading and amino acid distribution to the embryo. Interestingly, in *aap2* seeds total carbon (C) levels were unchanged while fatty acid amounts were elevated. Moreover, silique numbers per plant and seed yield were strongly increased. Together this suggests changes in N and C delivery, and subsequent modulations of sink development and seed metabolism. This is supported by expression studies of genes of N/C transport and metabolism in source and sink, and by metabolite analyses of *aap2* leaves. The importance of amino acid phloem loading for plant metabolism and productivity was further confirmed with pea (*Pisum sativum*) plants, in which a yeast amino acid transporter was expressed in the phloem. This work was supported by the National Science Foundation (IOB 0448506).

The Dynamics of the Symplasmic Transport in the Cambial Region

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Cell-to-cell transport via plasmodesmata, between the ray and fusiform cells in a cambial region, is regulated both in time and in space. The pattern of changes was found to be consistent for two different tree species: *Acer pseudoplatanus* and *Populus tremula* x *tremuloides*, thus indicating a general character of controlling mechanisms. The unlimited or restricted spreading of the small molecule tracer, carboxyfluorescein (CF), earlier applied to the vascular system, revealed seasonal differences in the level of symplasmic continuity between ray and fusiform cells. This was high in the dormant, and low in the active state of cambium (Sokołowska and Zagórska-Marek, 2007). The level of symplasmic communication depends upon the molecular weight of the transported cargo. Thus, in a series of further experiments it has been shown that in a dormant cambium, where the transport of small molecules appeared to be unlimited, the spreading of macromolecule tracers - like FITC-labelled dextrans - was restricted. In the active cambium, the symplasmic transport was spatially controlled. Generally, communication between the fusiform and ray cells was restricted. However, the degree of symplasmic isolation clearly changed along the axis of the cambial cylinder, alternately increasing or decreasing from the site of tracer application. The factors regulating seasonal and spatial changes of the symplasmic transport in cambium are still unknown. The differences in symplasmic communication in the cambial region in the study could be related to the strictly controlled orientation of transport between ray and fusiform cells. Thus symplasmic communication may be postulated as bidirectional in the dormant, and unidirectional in the active state of cambium.

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Genetic Regulation of Sucrose Transport into the Phloem

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Carbon partitioning is the process whereby photoassimilates are distributed from their site of synthesis in leaves to the rest of the plant. Control of carbon partitioning is crucial for plant growth and development, and underlies all aspects of crop yield. For most plants, carbon, in the form of sucrose, is loaded into the phloem and transported from leaves to non-photosynthetic tissues, such as roots and fruits. This process is well characterized at the physiological, biochemical and anatomical levels. Yet despite the obvious importance of carbon partitioning for plant growth and development, we still know very little about how it is regulated at the molecular level. The best characterized genes that directly function to load sucrose into the phloem are sucrose transporters (SUTs). Utilizing a reverse genetic approach to determine the biological functions of all SUTs in maize we discovered that SUT1 functions to load sucrose into the phloem. We are currently investigating the functions of the other SUT family members. In addition, my lab has been studying the functions of a second class of genes that control carbohydrate accumulation in maize leaves. *tie-dyed (tdy)* mutants display variegated leaves with chlorotic regions that hyperaccumulate carbohydrates. Cloning *Tdy1* revealed that it encodes a novel transmembrane protein highly conserved in grasses. Expression analyses determined that *Tdy1* mRNA is exclusively present in the phloem of all tissues. Additional forward genetic screens have identified a large collection of carbohydrate partitioning defective mutants, which hyperaccumulate starch and soluble sugars in their leaves. Through cloning and characterizing these mutants, we will provide a deeper understanding of the genes regulating sucrose entry into the phloem and carbohydrate partitioning throughout the plant.

Microelement Trafficking in Plants: A Multi-compartment Journey from the Rhizosphere to Seeds

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Essential microelements, such as Fe, Zn, Cu, Mn and Ni, are required throughout plant growth. They are needed both to support metabolic processes in vegetative tissues and for eventual deposition in seeds to support the start of the next generation. Because these elements can be toxic in excess, their absorption from the rhizosphere and transport throughout the plant must be carefully regulated to maintain adequate, but non-toxic levels. Thus, plants have developed a cadre of membrane transporters for metal ions (and/or for metal chelates) that are coordinately utilized to traffic metal ions into the roots, on into the xylem vasculature, to the shoot apoplasmic and symplasmic spaces, and eventually to the phloem compartment for delivery to various sink tissues, such as developing seeds. In this presentation we will introduce the various molecular mechanisms that have been identified to move metal ions across membranes and will discuss these mechanisms in the context of their role in moving metals to and throughout the long-distance vascular pathways. Specifically, we will describe how these transporters are arranged spatially and temporally to effectively move metals throughout the whole-plant continuum. We will discuss how shoot-to-root communication via the phloem pathway regulates root iron acquisition processes (and possibly the transport/uptake mechanisms of other metals). Furthermore, recent evidence for the involvement of transcription factors in the regulation of metal mobilization from source leaves to developing seeds (via the phloem pathway) will be presented. Finally, because of the multi-compartment, multi-process complexity of whole-plant metal transport, we also will focus on the missing pieces in this puzzle, thereby providing the audience with knowledge about the challenges and opportunities for further research in this area. And, continued research on this topic is critically needed, because a deeper understanding will allow us to effectively manipulate plant processes in ways that will enhance the micronutrient density of staple seed crops – a goal that is being targeted to combat global micronutrient deficiencies in human populations.

Role of Hexose Phosphorylating Enzymes in Vascular Development and Function

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Vascular tissues transport sugar but also consume part of it for their own development and function. Sucrose, the inert sugar transported in the phloem of many plants including tomato should be cleaved first and the hexoses yielded, glucose and fructose, must be phosphorylated for further metabolism. Hexose phosphorylation appears to be key regulatory step affecting many aspects of plant development. There are only two groups of glucose and fructose phosphorylating enzymes in plants, hexokinases (HXKs) and fructokinases (FRKs) with specific intracellular localizations and biochemical characteristics. Functional studies indicate that HXKs and FRKs have different roles. While HXKs function as sugar sensors regulating photosynthesis and hormones responses, FRKs are essential for vascular development. Individual and combined suppression of FRK genes uncovered that specific FRK genes have different roles in vascular development. Most interestingly is the discovery that a plastidial FRK also affects vascular function but mainly in mature leaves, with entirely different effects on plant development. It appears therefore that unlike HXK, FRKs are indispensible for vascular development and function.

A Snapshot of Xylem Vessel - Stem Tissue Interactions: New Perspectives on Mineral Nutrient Transport

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Lateral exchange of mineral nutrients between the xylem vessels and stem tissues helps to decouple the uptake from their utilization in all parts of the plant and may be important for the demand-specific distribution within the plant. Experimental difficulties have hindered the characterization of this exchange. We studied the lateral exchange and its dynamics in stems of bean (*Phaseolus vulgaris* cv. Fardenlosa Shiny) with a new microimaging approach. Stable isotope tracers for magnesium (^{26}Mg), potassium (^{41}K) and calcium (^{44}Ca) were delivered via the cut stem to the transpiration stream. Tracer distributions were subsequently mapped as "snapshots"in shock-frozen stem cross-sections with cryo-secondary ion mass spectrometry (cryo-SIMS) at spatial resolutions to at least 1µm. Exchange of the nutrients between xylem vessels and the specific stem tissues was very different. The xylem parenchyma showed a fast and strong exchange and the pith a fast exchange but for small fractions of nutrients only. These intense interactions suggest an important role of both tissues in regulating axial nutrient fluxes, possibly for a nutrient homeostasis and redistribution of nutrients from one vascular bundle to another to permit demand-specific nutrient distribution. In contrast, the cambium and phloem showed very slow and weak exchange of nutrients with the vessels, suggesting an apoplastic barrier refuses nutrient movement between xylem parenchyma and cambium. Water in contrast, traced with $H_2{}^{18}O$ showed free equilibration in all stem tissues. We found little difference in these distributions when nutrient tracers were applied directly to xylem of intact stems via a micro-capillary, suggesting that xylem tension had little effect on radial exchange of these nutrients, and that their movement was mainly diffusive. These results illustrate the potential of our new approach for investigating tissue-level nutrient exchange in plants to better understand the functional role of specific tissues for a coordinated nutrient distribution, homeostasis and demand-specific delivery. Furthermore, combining this methodology with radiotracer and MRI-techniques may shed new light on many old questions concerning the mechanisms of xylem and phloem function.

Phloem RNAs, Nutrient Stress, and Systemic Mobility

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Long-distance signaling via the phloem mediates many different processes during growth and development, pathogen defense, and the adaptation to adverse environmental conditions. One class of molecules that recently emerged as potential regulatory molecules are RNAs, and a wide range of mRNAs as well as small RNAs (sRNAs) has meanwhile been detected and identified in phloem sap of different plant species, including cucurbits, lupin and oilseed rape. Interestingly, the large set of sRNAs contains a range of micro RNAs (miRNAs) that are known to be nutrient deprivation-responsive in other tissues. We have recently demonstrated that these miRNAs accumulate in phloem sap under nutrient deficient conditions to levels even higher than in other tissues. Moreover, specific phloem-accumulating miRNAs can be indeed be transported over graft unions from shoots to roots and down-regulate the expression of their target genes there. Whether this translocation is of importance during nutrient stress responses and what functions it could have, given that the miRNAs can be synthesized in roots themselves, is one of the current focuses of our research. We propose that this inter-organ signaling is required to coordinate nutrient uptake with transport and assimilation pathways on the whole plant level. This talk will give an overview about the current knowledge on sRNAs in the phloem stream, will provide evidence for their mobility *in vivo*, and discuss the possible functions that long-distance translocation of these macromolecules could have, especially during nutrient-deficiency responses.

The Long-distance Transport of a Full-length mRNA Mediates Development

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BEL1-like transcription factors are ubiquitous in plants and interact with KNOTTED1-types to regulate numerous developmental processes. In potato, the RNA of several BEL1-like transcription factors has been identified in phloem cells. One of these, StBEL5, and its Knox protein partner regulate tuber formation by targeting genes that control growth. RNA detection methods and heterografting experiments demonstrated that *StBEL5* transcripts move across a graft union to localize in stolon tips, the site of tuber induction. This movement of RNA originates in leaf veins and petioles and is induced by a short-day photoperiod, regulated by the untranslated regions, and correlated with enhanced tuber production. Addition of the *StBEL5* untranslated regions to another *BEL1*-like mRNA resulted in its preferential transport to stolon tips and enhanced tuber production. Upon fusion of the untranslated regions of *StBEL5* to a GUS marker, translation in tobacco protoplasts was repressed by those constructs containing the 3′ untranslated sequence. The untranslated regions of the mRNA of *StBEL5* are involved in mediating its long-distance transport and in controlling translation. The 3′ untranslated sequence contains an abundance of conserved motifs that may serve as binding motifs for RNA-binding proteins. Because of their presence in phloem cells, their unique UTR sequences and their diverse RNA accumulation patterns, the family of *BEL1*-like RNAs from potato represent a valuable model for studying the long-distance transport of full-length mRNAs and their role in development. This research was funded by USDA-NRI award no. 2008-02806 and NSF-PGRP award no. 0820659 to DH.

Involvement of a MicroRNA in the Long-Distance Regulation of Potato Tuber Induction

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Flowering and tuber induction are controlled by long-distance signals produced in leaves in response to changes in photoperiod. These signals travel through the phloem to the shoot apical meristem to induce flowering and to underground stems (stolons) to induce tuber formation. In several plant species, the transcriptional regulator CONSTANS (CO) induces the expression of FLOWERING LOCUS T (FT), a protein that moves to the shoot apical meristem to promote flowering. In potato, the homeobox transcription factor BEL5 promotes tuberization. Long-distance movement of *BEL5* mRNA correlates with tuber induction. We have identified a potato *CO* gene (*StCO*) involved in the response of tuberization to photoperiod. In addition, we have shown that a microRNA, miR172, promotes tuberization and flowering when overexpressed in potato plants. Consistent with this, miR172 abundance is higher under tuber-inducing than under tuber-repressing photoperiods and increases in wild-type stolons in the early stages of tuber induction. Potato plants with reduced levels of the photoreceptor phytochrome B (anti-PHYB plants) tuberize under long days, a condition that represses tuberization in the wild type. Interestingly, leaf levels of *BEL5* mRNA and miR172 are lower in anti-PHYB than in wild-type plants, whereas stolon levels are higher in anti-PHYB than in wild-type plants. Therefore, high *BEL5* mRNA and miR172 accumulation in stolons correlates with the onset of tuberization. Grafting of miR172-overexpressing scions onto wild-type stocks induces tuber formation under long days, indicating that the effect of miR172 on tuber induction is graft transmissible. Furthermore, this miRNA is present in potato vascular bundles. Altogether, these results suggest that miR172 might be a long-distance signal for the regulation of tuberization. We have identified a potato gene containing a miR172 target site. We will present the effect of miR172 and PHYB on this gene and the effect of miR172 on *BEL5* mRNA levels. Currently, we are testing whether StCO affects miR172 and *BEL5* mRNA abundance. On the basis of our results, we will discuss a model for the long-distance control of tuberization by these factors.

Geminivirus-mediated Delivery of Florigen to Ancestral Cotton Uncouples Flowering from Photoperiod and Promotes Determinate Growth

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Cotton is the leading textile crop with a worldwide impact estimated at $500 billion annually. Ancestral cotton is a tropical perennial that flowers in response to short-day photoperiods. Modern cultivars in the U.S. are day neutral and are grown as annuals. Modern cultivars have restricted diversity while primitive accessions are a tremendous reserve for desirable traits, but differences in the onset of flowering complicate breeding and increase costs. We have modified a disarmed Cotton Leaf Crumple Virus (CLCrV) [1] to express the *Arabidopsis thaliana FLOWERING LOCUS T* gene (*FT*, encoding florigen) from the coat-protein promoter, and used biolistic bombardment to infect the ancestral cotton accession TX701. Under long-day, greenhouse conditions, where TX701 would not normally flower, TX701 infected with *pCLCrV::FT* initiated reproductive growth concurrently with day-neutral cultivars. The suitability of using "virus-induced flowering" to facilitate breeding between short-day ancestral lines and modern, long-day cultivars is being evaluated. When TX701 plants were grown under short-day conditions to naturally induce flowering, leaf morphology changed from a deeply lobed, five-fingered structure to a simple, single finger, concomitantly with the onset of reproductive growth. When plants were returned to long-day conditions, flowering quickly ceased, unfertilized flowers abscised, and new vegetative growth resumed formation of highly-lobed leaves. Simple, non-lobed leaves were also observed in *pCLCrV::FT* infected plants. These findings will be discussed in relation to an emerging model that places *FT* and florigen in a signaling network that controls the indeterminate / determinate nature of meristems and primordia, such that it should be considered a general growth hormone rather than a flowering hormone.

[1] **Tuttle JR, Idris AM, Brown JK, Haigler CH, Robertson D** (2008) Geminivirus-mediated gene silencing from Cotton Leaf Crumple Virus is enhanced by low temperature in cotton. Plant Physiol. **148:** 41-50

Photoperiodic Effect on Protein Profiles of Potato Petioles and Phloem

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In potato (*Solanum tuberosum*), *StBEL5* mRNA is a mobile RNA that is involved in the signaling system that activates tuber formation. Short-day (SD) conditions induce tuberization and long-day (LD) inhibits the process. The transcriptional source of this mobile RNA is leaf veins and leaf stalks designated, petioles. Transport of *StBEL5* RNA is induced by a SD photoperiod that leads to tuber formation. It is likely that the movement of *StBEL5* is facilitated by the formation of RNA-protein complex(s). Phloem is the important translocation stream for these RNA-protein complex(s). To further understand this proposed mechanism of downstream signaling we have undertaken a detailed proteomic analysis of proteins isolated from potato petioles (PP) grown under LD and SD photoperiod conditions using 2-dimensional gel electrophoresis (2-DE) followed by MALDI MS/MS and/or LC MS/MS. Shotgun proteomic approach was used to study the potato phloem. Proteins that were differentially expressed in response to changes in photoperiods were analyzed by *Progenesis SameSpots* software. Phosphoproteins and RNA-binding proteins were enriched using immobilized metal affinity chromatography and poly(U) Sepharose columns respectively from SD and LD PP protein extracts and similarly analyzed. In addition to establishing a catalog of PP proteins, we have so far identified nearly sixty-seven proteins that are differentially expressed in response to SD or LD photoperiods. Numerous other poly(U)-binding proteins which contain RNA recognition motifs have also been isolated and identified.

Long Distance Root-to-Shoot Signaling: Intersection between the *bps1* Mobile Signal and Auxin

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The Arabidopsis *bps1* mutant shows a root and shoot arrest phenotype. However, shoot arrest can be relieved if the mutant root is excised, or if plants are treated with inhibitors of carotenoid biosynthesis. Moreover, shoot arrest can be induced in a wild type plant if it is grafted to a *bps1* root. Thus the *bps1* root appears to be the source of a mobile signal that induces arrest within the shoot. Our goal is to understand the biology of this signaling molecule: what is it biochemically, and how does it act? To identify the signal, a bioassay has been developed which detects active metabolite extracts from the *bps1* mutants. Progress toward signal identification is also coming from genetic studies. The *bps1* mutant is epistatic to auxin over-production mutants and appears to interfere with TRP metabolism. These data are allowing us to compose models for biosynthesis and mode of action of this mobile signal.

The Role of Protein Folding in Cell-Cell Protein Trafficking

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Cell-to-cell communication plays critical roles in specifying cell fate and coordinating development in multi-cellular organisms. A new paradigm for such communication in plants is the selective trafficking of transcription factors through plasmodesmata (PDs), channels that traverse the cell wall and connect the majority of plant cells. We have taken an unbiased genetic strategy to dissect the mechanism of PD trafficking. The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PD, and its' trafficking appears to be important for its function in stem cell maintenance. A gain-of-function trafficking assay using trichome rescue in *Arabidopsis* was developed to demonstrate that the C-terminal region of KN1 is necessary and sufficient for trafficking in vivo. This system provides a simple and tractable model to understand how proteins traffic and to isolate mutants defective in trafficking. As a proof of concept for our strategy, a mutant with attenuated KN1 trafficking has been identified, and was found to be defective in a chaperonin gene. This chaperonin appears essential for PD trafficking of some but all non-cell-autonomous proteins, and biochemical evidence suggests a physical association between chaperonin and KN1. Proteins are thought to undergo partial unfolding during PD translocation, which makes the discovery of this chaperonin particularly exciting. A functional characterization of the role of chaperonins in will further our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking. In addition, it may give mechanistic insights into this elaborate protein folding machinery, which at a molecular level is not well understood in any system.

Identification of Putative Lipid Binding Proteins in the Phloem and Their Role in Plant Development and Stress Response

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The role of the phloem has changed from that of simple assimilate transport to a trafficking system for pathogen response and developmental regulators. We have obtained enriched phloem exudates from *Arabidopsis thaliana* and characterized proteins, metabolites and mRNAs. We discovered several fatty acids, lipids and lipid-binding proteins in the phloem. These phloem lipids are not artifacts from membranes but intrinsic to phloem exudates. As the phloem is an aqueous environment, the lipids may be bound to proteins to increase solubility. This is not without precedence in biological systems: human blood contains a variety of lipids. They are bound to proteins and transported to other tissues for use, storage, modification, or degradation (e.g. vitamins, cholesterol), or serve as messengers and have transcription factor activity. It raises the possibility that lipids and the respective lipid-binding proteins in the phloem serve similar functions in plants. One phloem lipid-binding protein contains a PLAT/LH2 domain. This domain is proposed to mediate interaction with lipids or membrane-bound proteins. Proteins containing the domain are typically induced in response to stress. We showed its localization using YFP labeling. LBP knock-out mutants have a low germination rate, delayed development of young leaves, and reduced seed set leading to a lower survival rate compared to wild-type plants. We will present the effect of LBP on development, ultrastructure, phloem lipid- and metabolite profiles as well as its localization and lipid-binding properties. This work is supported in part by MSU-Intramural grant #05-IRGP-313.

P-proteins in Sieve Elements: A Role in Macromolecular Trafficking?

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P-proteins are defined as idiosyncratic protein bodies observed in sieve elements. Depending on species, they can be in a crystalloid state, as forisomes found in faboid legumes, or they form filaments, in many species including pumpkin and Arabidopsis. Biochemical analyses showed various protein compositions of these bodies, depending on species. Therefore it is not clear whether all P-proteins have similar properties *in vivo*. Phloem proteins 2 (PP2) are one of two components of the filamentous P-proteins initially characterized in cucurbits. *PP2* genes were subsequently shown to belong to a large plant family, with members expressed in the phloem from unrelated species. In pumpkin, CbmPP2 was shown to be a dimeric lectin. CbmPP2 also bound to endogeneous or viral RNAs. These observations were consistent with a role in trafficking of macromolecules. To investigate the function of PP2 proteins in sieve elements, we studied the function of a close ortholog in Arabidopsis, PP2-A1. We first confirmed that PP2-A1 was associated to P-proteins, using immunogold labeling and transmission electron microscopy. Using a recombinant protein produced in *E. coli*, we demonstrated binding to three glycan types: N-acetyl-glucosamine oligomers, high-mannose N-glycans and 9-acyl-N-acetyl-neuraminic sialic acid. Fluorescence spectroscopy-based titration experiments revealed that PP2-A1 has two classes of binding site for N,N',N"triacetylchitotriose: a low-affinity and a high-affinity site, promoting the formation of protein dimers. PP2-A1 interacted with phloem sap proteins, as revealed by protein overlay experiments, suggesting that PP2-A1 might be involved in events requiring interactions with phloem sap glycoproteins. To test for such a role *in vivo*, RNAi lines affected in the expression of *PP2-A1* gene were characterized. The plants presented pleiotropic growth and developmental defects, suggesting that local and/or long distance signaling is affected. The observations suggested a role of PP2-A1 in signaling. Our biochemical findings also suggest that oligomeric state of the protein might be critical for PP2-A1 properties and therefore regulate its activity. As PP2-A1s are associated to P-proteins in sieve elements, a large proportion is expected to be assembled in larger complexes. Thus depending on their level of assembly, PP2-A1 binding properties might be limited to a particular cell type (i.e. companion cells *versus* sieve elements) or to a fraction of the proteins (i.e. mobile oligomers *versus* large complexes anchored into sieve elements). This remains to be determined. The subcellular localization of the protein and its assembly *in vivo* is currently analyzed using fluorescent tags.

‘Omics’ Approaches to Defining the Plasmodesmal Proteome

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Simple plasmodesmata (PDs) provide the pathways that allow cell-to-cell communication throughout higher plants. As such they are essential for plant growth and development and for defence against invading pathogens such as viruses. Despite these important roles, our knowledge of PD structure and function remains very rudimentary. A major hurdle to advance in this field is knowledge of the molecular components of PDs. Immunological techniques have identified components of the cytoskeleton and ER luminal proteins, and other proteins have been identified through their molecular associations with pathogen and host non-cell-autonomous molecules. Unfortunately our knowledge so far has been insufficient to propose networks of activity that might lead to a comprehension of how PD gating is controlled. This talk will review the progress we have made in using proteomics to define the PD membrane proteome. We have concentrated upon the membrane proteome because membranes are the most clearly distinguished features of PDs. From preparations of purified PDs we have identified some known and many new PD proteins. Combining information for new and known PD proteins with expression data from public datasets we are identifying such potential networks. These connect our identified proteins with further novel proteins and provide a platform of testable hypotheses about the structure of PD and the regulation of PD gating.

Three Mutations, *ise1,ise2, dse1*, Affect *Arabidopsis* Embryo Development and Control Plasmodesmata Structure and Function

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A genetic screen for altered PD mediated intercellular transport in mid-torpedo embryos of *Arabidopsis* identified 3 mutations, *increased size exclusion limit* (*ise*)*1* (1), *ise2* (2), and *decreased size exclusion limit* (*dse*)*1*. All three mutations reside in genes essential for different aspects of cellular homeostasis; thus, optimal cell health is critical to regulate PD function. A quantitative study of PD structure in the above mutants and following gene silencing in *Nicotiana benthamiana* reveals that loss of either ISE1, ISE2, (or DSE1, unpublished) significantly affects the frequency of secondary PD formation (3). Thus, all three genes affect PD function and structure. *ISE1* encodes a DEAD box RNA helicase that localizes to mitochondria; without ISE1 mitochondrial function is severely reduced and the cell accumulates reactive oxygen species. RNA helicases have essential roles in RNA synthesis and processing; mitochondrial RNA processing is severely abnormal in *ise1* mutants. *ISE2* encodes a DEVH box RNA helicase that localizes to cytoplasmic granules; such granules sequester and process mRNAs and siRNAs. We are comparing ISE2 granules to processing-body granules, stress granules, heat shock granules, and gene silencing granules (Burch-Smith, Rennie, PZ, unpublished). In contrast to *ise1* and *ise2*, *dse1* exhibits decreased intercellular transport during embryogenesis. *DSE1* encodes a plant specific WD40 protein (Xu, PZ, unpublished). WD40 repeats are among the most abundant identifiable protein domains found in a wide variety of eukaryotic proteins. They have a range of functions including adaptor/regulatory modules in signal transduction, pre-mRNA processing, cytoskeleton assembly, transcriptional activation, and cell cycle control. WD40 domains collude as repeats to form β-propeller structures that act as a platform for the stable or reversible association of binding partners. Currents efforts are to define DSE1 interaction partners. Strong mutant allelles of *ise1*, *ise2*, and *dse1* all exhibit severe developmental defects and embryos do not develop beyond the earliest stages. Thus, *ISE1*, *ISE2*, and *DSE1* are essential genes that control cellular homeostasis and PD structure and function.

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PLASMODESMAL GERMIN-LIKE PROTEIN 1 (PDGLP1) Functions in Selective Cell-to-Cell Trafficking of Non-cell-autonomous Proteins (NCAPs)

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In plants, a number of non-cell-autonomous proteins (NCAPs) as well as viral movement proteins (MP) are able to use plasmodesmata (PD) to traffic cell to cell. This unique process is based on specific interactions between the trafficking macromolecules and plasmodesmal components. Identification of the crucial PD components is essential if we are to develop an understanding of the mechanisms and regulation of intercellular trafficking. In this study, we used an antibody directed against a phloem NCAP, CmPP16-1 to perform co-immunoprecipitation (co-IP) experiments to identify interacting proteins contained within a plasmodesmal enriched cell wall preparation (PECP) isolated from tobacco BY-2 suspension cultured cells. Seven candidate proteins were identified and four of these candidate proteins, when fused with RFP, appeared to be co-localizated with both *Cucumber mosaic virus* (CMV)-MP:GFP and *Tobacco mosaic virus* (TMV)-MP:GFP being expressed in transgenic *Nicotiana benthamiana*. These results are consistent with their being components of the plasmodesmal NCAP pathway. One candidate, named *Nicotiana tabacum* PLASMODESMAL GERMIN-LIKE PROTEIN1 (NtPDGLP1), was chosen for further study. NtPDGLP1 appears to be targeted to plasmodesmata through the secretory pathway in a Bredfeldin A-sensitive manner. The N-terminal signal peptide on NtPDGLP1 was necessary and sufficient for this PD targeting. The GST-pull down assay shows that NtPDGLP1 directly interacted with CmPP16 and that the C-terminal region of NtPDGLP1 is involved in CmPP16 binding. NtPDGLP1 has 29 Arabidopsis orthologs and 2 Arabidopsis orthologs, named Arabidopsis PDGLP1 and PDGLP2 (AtPDGLP1 and AtPDGLP2), exhibited a similar PD localization pattern; however, the other members of this gene family accumulated along the length of the plasma membrane. PDGLP overexpression plants displayed a somewhat enhanced growth rate compared with wild-type plants. PDGLP also appeared to interact with a range of PECP proteins indicating that it may function within PD as a larger complex. These findings will be discussed in terms of the role of the PDGLP in NCAP trafficking and the strategy for building a comprehensive model of the NCAP pathway. Acknowledgments: This work was funded by NSF grant IOS-0918433.

Roles of RESTRICTED SUCROSE EXPORT1 Pectate Lyase in Sucrose Translocation and Secondary Plasmodesmal Biogenesis in the Leaf Vein of Arabidopsis Source Leaves

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Sucrose, the primary assimilate by photosynthesis, is translocated from source leaves to sink organs via the phloem. The companion cell (CC)-sieve element (SE) complex plays the most important role in sucrose translocation. CC and SE ontogenetically share the symplast via primary plasmodesmal connections. However, when source leaves acquire sucrose translocation capacity during the sink-to-source transition of leaves, secondary plasmodesmata with branches and other characteristic structural features develop in the cell wall between CC and SE. Although such secondary plasmodesmata are thought to increase the capacity of sucrose translocation, the exact role and mechanism of secondary plasmodesmal biogenesis remain to be elucidated. We previously showed that *restricted sucrose export 1* (*rsx1*) mutants showed unusual starch accumulation in CC and incomplete formation of the secondary plasmodesmata in the cell wall between CC and SE (Plant Vascular Biology 2007). We herein report the characterization and subcellular localization of RSX1 proteins to elucidate its roles in sucrose translocation and secondary plasmodesmal biogenesis.We showed that a recombinant RSX1 protein exhibit the pectate lyase activity in bacterial expression system. Non-methylated pectate served as better substrates than methylated substrates, suggesting that *RSX1* encodes a pectate lyase rather than a pectin lyase. This is consistent with our hypothesis that RSX is required for the breakdown of pectate in the middle lamellar of cell walls. We constructed a recombinant gene for an RSX1-sGFP fusion protein and showed that the expression of RSX1-sGFP complements *rsx1* phenotypes. Confocal laser scanning fluorescence microscopy revealed that RSX1-sGFP is expressed in CC-like cells in the leaf veins. Immunogold labeling using anti-GFP antibodies revealed that gold particles are enriched in some plasmodesmata. We will also report the results of translocation assays for photosynthetic assimilates using $^{14}CO_2$-fed *rsx1* and other transgenic plants.

Callose Synthase, A Fine Tuner of Intercellular Movement of Signaling Molecules through Plasmodesmata

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Plant cells evolved a unique intercellular information channel termed plasmodesmata (PD). PD allow not only the movement of small molecules such as ions, water, nutrients, but also macromolecules (proteins, RNA). PD permeability can be mediated by callose, which is deposited at the neck region of PD. However, how this callose deposition is controlled at the molecular level and its biological function remain to be elucidated. We isolated and characterized a mutant (*glucan synthase–like 8*, *gsl8*) that lacks callose deposition at PD. Our data revealed that these mutants and an RNAi line(s) have dramatically reduced callose at PD and increased PD permeability as judged by enhanced movement of GFP. *GSL8* RNAi lines displayed a defect in cell elongation and tropisms (gravitropism and phototropism). Auxin gradients regulate differential cell elongation in tropic processes; interestingly, the pattern of auxin distribution was changed significantly in these *GSL8* RNAi lines. In summary, our data suggest that callose deposition within PD plays a crucial role to control both the movement of both small molecules and proteins, such as TFs. Our studies have revealed the power of non-cell-autonomous TFs on plant cell communication and the crucial role played by callose in PD gating. Acknowledgments: Supported by EB-NCRC, BK21, NRL and WCU programs.

Long-distance Chemical Signalling Facilitates Autoregulation of Nodulation in Legumes

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Legumes, such as soybean, regulate the number of nitrogen fixing root nodules via the Autoregulation Of Nodulation (AON), which commences following rhizobia-inoculation, or nitrate-treatment, with the production of a root-derived signal called Q. We used molecular physiology, functional genomics, computational modeling, next-generation sequencing (Illumina GAIIx) and the available soybean genome (www.phytozome.net/soybean) to analyse the systemic, long-distance signaling during AON. Computational modeling allowed predictions to be developed on plant organ involvement in long distance signaling. We identified novel soybean genes that are differentially expressed in the zone of root hair emergence following rhizobia-inoculation. We have identified soybean Q candidate genes encoding CLAVATA3/ESR related (CLE) peptides that exhibit increased expression following rhizobia inoculation or inhibitory nitrate treatment. Overexpression of these genes significantly reduces soybean nodule numbers. Nitrate-induced Q peptide appears to act locally, whereas rhizobia-induced Q is transported to the shoot where it, or a product of its action, is perceived by a LRR receptor kinase called NARK. We used next-generation sequencing to identify a number of soybean components acting downstream of NARK in the leaf. One factor produced in the shoot following Q perception by NARK is a novel signaling compound called the Shoot-Derived Inhibitor (SDI). Once synthesised, SDI is subsequently transported from the shoot to the root where it inhibits further nodule induction. We found SDI to be NARK- and Nod factor-dependent, heat stable, small, and likely not a peptide or RNA molecule. Findings regarding our progress in identifying and characterising the abovementioned nodulation factors will be presented.

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Differential Vascularization of Nematode-induced Feeding Sites

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Sedentary nematodes are destructive plant pathogens that cause significant yield losses. In the roots of their host plants, cyst nematodes (CNs) and root-knot nematodes (RKNs) induce different, highly specialized feeding sites, syncytia or giant cells (GCs), respectively, to optimize nutrient uptake. We compared the mechanisms by which nutrients are delivered from the model host plant, Arabidopsis, to GCs induced by the RKN *Meloidogyne incognita* or to syncytia induced by the CN *Heterodera schachtii*. From previous work, syncytia were known to be symplastically connected to newly formed host phloem composed of sieve elements (SEs) and companion cells (CCs). Here we studied the formation of plasmodesmata (PD) during GC and syncytia development by monitoring a viral movement protein that targets branched PD and the development of host phloem during GC formation by applying confocal laser scanning microscopy (CLSM) and immunocytochemistry. Analyses of plants expressing soluble or membrane-anchored green fluorescent protein in their phloem demonstrated symplastic isolation of GCs. GCs were found to be embedded in a tissue that consists exclusively of SEs. These *de novo*-formed SEs contained nuclei and were interconnected by secondary PD. Distinct cells within the vasculature surrounding the feeding sites show auxin accumulation. Therefore a role of auxin in the pathogen induced the novo development of vasculature is suggested. A similar interconnection of SEs was observed around syncytia. However, these secondary PD were also present at the SE-syncytium interface, demonstrating the postulated symplastic connection. Our results show that CNs and RKNs, despite their close phylogenetical relatedness, employ fundamentally different strategies to withdraw nutrients from host plants.

Phloem-feeding Whiteflies and the Evasion of Plant Defense

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In response to herbivore feeding, plants perceive signals generated in response to tissue damage and insect oral secretions to activate and/or suppress defense-signaling pathways. The genetic and genomics resources from *Arabidopsis thaliana* were used to identify the defense pathways that control basal resistance to the silverleaf whitefly (*Bemisia tabaci* biotype B). In addition, electropenetration graph (EPG) studies are determining the insect behaviors on *Arabidopsis* defense mutants. Sentinel defense-gene RNAs were quantitated after *B. tabaci* infestation and revealed that responses to *B. tabaci* were biphasic and that adults and nymphs elicited distinct reponses. During the first 18 hrs of *B. tabaci* adult feeding, the jasmonic acid (JA)- and ethylene (ET)-responsive *PDF1.2* RNA levels increased. In contrast, salicylic acid (SA)-responsive RNAs were repressed relative to non-infested plants. After 24 hr of feeding, a shift in defense-signaling pathways occurred. At these later times after adult feeding and during nymph feeding (7 to 24 days infestation), SA-regulated gene RNAs and free and glucose-conjugated SA accumulated locally and systemically. In accordance with the cross-talk between the JA and SA signaling pathways, JA- and ET-responsive gene RNA levels declined or were not modulated in *B. tabaci*-infested leaves. To determine if the SA- and JA-regulated defense pathways influenced *B. tabaci -Arabidopsis* interactions, insect no-choice studies with mutants/transgenic lines that activated or impaired SA-regulated defenses (*npr1,* NahG*, cim10*) or JA-regulated defenses (*coi1, cev1, jar1*) were performed. Nymphal development was accelerated in the mutants that activated SA-regulated or impaired JA-regulated defenses (*cim10* and *coi1,* respectively). Reciprocally, lines that activated JA-regulated (*cev1*) or impaired SA-regulated (*npr1,* NahG) defense gene expression slowed nymphal development. Finally, when *npr1* plants, which do not activate SA-regulated defenses, were treated with MeJA, a dramatic delay in nymphal development was observed. Unlike the aphids, *B. tabaci* performance was similar on wild-type, *ein2* and *pad4* mutants. Collectively during the 2nd phase of infestation *B. tabaci* induce decoy defenses– and repress JA-regulated defenses, which deter nymphal development. In this manner, *B. tabaci* adjust plant defense to make Arabidopsis a better host for nymphal development. EPG studies are underway with *B. tabaci* adults and 2nd instar nymphs to examine the behavioral response on *Arabidopsis* lines that accelerate or interfere with *B. tabaci* nymphal development.

Influence of a Fatty Acid Desaturase in Tomato on Plant Defenses against a Phloem-feeding Herbivore

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This study demonstrates that survival and reproduction of the potato aphid (*Macrosiphum euphorbiae*) on tomato (*Solanum lycopersicum*) is significantly reduced by the *spr2* mutation, which abolishes function of LeFAD7, a chloroplast-localized ω-3-fatty acid desaturase. Loss of function of LeFAD7 reduces synthesis of linolenic acid and its derivatives, including jasmonic acid (JA). However, the impact of the *spr2* mutation on aphids appears to be independent of its impact on JA synthesis, because aphid population growth is not significantly impacted by other mutations that impair JA synthesis or perception. Induction of salicylic acid (SA) accumulation and upregulation of pathogenesis-related (PR) gene expression by aphid feeding is significantly greater in the *spr2* mutant than in wild-type plants. Furthermore, double mutants impaired in SA accumulation (*spr2 NahG*) are more susceptible to aphid feeding than the *spr2* mutant; thus, loss of function of LeFAD7 appears to enhance SA-dependent defenses against aphids. In addition, this mutation modifies constitutive and stress-responsive oxylipin profiles in the plant, which may also play a role in enhancing aphid resistance.

SA, Methyl Salicylate, Lipids, and Systemic Acquired Resistance – the Plot Thickens

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For over a century naturalists and scientists have observed that plants which survive an initial pathogen attack often develop enhanced resistance to subsequent infections. Kenneth Chester in his 1933 review of 200 publications covering this phenomenon termed it physiological acquired immunity (Q. Rev. Biol. 8:275-324). Systematic studies by Frank Ross in the early 1960's demonstrated that prior infection of tobacco plants by Tobacco Mosaic Virus (TMV) enhanced resistance in the distal systemic tissue to subsequent challenge by TMV or other pathogens, which he termed Systemic Acquired Resistance (SAR; Virology 14:340-358). Kuc and others showed that development of SAR required movement of a signal made in the primary infected tissue through the phloem to the distal systemic tissue more than a quarter of a century ago (Phytopathology 69:753-756, 1979). Our studies on salicylic acid (SA) – mediated signal transduction have shown that methyl salicylate (MeSA) is a critical phloem-mobile signal required for SAR in tobacco (Science 318:113-116, 2007). MeSA is biologically inactive; it is converted by the MeSA esterase activity of salicylic acid-binding protein 2 (SABP2) to SA, a key hormone for activating host defenses to many plant pathogens. Results of grafting studies indicate that SABP2's MeSA esterase activity is required in systemic tissue. A mutation, which destroys SABP2's SA-binding activity and the resulting feedback inhibition leading to unregulated MeSA esterase activity, compromises SAR if expressed in primary infected tissue that generates the SAR signal. MeSA levels increase in primary infected leaves, phloem exudates from these leaves and systemic leaves of control plants but not in these tissues of transgenic tobacco expressing the unregulated SABP2 in the primary infected leaves. SAR also is blocked when SA methyl transferase, which synthesizes MeSA from SA, is silenced in primary infected leaves. Current studies suggested that MeSA is also an SAR signal in Arabidopsis (Plant J. 56:445-456, 2008) and potato (MPMI, 2010 in press). However, a recent *Plant Cell* (21:954-971, 2009) paper from Jürgen Zeier's group argues that MeSA is not a mobile SAR signal in Arabidopsis. They employed two different KO mutants in *Benzoic acid-SA Methyl Transferase 1* (*BSMT1I*) and found that although these mutants were unable to produce elevated levels of MeSA after infection, they were still able to develop SAR. In contrast, we have found that a similar KO mutant in *BSMT1* has suppressed levels of MeSA and is compromised for SAR. Moreover, SAR can be restored in the *bsmt1* KO by treatment with MeSA or phloem exudate from infected wt, but not *bsmt1* KO, plants (MPMI, 23:82-90, 2010). In addition, recently several groups have identified lipid-derived mobile SAR signals. These include azelaic acid (*Science*, 324:87-91, 2009) and a diterpenoid (Jyoti Shah per. comm.). In summary, SAR is a complex process that involves multiple mobile signals. These signals appear to interact directly or indirectly with each other and may be used during different developmental stages of the plant.

Photoreceptors and Resistance Protein-mediated Signaling against Turnip Crinkle Virus in Arabidopsis

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Light harvested by plants is essential for the survival of most life forms. This light-perception ability requires the activities of proteins termed photoreceptors. In addition to various growth and developmental processes, light also plays a role in plant defense against pathogens and is required for activation of several defense genes and regulation of the cell death response. However, the molecular or biochemical basis of light modulated regulation of defense signaling is largely unclear.

Previously we have shown that incompatible interaction between Arabidopsis-Turnip Crinkle Virus (TCV) and tobacco-Tobacco Mosaic Virus pathosystems are dependent on light (Chandra-Shekara et al., 2006). Resistance to TCV is dependent on the Resistance (R) protein HRT, which contains coiled coil, nucleotide binding, and leucine-rich-repeat domains. To determine the genetic, molecular and biochemical basis of light-dependent defense pathway, we studied the role of various photoreceptors in *HRT*-mediated resistance to TCV, HRT protein levels and its localization (Jeong et al., 2010). Interestingly, mutation in blue-light photoreceptors led to degradation of HRT via a proteasome-dependent pathway and resulted in susceptibility to TCV. Exogenous application of salicylic acid induced transcription of *HRT*, which restored HRT levels in some, but not all, mutant backgrounds. These results show that different photoreceptors function distinctly in maintaining post-transcriptional stability of HRT. The current focus is to determine if these photoreceptors undergo direct or indirect interactions with HRT and if they are generally required for other R-mediated defense pathways.

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Identification of a Diterpenoid as a Vasculature Translocated Signal Associated with the Activation of Systemic Acquired Resistance

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Systemic acquired resistance is an inducible defense mechanism that confers enhanced resistance against a broad-spectrum of pathogens. SAR is activated in the distal organs of a plant that has been locally exposed to a pathogen. SAR requires the translocation through the vasculature of a signal(s) from the pathogen-inoculated organs to the distal organs, where it stimulates salicylic acid signaling. Methyl-salicylate and jasmonic acid have been suggested as vasculature translocated long-distance signals in SAR. Azelaic acid is another compound that has been shown to prime the activation of salicylic acid signaling in the SAR expressing organs. However, the importance of methyl-salicylate and jasmonic acid in SAR has been questioned by other studies, since they are not always required for SAR. Genetic studies with the *dir1*, *sfd1* and *fad7* mutants in Arabidopsis had indicated that a hydrophobic molecule that is present in the petiole exudates collected from pathogen-inoculated leaves is critical for SAR. We have purified this hydrophobic SAR inducing activity from Arabidopsis petiole exudates. MS analysis and pharmacological studies have confirmed that the diterpenoid compound, dehydroabietinal is a potent activator of SAR in Arabidopsis. Picomolar concentrations of dehydroabietinal were sufficient to induce SAR. Dehydroabietinal is rapidly translocated long-distance in plants and functions upstream of SA signaling in SAR. Dehydroabietinal is produced by a variety of plants, and is a potent activator of SAR in plants other than Arabidopsis, indicating that its role in SAR is conserved in plants.

RPG1-B Derived Resistance to *AvrB* Expressing *Pseudomonas syringae* Requires RIN4-like Proteins in Soybean

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Soybean RPG1-B mediates species-specific resistance to AvrB expressing *Pseudomonas syringae*, similar to the non-orthologous RPM1 in Arabidopsis. RPM1-derived signaling is presumably induced upon AvrB-derived modification of the RPM1-interacting protein, RIN4. Similar to RPM1, RPG1-B does not directly interact with AvrB. However, RPG1-B associates with RIN4-like proteins from soybean. Unlike Arabidopsis, soybean contains at least four RIN4-like proteins (GmRIN4a-d), all of which bind AvrB. In contrast, GmRIN4b, c, and d bind RPG1-B, but GmRIN4a does not. Silencing either GmRIN4a or b abrogates RPG1-B-derived resistance to *P. syringae* expressing AvrB. Binding studies show that the various GmRIN4 isoforms interact with each other. The lack of functional redundancy amongst the GmRIN4 proteins and their abilities to interact with each other, suggests that these proteins might function as a heteromeric complex in mediating RPG1-B-derived resistance. The GmRIN4 proteins also participate in soybean basal defense, since silencing *GmRIN4a* or *b* enhances basal resistance to virulent strains of *P. syringae* and the oomycete *Phytophthtora sojae.* Interestingly, although both GmRIN4a and b function to monitor AvrB in the presence of RPG1-B, GmRIN4a, but not GmRIN4b, negatively regulates AvrB virulence activity in the absence of RPG1-B.

Virus Transport: Diversity in Motion

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Viruses as obligate parasites require host factors to accumulate and spread in their hosts. Fluorescence co-localization studies with specific proteins from RNA- and DNA-based viruses show an association of these proteins with actin microfilaments (MFs) and in some instances, their intracellular trafficking along these MFs. For *Tobacco mosaic virus* (TMV), intracellular trafficking of cytoplasmic bodies formed by the viral 126 kDa protein fused with GFP during ectopic expression or by virus replication complexes (VRCs) labeled with GFP during infection is along MFs. Results from pharmacological and host gene knockdown studies in *N. benthamiana* surveying multiple RNA viruses indicate that virus intracellular and sustained intercellular movement can be influenced by the host actomyosin-based motility system (e.g. Harries et al. PNAS 106:17594-17599). Interestingly, however, *Tobamovirus* genus members substantially differ in their requirement for MFs for sustained intercellular movement: TMV and not *Turnip vein clearing virus* (TVCV), which does not form cytoplasmic inclusion bodies, requires MFs. TVCV also does not require intact microtubules for sustained intercellular spread and thus has no apparent cytoskeletal requirement for its movement. For TMV, sustained intercellular transport requires a specific myosin motor protein, XI-2, in addition to MFs. Other RNA viruses that require intact MFs for sustained intercellular spread, *Potato virus X* and *Tomato bushy stunt virus*, do not require myosin XI-2 for this activity. These findings indicate that plant viruses use a variety of methods for intercellular transport which are not correlated with their predicted phylogeny. While these findings are informative, they are insufficient to allow a full understanding of the mechanisms of virus intra- and inter-cellular movement. We are addressing these issues by investigating whether the myosin XI-2 requirement for sustained intercellular TMV movement correlates with an XI-2 requirement for intracellular transport of 126 kDa protein-GFP fusions or VRCs. We also are identifying domains within the TMV 126 kDa protein required for cytoplasmic body formation and MF-mediated transport through construction of chimeras between the 126 kDa protein and the TVCV 125 kDa protein homolog that does not form cytoplasmic bodies or associate with MFs. Lastly, we are studying the importance of myosin for virus spread between host species. Through these studies we will better understand the relationship of cytoplasmic body formation with actomyosin-mediated transport, the impact of intracellular virus movement on sustained intercellular virus movement and the similarity of intercellular movement mechanisms for an individual virus between plant species. Model(s) for virus movement will be presented.

The Cell Nucleus and Long-distance Plant Virus Movement

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The nucleolus and Cajal bodies are prominent interacting subnuclear domains involved in crucial aspects of cell function such as RNA metabolism, the cell cycle and aging. Certain viruses interact with these compartments but the functions of such interactions are largely uncharacterized. In this work, we show that the ability of the groundnut rosette virus ORF3 protein to move viral RNA long-distances through the phloem and mediate systemic infection strictly depends on its interaction with Cajal bodies, the nucleolus and the major nucleolar protein, fibrillarin. The ORF3 protein targets and re-organizes Cajal bodies into multiple Cajal body-like structures and then enters the nucleolus by causing fusion of these structures with the nucleolus. This process is mediated by the interaction between ORF3 protein and fibrillarin leading to the formation of ring-like complexes. A model of structural organization of fibrillarin-ORF3 protein complexes will be presented. It is suggested that ORF3 protein and fibrillarin can move from the nucleus to the cytoplasm in a form of these ring-like complexes. Furthermore, in the cytoplasm, these rings formed by both ORF3 and fibrillarin proteins interact with viral RNA encapsidating it and re-organizing it into helical structures, and thereby play a key role in the assembly of umbraviral RNP complexes capable of long-distance movement and systemic infection. These results demonstrate novel functions for plant fibrillarin as an essential component of translocatable viral RNPs and may have functional and mechanistic implications for movement of other plant viruses, which will be discussed.

Multiple Factors Coordinate the Movement of Satellite RNA Associated with *Bamboo mosaic virus* in Infected Plants

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Satellite RNA of Bamboo mosaic virus (satBaMV) is a single-stranded positive-sense RNA and contains an open reading frame for a nonstructural 20-kD protein (P20). SatBaMV depends on BaMV for replication and encapsidation. To understand how satBaMV RNA moves in the infected plants, we analyzed factors involving helper BaMV, satBaMV itself and host plants. SatBaMV RNA alone could not move cell-to-cell in *Nicotiana benthamiana* leaves, except on co-infection with BaMV or in transgenic plants expressing the whole BaMV RNA genome. Long-distance trafficking of satBaMV RNA occurred when BaMV or satBaMV was inoculated in separate leaves of *N. benthamiana* by *Agrobacterium tumefaciens* agroinfiltration. Increased efficient trafficking of satBaMV was detected when BaMV was agroinfiltrated in lower leaves. The satBaMV-encoded P20 protein, not required for the cell-to-cell movement, facilitated the long-distance trafficking of satBaMV RNA in BaMV-co-infected *N. benthamiana*. By serial mutation analysis of P20, the functional domain was mapped at the C-terminus. Three *N. benthamiana* proteins interacting with P20 protein were identified by the yeast two-hybrid system. With virus-induced gene silencing of P20-interacting proteins, the long-distance trafficking of satBaMV was greatly reduced in plants. Thus, the movement of satBaMV RNA in *N. benthamiana* requires a coordinated orchestration of multiple players: helper BaMV, satBaMV and host factors.

Cytoplasm-tethered Transcription Activators Are Implicated in the Cell-to-Cell Movement of *Sonchus yellow net virus*

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Nicotiana benthamiana is the most widely used host in plant virology given its susceptibility to many genetically diverse viruses. To identify host factors that play critical roles in viral processes such as replication and cell-to-cell movement, we have constructed and validated a high-resolution yeast two-hybrid library for *N. benthamiana* (NbY2H). The library was screened with the putative movement protein (sc4), nucleocapsid (N), and matrix (M) proteins of *Sonchus yellow net virus* (SYNV), a member of the plant-infecting Rhabdoviridae family. This resulted in the identification of 31 potential host factors. Bimolecular fluorescence complementation assays were used to validate the two-hybrid interactions. Also, steady-state localization studies using autofluorescent protein fusions to full-length clones of interactors were conducted in transgenic *N. benthamiana* marker lines. One of the sc4 interactors, sc4i21, localized to microtubules. The N interactor, Ni67, localized to punctuate loci on the ER. These two proteins are 84% identical, homologues of the Arabidopsis phloem-associated transcription activator AtVOZ1, and contain functional nuclear localization signals. Another sc4 interactor, sc4i17 is a likely microtubule-associated motor protein. The M interactor, Mi7, is a nuclear-localized transcription factor. Combined with a binary interaction map for SYNV proteins, our data support a model in which the SYNV nucleocapsid is exported from the nucleus and moved cell-to-cell by transcription activators tethered in the cytoplasm.

An N-terminal Domain of *Potato mop-top virus* TGB1 Protein Mediates Nucleolar Targeting and Is Essential for Long-distance Movement of Viral RNAs

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Potato mop-top virus (PMTV) gene products have been shown to interact with a wide range of cellular components including the plant endocytic pathway, chloroplasts, the nucleus and cytoskeletal components. However, the function of these interactions is still poorly understood with regard to local and systemic movement and to transmission by the plasmodiophorid vector *Spongospora subterranea.*

PMTV encodes a triple gene block (TGB) of proteins involved in movement of the viral genome. TGB2 and TGB3 are integral membrane proteins that co-localize to compartments of the endocytic pathway. TGB1 has been shown to bind RNA and current models suggest that, by co-operation with TGB2 and TGB3, the three proteins allow movement of the viral RNA. The temporal and spatial localization of the TGB1 protein and its function in viral pathogenesis has been studied using a full-length infectious clone of PMTV encoding TGB1 fused to fluorescent reporter proteins. In addition, transient expression systems have been employed to study ectopic expression of the TGB1 protein in isolation. Recent data has shown that, like other TGB proteins, TGB1 localises to a number of discrete compartments during different phases of viral pathogenesis. However, to date, this is the only PMTV protein to be found to target the nucleolus and microtubules. Deletion of nucleotides at the N-terminus of the TGB1 protein or fusion of a fluorescent protein to the N-terminus prevents virus long-distance movement, although cell-cell movement is unaffected. We have recently shown that the N-terminal deletion mutant fails to target the nucleolus and loss of this phenotype is associated with the loss of functional long-distance movement of the virus. This is the first report of nucleolar and microtubular association of a TGB movement protein and the results suggest that PMTV TGB1 requires interaction with nucleolar components and possibly microtubues for systemic infection. In order to study the interaction of viral proteins with host proteins to allow phloem transport, we are attempting to use three-dimensional laser microdissection of flash-frozen tissue to harvest phloem tissue in sufficient quantities to create high complexity cDNA libraries to allow us to study changes in gene expression in the phloem in response to virus infection. This approach relies on the production of statically relevant 3D models of the phloem to guide lasers to dissect out phloem tissue from frozen petiole samples. This approach is in development at present and an overview of this technique will be presented.

Sucrose Transporters Play a Role in Phloem Loading of CMV-infected Melon Plants that Are Defined as Symplastic Loaders

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By general consensus, there are two main mechanisms for loading sugar into the sieve element-companion cell complex of higher plants: apoplastic and symplastic. Based on the high density of plasmodesmata interconnecting the intermediary cells and their neighboring phloem parenchyma or bundle-sheath cells, and insensitivity to the sucrose transport inhibitor *p*-chloromercuribenzenesulfonic acid (PCMBS), cucurbits have been concluded to be symplastic loaders. In the present study, we provide the first full-length sequence of three sucrose transporter genes from melon (*Cucumis melo* L. cv. Hale's best jumbo): *CmSUT1*, *CmSUT2* and *CmSUT4*. Functioning of the encoded proteins was examined *in vitro*. Cucumber mosaic virus (CMV) infection caused significant upregulation of *CmSUT2* and *CmSUT4* transcripts, which was associated with elevated sucrose content in phloem sap collected from source-leaf petioles. This effect was also observed in CMV-movement protein (MP)-expressing melon source leaves. No differences in *CmSUT1* expression were found between healthy, CMV-infected and CMV-MP-expressing source leaves. Pretreatment with PCMBS resulted in a sharp decrease in phloem-sap sucrose and in [^{14}C]sucrose uptake into leaf discs, suggesting that a sucrose transporter(s) is also active in melon plants and that this activity is higher in CMV-infected plants. Tissue specific expression of the three *CmSUT* transcripts was verified using a laser capture microdissection system.

The possible role of CmSUT2 and CmSUT4 in CMV-infected and CMV-MP-expressing plants is discussed in terms of the mechanisms of phloem loading and long-distance signaling.

How Fast Can Phloem Sap Flow?

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Very few studies deal with phloem sap flow velocity because it is exceedingly difficult to measure. Such data as are available suggest that sap flow velocities in the transport phloem (i) are low, with flow velocities roughly between 0.25 mm/s and 0.40 mm/s, (ii) are similar between different plant organs and species (even in species with exceptionally wide sieve tubes), and (iii) tend to remain constant over the diurnal cycle (Windt *et al*., 2006; Windt *et al* 2009; Mullendore *et al* 2010).

To explain these observations, two hypotheses have been formulated. 1) Phloem architecture limits maximum sap flow velocity. If flow velocities would become too high, shear forces might cause parietal organelles in the phloem to be flushed away, thus damaging and clogging up the sieve tubes. 2) The phloem is built and rigorously controlled to maintain a constant phloem flow velocity. Beside mass flow of carbon, one of the purposes of the phloem is long distance signaling. To ensure that communication occurs at predictable speeds it might be important to keep translocation speeds constant. Here we tested these hypotheses.

In three model plant species (castor bean, poplar and maize) the phloem flow conducting area was reduced progressively by partial phloem girdling (0% of stem circumference girdled, 50% and 75% girdled), with the sources and sinks left un-modified. Phloem flow velocities were measured by means of MRI velocimetry. After partial phloem girdling the phloem flow velocity in the remaining phloem increased in all three species, demonstrating that it must not necessarily stay constant. Instead, the loss of flow conducting area was partially compensated by an increase in flow velocity, thus minimizing the loss in mass flow. However, even after severe reductions in phloem area, the flow velocity in the remaining phloem area did not become higher than 1 mm/s, and the total solution flow was never as high as before girdling. This suggests that there may indeed be an upper limit, of around 1 mm/s, to the flow velocity in the phloem.

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Towards an Elucidation of the Three-dimensional Structures of Complex and Flexible Biological Materials

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Solving structure of protein subunits or oligomers is a difficult task in many cases of macromolecular complexes. Indeed, changes in cellular environment may induce changes in conformation and assembly, critical for protein function. Therefore new structural approaches are required. Analyses by small angle scattering of biological materials have been rapidly evolving and promise to move structural analysis to a new era. Recent innovations in small angle scattering data analyses allow *ab initio* shape predictions of biological macromolecules in solution. Large complexes, flexible proteins or complex polysaccharides are not easily amenable for crystallography, but pieces of complexes, rigid portions of flexible proteins or polysaccharide are. Due to the relative ease and speed of small angle scattering sample preparation and data collection, this technique can be extremely powerful and constitutes a complementary method towards the elucidation of three dimensional low resolution structures in case of biological materials for which crystallography assays have failed. One first example is the resolution of the structure of a complex polysaccharide, the arabinogalactan-peptide from Acacia gum, for which we demonstrated, based on small angle neutron scattering experiments, *ab initio* calculations and microscopy, that this polysaccharide is a porous disk-type macromolecule with a diameter of ~20 nm and a thickness of ~1.9 nm with an inner dense fractal structure. This model is a breakthrough in the field of arabinogalactan-protein type polyelectrolytes. Concerning the site of biosynthesis of these macromolecules, the structural dimensions obtained were found in agreement with a long distance transport through plant vasculature. The second example is the analysis of the PP2-A1 phloem protein from *Arabidopsis*. PP2s, as components of P-proteins, are potential filamentous proteins. Secondary structure predictions revealed a large flexible and unstructured N-terminal domain, though to be in part responsible for unsuccessful crystallisation attempts. In this protein, a carbohydrate binding site was predicted in the core of the protein, folded into b-strands. Studies using scattering experiments and *ab initio* calculations revealed that PP2-A1 is present in solution as an oligomer, the degree of oligomerization depending on pH. We proposed from these structural data that filament formation of these proteins may result from a nucleation and growth process, the nucleation step being the formation of oligomers. Further structural studies, in particular the elucidation of both structure of PP2-A1 monomer and events leading *in vitro* in filament formation, would allow unambigously to conclude about the mechanism of PP2-A1 phloem protein self-assembly.

Tracing Cadmium from Culture to Spikelet: Noninvasive Imaging of Long-distance Transport of Cadmium in Rice Plant

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Contamination of crops, particularly irrigated rice, with cadmium (Cd) is one of the most serious agricultural problems in the world. In this study, we characterized the absorption and short-term translocation of Cd in rice quantitatively using a positron-emitting tracer imaging system (PETIS). PETIS is one of the radiological and noninvasive imaging techniques for plant science and has been widely employed in this decade to visualize transport of various nutrients. We fed a positron-emitting ^{107}Cd (halflife of 6.5 h) tracer to the hydroponic culture solution and obtained quantitative serial images of Cd distribution in intact rice plants at the vegetative stage and at the grain-filling stage every 4 min for 36 h. The results and conclusions were as follows. The rates of absorption of Cd by the root were proportional to Cd concentrations in the culture solution within the tested range of 0.05 to 100 nM. The radial transport from the culture to the xylem in the root tissue was completed in less than 10 min. The velocities of Cd movement through the shoot organs were a few centimeters per hour at both stages, which were obviously lower than that of transpiration stream. Finally, Cd arrived at the panicles 7 h after feeding and accumulated there constantly, although no Cd was observed in the leaf blades within the initial 36 h. The nodes exhibited the most intensive Cd accumulation in the shoot at both stages, and Cd transport from the basal nodes to crown root tips was observed at the vegetative stage, which indicated phloem transport. We concluded that the nodes are the central organ where xylem-to-phloem transfer takes place and play a pivotal role in the transport of Cd from the soil to the grains at the grain-filling stage.

Phloem Loading in Gymnosperms and the Functional Analysis of Plasmodesmata

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The key step in phloem transport is the loading of sugars. Two strategies in herbaceous plants are well established: apoplastic loading, involving membrane transport of sucrose, and symplasmic loading, involving synthesis of raffinose family sugars. Both are accompanied by energy investment in the companion cells (CC) of source phloem. In addition, recent investigations combining ^{14}C-compound uptake experiments, solute concentration measurements and plasmodesmata (PD) counting indicated a third mode of phloem loading present in angiosperm tree species. It does not depend on energy investment in CC and has therefore been termed passive symplastic loading. Connectivity along the pre-phloem pathway, which should be low in apoplastic loaders and high in symplastic loaders, has never been tested functionally. Electron microscopy allows measuring the frequency of plasmodesmata between cells, but not their functionality. For functional analyses, the preparation of leaf tissue is a challenge, because it can easily lead to pressure loss in the phloem system, thereby causing PD, which can be considered as pressure valves to open. We used whole-plant live-cell imaging in order to quantify connectivity between cells in source leaves. Functional connectivity along the pre-phloem pathway could be tested non-invasively by activating caged fluorescein in the target cell with a UV laser. This technique was able to identify the loading mode in different herbaceous plant species. Phloem loading is an enigma in gymnosperms because of their specific phloem anatomy. Mapping the connectivity of the pre-phloem pathway in pine needles indicated a symplastic loading mode, resembling that of many angiosperm trees. However, presence of an endodermis around the vascular tissue and isolation of the phloem from the mesophyll by transfusion parenchyma and transfusion tracheids indicate yet another mode of phloem loading in gymnosperms. This might have evolved to optimize water access inside the endodermis, needed for creation of pressure in the sieve element system, while preventing water loss to the outside. It can be speculated if the high efficiency of this loading mode led to the fact that gymnosperms hold the size record of tree species with heights of more than 100m. The results show the importance of addressing the phloem as an intact system and demonstrate the suitability of advanced live-cell imaging for quantification of cell connectivity.

Posters

P1

Carbohydrate partitioning defective1 Is Essential for Phloem Function and Plant Viability

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In plants, transport of carbohydrates from photosynthetic source tissues to non-photosynthetic sink tissues takes place in a specialized group of vascular cells called the phloem. To better understand the genetic regulation of carbon translocation, we screened mutagenized maize populations and have identified a class of mutants that are defective in carbohydrate partitioning and phloem transport. *Carbohydrate partitioning defective1* (*Cpd1*) is a unique semi-dominant mutant isolated from this screen. *Cpd1* hyperaccumulates carbohydrates in the leaves when heterozygous for the mutation and is seedling lethal when homozygous. Heterozygous mutant plants strongly resemble the previously characterized *sut1* mutant of maize, where leaves hyperaccumualte starch, undergo rapid chlorosis and senescence, and have reduced vegetative and reproductive development. Interestingly, homozygous mutant plants do not accumulate carbohydrates in the leaves and die before leaf four emerges. Aniline blue staining revealed that the heterozygous mutant plants accumulate large aggregates within the sieve elements and at the sieve plate interfaces. These aggregates presumably disrupt phloem transport leading to a build up of carbohydrates in the leaves. The severity of the heterozygous phenotype appears to be temperature dependent; high temperatures exacerbate the phenotype and lower temperatures suppress the phenotype. We mapped the gene to the short arm of chromosome 7. No known genes that function in carbohydrate metabolism or transport have been mapped to this region. Current progress toward cloning the gene will be described.

P2

Metabolite-assisted Early Selection of *Pinus densiflora* Families with Fast-growing Traits

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Metabolic information can often reflect biological endpoints more accurately than transcript or protein analysis and has thus become an important method in assessing genotypic and phenotypic diversity in plants. Here we report on the identification of several metabolic determinants for early selection of families of *Pinus densiflora* with fast-growing traits and on its integration with other profiling tools. We used a retrospective approach where 12 open-pollinated families were grown and ranked in field trials to age 35-years. Then we compared the growth of their seedlings (3- to 6-month-old) in a nursery trial with the age 35-year growth index. Interestingly, the growth index of age 35-year trees was significantly correlated with both stem dry weight and height of seedlings. Additionally, stem tissue GA_{20} levels and *PdGA20OX* expression in seedling shoots also showed significant correlations with growth performances of nursery seedlings and age 35-year trees across the 12 families. Other potential metabolic markers will be also discussed. The integration of phenotypic, metabolic and genomic profiling in very young seedlings with age 35-year performance in a retrospective manner provides important insight into the usefulness of metabolite-assisted breeding as a valuable tool to accelerate the selection process for fast-growing traits in *P. densiflora*.

P3

A Dynamic Model to Couple Carbon and Nitrogen Metabolism with Transport in Whole Plants

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Transport of metabolites in plants consists of movement at two scales of distance. Short distance transport within plant tissues is governed by diffusion of water and/or active transport of metabolites. In the model plant, tissues are divided into the compartments: cytoplast, xylem, phloem, and apoplast. The movement among compartments within each tissue is rapid, which leads to a steady state distribution of water and metabolites. Long distance transport corresponds to a flux of water and solutes in xylem and phloem between organs or tissues of a plant. This movement is driven by hydrostatic pressure, related to water potential gradient in the xylem, and to turgor pressure in the phloem. In the model plant, tissues are defined as root, stem, and leaf, and a conductance is defined for movement in xylem and phloem between each tissue. The flux of water is determined by conductance and pressure difference between tissues. Long-distance flux of metabolites is calculated from the concentration in xylem or phloem times the flux of water. All these processes were programmed as finite difference equations using the VENSIM visual dynamic simulation modeling tool. The model was used to examine effects due to diurnal variation in transpiration, and in water potentials. There was a diurnal variation in both solute concentration and flow rate in the xylem, because there is a greater change in water movement than in uptake of solutes. Changes in flow in the phloem depended on the apparent conductivity to water flow. At high conductivity, flow from leaf to root was reversed in the morning. Water potential in leaves decreased faster due to transpiration than it did in the phloem due to accumulation of sugars. If conductivity in phloem was sufficiently low, the flow varied relatively little from day to night, because sugar concentration remained high throughout. The diurnal variation in long distance transport altered the availability of nitrate in leaves and sugars in roots.

P4

Functional Cytology of Minor Vein Phloem in Dicots: A Nomenclature Integrating Structural and Functional Features

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To date, the most comprehensive analysis of the diversity of phloem companion cells found in minor veins of dicotyledonous plants has been performed by Gamalei (Gamalei, 1990). In those studies, over 800 species were analyzed. Based on these data, "open" (1, 1-2a) and "closed" (2a, 2b) types of minor veins were described on the basis of two features, namely abundance of symplastic connections between companion cells and bundle sheath, and presence of cell wall ingrowths in companion cells (Gamalei, 1989; 1991). However, attribution of a species to a certain type was sometimes difficult because of the continuous nature of the first characteristic. In the present work, a new classification was developed on the basis of three discrete cytological characteristics: (1) presence vs. absence of plasmodesmal fields connecting companion cells to the bundle sheath; (2) presence vs. absence of cell wall ingrowths in companion cells; (3) plastid type in companion cells (leucoplasts vs. chloroplasts). Eight different combinations of these traits have been used to designate companion cell types, and, with one exception, all these types have been found in plants. Organization of the phloem endings in leaf minor veins was analyzed on the basis of (i) the types of companion cells present in minor vein phloem as outlined above, (ii) vein symmetry and (iii) the presence and position of phloem parenchyma cells. Altogether, 12 types of leaf minor vein architecture were described in this way comprising about 98 % of all species studied, which makes it unlikely that some entirely novel structural types will be discovered. This upgraded classification highlighted that in many species with "open" minor vein cytology, i.e. putative symplastic phloem loaders, sieve-element-companion cell complexes specialized for apoplastic loading are very often present as well. However, evidence for functional specialization of different parts of the minor vein phloem has been missing. We demonstrated for the first time that the expression of a stachyose synthase gene, *AmSTS1*, and localization of a sucrose transporter, AmSUT1, locate to structurally different elements in minor veins of the stachyose-translocating plant *Alonsoa meridionalis*. As stachyose synthesis is a marker for symplastic loading, and the presence of a sucrose/proton symporter is a marker for for apoplastic loading, respectively, the data clearly show that apoplastic loading is indispensable even in species with "open" minor veins, at least in a large part of them. As apoplastic loading is the only mechanism known at present which allows the creation of a water potential difference between phloem and the surrounding tissues, driving the phloem transport of solutes, these data point at common principles of transport energization in dicot species with a large spectrum of minor vein types.

P5

The Impact of *Phytophthora ramorum* on Net Photosynthesis, Stomatal Conductance, Water Usage, and Stem Hydraulic Conductivity in Tanoak *(Notholithocarpus densiflorus)*

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Phytophthora ramorum, an oomycete plant pathogen, is the causal agent of sudden oak death and has killed over one million trees in California and Oregon over the last decade. The cause of host mortality is poorly understood but previous studies have implicated two possible mechanisms of pathogenesis. The first of these mechanisms suggests that *P. ramorum* interferes with stem water transport, causing trees to die from water stress. The second mechanism implicates a class of small molecular weight secreted proteins, known as elicitins. Host leaf tissue exposed to a purified elicitin solution showed reduced chlorophyll functionality. When *Rhododendron macrophyllum* plants were artificially inoculated with *P. ramorum,* the first observed physiological response was a reduction in the net rate of photosynthesis. In this study we investigated the impact of *P. ramorum* infection on tanoak sapling net photosynthetic rate and whole plant water usage over a five-week infection period. The earliest effects of inoculation were reduced specific hydraulic conductivity of the stem, and reduced whole plant water usage. These effects occurred before a reduction in net photosynthetic rate and stomatal conductance were observed, suggesting that early pathogenesis involves interference with stem water transport. This study provides strong evidence that the earliest physiological impact of infection by *P. ramorum* in tanoak is on stem water transport.

P6

Formation and Three-dimensional Structure of Stem in Two Tropical Lianas with Successive Cambia (*Sericostachys scandens,* Amaranthaceae and *Salacia reticulata*, Hippocrateaceae)

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Plant stems formed by successive cambia contain alternating layers of vascular increments, embedded in conjunctive tissue. The interpretations of the origin of successive cambia and the pattern of secondary growth (e.g., whether multiple cambia are simultaneously active, or only the outermost cambium is active) vary in different studies and among species investigated. Moreover, the functional significance of the stem architecture with alternating vascular bands is little known. In *Sericostachys scandens*, similar to other Amaranthaceae species, there is a lateral meristem (or master cambium) that forms conjunctive tissue (radially aligned axial parenchyma cells). In *Salacia reticulata*, the successive cambia originate directly from cortical parenchyma cells. In both species, successive cambia and new vascular increments were initiated at a similar distance from the recently-formed phloem, and subsequent increments of secondary phloem and conjunctive tissue had identical structure and thickness. In both species, only the outermost cambium was mitotically active, which differs from reports for other species with successive cambia. Radial connections between xylem and cambium within one vascular increment were established via frequent tangential-wall pitting at the xylem/cambium interface. Moreover, the successive vascular increments were inter-connected along the stem. Such 3-D network of subsequent vascular increments would confer an improved safety for the long-distance vascular transport in the case of injuries or dysfunction of portions of the vasculature. Furthermore, it has been suggested that embedding of phloem and parenchyma within the rigid xylem promotes the flexibility of the stem and preserves the functionality of xylem in the case of stem twisting. In addition, the occurrence of internal phloem and conjunctive parenchyma greatly increases the abundance and proximity of tissues that can function for water and carbohydrate storage, wound repairs and defenses against pathogens, as well as to meet local demands for growth and maintenance. The stem of *S. scandens* contains large vascular rays that are formed by successive cambia. Radially oriented vascular bundles consistently occurred within each wide ray of this species. The xylem in the radial bundles consisted of tracheids and narrow vessels which differed from the axial primary and secondary xylem cells. The radial xylem was continuous across successive vascular increments and ended within the inner layers of the cortex. To our knowledge, this is the first report on radially aligned xylem in vascular rays, which appears different from adventitious shoots or roots but is continuous across the cambium from xylem to inner bark.

P7

The Sucrose Transporter Gene Family in Sorghum

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Sucrose transporter (SUT) proteins play an important role in the accumulation of sucrose in stems of sweet *Sorghum bicolor* varieties. Sweet sorghum can accumulate up to four times the sugar concentration in the stem juice, as compared to grain sorghum, at the milky dough stage. The transcript levels of all six Sorghum SUTs in grain (BTX623) and sweet Sorghum (Rio), were compared prior to and during anthesis, and differing patterns of expression were observed. Three of the six SUTs, designated SUT2 (Sb04g038030), SUT4 (Sb08g023310) and SUT5 (Sb04g023860), were isolated from both BTX623 and Rio. The sequences of SUT2, SUT4 and SUT5 from BTX623 were identical to the previously published genome sequence, but SUT2 and SUT5 genes isolated from Rio differed in sequence compared with the corresponding genes from the grain sorghum variety. The three SUTs, isolated from both BTX623 and Rio, were expressed in *Saccharomyces cerevisiae* and functional transport characteristics compared. The cellular location of the SUTs was investigated using two antibodies. The PEP2 antisera (Bagnall *et al*., 2000), raised against a highly conserved region of sucrose transporters between loops 2 and 3 corresponding to amino acids 87 to 106 of the potato StSUT1, was considered likely to bind to five of the six SbSUTs, based on peptide sequence homology. The second antibody raised against ShSUT4, was expected to bind specifically to the remaining Sorghum SUT, SbSUT4. Both StSUT1 PEP2 and ShSUT4 antibodies were localized to the plasma membranes (PM) of thin walled sieve elements (SE) in the phloem of intermediate veins of mature leaves and in stem tissue; in the PMSE of elongating stem tissue and the PM of storage parenchyma cells. The influence of transcript levels and sucrose transport rates on accumulation of sucrose, in BTX623 verses Rio, will be discussed.

Reference: Bagnall, N., Wang, X-D., Scofield, G. N., Furbank, R. T., Offler, C. E. and Patrick, J. W. (2000). Sucrose transport-related genes are expressed in both maternal and filial tissues of developing wheat grains. Australian Journal of Plant Physiology. 27: 1009-1020.

P8

Optimality of the Münch Mechanism

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The sugar translocation in plants, which is responsible for the growth, takes place in the sieve elements in the phloem. It is believed that the flow is driven by osmotic pressure differences caused by sugar loading and unloading. We have made a simple model for a plant consisting of a sugar loading zone (a leaf), a translocation zone (the stem) and an unloading zone (the root). With this simple model we are able to account quantitatively (and in some limits even analytically) for observed flow rates and their dependence on the parameters, i.e., tube radius, stem length and leaf size. A wide tube will have a small resistance to flow along the tube, but the osmotic driving force, being a surface effect, will be ineffective. In a very narrow tube the osmotic driving force will be large, but the flow resistance will be large as well. We thus expect an optimal tube radius rc , where the phloem flow is maximal, and our model allows us to compute the scaling of rc with the other parameters, notably the stem length and the leaf size. Our predictions are in agreement with data from plants ranging in length from 10 cm to 40 m.

P9

Molecular Analysis of Poplar Cambium Dormancy Break Using Plant miRNAs Microarrays

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Trees in temperate zone experience an alternative cold to warm transition during their whole life and thus evolved a mechanism to rest meristem activity under rigorous winter. Investigation on the mechanisms underlying this phenomenon would help us to understand the regulation of cambium activity and optimize trees growth characteristics eventually. Dormant phases in poplar were subdivided to Quiescence Ⅰ, Rest and then Quiescence Ⅱ along time course. Quiescence is distinguished from Rest by the capacity of trees in this phase to respond exogenous auxin treatment. miRNAs are a class of endogenously encoded small RNAs in eukaryotes. Through post-transcriptional or translational regulation of their target genes, miRNAs plays mighty roles in various cellular and developmental processes. Here we reported our primary analysis of miRNAs expression profile during the course of dormancy breaking in short-day induced dormant poplar 84k clones. Briefly, we induced dormancy of 84k clones by 2 months short-day treatment. After dormant 84k clones were transferred to cold environments (4℃, dark growth chambers), cambial tissues were sampled at 0, 2 and 5 weeks. Setting up 84k clones with active cambium as growth status controls, we exploited a miRNAs microarray analysis to investigate expression profiles during Rest to Quiescence Ⅱ phase transitions. The microarray was designed mainly based on plant miRNAs information deposited in Sanger mirbase (Release 13.0). Several families of differentially expressed miRNAs have been identified, including miR166 (vascular development), miR171 and miR172 (meristem activity), miR156 (developmental phase transition), miR168 (miRNAs biogenesis), miR160 (auxin response) and miR399 (phosphorus metabolism). Several stress related small RNAs and some computationally predicted miRNAs were also found expressed phase specifically. Prediction of targets of corresponding miRNAs and subsequent biochemical verification are undergoing. Over-expression of candidate miRNAs genes in poplar 84k clones and genetic work in Arabidopsis would be done.

P10

Virus-induced Gene Silencing in *Nicotiana benthamiana* is a Potential Tool for Revealing Epigenetic Regulation in Plant Vascular Development

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Wood, the direct product of secondary growth of trees, is economically important for many aspects of human life. However, long life span and lack of genetic mutants of trees slow our investigation on the molecular regulation on wood formation. Virus-induced gene silencing (VIGS) in *Nicotiana Benthamiana* is a widely-used genetic tool in plant virological research. Here, we used this system to investigate epigenetic regulation in vascular development in *N. benthamiana*. Silencing of plant Polycomb Repressive Complex 2 component genes (homologs of *FIE* and *MSI1* genes in *N. benthamiana*) generally lead to irregular phenotypes in leaf shape, shoot branching, shoot apical meristem determinacy and impaired secondary growth in stems. Silencing of *NbFIE1.1* and *NbFIE1.2* genes resulted in lack of differentiation to tracheary elements and fibers in the secondary xylem, while downregulation of *NbMSI1* gene leads to disorganization of xylem-phloem radial pattern in newly-formed stem and also loss of abaxial-adaxial polarity of newly occurring leaves. Analysis of tobacco full genome microarrays revealed that dramatically differentially expressed genes were categorized to diverse genetic pathways, including secondary cell wall formation, flowering time control, meristem specific genes, plant hormone biosynthesis and signaling, programmed cell death, chloroplast functions, cellular metabolism, and plant defense responses. Persistent phenotypes rather than fading of silencing effects would make VIGS in *N. benthamiana* be a potential fast genetic approach to investigate the epigenetic regulation in postembryonic vascular development.

P11

Genetic and Functional Characterization of the RTM-mediated Resistance

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The RTM-mediated resistance was identified to restrict long distance movement of the potyviruses, *Tobacco etch virus* (TEV), *Lettuce mosaic virus* (LMV) and *Plum pox virus* (PPV) in *Arabidopsis thaliana* (Whitham et al., 1999, Decroocq et al., 2006). At least three dominant genes named *RTM1*, *RTM2* and *RTM3* are involved in this resistance process. Remarkably, a mutation in anyone of these three genes is sufficient to abolish the restriction of the potyvirus long distance movement, suggesting that these genes act in an interdependent way to block the generalized invasion of the plant. *RTM1* encodes a lectin-like protein (Chisholm et al., 2000) and *RTM2* encodes a protein with similarities to small heat shock proteins (Whitham et al., 2000). Both of these genes are expressed in phloem-associated tissues (Chisholm et al., 2001). Recently we showed that the potyviral coat protein is the determinant necessary to overcome the RTM resistance (Decroocq et al., 2009). In order to elucidate the RTM-mediated resistance mechanism, we have undertaken several kinds of experiments: (1) Cloning of *RTM3* which encodes a MATH domain-containing protein, (2) protein-protein interaction experiments between the different RTM proteins and the CP of potyviruses and (3) analyse the natural variation of the three RTM genes among 30 Arabidopsis accessions to identify RTM susceptible alleles and thus amino acids necessary for the resistance process.

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P12

Metabolic Engineering of Raffinose-family Oligosaccharides in the Phloem Reveals Alterations in Carbon Partitioning and Deters Aphid Feeding

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Phloem transport is along hydrostatic pressure gradients generated by differences in solute concentration between source and sink tissues. Numerous species accumulate raffinose-family oligosaccharides (RFOs) in the phloem of mature leaves to accentuate the pressure gradient between source and sinks. In this study, metabolic engineering was used to generate RFOs at the inception of the translocation stream of *Arabidopsis thaliana*, which transports predominantly sucrose. To do this, three genes, *GALACTINOL SYNTHASE*, *RAFFINOSE SYNTHASE* and *STACHYOSE SYNTHASE*, were expressed from promoters specific to the companion cells of minor veins. Two transgenic lines homozygous for all three genes (GRS63 and GRS47) were selected for further analysis. Sugars were extracted and quantified by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), and 21-day old plants of both lines had levels of galactinol, raffinose, and stachyose approaching 50% of total soluble sugar. All three exotic sugars were also identified in phloem exudates from excised leaves of transgenic plants whereas levels were negligible in exudates from wild type leaves. Differences in starch accumulation or degradation between wild type and GRS63 and GRS47 lines were not observed. Similarly, there were no differences in vegetative growth between wild type and engineered plants, but engineered plants flowered earlier. Finally, since the sugar composition of the phloem translocation stream is altered in these plants, we tested for aphid feeding. When green peach aphids were given a choice between WT and transgenic plants, WT plants were preferred. When aphids were reared on only WT or only transgenic plants, aphid fecundity was reduced on the transgenic plants. Tests of aphid fecundity on artificial media with and without RFOs are being employed to establish if this resistance to aphid feeding is a direct or indirect effect of the exotic sugars.

P13

Polypyrimidine Tract Binding (PTB) Proteins of Potato: Candidates for Chaperones to Mobile RNAs

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Polypyrimidine tract binding (PTB) proteins are RNA-binding proteins that are involved in multiple aspects of RNA metabolism including alternative splicing, polyadenylation, translation, localization and stability. PTB proteins contain four RNA recognition motifs (RRMs) separated by three linker regions and bind to polypyrimidine (CU) tracts in target mRNAs. Recently, it has been demonstrated that a PTB protein of pumpkin, CmRBP50, functions as the core protein of a RNA/protein complex that transports RNA molecules within the phloem translocation stream. In potato (*Solanum tuberosum*), three cDNAs encoding full-length PTB proteins have been isolated, designated *StPTB1*, *StPTB6* and *StPTB7*. A comparison of the amino-acid sequences of PTB proteins of plants reveals two clades. One group includes RPB50, StPTB1 and StPTB6, whereas, the second group includes StPTB7. We have also identified several truncated forms of StPTB1 and StPTB6. These variants contain only one or two RRMs instead of the normal set of four and have been characterized as full-length cDNAs with untranslated sequences and poly (A)+ tails. Expression analysis by RT-PCR showed that although all *StPTB* transcripts are present in most of the organs tested, the levels of *StPTB* transcripts are most abundant in petioles. Using yeast three-hybrid and electrophoretic mobility-shift assays, binding of StPTB proteins to select RNA sequences containing polypyrimidine tract motifs was confirmed. In summary, our results suggest that similar to CmRBP50, StPTB proteins may play important roles in regulating RNA metabolism including long-distance transport through the phloem. The presence of truncated forms of StPTB cDNAs suggests the existence of an elaborate network of PTB proteins that bind RNAs to regulate post-transcriptional processes.

P14

Photosynthetic Carbon Metabolism and NO-signalling

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Photosynthetic activity of leaf is closely linked to nitrogen assimilation, to temperature, light and the stage of growth.In addition, it is connected with transport function of leaf. There are many findings about diverse influence of nitrogen supply (ammonium or nitrate) on plant growth and development. Supplying the shoot of *Linum usitattisivum* by solution of nitrate in model condition (without root) led to inhibition of photoassimilate outflow from leaf as it was shown earlier in our laboratory.Administration of liquid was attained by special device our scientific group had developed. The nitrate solution (50 mM) was dipped under the 10^4 Pa pressure when fibre flax was in the end of fast growth phase and was almost 20 inches high. As control the shoot loaded by distilled water was taken. Nitrate decreased the assimilation of CO_2. We obtained that the most part of assimilates containing ^{14}C entered the upper part of experimental shoot, in contrast to control shoot. Also direction of photosynthesis has changed (predominance of non-carbohydrate direction). Then we decided to research the influence of nitrate supply on native plant of *Linum usittatisivum* (with root). The plants were irrigated by nitrate (50 mM) the day before the experiments. And it was shown that the effects in native plants were the same.

There are a lot of literature data about NO formation from nitrate and the mechanism by which nitrate impacts on evacuation of photoassimilates from leaves may be through NO-signaling system. We compared the action of nitrate (50 mM) and *sodium nitroprusside (50 mkM, 100 mkM)* (donor of NO) on photosynthesis and transport of assimilates in Linum isitattisivum. Nitrate was brought as described as above. Following the 60 minutes after introduction of solutions leaves of flax were supplied by $^{14}CO_2$ during 2 minutes. Was analyzed photosynthesis and post-photosynthetic changes. Both solutions (nitrate and nitroprusside) led to inhibition of the assimilation of CO_2 by 50% and 12%, accordingly and enhanced inclusion of ^{14}C to the products of glycollat pathway, and in post-photosynthetic period ^{14}C-label accumulated in sucrose in leaf. The analysis of ultrastructure showed that the introduction of nitrate led to appearance of a big vacuole in the companion cells. The same changes occurred at introduction of sodium nitroprusside. Thus, the results give evidence about similar of influence of nitrate and nitroprusside. Triggering of NO-signalling system may be due to high concentration of nitrate in the vascular system and the apoplast. These results allow to understand mechanism by which nitrate influence on carbon metabolism and decrease using of nitrate in agriculture.

P15

Investigation of Phloem-mobile *Aux/IAA* Transcripts in Arabidopsis

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The plant hormone auxin is a key regulator of virtually every aspect of plant growth and development. The timing and site of auxin action is highly regulated for precise developmental control. It has been thought that this temporal and spatial restriction of auxin action is achieved by Aux/IAA transcription factors which enable the rapid auxin response and polar auxin transport which generates auxin maxima and gradients within tissues, respectively. The molecular mechanism of IAA protein has been well characterized at the cellular level. However, to expand our understanding regarding the role of phloem-mobile transcripts associated with auxin action, heterografting studies were performed with melon; these experiments demonstrated long-distance translocation of two *IAA* transcripts (Omid et al., 2007, J. Exp. Bot. 58:3645-3656). This supports the notion that local auxin action depends on not only auxin distribution but also the long-distance transport of *IAA* transcripts; i.e., *IAA* could make a contribution to both temporal and spatial auxin action. Since the identified melon *IAA* transcripts have high similarity with Arabidopsis *IAA14*, we selected *IAA14* as a candidate for further studies in Arabidopsis. First, we tested the long-distance movement capacity of the *IAA14* transcripts in tobacco and tomato using an *IAA14:GFP* fusion construct (Wolf lab). To investigate the biological significance of *IAA* transcript movement in the phloem, we also tested the graft-transmissibility of the action of *IAA14* gene. These studies were carried out using the Arabidopsis *IAA14* gain-of-function mutant, named *slr*. Micrografting experiments were conducted to test whether the dominant effect of an *slr-1* donor scion was transmissible to a wild-type recipient root-stock. Unexpectedly, the wild-type root-stock grafted with the *slr-1* mutant shoot did not show any *slr-1* dominant phenotype. This finding suggested that there could well be other phloem mobile *IAA* transcripts that have important functions in Arabidopsis. Currently, we are screening additional phloem-mobile *IAA* transcripts to identify potential candidates for study. This work is supported by a Binational Science Foundation grant (to S.W. and W.J.L.); M.N. is the recipient of a Japan Society for Promotion of Science Postdoctoral Fellowship for Research Abroad.

P16

Entry of *C. moschata* FLOWERING LOCUS T-LIKE2 (Cmo-FTL2) into the Phloem Translocation Stream

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Cmo-FTL2, a component of the florigenic signaling system in the short-day flowering plant *C. moschata*, moves long- distance from source leaves to the vegetative apex where it mediates floral induction. However, the mechanism of entry into sieve tube from the neighboring companion cells has not yet been elucidated. To address this important question, microinjection experiments were performed. These studies established that Cmo-FTL2 moves cell to cell in a manner equivalent to Cm-PP16; i.e., it induces an increase in plasmodesmal size exclusion limit and can itself traffic through plasmodesmata into surrounding mesophyll cell of *N. benthamiana.*Controls for these experiments were performed using 10 kDa FITC-labeled dextran, GST, and GFP; none of these control probes moved in our microinjection experiments. Mutant studies are currently being performed on CmoFTL2 in order to identify the motifs required for cell-to-cell movement of this protein through the companion cell-sieve element plasmodesmata. In parallel studies, two Cmo-FTL2-specific protein bands were detected from vascular strip of source leaves in co-immunoprecipitation experiments using CNBr-activated affinity chromatography. These bands were consistently detected in both inductive short-day and non-inductive long-day conditions, demonstrating that these putative Cmo-FTL2-interaction proteins are not regulated by photoperiod. Once these putative Cmo-FTL2-interaction proteins have been identified by LC-MS/MS their role in CmoFTL2 function will be further investigated. These studies will be discussed in terms of the mechanisms that evolved to mediate transmission of the florigenic signal from the source region of the plant to the vegetative apex. This work is supported by NSF grant IOS-0752997.

P17

The Nitrate Influence on Photosynthesis and Transport in Symplastic Plant *Chamerion angustifolium* (L.) Holub and Apoplastic plant *Linum ussitatissimum*

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It was known, that high nitrogen feeding represses assimilate efflux from leaves. But nobody knows how it occurs. In apoplastic plants, one of explanations is the probable increase of acid cell wall invertase activity in apoplast by nitrate that destroys sucrose into fructose and glucose. These sugars cannot go to phloem and they come back to mesophyll cell. Would the assimilate transport be repressed in symplastic plant, where the stage of assimilate crossing the apoplast is absent or rare? To study the influence of nitrate on distribution of ^{14}C in symplastic and apoplastic plants under N-conditions we chose *Chamerion angustifolium* L.Holub as a plant with symplastic loading of phloem and *Linum ussitatissimum* as that with apoplastic one. It was shown that under nitrate influence repressing of assimilate efflux is occurred. This process is independent from the type of assimilate transport to phloem cells. Nitrate facilitate transport of assimilates into upper parts of the plant. Analysis of ultrastructure alterations of minor leaf vein showed that repressed assimilate efflux from apoplastic plant (flax) was accompanied by vacuolization of companion cells in sieve element-companion cell complex. There is accumulation of polymer fibrillar inclusions in the vacuoles of all cells of conducting system in symplastic plant (*Chamerion angustifolium* L.Holub). We expect that plant have multicomponent system of transport regulation by nitrate. One of components is invertase system, the other one is conductance alteration of phloem.

P18

Long-distance Movement of Auxin-responsive Transcript in Mediating Auxin Response Signaling

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The role of the phloem as a tissue responsible for the distribution of photoassimilates and nutrients among the various organs of higher plants has long been recognized. Recent studies have established that numerous proteins and mRNA molecules are present in the phloem translocation stream. An EST database obtained from melon phloem sap provided the foundation for a study aimed at exploring the biological role of specific mRNA molecules that are trafficking long-distance via the phloem. Interestingly, our analysis of the phloem sap transcriptome revealed the presence of three transcripts that have been previously shown to be related to auxin signaling. The first of these transcripts, F-308, was an *Aux/IAA* with its closest *Arabidopsis* and tomato homologs being *IAA14* (4e-82) and *IAA9* (1e-54), respectively. Grafting experiments provided direct evidence that the auxin-related transcripts move in the phloem from the source region of the plant to distantly located sinks, such as developing leaves and meristems. The sites of cellular accumulation of F-308, in melon leaves was established using a laser capture microdissection system (LCM). To further explore the physiological role of the phloem-delivered *Aux/IAA* transcripts, a mutant version of the *AtIAA14* gene fused to *GFP* was inserted to transgenic tomato plants under the control of the phloem-specific galactinol synthase (GAS) promoter. Earlier publications demonstrated that Arabidopsis mutants (mutated *IAA14*) completely lack lateral roots and are also defective in root hair formation and gravitropic responses of their roots and hypocotyls. These mutants were named *solitary roots* (*slr*). Phenotypic analyses of our transgenic tomato plants established that expression of mutated *Aux/IAA* gene in the phloem caused significant changes in root length, fruit set and shoot growth. Collectively, our results indicate that the presence of *Aux/IAA* transcripts in the phloem translocation stream plays an important role in the control of plant development.

P19

Clusters of Positively Charged Amino Acids of Hordeivirus TGB1 Movement Protein Indispensable for Virus Long-distance Transport Contain Presumable NLS and Interact with Nucleolar Protein Fibrillarin

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Hordeivirus movement protein encoded by the first gene of the triple gene block (TGBp1) is the major protein that binds viral genomic RNA forming RNP particles, possible transport complex competent for cell-to-cell and long-distance transport in infected plants (Lim et al., 2008). A double mutation in N-terminal part of TGBp1 of *Poa semilatent virus* (PSLV) that disrupts two clusters (A and B) of positively charged amino acids responsible for RNA binding blocks long-distance but not cell-to-cell transport of viral infection (Kalinina et al., 2001). These clusters encompass the part of TGBp1 intrinsically unstructured N-terminal domain (NTD) (Makarov et al., 2009). The possible role of these clusters for viral long-distance transport was studied in this work. According to the computer predictions a presumable NLS localizes at the same region of NTD and overlaps one of these clusters. In agreement with this prediction TGBp1-GFP co-localizes with mRFP-fibrillarin in the nucleolus. Moreover co-expression of TGBp1 and fibrillarin as fusions to halves of YFP in *N. benthamiana* plants using an *Agrobacterium* system shows that both proteins interact with each other and localize to the nucleolus, Cajal bodies and inclusions of different sizes in the cytoplasm. BiFC assays also reveal that the deletion mutants retaining the intact NTD still interact with fibrillarin. The PSLV TGBp1 has also been shown to bind fibrillarin (AtFib-2 from *Arabidopsis thaliana*) *in vitro* in Far-Western assay and sites of interaction have been mapped within the GAR domain of fibrillarin and NTD of TGBp1, respectively. Interestingly, substitution of basic amino acid residues for alanine in the A and B clusters abolish this interaction. Taking into account that in the presence of TGBp1 fibrillarin re-localizes from the nucleolus to the cytoplasm, we hypothesize that fibrillarin could assist hordeivirus TGBp1 in forming a long-distance transport-competent RNP complex as it does in a concert with the groundnut rosette virus ORF3 protein (Kim et al., 2007a,b). This work is supported by the Russian Foundation for Basic Research (09-04-91285) and The Russian Office of Education and Science (contract 02.740.11.5145).

P20

A Serine/Threonine Kinase Phosphorylates Pumpkin RBP50 and Modulates RBP50 Based Ribonucleoprotein Complex Formation

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Our previous study identified a polypyrimidine tract binding protein, RBP50, which formed the basis of a ribonucleoprotein (RNP) complex in pumpkin (*Cucurbita maxima* cv Big Max) phloem sap. It also showed that this protein appeared as a phosphoprotein in the phloem sap, and furthermore, this post translational modification was found to be essential for binding to its phloem interaction partners. However, the location and mechanism underlying RNP complex formation remained to be established. To address this aspect of RBP50 function, experiments were performed to characterize the phosphorylation residues and the cognate protein kinase that carries out this essential posttranslation modification. To this end, we mapped *in vivo* phosphorylation sites on RBP50 by LC-MS/MS from phloem purified native RBP50. Four serines, S223, S438, S440 and S444 were identified as phosphor-residues. Site-directed mutagenesis was employed to re-engineer S223A (Serine 223 to Alanine), STripleA (Serine 438, 440, 444 to Alanine), SQuardA (Serine 223, 438, 440, 444 to Alanine) RBP50, and mutated proteins were purified from pumpkin using the *Zucchini yellow mosaic virus* (ZYMV) vector system. Protein overlay assays and co-immunoprecipitation experiments confirmed that the replacement of these four serines abolished the binding to its phloem interaction partners. Next, in-gel kinase assays were performed to identify the protein kinase responsible for these phosphorylation events. A serine/threonine kinase was identified as a strong candidate from pumpkin vascular bundle proteins. Molecular cloning and biochemical approaches are currently being conducted in order to confirm the specificity between this kinase and RBP50. These findings will be discussed in terms of the role of the CmRBP50-based RNP complex as an effective long-distance signaling mechanism in plants. This project was supported by Research Initiative Grant 2006-35304-17346 (W.J.L.) from the USDA Cooperative State Research, Education and Extension Service.

P21

Phloem Metabolomics: Metabolite Network Analysis of the Cucumber (*Cucumis sativus* var. *sativus L.*) Phloem Translocation Stream

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The phloem translocation stream functions in the delivery of photosynthate, amino acids, and essential mineral nutrients to developing tissues and organs of the plant. Recently, studies on the phloem have expanded the content of the cargo delivered by the phloem to include a complex population of proteins, as well as RNA, including both polyadenylated transcripts and small interfering/micro-RNA species. Proteomics studies performed on the phloem translocation stream of the cucurbits, plants from which analytical quantities of sap can readily be collected, unexpectedly identified a wide range of enzymes. This discovery raised the question as to whether the enucleate sieve tube system might have evolved the capacity to function as a unique metabolic compartment that runs the length and breadth of the body of the plant. This notion gains support from the earlier finding that the companion cell-sieve element system, within the mid-vein and long-distance region of the translocation stream, functions as an autonomous symplasmic domain. To test this metabolic compartment hypothesis, phloem sap was first collected from cucumber, a cucurbit species for which the genome was recently published. These samples were processed for LC-MS/MS analysis in order to develop a phloem proteome for the specific plants on which a comprehensive metabolic profile would also be developed. Vascular bundles were extracted from a parallel set of cucumber plants and samples were similarly processed to develop a correlated vascular proteome and metabolic profile. These databases are being employed to develop metabolic networks for both the vascular tissue (comprised of phloem, cambium and xylem parenchyma) and the contents of the sieve tube system. In the next phase of this project, vascular tissue and phloem sap will be collected from sink regions of the plant to permit equivalent proteomics and metabolomics analyses. These studies will be discussed in terms of the functioning of the enucleate sieve tube system as a novel "*metabolic interactome*" that serves to supply sink regions of the plant with metabolic resources to optimize processes of growth and development and interactions with abiotic and biotic inputs. This work was supported by grants from DOE and NSF (to W.J.L.)

P22

Specific Companion Cell Profiling to Investigate Plant Virus Long Distance Movement

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Poleroviruses and potyviruses are agronomically important plant viruses that infect a wide range of plants. They highly differ in their morphology, genome organisation and tissue tropism, but both use the phloem vasculature to traffic over long distances through the host. The mechanism underlying this transport has never been extensively investigated. By comparing these two viruses, we expect to identify some common mechanisms in viral systemic movement but also to characterise specific features to different viruses. The aim of this project is to identify phloem specific genes, especially those from companion cells (CC) that are deregulated by viral infection. This project is based on the specific isolation of CC initially by laser capture microdissection, to perform a comparative transcriptional analysis of *Arabidopsis thaliana* using healthy and CC infected by *Turnip yellows virus* or TuYV (polerovirus) or *Lettuce mosaic virus* or LMV (potyvirus). By avoiding induction of stress, laser capture microdissection is the method of choice to specifically collect CC from Arabidopsis plants expressing GFP under the control of a phloem specific promoter. We first tested different fixation methods and then assayed several techniques to embed the plant tissue and isolate RNA from sectioned tissue. When paraffin embedding was used (manual or robotised technique) we had to face the loss of integrity of the extracted RNA. We also tried a cryosectioning method that gave good quality RNA, but is technically less adapted to our material. Therefore, we developped an alternative method based on the collection of protoplasts from fluorescent CC after cell wall digestion. We obtained protoplast suspensions containing about 7% of fluorescent protoplasts, which were then sorted by FACS (Fluorescent Activated Cell Sorter). The goal is now to obtain few micrograms of RNA extracted from sorted healthy CC or TuYV- or LMV- infected CC in order to do deep sequencing and comparative transcriptional analysis to investigate the phloem gene expression changes upon virus infection.

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P23

CLE Peptides Negatively Regulate Protoxylem Vessel Formation via Cytokinin Signaling

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Plant vascular systems are composed of xylem, phloem, and procambium/cambium, which gives rise to xylem and phloem cells. The xylem includes two types of vessels, protoxylem vessels, which have annular or spiral secondary wall thickenings, and metaxylem vessels, which show the reticulate or pitted thickening pattern of secondary walls. In *Arabidopsis* roots, two protoxylem vessels are always located at the outer position of two to four metaxylem vessels. Therefore, xylem differentiation must be strictly controlled to maintain the spatial arrangement of two different vessels in the root. Plant hormones such as auxins, brassinosteroids, and cytokinins are crucial intercellular signaling molecules that control differentiation and/or proliferation of vascular cells. Of them, cytokinins play crucial roles in the spatial arrangement of vessels through the suppression of protoxylem vessel differentiation. However, little is known about the control mechanisms of cytokinin signaling in vascular development. Screening for extracellular factors governing xylem differentiation using *Zinnia* cultured cells has revealed tracheary element differentiation inhibitory factor (TDIF), which is a dodeca-amino acid secretory peptide regulating spatial arrangement of vascular cells (Ito et al., 2006). TDIF belongs to the CLAVATA3/EMBRYO SURROUNDING REGION-related (CLE) family, which contains 32 members in *Arabidopsis*. However, the function of most CLE peptides is largely unknown. We expected that not only TDIF but also other CLE peptides may play some roles in cell communication involved in vascular development. In this report, we examined if CLE peptides other than TDIF act at vascular development in *Arabidopsis*. For this purpose, we treated *Arabidopsis* seedlings with chemically synthesized 26 CLE peptides and observed their effects on vascular development in roots. This comprehensive analysis revealed some CLE peptides, including CLE10 suppressed protoxylem vessel formation. These peptides also inhibited root growth. Next, we performed microarray analysis of CLE10-treated roots using CLE25-treated roots as a control, because CLE25 inhibits root growth but not protoxylem vessel formation. This analysis revealed that CLE10 specifically suppressed the expression of some type-A *ARRs*. Genetic and physiological analyses with various cytokinin mutants including loss-of-function mutants for type-A *ARRs* revealed the suppression of protoxylem vessel formation by the CLE peptides resulted from the activation of cytokinin signaling. Our data indicate for the first time a cross talk between the CLE peptide signaling and the cytokinin signaling during vascular development.

P24

Internal Signal from the Vascular system is Required for Rosette Leaf Initiation in PAT-impaired *Arabidopsis* Plants

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Organogenesis at the shoot apical meristem (SAM) is induced by auxin, which is acropetally transported to the organogenic zone in epidermis and the L1 layer with contribution of PIN1 proteins, in the process of polar auxin transport (PAT; Reinhardt 2005). The damage of PAT in *Arabidopsis* inhibits organogenesis in a generative stage of development but it does not block leaf initiation in a vegetative rosette. This suggests that in a vegetative stage auxin can be transported to the SAM in a different pathway. Involvement of the vascular system in organogenesis was suggested earlier (Dengler 2006), therefore the hypothesis that auxin signal, inducing leaf initiation in PAT-defective plants, can be transported by xylem to the organogenic zone was tested. Results show that in PAT-impaired plants coincident with formation of pin-like stems devoid of organs, differentiation of the vascular system is delayed, causing an increase in distance of the youngest protoxylem from the SAM. Contrary, organogenesis in vegetative shoots of these plants is not inhibited and, at the same time, protoxylem reaches the organogenic zone. The interrelation between the meristem ability to form lateral organs and location of mature protoxylem close to the SAM organogenic zone was confirmed in different experimental systems. Furthermore, gibberellin induced elongation of rosette internodes resulted in the delay of vascular differentiation already in a vegetative stage and thus, leaf initiation was blocked and rosettes were not formed. These results provide evidence that in PAT-defective plants leaf initiation depends on the presence of a putative auxin signal, which can be transported acropetally via xylem of the vascular system. Such a signal is absent in the meristem in a generative stage due to delay of vascular system differentiation in this developmental stage. This suggests that organogenesis can be inhibited only when the meristem is deprived of both auxin sources – the superficial signal as well as the signal from the vascular system. If only one pathway of signal transport is dysfunctional, the meristem is able to induce organ primordia.

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P25

Cellular Factor-Viroid Interaction in Systemic Trafficking

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Systemic RNA trafficking serves as a novel layer of regulations impacting plant development, global gene regulation, virus/viroids infection and plant defense. How non-cell autonomously acting RNAs interact with the cellular machinery to achieve the long distance trafficking remains mostly unknown. Viroids, as non-coding and self-replicating pathogenic RNAs that can systemically infect plants, serve as excellent models to study the mechanisms of RNA long distance trafficking in plants. We have found that *Potato spindle tuber viroid* (PSTVd) can interact with transcription factor IIIA (TFIIIA) both *in vitro* and *in vivo*. Furthermore, our preliminary data suggest that TFIIIA traffics with PSTVd RNA systemically in infected *Nicotiana benthamiana* plants. We will present our analyses and discuss the potential implications of TFIIIA-PSTVd interaction in systemic RNA trafficking.

P26

Characterization of Arabidopsis Phloem Filament Proteins

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Phloem filament proteins form high molecular weight aggregates that occlude sieve pores in response to sieve element damage. Several monoclonal antibody lines have been shown to recognize an apparent 89 kDa protein that localizes to phloem filaments in sieve elements of Arabidopsis. Previous studies with the mabs suggest that the epitope is highly conserved among brassicas and other species. Peptide sequences obtained by mass spectrometry identified proteins of 94 kDa and 85 kDa corresponding to two contiguous genes in *Arabidopsis*. Sequence similarity combined with the intron/exon patterns support a gene duplication event. The ratio of synonymous to nonsynonymous nucleotide substitutions indicates that these genes are under purifying selection and are not in the process of evolving novel functions. Promoter-GUS fusions from both genes directed beta-glucuronidase activity in the phloem of transgenic plants. Quantitative RT-PCR experiments showed that transcripts of both genes are present in approximately equal proportions. Disruption of either gene in T-DNA tagged mutant lines results in the inability to detect the corresponding gene-specific mRNA. Unexpectedly, the disruption of either gene also resulted in the complete loss of antigenicity in immunolocalization experiments. Complementation experiments using GFP-tagged proteins in T-DNA insertion mutants will aid in understanding the interactions of these putatively redundant proteins.

P27

Phloem-specific Virus-induced Gene-silencing Using a Recombinant Polerovirus

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Virus Induced Gene Silencing has largely proven to be a powerful tool to study gene functions in plants. We have taken advantage of the phloem restriction of poleroviruses to engineer a chimeric virus carrying a cDNA fragment of an endogenous gene to silence specifically the corresponding mRNA in vascular tissues. As poleroviruses move i*n planta* in an encapsidated form, a maximum of 108 nucleotides was inserted into the 3' non-coding region of *Turnip yellows Virus* (TuYV). We evaluated the silencing potential of sense (S), antisense (AS) sequences and inverted-repeats (IR) of the CHLI1 gene required for chlorophyll biosynthesis. While wild-type TuYV remained asymptomatic on *Arabidopsis thaliana*, all chimeric viruses developed a vein chlorosis as early as 8 days pi and the effect intensified during the following weeks but remained restricted along the vasculature. When similar constructs were introduced into a P0 silencing suppressor knock-out virus, the same phenotype was observed with a similar kinetic of appearance, indicating that P0 does not interfere significantly with endogenous transcriptional silencing of the CHLI1 gene.
Viruses containing the IR sequence developed a less durable silencing phenotype than the S and AS containing viruses. This observation could be correlated to an increased instability of the IR construct determined by analyzing the sequence of the progeny. Genome stability and encapsidation of the S construct were confirmed in aphid transmission tests.

This study indicates that a recombinant polerovirus TuYV containing a partial sequence of an endogene can be used to efficiently silence genes specifically expressed in the phloem. It constitutes the first example of a labeled and systemically infectious polerovirus which progression *in planta* can be visually monitored spatially and temporarily.

P28

Determining the Role of Small RNAs in Phloem-localized Aphid Resistance

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Phloem-localized resistance in melon against *Aphis gossypii* (melon aphid) is primarily attributed to the presence of a single dominant gene *Vat*, which is a member of the NBS-LRR super-family of resistance genes. Small RNA (smRNA) libraries are being constructed and analyzed from *Aphis gossypii* and *Vat*$^{+}$ melon lines to determine whether smRNAs play a role in this resistance mechanism. Previous studies have shown distinctive patterns of differential gene expression during the early and late stages of aphid infestation in *Vat*$^{+}$ melon lines. To gain a better understanding of the temporal relationships between these gene expression patterns and the accumulation of smRNAs in this interaction, two smRNA libraries were constructed from aphid-infested melon leaf tissue collected at different time points (early: 2h, 4h, 6h & late: 8h, 10h, 12h) post infestation. A library from non-infested melon leaf tissue was also constructed, and each library was analyzed by Illumina deep sequencing. Sequences were filtered against rRNA and tRNAs, and reads of 18-30 nucleotides were mapped against the Melon unigene database to identify smRNA precursors. Further bioinformatic analysis of the hairpin structure of these precursors will result in a list of conserved and novel melon smRNAs. Dual assay systems are being developed to test the hypothesis that plant smRNAs can affect gene expression in aphids and to serve as validation tools to test the biological significance of smRNAs in *Vat*-mediated resistance. An artificial feeding assay exposes insects to varying concentrations of smRNAs in their diet followed by qRT-PCR determination of altered gene expression. A transgenic plant assay targeting smRNAs to the phloem will determine whether the growth and development of insects feeding on these plants can be influenced by modifying the expression of target genes in the insect.

P29

Structure and *in vitro* Self-assembly of Phloem Protein 2 (PP2) from *Arabidopsis thaliana*

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Dicotyledonous sieve elements typically contain protein inclusions, called P-Proteins (for Phloem Proteins). Three types of P-protein have been described: PP1 and PP2 identified from the filamentous P-proteins in Cucurbitaceae and SEO identified from the forisomes in Fabaceae. Based on observations in electron microscopy, an hypothesis has been proposed about a role of P-proteins in the regulation of sap flow by occlusion of sieve elements, in response to phloem injury. Moreover, forisomes were shown to plug sieve elements by undergoing calcium dependent conformational switch. This indicated that changes in conformation and/or assembly properties of P-proteins might be critical for regulation of sap flow. In *Arabidopsis thaliana*, P-proteins are found as filaments and immunolocalization studies had confirmed that PP2s are indeed associated to P-proteins. Two *PP2* genes were shown to be expressed specifically in the phloem: *AtPP2-A1* and *AtPP2-A2*. Our hypothesis is that PP2s, as components of P-proteins, might participate in the regulation of sap flow by self-assembly. Recombinant proteins were produced in *E. coli* and their structure and self-assembly properties were studied under different physical chemical conditions, close to key components of phloem sap, to determine whether these proteins could have a role in sap flow regulation. PP2-A1 and PP2-A2 were present as oligomers in solution, whatever the pH was, with a degree of oligomerization N being higher for PP2-A2 than for PP2-A1. The oligomers displayed elongated shapes, with a maximum of elongation for PP2-A2 in agreement with its higher N value. These results, obtained by small angle X-ray scattering (SAXS), together with observations using transmission electron microscopy, suggested that these proteins self-associate with an atypical arrangement of filamentous and twisted structures. This peculiar spatial arrangement was amplified by an increase of ionic strength with the presence of interspersed thicker filaments forming a network-like organization. Moreover, it appeared that calcium or sucrose had no effect on the self-assembly of PP2-A1 and PP2-A2. Moreover, the effects of shear were investigated on PP2-A1 protein solutions. We found that PP2-A1 in solution displayed displayed shear-thickening and Newtonian behavior at, respectively, low and high shear rates. In addition, protein association/dissociation phenomena, as probed by rheo-SAXS, were insensitive to transitions in shear rate. These results suggested that the presence of PP2 proteins within phloem sieve tubes should not compromise mass flow by changes in their structure and their subsequent hydrodynamic properties, unless pressure or other biological partners might change drastically their self-assembly properties.

P30

Autoregulation of the *StBEL5* Promoter Is Based on the Long-distance Transport of Its mRNA

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BEL1- and KNOTTED1-type proteins are large families of transcription factors from the TALE superclass that are ubiquitous among plants. They interact in a tandem complex to regulate transcription of target genes. In potato these transcription factors are involved in regulating growth, specifically tuber formation. By using laser capture microdissection and in situ hybridization, the mRNA of *StBEL5* has been detected in phloem. Heterografting experiments verified that its mRNA was transported in a rootward direction, from leaves to stolon tips in response to short days. Transcription of *StBEL5* is regulated by different signals in different organs. In leaves, it is induced by light, but not affected by photoperiod, whereas in stolon tips, growing in the dark, promoter activity is enhanced by short days. As a result, the accumulation of *StBEL5* mRNA in stolons increases under short days. Promoter activity is completely repressed in stolons that grow in the light. Gel shift assays have shown that the dimer of StBEL5 and POTH1 (a KNOTTED1-type transcription factor) can bind to a tandem TTGACTTGAC motif and is essential for regulating transcription. The discovery of an inverted tandem TTGAC motif (GTCAATGCTTGAC) in the *StBEL5* promoter with TTGAC motifs on opposite strands may explain the enhanced levels of *StBEL5* promoter activity in stolon tips under short days. Using transgenic potato lines, deletion of one of the TTGAC motifs from the *StBEL5* promoter results in reduction of GUS activity in stolon tips, new tubers, and roots. Based on these results, it appears that autoregulation of the *StBEL5* promoter in stolons and roots is controlled via transcript mobility.

P31

In vivo Proteasome Activity Imaging and Profiling Characterizes Bacterial Effector Syringolin A

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The proteasome is involved in many biological processes. The activity of the proteasome depends on developmental stage as well as physiological conditions but crucial tools to study where and when the proteasome is active inside a living cell are still limited. These tools are necessarily, e.g. to study the action of Syringolin A (SylA), a small molecule proteasome inhibitor produced *by Pseudomonas syringae* pv. *syringae* during infection. We introduce activity-based protein profiling (ABPP) to image proteasome activities *in vitro* and *in vivo* in plants. ABPP makes use of probes that label the catalytic residues of the proteasome in an activity-dependent manner. Probes carrying fluorescent reporters enable visualization of labelled proteasome subunits after biochemical separation, but are also used to image enzyme activity in living cells with microscopy techniques. The aim of this study was to establish activity - based imaging to reveal proteasome activities in living plant cells. ABPP with proteasome-specific probes carrying vinyl sulfone, epoxyketone and syrbactin reactive groups on Arabidopsis cell cultures revealed that all three catalytic subunits of the proteasome can be labelled. Comparative studies show that the probes differ in selectivity for the different proteasome catalytic subunits, and that the novel probes exclusively labels the proteasome, in contrast to vinyl sulfone probes, which also label cysteine proteases, especially in living cells. The exclusive proteasome labeling by epoxyketone and syrbactin probes facilitates *in vivo* imaging by microscopy. These studies revealed that SylA targets the β2 and β5 catalytic subunits of the proteasome in the nucleus of plant cells, pointing at new biological roles of nuclear targeting and subunit selectivity by the bacterial effector SylA.

P32

Alternatively Spliced Transcripts Arising from Polypyrimidine Tract Binding Genes of Potato

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Polypyrimidine tract binding (PTB) proteins are a family of RNA-binding proteins known to mediate post-transcriptional processes by binding CU-runs within target transcripts. Most PTB proteins contain four RNA recognition motifs (RRM) that facilitate binding to RNAs. Recently, it has been demonstrated that a PTB protein of pumpkin, CmRBP50, functions as the core protein of a RNA/protein complex that transports RNA molecules within the phloem translocation stream. To investigate the role of related PTB proteins in potato (*Solanum tuberosum*), transcripts of PTB proteins were identified by using conserved RRM sequence and available EST libraries. By performing 5′ and 3′ RACE, several polyadenylated RNAs containing both coding sequences and untranslated regions were identified. These RNAs were generated from three PTB genes of potato, designated *StPTB1*, *-6*, and *-7*. Exons from all three of these genes contained four RRMs. Several variant transcripts for two of the three potato homologs (StPTB1 and StPTB6) were discovered, suggesting alternative splicing of these genes. Alignment of full-length cDNAs with genomic sequences revealed truncated, splicing variants that contained only RRM1 or RRM1 and -2 domains. Conserved sequences indicating the putative sites for splicing and polyadenylation have also been identified. Although alternative splicing of PTBs has been described extensively in other, biological systems, the function of these truncated forms in plants is currently unknown. Similar truncated forms of StPTB6-like proteins have been identified in tobacco and tomato EST databases. Experiments are ongoing to investigate expression patterns of these PTB genes and to identify any putative protein partners using the yeast two-hybrid system.

P33

Construction of Reticulate Wall Ingrowth Labyrinths of Transfer Cells

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Transfer cells (TCs) are plant cells specialized for membrane transport of nutrients. They have an intricately-invaginated wall ingrowth (WI) labyrinth ensheathed by an amplified plasma membrane, enriched in a suite of nutrient transporters. In cotyledons of Faba bean, abaxial epidermal cells *trans*-differentiate to a TC morphology *in planta* and, when cotyledons are cultured, adaxial epidermal cells are induced to form functional TCs. This induction system provides an opportunity to explore the sequence of events associated with the processes orchestrating WI construction. On TC induction, a new uniform wall layer is deposited within less than three hours. The first step in WI construction is deposition of small papillate projections at discrete loci. These WI papillae are first visible as patches of cellulosic microfibrils that form a scaffold for deposition of other wall components. Significantly, cellulose microfibril orientation switches from parallel in the uniform wall to arcs or whorls as papillae develop. This changed orientation coupled with the cylindrical shape of WI papillae is indicative of apical growth driven by wall building confined to their leading tips. We hypothesized that whorled microfibrils are a consequence of cellulose synthase complexes being constrained at the leading tips of developing papillae by cortical microtubules. To explore this hypothesis, we examined the spatial relationship between the cortical microtubule network and forming WIs. Microtubule arrays were visualized by immunofluorescence confocal microscopy of epidermal peels of cultured cotyledons and WI deposition by scanning electron microscopy. On induction, the parallel-aligned network of microtubules became displaced and bent around forming WI papillae. By 24 h of culture, microtubules were randomly oriented and "holes" (areas of no fluorescence), of comparable diameter to that of WI papillae, were evident in the microtubule network. Surprisingly, when cotyledons were cultured for 24 h in the presence of 5 μM taxol or 20 μM oryzalin, microtubule stabilizing and depolymerizing drugs respectively, WI deposition was not affected in 100 and 90 % of induced cells respectively. For oryzalin-treated cotyledons, the remaining 10 % of cells exhibited both normal WIs and aberrant clumps of papillae. These aberrant WIs most likely represent those known to form in 10% of cells by 3 h in culture and prior to apparent re-organization of tubulin. These results predict a novel role for cortical microtubules during switching of cellulose microfibril orientation from parallel to whorled to initiate the construction of WI papillae. A model for their role will be advanced.

P34

Non-cell-autonomous Transcription Factors, Action beyond their Expression Site

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Plants developed a sophisticated signaling pathway through intercellular symplasmic channels, termed plasmodesmata (PD), for the coordinate development of different cells/tissues. PD allow not only the movement of small molecules such as ions, water, nutrients, but also macromolecules (proteins, RNA). Transcription factors (TF) among cellular proteins are *bona fide* intercellular trafficking signal molecules. PD can exhibit different behaviors in their size exclusion limits and directionality of macromolecular trafficking in a given cell layer. To study intercellular trafficking of macromolecules through PD, we developed a GAL4/UAS trans-activation-driven promoter-reporter system in the Arabidopsis root. A set of Arabidopsis promoters was selected for detailed study, in terms of their functioning across specific cellular boundaries. Transgenic plants were generated in which GAL4 expression was achieved in the domain of the specific promoter; this GAL4 represented a cell-autonomous agent. These lines also carried a GFP-ER construct with a 5xUAS element in the promoter. This cell-type specific promoter was used, in conjunction with ectopic expression of candidate proteins (as fusions with mCherry and driven by the same GAL4 system), to screen for their ability to move through PD. This system was used to screen and categorize over 100 transcription factors (TFs). Of these 30 TFs were found to move beyond their expression domains. These non-cell-autonomous TFs displayed various properties, including directionality, restricted or extensive cell-to-cell movement. The nature and mechanisms (diffusion based and selective movement) underlying this movement will be discussed. Acknowledgments: Supported by EB-NCRC, BK21, NRL and WCU programs.

P35

Source-to-Sink Translocation of Sucrose in Arabidopsis *rsx1* Mutants

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Sucrose, the primary assimilate of photosynthesis, is translocated from photosynthetic organs (the source) to nonphotosynthetic or photosynthetically immature organs (the sink) via the phloem. In developing source leaves, secondary plasmodesmata (2°-PD) develop in the pre-existing cell wall between companion cells (CCs) and sieve elements (SEs). Development of 2°-PD may be required for efficient sucrose export from source leaves. However, little is known about the mechanism of 2°-PD development. We previously showed that mature leaves of *restricted sucrose export 1* (*rsx1*) showed unusual starch accumulation in CCs and incomplete formation of 2°-PD between CCs and SEs. To investigate whether incomplete 2°-PD leads to restricted sucrose translocation in *rsx1* mutants, we fed $^{14}CO_2$ to a source leaf and then incubated the labeled plants for 2 and 4 h to monitor translocation of labeled assimilates. The results showed that $^{14}CO_2$ fixation was reduced in *rsx1* mutants compared with the wild type and the translocation of labeled assimilates from the source leaf to sink organs (such as stems and sink leaves) was significantly restricted. When RSX1-sGFP was expressed in the *rsx1* mutant under the control of the own promoter, $^{14}CO_2$ fixation and translocation of labeled assimilates were fully recovered as in the wild type plant. These results indicated that the mutant phenotype in sucrose translocation is caused by malfunction of the *RSX1* gene. This supports our hypothesis that the *RSX1* gene is involved in the construction or establishment of sucrose translocation pathways in source leaves.

P36

Roles of *Arabidopsis thaliana* Myosins in Sustained *Tobacco mosaic virus* Intercellular Movement

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Cell-to-cell movement of *Tobacco mosaic virus* (TMV), a member of the genus *Tobamovirus*, is a highly orchestrated process that requires specific proteins encoded by the virus and its host plant. Although our knowledge of the functions of TMV-encoded proteins during movement has increased in the past two decades, the function of host proteins and other factors in this process has not kept pace. Through studies using pharmacological reagents multiple research laboratories demonstrated that the microfilaments/ER network has an important role in the intercellular movement of many plant RNA viruses, including TMV. Using a similar approach, Harries et al (*Proc. Natl. Acad. Sci., USA* (2009) 106:17594-17599) showed that disruption of microfilaments inhibited the sustained intercellular movement in *Nicotiana benthamiana* by TMV, *Potato virus X* and *Tomato bushy stunt virus*, but not of *Turnip vein-clearing virus* (TVCV), a different member of the genus *Tobamovirus*. The same study also determined that a specific myosin, myosin XI-2, controlled the sustained intercellular movement of TMV, but not of the other three RNA viruses. To investigate the role of myosins in *Tobamovirus* intercellular trafficking in different host plants, we inoculated *Arabidopsis thaliana* single myosin T-DNA insertion mutants with TMV (U1 strain) and TVCV followed by analysis of viral RNA and coat protein accumulation in non-inoculated (petioles of the inoculated leaves and floral stems) tissues at 3, 4, 5 and 7 days post inoculation. Both TMV and TVCV induced systemic symptoms (reduced growth and distorted floral stem) in the single myosin knockout lines. Viral RNA and coat protein levels in petioles and floral stems were similar in the myosin XI-2 knockout line and the parental Arabidopsis plants. We are analyzing virus accumulation in the inoculated leaf lamina, repeating these studies and analyzing other myosin knockout mutants. The current findings, however, suggest that TMV requires different myosins in different hosts for its sustained movement or utilizes multiple myosins, implying their redundant function. If the latter possibility proves true, our previous findings on TMV movement in *N. benthamiana* may be explained by unanticipated silencing of additional unknown myosins beyond myosin XI-2, leading to decreased TMV movement.

P37

Genomic Approaches to Understanding mRNA Movement between Hosts and Parasitic Plants

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Parasitic plants have unique morphological and physiological features, the most prominent of these being the haustorium, a specialized organ that functions to connect them with their host's vascular system. We are studying two parasitic plant species that have haustoria capable of forming close connections to host phloem, but which represent two distinct evolutionary lineages of parasitism. *Cuscuta pentagona* is an obligate parasite of plant stems, whereas *Orobanche aegyptiaca* is an obligate parasite of roots. Our research has demonstrated that specific phloem-mobile mRNAs are trafficked from host plants to *Cuscuta*. It is not clear whether host-to-*Cuscuta* mRNA trafficking is unique to this interaction or is a general phenomenon of parasitic plant interactions. Nothing is known about the mechanisms regulating cross-species transfer or its biological significance to the parasite. We are addressing these questions with a genomics approach that uses Illumina/Solexa sequencing to assess the presence of host mRNAs in the parasite (and potentially parasite mRNA in the host). *Cuscuta* is being grown on Arabidopsis and tomato hosts in order to facilitate identification of transcripts moving between the plants. We are focusing on three regions of tissue: the *Cuscuta* alone, the point of attachment between *Cuscuta* and the host plant, and the host stem adjacent to the attachment site. A similar approach is available as an offshoot of the Parasitic Plant Genome Project, which is sequencing *Orobanche* ESTs as part of a larger gene discovery activity. *Orobanche* tissues harvested from near – but not contacting – host (*Nicotiana tabaccum*) tissue was analyzed to identify potentially host-derived transcripts and several candidate mobile mRNAs have been identified. We expect the comparative genomics approach to provide insight into mechanisms of mRNA mobility and parasite biology. This research is also relevant to ideas of using gene silencing transmission through haustrorial junctions as a practical approach to engineering crops for resistance to parasitic weeds.

P38

Characterization of Phloem-mobile mRNA Uptake to a Parasitic Plant (*Cuscuta* spp.) using Quantum Dots

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The obligate parasitic plants of the genus *Cuscuta*, commonly known as dodders, have a wide host range and are pests to crops worldwide. Their uptake of water and nutrients depends on a unique organ, the haustorium, which penetrates the host tissue and forms connections to host vascular tissue. Dodders have especially open connections to the host phloem and have been shown to traffic simple sugars, phloem-specific dyes, proteins, and viruses from their hosts. The ability of dodder to form connections to taxonomically distant host plants simplifies identification of host mRNAs and twenty-two tomato mRNA transcripts have been confirmed as mobile into dodder, including the putative transcriptional regulator *GAI* (Gibberelic Acid Insensitive), which has been previously shown to be translocated in the phloem of tomato, and a cathepsin D proteinase inhibitor, which may function in defense or metabolic regulation. Real time quantitative PCR of these mRNAs has demonstrated that as the distance from the haustorial connection increases, mRNA quantities detected in the dodder stem decline, prompting questions about the fate of these mRNAs in the parasite. Current research is focused on *in situ* characterization of these phloem-mobile transcripts by tagging oligonucleotide sequences complementary to the mRNA of interest with quantum dots. Quantum dots are persistent, strong flurophores with many potential applications in plant science applications because of their stability, resistance to photobleaching and high tunability. This research aims to better understand the nature of the vascular connection by characterizing structures involved and the mechanisms for mRNA translocation between host and parasitic plants.

P39

Functional Analysis and Subcellular Localization of Alternative Splice Forms of a Rice Polyamine Transporter

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Polyamines are nitrogenous compounds found in all eukaryotic and prokaryotic cells and absolutely essential for cell viability. In plants, they regulate several growth and developmental processes and the levels of polyamines are also correlated with the plant responses to stresses such as drought, salinity and temperature. In plant cells, polyamines are synthesized in plastids and cytosol. This biosynthetic compartmentation indicates that the specific transporters are essential to transport polyamines between the cellular compartments. In the present study, a phylogenetic analysis was used to identify candidate polyamine transporters in rice. A full-length cDNA rice clone AK071314 was heterologously expressed in the yeast spermidine uptake mutant *agp2Δ*. Radiological uptake and competitive inhibition studies with other polyamines indicated that rice gene encodes a protein that functioned as a spermidine preferential transporter. The computational analysis identified AK062252 as another splice form of AK071314. ARAMEMNON predictions suggest that AK062252 is localized to the plasma membrane and AK071314 has an N-terminus mitochondrial targeting sequence. Transient expression assays in onion epidermal cells confirmed the localization of AK071314 to the mitochondria. Experiments are underway to determine the subcellular localization of the isoform, AK062252. The confirmation of the differential expression of the alternative splice variants suggest that alternative translational start sites may be a common mechanism that plants use to direct the same transporter to different subcellular compartments.

P40

Movement of *Tobacco mosaic virus* 126 kDa Protein Depends on Myosin XI-2

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The plant cytoskeleton is a dynamic structure that has an important role in the intracellular transport of macromolecular complexes, including vesicles and organelles. For some plant viruses there is evidence that their intracellular and sustained intercellular movement depends on actin microfilaments. For example, our laboratory previously determined that sustained intercellular movement of *Tobacco mosaic virus* (TMV), *Potato virus X* (PVX) and *Tomato bushy stunt virus* (TBSV) was inhibited by latrunculin B (LatB) treatment: LatB being an inhibitor of actin polymerization (Harries et al. PNAS 106:17594-17599). In addition, sustained movement of TMV was inhibited by knocking down myosin XI-2 expression through virus-induced gene silencing (VIGS). Interestingly, the movement of PVX and TBSV were unchanged when XI-2 was silenced. Since TMV 126 kDa protein, which is required for TMV intercellular movement, is known to co-localize and traffic along microfilaments, we wanted to determine if the same myosin that inhibits sustained TMV intercellular movement inhibits ectopically expressed TMV 126 kDa protein intracellular movement. Myosins XI-2 and XI-F were silenced through VIGS in 126kDa-GFP expressing transgenic *Nicotiana benthamiana*. Plants inoculated with *Tobacco rattle virus* without myosin inserts served as controls. In XI-2 silenced plants, only 24 % (±5%) of 126 kDa protein-GFP containing cytoplasmic bodies moved compared with 42% (±4.5%) in controls and 37% (±4.2%) in XI-F silenced plants. This specific reduction in 126 kDa protein-GFP body movement in the XI-2 silenced plants suggests that myosin-mediated sustained TMV intercellular movement is mediated through a requirement for 126 kDa protein intracellular movement, but this is based only on correlation. We are further exploring this correlation by analyzing the movement of the 126 kDa protein-GFP fusion expressed through agroinfiltration (i.e. transient expression) in myosin silenced plants and after silencing additional myosins.

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Downregulating the Sucrose Transporter *VpSUT1* in *Verbascum phoeniceum* Does Not Inhibit Phloem Loading

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Phloem loading is the initial step in photoassimilate export and the one that creates the driving force for mass flow. Two active, species-specific loading mechanisms have been proposed. One involves transporter-mediated sucrose transfer from the apoplast into the sieve element-companion cell complex, so-called apoplastic loading. In species that translocate carbohydrate primarily as raffinose family oligosaccharides (RFOs), it has been proposed that loading occurs symplastically. Several sucrose-transporting plants have been shown to be apoplastic loaders by downregulating *sucrose transporter 1* (*SUT1*), leading to accumulation of sugars and leaf chlorosis. In this study we compared the effect of downregulating *SUT1* in *Nicotiana tabacum*, a sucrose transporter, and *Verbascum phoeniceum*, a species that transports raffinose and stachyose. To test the effectiveness of RNAi downregulation, we measured *SUT1* mRNA levels and sucrose-H^+ symport in leaf discs. Mild *NtSUT1* downregulation in *N. tabacum* resulted in the pronounced phenotype associated with loading inhibition. In contrast, no such phenotype developed when *VpSUT1* was downregulated in *V. phoeniceum*, leaving minimal sucrose transport activity. Only those plants with the most severe *VpSUT1* downregulation accumulated more carbohydrate than usual and these plants were normal by other criteria: growth rate, photosynthesis, and ability to clear starch during the night. The results provide direct evidence that the mechanism of phloem loading in *V. phoeniceum* does not require active sucrose uptake from the apoplast and strongly supports the conclusion that the loading pathway is symplastic in this species.

P42

Real-time Imaging of Zinc Uptake and Translocation in an Intact Plant Using ^{65}Zn

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Zinc is an essential element for plants. Real-time imaging of zinc dynamics has been a powerful tool for elucidating how plants regulate zinc uptake and translocation. In the past decade, we have employed a positron-emitting tracer imaging system (PETIS), which provides serial time-course images of the two-dimensional distribution of a radioisotope (e.g. ^{11}C, ^{13}N, ^{52}Fe, ^{64}Cu, ^{107}Cd) in an intact plant without contact. Real-time imaging of zinc by PETIS has been conducted using ^{62}Zn (half-life: 9.2 hours), which was produced by ourselves in the facility with a cyclotron. In this study, we demonstrate that real-time imaging of zinc is also possible using commercially available radioisotope, ^{65}Zn (half-life: 244 days). The tracer solution containing ^{65}Zn was fed to a rice plant and serial images of ^{65}Zn distribution were successfully obtained by PETIS. Uptake kinetics (Km/Vmax) and translocation velocity of zinc were determined from the image data. Furthermore, we observed zinc translocation for several weeks by taking advantage of the long half-life. These results indicate that ^{65}Zn is widely useful for the analysis of zinc dynamics in plants.

P43

Progress Towards Identifying the *bps1* Signal

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Our goal is to identify the mobile root-derived signal '*bypass1* (*bps1)* signal'. A root-derived signal was implicated in the *bps1* mutant from grafting and root excision experiments. When roots are intact, *bps1* mutants show arrested leaf growth but once we cut off the roots, leaf development is restored. This implies that *bps1* mutant roots produce a mobile compound that moves up to the shoot. To identify the *bps1* signal, we have developed a bioassay to test different metabolite fractions. This bioassay is based on CyclB:GUS which is expressed in G_2/M phase of cell cycle. The *bps1* mutants have very few dividing cells, so we expect that the number of dividing cells in CyclB:GUS (WT) treated with a mutant metabolite fraction is also reduced. We are applying different metabolite fractions from mutant and WT seedlings to CyclB:GUS (WT) and comparing the number of cells in the G_2/M phase. Because this approach requires a large amount of *bps1* signal-producing tissues, the tiny Arabidopsis *bps1* mutant roots are impractical. Instead, we are generating tomato (*Solanum lycopersicon*) plants in which we can induce synthesis of the *bps1* signal. To generate tomato root-system producing *bps1* signal, we have designed several artificial microRNAs to deplete SlBPS (Tomato BPS) mRNA. In my poster, I will describe our strategies for the Sl*BPS1* mRNA depletion, metabolite extractions and the bioassay for *bps1* signal identification.

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3D-structured Illumination Microscopy (3D-SIM) and Photoactivation Localisation Microscopy (PALM): New Tools in the Study of Phloem Structure

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The spatial resolution afforded by conventional lens-based light microscopy leaves many plant cell structures beyond the limit of detection. We have been exploring new 'super-resolution' approaches to obtain sub-diffraction images of the phloem and plasmodesmata (PD). Two recent super-resolution imaging protocols, 3D structured illumination microscopy (3D-SIM)[2] and Photoactivation Localisation Microscopy (PALM)[3], appear to offer considerable potential in the study of plant cell substructure. PALM uses photo-activatable probes that can be switched between fluorescent 'on' and 'off' states, by repeating activation cycles an image is reconstructed. Lateral (x-y) resolution of 10nm has been reported[3]. 3D-SIM, through applying patterned excitation light, offers a two-fold increase in x-y resolution (100nm) and an almost 5-fold increase in axial (z) resolution (125nm) versus confocal laser scanning microscopy (CSLM). 3D-SIM has the additional advantage that it can use conventional fluorochromes and fixation protocols that are compatible with CLSM. Using the 3D-SIM microscope we employed a combination of probes to label the central cavity and the callose collar of PD. We were able to spatially resolve the collars from central cavities and establish a mean collar width that was consistent amongst simple PD pores. Within the phloem, using the same probes, we resolved single sieve-plate pores that were beyond the limit of CLSM. Optical sections through the entire sieve element companion cell complexes revealed an intricate network of sieve-element reticulum tubules (<100nm diameter) that interconnect individual PD within the phloem. Although PALM has limited optical sectioning capacity, we have been able to produce the first PALM images of plant cells using the photoactivatable probe Dronpa. The relative merits of PALM *versus* 3D-SIM will be discussed.Acknowledgements: Karen Bell is funded by a University of Edinburgh Staff Scholarship.

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A Knotted1-type Transcription Factor of Potato: A Potential Mobile Signal Involved in Plant Development

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Knotted1-type transcription factors interact with BEL1-like types to regulate several developmental processes in plants. In potato (*Solanum tuberosum* L.), POTH1, a Knotted1-type, and its BEL1-like partner, StBEL5, regulate tuber development. POTH1 overexpression transgenic lines of *S. tuberosum* display a mouse-ear leaf phenotype, altered hormonal levels and increased tuber yields. In situ hybridization and RNA detection methods confirm the presence of *POTH1* RNA in phloem cells. Preliminary heterografting experiments demonstrated that *POTH1* transcripts move across a graft union through the stem. A 1.7 kb upstream sequence of the *POTH1* gene has been isolated by genome walking. The *POTH1* promoter has several motifs known to be present in promoters of numerous other light-regulated genes. Transgenic *S. tuberosum* plants with POTH1 promoter::GUS constructs demonstrate that the promoter is active in the shoot apical meristem, axillary meristems and the lamina of young leaves. In addition, full length POTH1-His tagged transgenic lines have been produced to elucidate localization of the POTH1 protein. To examine the role of untranslated regions (UTR) of *POTH1* mRNA in the regulation of translation, tobacco protoplasts were transfected with POTH1-UTR::GUS fusion constructs and GUS protein expression was analyzed. The *POTH1* transcript contains several polypyrimidine tract (CU) and ten poly(U) motifs, suggesting the possibility of interactions with RNA-binding proteins. Overall, our results indicate that *POTH1* mRNA functions, along with *StBEL5* RNA, as another long-distance mobile signal of potato.

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PDLP5: A Plasmodesmata-localized Protein Involved in Cell Death and Defense

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Plasmodesmata (PD) are specialized, membrane-lined pores that symplastically connect and allow neighboring cells to directly communicate across the cell wall. In conjunction with their role in sharing critical developmental signals and nutrients between adjacent cells, it has been speculated that the precisely controlled spread of programmed cell death (PCD) that takes place during the hypersensitive response (HR) in plant defense may require regulation of defense signals through PD. However, the molecular mechanisms by which PD may control the intercellular movement of these signals remain unknown. Our lab recently characterized a novel member of a PD-localized protein family, PDLP5, and found that it is upregulated by defense hormones salicylic acid (SA) and methyl jasmonate (MeJA). Expression profiling of knockouts of critical SA defense pathway genes revealed that eds1, ics1, and npr1 had decreased basal and SA-induced expression of PDLP5, establishing the importance of the SA defense pathway in PDLP5 induction. Loss- or gain-of-function changes in critical defense pathway genes can sometimes lead to constitutive activation of defense genes and trigger PCD. To test if the expression level of PDLP5 had any impact on defense gene regulation, we next studied how PDLP5 loss and overexpression affected plant phenotype and defense gene expression. Transgenic Arabidopsis plants constitutively overexpressing PDLP5 were chlorotic and developed spontaneous HR-like lesions in leaves. Consistent with the positive role of PDLP5 in cell death associated with defense responses, overexpression of PDLP5 was sufficient to induce or upregulate pathogenesis related markers such as such as PR1 and PDF1.2. The model as to the role of PDLP5 in modulating PD permeability and defense responses will be discussed. This research was funded by the National Institute of Health (P20 RR15588 to JYL).

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Root Growth under Anaerobic Conditions as Affected by Radial Oxygen Loss and Root Cortical Traits in Two Species of the Flooding Pampa Grasslands (Argentina)

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The anoxic environment associated with flooding induces a sequence of plant changes that comprise physiological, anatomical and morphological adjustments related to their survival under anaerobic conditions. The most common responses include adventitious rooting along with increases in aerenchyma tissue, which facilitate root aeration by providing a low resistance pathway to oxygen diffusion from aerial shoots to submerged roots. However, root growth under anaerobic conditions could be constrained due to the reduced apex oxygenation, which in turn could depend on the amount of radial oxygen loss from the roots toward the rhizosphere (hereafter ROL). The aim of this work was to investigate the anatomical traits and physiological responses defining the adventitious root growth under aerobic and anaerobic conditions of two species with contrasting types of aerenchyma of the Flooding Pampa Grasslands of Argentina: the graminoid *Cyperus eragrostis* ("spiderweb" aerenchyma) and the dicotyledonous *Rumex crispus* ("honeycomb" aerenchyma). Plants of both species were grown under hydroponics culture (half-strength Hoagland nutrient solution) under two treatments: aerated (7.9 $mg.O_2.L^{-1}$) and stagnant (0.5 $mg.O_2.L^{-1}$). We assessed the proportion of aerenchyma tissue, the pattern of ROL along roots (0.5, 1, 2, 3, 4 and 5 cm from the apex), suberin deposition in the cells of the outer cortex by UV fluorescence, root growth rate and final root length. Results showed that the proportion of aerenchyma tissue was higher under anaerobic than aerobic conditions for both species (54.1% *vs.* 18.1% in *R. crispus* and 38.5% *vs.* 16.9% in *C. eragrostis*). In *R. crispus*, ROL was similar between treatments (136 vs. 127 $ng.cm^{-2}.min^{-1}$; p=0.68) without differences along the root. By contrast, *C. eragrostis* showed a differential ROL pattern along the root, being higher near the apex than towards the root base. Interestingly, anaerobic conditions determined a ROL reduction at root basal positions (106 *vs.* 44 $ng.cm^{-2}.min^{-1}$), suggesting the induction of a "ROL barrier" in response to anoxia as the higher fluorescence intensity measurements revealed. The root growth rate and final root length of *R. crispus* was lower under anaerobic than under aerobic conditions (25.2% and 54.3%, respectively; p=0.02), while root growth of *C. eragrostis* was not affected by anaerobic conditions (p=0.9). In conclusion, although both species noticeably increased root aerenchyma under anoxia, the ability of the graminoid *C. eragrostis* in generating a "ROL barrier" seems to bring an advantage for maintaining root growth and soil exploration under anaerobic conditions.

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Differential Vascularization of Nematode-induced Feeding Sites

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Sedentary nematodes are destructive plant pathogens that cause significant yield losses. In the roots of their host plants, cyst nematodes (CNs) and root-knot nematodes (RKNs) induce different, highly specialized feeding sites, syncytia or giant cells (GCs), respectively, to optimize nutrient uptake. We compared the mechanisms by which nutrients are delivered from the model host plant, Arabidopsis, to GCs induced by the RKN *Meloidogyne incognita* or to syncytia induced by the CN *Heterodera schachtii*. From previous work, syncytia were known to be symplastically connected to newly formed host phloem composed of sieve elements (SEs) and companion cells (CCs). Here we studied the formation of plasmodesmata (PD) during GC and syncytia development by monitoring a viral movement protein that targets branched PD and the development of host phloem during GC formation by applying confocal laser scanning microscopy (CLSM) and immunocytochemistry. Analyses of plants expressing soluble or membrane-anchored green fluorescent protein in their phloem demonstrated symplastic isolation of GCs. GCs were found to be embedded in a tissue that consists exclusively of SEs. These *de novo*-formed SEs contained nuclei and were interconnected by secondary PD. Distinct cells within the vasculature surrounding the feeding sites show auxin accumulation. Therefore a role of auxin in the pathogen induced the novo development of vasculature is suggested. A similar interconnection of SEs was observed around syncytia. However, these secondary PD were also present at the SE-syncytium interface, demonstrating the postulated symplastic connection. Our results show that CNs and RKNs, despite their close phylogenetical relatedness, employ fundamentally different strategies to withdraw nutrients from host plants.

P49

Geometrical Modelling for 3D Laser Microdissection of Phloem Tissue

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Phloem tissue, responsible for trafficking many molecules throughout the plant, is deeply embedded in ground tissue and is therefore difficult to extract for study. We have been working towards creation of a 3-dimensional laser microdissection (LDM) instrument capable of sampling phloem from flash-frozen plant tissue in sufficient quantities to create high complexity cDNA libraries to allow us to study changes in gene expression in the phloem in response to virus infection and environmental stresses. 3D LDM offers a number of advantages compared to traditional 2D LMD, allowing dissection of whole tissues rather than fixed sections, and capture of samples large enough for molecular and biochemical studies. To date, no 3-D LMD device is available but we are collaborating in the development of such an instrument with Molecular Machines and Industries, a company based in Munich, Germany, and Prof. Dr. Udo Seiffert of the Fraunhofer-Institute in Magdeburg, Germany. One prerequisite is the availability of a 3D geometrical models with sufficient spatial and histological resolution, typically based on large stacks of high-resolution serial sections. In order to assess the inherent inter-individual variability of the biological samples, the final geometrical model has to statistically reflect data from a number of different yet representative individuals. Petiole tissue has been selected for modeling due to its lack of radial symmetry, and preliminary datasets have been created to test the applicability of this approach for phloem sampling in future. High resolution, manually-segmented serial sections are used to 'teach' the computer system histologically-correct assignments of plant tissues and allow the creation of 3D models that can be used to accurately predict the location of phloem within the tissue. These models then guide a laser to dissect out the required regions of tissue for downstream analysis. An overview of the process will be provided and examples of prototype models will be presented.

P50

Regulation of KNOTTED1 Cell-to-Cell Trafficking by Chaperonins

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Cell-to-cell communication plays critical roles in specifying cell fate and coordinating development in multi-cellular organisms. A unique paradigm for such communication in plants is the selective trafficking of transcription factors through plasmodesmata (PDs), channels that traverse the cell wall and connect neighboring cells. The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PD, and its trafficking appears to be important for its function in stem cell maintenance. To understand how KN1 traffic through PD, an unbiased genetic approach was taken using a gain-of-function trafficking system in Arabidopsis. A mutant with attenuated KN1 trafficking has been identified as a gene encoding a chaperonin subunit. This chaperonin subunit also appears essential for the trafficking of other but all non-cell-autonomous proteins tested. Genetic interaction data suggest functional relevance for chaperonins during KN1-related activities in stem cell maintenance. Chaperonins are a special group of cytosolic chaperons functioning in assisting the folding of certain substrate proteins. Downregulation of other chaperonin subunits suggests that KN1 trafficking requires the whole chaperonin complex function, likely its folding activity. Furthermore, tissue-specific complementation assays point to a specific role for chaperonins during post-trafficking re-folding process. In line with previous reports that proteins undergo partial unfolding during PD translocation, our data highlight the importance of confirmational changes and chaperone activities in protein tranfficking through PD.

P51

The Role of miRNAs in Systemic Responses to Abiotic Stresses

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Plants frequently have to cope with environments where sub-optimal nutrient availability persists. Under such deficiency conditions, nutrient availabilities and needs have to be tightly coordinated between organs to ensure a balance between uptake, and consumption for metabolism, growth, and defense reactions. To achieve such a fine tuning, information about the nutrient status has to flow from cell-to-cell, but also between distant organs via the long-distance transport tubes of xylem and phloem. An increasing set of evidence suggests that systemic signaling of the nutrient status from the shoot to the root is an important component of the network regulating nutrient uptake by the root. Recently, micro RNAs (miRNAs) have been shown to be involved in nutrient deficiency responses and they have also been implicated with systemic signaling (1). However, whether miRNAs are directly involved in the information transfer is unknown and whether miRNAs are mobile between cells and even long distance in living plants is still a matter of debate. While short interfering (siRNAs) are generally assumed to be mobile between cells and also systemically, most miRNAs seem to act cell autonomously under normal growth conditions in adult tissues as demonstrated, for example, for miR171 (2). Recently, we showed by high-throughput sequencing and miRNA microarray hybridizations that phloem sap contains a specific set of small RNAs (sRNAs) of fewer than 30 nucleotides (siRNAs, miRNAs and a set of unknown sRNAs) that is distinct from leaves and roots, and that specific nutrient deficiency-responsive miRNAs accumulate in this compartment under the respective deficiency conditions (3,4). We and others could also recently demonstrate that the phosphate-responsive miR399 is indeed phloem-mobile from shoots to roots in grafting experiments between fully nutritioned overexpressor and wildtype plants (5,6). Here, it down-regulates its target mRNA, a putative ubiquitin E2 conjugase. Similarly, we further demonstrated that under nutrient starvation miR399 and miR395 can be translocated through graft unions from wild type scions to rootstocks of the miRNA processing *hen1-1* mutant to negatively affect the expression of their targets. In contrast, miR171 was not transported (4). From the data it is tempting to assume that the translocatable miRNAs have a physiological function in long-distance signaling. Although this could well be the case, we will also critically discuss the limitations of grafting studies to convincingly assign such a function.

(1) BC Yoo et al., 2004, Plant Cell; (2) EA Parizotto et al., 2004 Genes Dev; (3) A Buhtz et al., 2008, Plant J; (4) A Buhtz et al., 2010, BMC Plant Biol; (5) BD Pant et al., 2008, Plant J
(6) SI Lin et al., 2008, Plant Physiol

P52

Identification and Isolation of Sieve Elements Using GFP

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The phloem tissue is responsible for the transport of photosynthesis products throughout the entire plant and consists of conducting cells known as sieve elements. Forisomes are sieve element specific proteins in legumes that contract and expand through chemical stimulation to stop the flow of photosynthates in a damaged region of phloem tissue and prevent nutrient loss. Previous studies have determined that a specific family of genes, sieve element occlusion genes, encode for the proteins that make up a forisome. We have isolated promoters for SEO genes 1 and 2 to study their function and determined, with the use of green fluorescent proteins and confocal laser scanning microscopy, that they are active exclusively in the sieve elements and not in companion cells or other plant tissue. Since the SEO promoters are the only known promoters found to be active exclusively in young sieve elements, we have used them in conjunction with GFP to isolate sieve element protoplasts for further study. The GFP studies have also shed light on the construction of the forisome, indicating that peptide synthesis and protein construction is most likely done in the young developing sieve elements.

P53

Calcium is a Component of the Signalling Cascade Leading to Wall Ingrowth Formation in Transfer Cells

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Transfer cells (TCs) are morphologically-specialized plant cells that facilitate apoplasmic nutrient transport and hence plant growth. TCs are characterized by invaginated wall ingrowths ensheathed in an amplified area of plasma membrane available for nutrient transport. In Faba bean cotyledons, only abaxial epidermal cells develop into TCs *in planta*. However, adaxial epidermal cells also form functional TCs when cotyledons are cultured. We are using this system to study signalling cascades leading to induction of the initial papillate wall ingrowths. We have visualized a polarized Ca^{2+} signal in adaxial epidermal cells during wall ingrowth formation using laser scanning confocal microscopy and the Ca^{2+}-sensitive dye Oregon Green BAPTA-AM ester and quantified wall ingrowth deposition using scanning electron microscopy. Exposure of cultured cotyledons to a Ca^{2+} chelator, BAPTA, eliminated the Ca^{2+} signal and wall ingrowth formation, indicating that an extracellular Ca^{2+} signal is a component of the inductive signalling cascade. The Ca^{2+} ionophore, A23187, abolished both the polarized intracellular Ca^{2+} signal and localized wall ingrowth formation but not a uniformly-thickened polarized wall deposition that precedes formation of wall ingrowth loci. This response demonstrates that wall ingrowth formation is dependent on a localized Ca^{2+} influx while polarity of cell wall deposition is determined by other processes. Treatment with a series of universal and specific Ca^{2+} channel blockers indicated that a L-type two-pore Ca^{2+} channel (TPC) delivers the localized Ca^{2+} influx. We have cloned a gene encoding a putative TPC (*VfTPC1*) and established that its spatio-temporal expression pattern follows that of wall ingrowth formation. Expression of VfTPC1 was suppressed 50% by the reactive oxygen species (ROS) inhibitor, DPI, but was not affected by the ethylene synthesis inhibitor, AVG. A model describing the TC inductive signalling cascade will be presented. It is envisioned that localized Ca^{2+} influxes, mediated by plasma membrane Ca^{2+} channels, identify loci for papillate wall ingrowth deposition. Channel activities are finely regulated by a ROS signal cascade operating at both transcriptional and post translational levels.

P54

Effect of Grafting and Xylem Vessel Anatomy of Two Avocado Tree Genotypes on Water Flow

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Xylem vessel features and leaf stomatal conductance were studied in two-year-old non-grafted and grafted avocado (*Persea americana* Mill.) trees. Vessel size, vessel number and total vessel area were determined histologically in the in the stem of clonal 'Duke 7' (D7), clonal 'Hass' (H) and 'Hass' grafted onto clonal D7 (H/D7) rootstocks. Mid-day stomatal conductance was measured on each plant with Decagon SC-1 leaf porometer. Significant differences in the leaf stomatal conductance were found among the genotypes, where 'Hass' had a 13 % higher mid-day stomatal conductance means then 'Duke 7' (non-grafted trees) and 29 % higher mid-day stomatal conductance means then H/D7 (grafted trees). There were also differences among clonal trees in xylem vessel features in the stem. 'Hass' had wider and fewer vessels then D7. Also, 'Hass' had a 23 % higher conduit area then D7. These results suggest that the differences in xylem vessel features and the grafting technique may largely control water flow in the xylem.

P55

Variation of Ureide (Nitrogen transport metabolites) Levels in Soybean Tissues

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Ureides (allantoin and allatoic acid) are important nitrogen compounds in soybean. They are synthesized in soybean nodules and transported from root to shoot tissues for plant growth and development. Plant extracts from nodule, stem and leaf tissues were separated and analyzed using high-performance liquid chromatography (HPLC)-electrospray ionization (ESI) linear ion trap mass spectrometry (LTQ, ThermoFisher Scientific). In all tissue types tested (younger leaf, older leaf, stem and nodule), allantoic acid levels were at least two-fold higher than that of allantoin. Ureide levels were similar in nodules, stems and younger leaves. In older leaves, ureide levels were less than 50% of those in three other tissues. Understanding of ureide transport mechanisms in soybean plants is critical to increase soy yield by improving nitrogen transport and assimilation mechanisms.

P56

Loop 6 of *Potato spindle tuber viroid* Is an Essential RNA Structural Motif Regulating RNA Intercellular Trafficking

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How an RNA itself directly regulates its trafficking between cells remains poorly understood, though the importance of inter-cellular RNA trafficking has been well-established based on the studies on both endogenous and infectious RNAs in plants. Viroids, small but highly structured non-coding RNAs, are excellent models to investigate the molecular mechanism of RNA trafficking. Using *Potato spindle tuber viroid* (PSTVd) infection of *Nicotiana benthamiana* as the experimental system, we demonstrated that various RNA loop structures, consisting of non-Watson-Crick base pairs, were essential for PSTVd systemic trafficking. Here, we present our analyses on the role of loop 6, a conserved motif comprising six nucleotides, in regulating inter-cellular RNA trafficking. The tertiary structural model of loop 6 was inferred by comparisons with the X-ray crystal structures of similar motifs in other RNAs. Extensive mutational analyses supported the structural model. Cellular analyses revealed that the maintenance of the tertiary structure of loop 6 is essential for PSTVd to traffic between specific cell types. Our findings support the hypothesis that unique RNA structural motifs mediate trafficking across distinct cellular layers. Furthermore, our approaches should be useful in characterizing the structure-function relationships for other RNA motifs.

P57

The Role of Apoplasmic Sugars in the Uptake of Carbohydrate into Kiwifruit (*Actinidia deliciosa*)

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This work investigates the role of apoplasmic sugars in cell carbohydrate accumulation in kiwifruit berries. We focus on the kinetics of sugar membrane transport and how sugar uptake and metabolism interact with cell water relations to drive fruit growth and development. Phloem sap flow into the fruit is dependent upon unloading rates within the fruit, membrane transport, metabolic conversion and compartmentation of solutes. Understanding carbohydrate accumulation in a developing fruit requires knowledge of the metabolic processes and water relations driving the supply and demand of solutes into the fruit at the cellular level. Radio-labelled sugar uptake rates and physiological apoplasmic sugar concentrations were used to calculate the potential apoplasmic uptake rates of the outer pericarp tissue of kiwifruit berries sampled between 35 and 185 days after anthesis. Inhibitors of active membrane uptake (CCCP and PCMBS) were used to investigate the mechanism of sugar uptake from the apoplasm. Tissue solute potentials and cell turgor pressure were calculated from fruit slices. Our results indicate that uptake of sugars from the apoplasm into outer pericarp cells can account for the berry dry matter accumulation rates observed in the orchard. The mechanism of uptake may depend upon the concentration gradient across the cell membrane. When apoplasmic glucose concentration was low, uptake rates showed Michealis-Menten type kinetics, and CCCP and PCMBS inhibited cellular uptake. Whereas, at high apoplasmic glucose concentrations, the uptake rate was linear with no effect of inhibitors of active membrane uptake. The apoplasmic solute potential was closely correlated to cell turgor pressure. Thus, low solute potential observed in fruit could be an important factor in maintaining a low hydrostatic pressure at the point of phloem unloading within the fruit. We conclude that the apoplasm can play an important role in 1) the uptake of sugars into cells along the post-phloem transport pathway of the kiwifruit and 2) maintaining a source/sink pressure gradient promoting a high solution flow into the fruit.

P58

Cell-cell Communication during Vascular Development in *Arabidopsis thaliana*

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Unlike the animals where organ patterning and morphogenesis are achieved by directed cell migration, plants lack cell mobility due to their glued rigid cell walls. Therefore, the plant tissue identities and patterning are determined by their relative position in embryos during post-embryonic development. This cell-cell communication involves transfer of positional information through mobile signaling molecules such as hormones, mRNA, proteins and small RNAs. The plant vasculature connects all parts of the body and forms the major transport system for water, nutrients and signaling molecules. Previous studies in our laboratories has identified two novel *Arabidopsis* mutants, *distorted root vascular pattern 1* (*dva1*) and *dva2* which display mis-expression of phloem-marker gene and show SHORT ROOT (SHR) mediated vascular tissue patterning defects. *dva2* carries a semi-dominant mutation in a member of glycosyl transferase gene family and display ectopic metaxylem formation and inefficient SHR movement out of the stele. The temporally and spatially controlled expression of *dva2* in ground tissues results in increased callose accumulation in the cell wall, particularly near plasmodesmata and shows increased transcript levels of *PHB* which might be due hindered trafficking of miR165/166. To gain more insights into other regulators for cell-cell communication, we have screened for the suppressors for *dva2* which will be described in detail. Thus, we are presenting the data towards our goal to understand the role of non-cell autonomous action of signaling molecules in the roots of *Arabidopsis thaliana.*

P59

The Mobile and Stationary Transcriptome of *Arabidopsis thaliana* Phloem

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The phloem network plays a major role in plant nutrition, development, and signaling. Besides primary and secondary metabolites, RNA and proteins are channeled via this system. Mobile RNAs and proteins have been associated with long distance signaling between functional units of the plant, such as leaves and the apex. We aimed to identify transcript profiles of the phloem of *Arabidopsis thaliana* shoots. Initially, phloem mRNA present in sieve elements was isolated from sieve tube exudates. The origin of the leaf exudate samples was controlled on the basis of the phloem-specific metabolite pattern and mineral composition. To exclude contaminations with non-phloem derived transcripts, mesophyll- and epidermis-specific marker genes served as negative control (Deeken et al., 2008). Secondly, applying the Laser Microdissection Pressure Catapulting (LMPC) we isolated transcripts expressed in phloem parenchyma and companion cells. Thirdly, a collection of companion cell transcripts was identified from an EST-library, generated from isolated GFP-expressing companion cell protoplasts. Applying Patch Clamp and quantitative RT-PCR analyses to this protoplast population, distinct K^+ channel types and a protein phosphatase known to be associated with the phloem were identified (Ivashikina et al. 2003). Following Affymetrix microarray analyses we could differentiate between transcripts associated with the sieve tube sap and parenchyma/companion cell of the shoot phloem. Furthermore differential analysis allowed us to identify mobile and non-mobile phloem mRNA species. Currently we are analyzing the transcriptome of the pathogen-infected phloem (Larisch et al. 2010). On the meeting we will present phloem mRNAs very likely involved in systemic acquired resistance.

Deeken et al. (2008) Identification of *Arabidopsis thaliana* phloem RNAs provides a search criterion for phloem-based transcripts hidden in complex datasets of microarray experiments. *Plant J.* 55(5): 746-59

Ivashikina et al. (2003) Isolation of AtSUC2 promoter-GFP-marked companion cells for patch-clamp studies and expression profiling. *Plant J.* 36(6): 931-45.

Larisch et al. (2010) Pathogen-induced phloem transcripts of *Arabidopsis thaliana* involved in SAR. *In prep.*

P60

The Mechanism of Boron Mobility in Wheat and Canola Phloem

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Low molecular weight borate complexes were isolated from canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L) phloem exudates, as well as the cytoplasm of the fresh-water algae, *Chara corallina* (Klein ex Will. Esk. R.D. Wood) and identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). Phloem exudate was collected from field-grown canola inflorescence stalks by shallow incision, while wheat phloem exudate was collected by aphid stylectomy. *Chara* cytoplasm was collected by careful manual separation of the cell wall, vacuole and cytosolic compartments. MALDI-TOF-MS showed the presence of isotopic borate complexes, at m/z of 690.22/691.22 in the canola and wheat phloem and at 300.11/301.11 in canola phloem and *Chara* cytoplasm. Using reference compounds, the borate complexes with m/z 690.22/691.22 was identified as a *bis*-sucrose borate complex in which the 4,6 hydroxyl pairs from the two α-glucopyranoside moieties formed an $[L_2B]^{-1}$ complex. Further investigation using liquid chromatography electrospray ionisation triple quadrupole mass spectrometry (LC-ESI-QQQ-MS) analysis confirmed the presence of the bis-sucrose borate complex in wheat phloem with a concentration up to 220 μM. The 300.11/301.11 complex was putatively identified as a bis-*N*-acetyl-serine borate complex but its concentration was below the detection limits of the LC-ESI-QQQ-MS so could not be quantified. The presence of borate complexes in the phloem provides a mechanistic explanation for the observed phloem boron mobility in canola and wheat and other species that transport sucrose as their primary photo-assimilate.

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Sieve Tube Geometry in Relation to Phloem Flow

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Sieve element transport and translocation velocities of photoassimilates are not well understood. Several models have been developed to better understand photoassimilate movement throughout the plant body. Due to current microscopy techniques, phloem flow models rely on low-resolution imagery of sieve tubes and plates to calculate phloem flow. We have developed a method to clear cytoplasmic constituents from cells in order to image phloem cell walls by Scanning Electron Microscopy. This method allows for precise measurements of sieve tube geometry required to calculate and model phloem flow. Sieve tube specific conductivity was calculated and compared with translocation velocities taken from Magnetic Resonance Imaging velocimetry for green bean (*Phaseolus vulgaris)*, squash (*Cucurbita maxima)*, castor bean (*Ricinus communis*), and tomato (*Solanum lycopersicum*). Phloem sap velocity measurements indicate that a faster translocation velocity does not accompany an increase in sieve tube conductivity.

P62

Bremsstrahlung Measurement of *in vivo* ^{14}C Phloem Translocation within Whole Plants

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This aim of this project was to develop a non-destructive method for monitoring ^{14}C phloem translocation *in planta*. The standard method for observing phloem transport of ^{14}C-labelled photosynthate requires destructive sampling and this is of little use for understanding the flow dynamics of partitioning. *In vivo* translocation has been measured using ^{11}C-labelled photosynthate, but this is limited because of the need for on-site generation of the short lived isotope, ^{11}C. This project demonstrates that the tracer ^{14}C can be monitored *in vivo* within whole plants by observing the Bremstrahlung x-rays generated by scatter of the emitted beta particle as it is thermalised. To our knowledge the approach has only once been used by one plant physiological group (Sowinski *et al*. 1990) but in a very limited way. In a series of experiments using a single pulse label of ^{14}C-labelled CO_2 to a source leaf we have been able to monitor tracer flows over four days between the source and a number of competing sinks in kiwifruit and grapevine. Changes in the relative partitioning of C were observed when either a competing sink was removed or when the plant defence activator MeJA was applied to the source leaf.

Sowinski P, Bednarek B, Jelen K, Kowalski TZ, Ostrowiski W. 1990. An *in vivo* method for the transport study of assimilated substances using 14C-isotope and x-ray proportional counters. *Acta Physiologiea Plantarum* **12**,139-148.

P63

Identification of Pathogenicity Factors in the Xylem-invading Pathogen *Xanthomonas albilineans* by Transposon Mutagenesis

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Xanthomonas albilineans is a systemic, xylem-invading pathogen that causes sugarcane leaf scald. Leaf symptoms vary from a single, white, narrow, sharply defined stripe to complete wilting and necrosis of infected leaves, leading to plant death. *X. albilineans* produces the toxin albicidin that blocks chloroplast differentiation, resulting in disease symptoms. Albicidin is the only previously known pathogenicity factor in *X. albilineans*, yet albicidin-deficient mutant strains are still able to efficiently colonize sugarcane. We used Tn*5* (transposome) mutagenesis in an attempt to identify additional *X. albilineans* pathogenicity factors. Sugarcane cultivar CP80-1743, moderately susceptible to leaf scald, was inoculated by the decapitation method with 1,216 independently derived Tn*5* insertions in Florida strain XaFL07-1. Leaf scald symptoms were recorded on emerging leaves one month after inoculation, and stalk colonization by the pathogen was determined two months after inoculation. In addition to the previously identified albicidin biosynthesis gene cluster mutations, 33 new loci were identified in which insertions were correlated with reduced pathogenicity. These insertions affected genes predicted to encode proteins involved in a variety of functions, including exopolysaccharide and lipopolysaccharide biosynthesis, fatty acid biosynthesis, regulatory and cell signaling, and secretion systems. Several of these have been associated with virulence in other bacterial plant pathogens that invade the xylem. However, some loci were identified that are predicted to encode previously unrecognized and apparently essential pathogenicity factors, at least for sugarcane leaf scald, including an OmpA family outer membrane protein. Five independent Tn*5* insertions in OmpA locus XALc_0557 of *X. albilineans* strain XaFL07-1 produced no or very few leaf symptoms. These mutants produced albicidin *in vitro* and were able to multiply in sugarcane leaf tissue to levels similar to the wild-type strain, but did not efficiently colonize the sugarcane stalk. These *ompA* mutants were also affected in growth rate, motility and biofilm formation *in vitro*.

P64

CmPSRP1-Like Proteins in Arabidopsis Function in Long-distance Trafficking of Small RNA

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Small RNA molecules ranging of 20-30 nucleotides have been shown to function as critical regulators of gene expression during both plant development and antiviral defense. Although it is well known that short interfering RNA (siRNA) molecules mediate in the systemic transmission of RNA silencing, the mechanism underlying this process remains poorly understood. Previously, our lab characterized *C. maxima* Phloem SMALL RNA BINDING PROTEIN1 (CmPSRP1), a unique component of the protein machinery probably involved in small RNA trafficking in pumpkin. In order to further study the mechanism of small RNA trafficking, we searched the Arabidopsis genomic database for CmPSRP1-like proteins. Based on these studies, we identified two Glycine-rich proteins (here designated as AtPSRP1 and AtPSRPL2) that have the capacity to bind small RNA probes. To ascertain whether these genes are expressed in the phloem of mature source leaves, we are presently generating transgenic lines expressing a *GUS:GFPer* constructs driven by the *AtPSRP1* or *AtPSRPL2* promoter. Single mutants of *AtPSRP1* and *AtPSRPL2* displayed interesting phenotypes, including abnormal shoot apical meristem development and late flowering. Currently, double mutants and overexpression lines for these two genes are being generated to further explore the effects on plant development. These studies should provide insight into the mechanism of small RNA movement in Arabidopsis and may provide the means to identify functional orthologues in other plant species.
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In Vivo Role for Flavonoids in Auxin Transport and Gravity Responses

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Flavonoids are bioactive plant secondary metabolites with a myriad of important functions, which include regulation of plant development (Buer and Djordjevic 2009; Buer et al., 2010). Our *in situ* flavonoid localisation techniques and grafting experiments previously showed that certain flavonoids could move long distances in *Arabidopsis thaliana* with shoot-to-root movement appearing to occur in the vasculature (Buer et al., 2007; Buer et al., 2008).

Here we used the *A. thaliana transparent testa* (*tt*) mutants compromised in various structural and regulatory genes of the flavonoid pathway to investigate the physiological consequences of abnormal flavonoid distribution in the roots. Several *tt* mutants have altered levels of flavonoid accumulation in the roots when compared to wild-type roots (Buer et al., 2010). We tested if this differential flavonoid accumulation affected auxin transport and root gravity responses. The mutants that accumulated quercetin (*tt3*, *tt8*, *tt10*, and *ttg1*) were affected in gravity responses, root elongation and auxin transport. Increased quercetin accumulation caused rapid gravitropic curvature, faster root elongation rates and higher auxin transport inhibition. Those mutants with no or low quercetin accumulation in roots (*tt4*, *tt5*, and *tt6*) were inhibited in gravity responses and had increased auxin transport rates confirming earlier experiments (Buer and Muday, 2004). These *in vivo* assays are supported by previous *in vitro* assays (Jacobs and Rubery, 1988) that show that flavonoids inhibit auxin transport with quercetin showing the greatest effects. We hypothesise that the co-localisation of quercetin and auxin in the vascular tissue may enable regulation of auxin transport and that elevated quercetin accumulation in roots affects localised auxin movement and gravity responses by possible competitive inhibition at the naphthylphthalamic acid binding site.

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The Rise and Evolution of the Cambial Variant in Bignonieae (Bignoniaceae)

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Cambial variants represent a form of secondary growth that creates great stem anatomical diversity in lianas. Despite the importance of cambial variants, nothing is known about the developmental mechanisms that may have led to the current diversity seen in these stems. Here, a thorough anatomical analysis of all genera along the phylogeny of Bignonieae (Bignoniaceae) was carried out in order to detect when in their ontogeny and phylogeny there were shifts leading to different stem anatomical patterns. We found that all species depart from a common developmental basis, with a continuous, regularly growing cambium. Initial development is then followed by the modification of four equidistant portions of the cambium that reduce the production of xylem and increase the production of phloem, the former with much larger sieve tubes and an extended lifespan. In most species, the formerly continuous cambium becomes disjunct, with cambial portions within phloem wedges and cambial portions between them. Other anatomical modifications such as the formation of multiples of four phloem wedges, multiple-dissected phloem wedges, and included phloem wedges take place thereafter. The fact that each novel trait raised on the ontogenetic trajectory appeared in subsequently more recent ancestors on the phylogeny suggests a recapitulatory history. This recapitulation is, however, caused by the terminal addition of evolutionary novelties rather than a truly heterochronic process. Truly heterochronic processes were only found in shrubby species, which resemble juveniles of their ancestors, as a result of a decelerated phloem formation by the variant cambia. In addition, the modular evolution of phloem and xylem in Bignonieae seems to indicate that stem anatomical modifications in this group occurred at the level of cambial initials.

The Arabidopsis Biological Resource Center (ABRC)

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The ABRC was established in 1991 with the mission to collect, preserve and distribute seed and other resources useful to the Arabidopsis community. The current collection is comprised of almost 1 million accessions. Approximately half of these are Arabidopsis seed stocks. These resources have been donated by researchers from 27 countries and all seed stocks are exchanged with the European Arabidopsis Stock Centre (NASC). The seed collection includes natural accessions, characterized mutants, transgenic lines, T-DNA and transposon insertion mutant populations, and recombinant inbred populations. Other resources include cDNA and genomic clones, phage and plasmid libraries, colony blot filters, cell cultures, protein chips and resources from related species. Each year approximately 100,000 stocks are shipped to laboratories in more than 60 countries.

ABRC stock data are housed at The Arabidopsis Information Resource (TAIR, http://arabidopsis.org). Stock searching, on-line order and payment tools are provided as well as catalog pages for browsing, full stock details and stock order histories. ABRC staff curate upload stock data to TAIR and participate in the design of stock related tools and detail pages. ABRC is implementing a new search by AGI number to allow researchers to find a comprehensive list of resources associated with a gene of interest. The search results will display as a list of seed lines, clones and other stocks categorized by stock type. This feature will be made available through a new ABRC database, and the data will be synchronized with TAIR.

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LRD3 Controls *Arabidopsis* Root System Architecture and the Distribution of Root Phloem Content

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Plants develop the bulk of their root systems post-embryonically through the continued elongation of the primary root, and the growth of lateral roots that are established along the length of the primary root. Here we describe a mutant, *lrd3* (*lateral root development 3*), that shows an increase in lateral root development and a decrease in primary root growth compared to wild-type plants. We have confirmed that *lrd3* is a mutant allele of a previously-uncharacterized gene, which is expressed in phloem cells throughout the plant including primary and lateral roots. Through the use of phloem tracer molecules, we have discovered that *lrd3* plants fail to unload phloem content at root tips which is tightly correlated with decreased root growth. Intriguingly, both phloem delivery and root growth recover in either the primary root or an adventitious or lateral root of *lrd3* plants later in development. Decreased phloem unloading at *lrd3* root tips could be due to increased callose formation at sieve plates in the root tip unloading zone, which would impede phloem transport. However, we observed that callose content in *lrd3* root tips was dramatically decreased compared to wild-type, as evidenced by aniline blue staining. In addition, the typical pattern of callose accumulation at sieve plates and plasmodesmata is disrupted at poorly-growing *lrd3* root tips, suggesting that sieve tube or plasmodesmal development may be altered. Therefore, we have identified *LRD3* as a novel, phloem-expressed component of phloem development, long distance delivery of phloem content, and proper maintenance of *Arabidopsis* root system architecture.