



# ICAR

International Conference



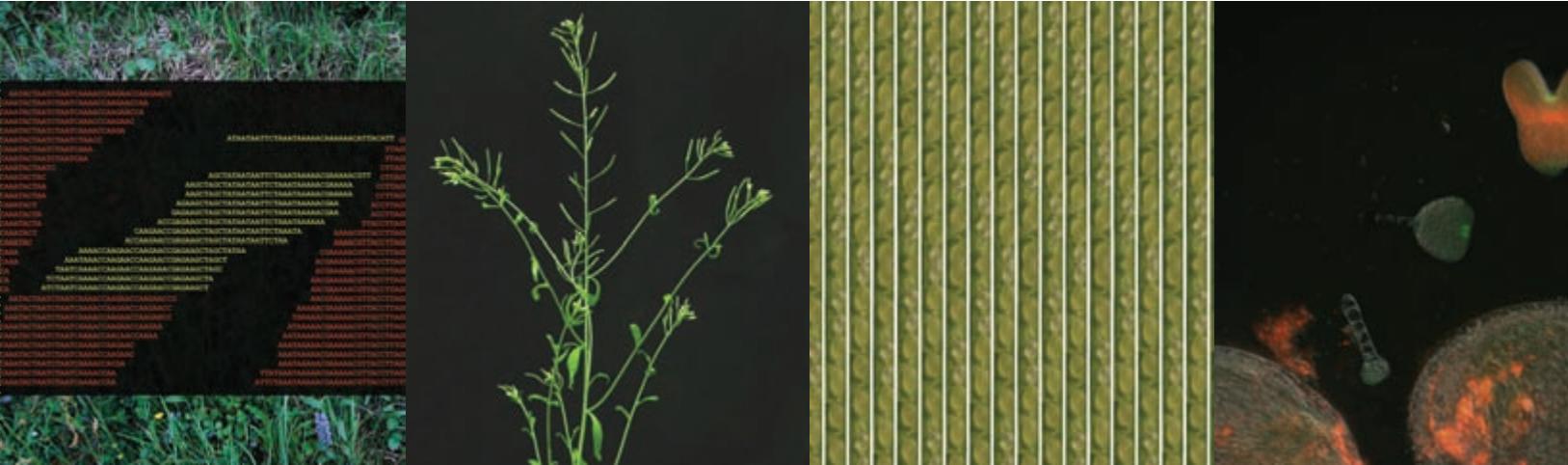
# 2011

on Arabidopsis Research

## 22ND INTERNATIONAL CONFERENCE ON ARABIDOPSIS RESEARCH

UNIVERSITY OF WISCONSIN, MADISON  
USA

JUNE 22-25, 2011



# WinRHIZO Arabidopsis

An outstanding Image Analysis System  
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## Seeds Germination

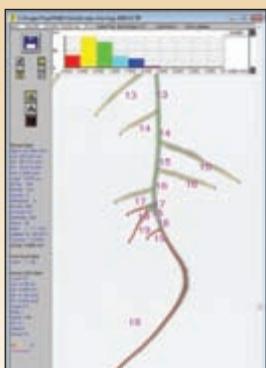


- ✓ Counting
- ✓ Leaf area
- ✓ Root length

## Mature Plants



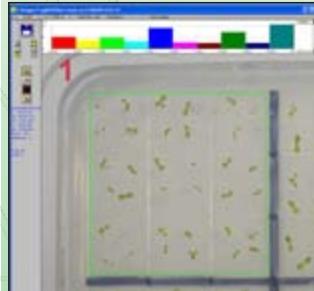
- ✓ Plant height and width



- ✓ Root link analysis

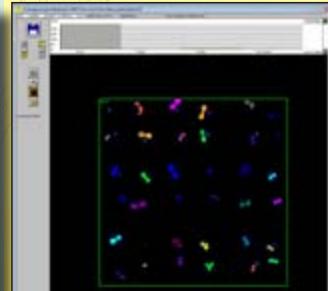
## Seedlings Growth

### Global Analysis



- ✓ Leaf area of seedlings grown in Petri dish

### Individual Analysis



- ✓ Leaf area - leaf/hypocotyl distinction
- ✓ Root morphology, topology, and developmental analysis

Celebrating  
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of Image Analysis  
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## PROGRAM & ABSTRACTS

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UNIVERSITY OF WISCONSIN – MADISON  
JUNE 22–25, 2011

Throughout this **PROGRAM**, the numbers next to abstracts refer to abstract numbers, not the page number in the **ABSTRACT** part of this book.

## SESSION OVERVIEW

### **WEDNESDAY, JUNE 22**

6:00 - 7:30 pm  
7:30 - 10:00 pm

Keynote Address  
Opening Reception

Union Theater  
Tripp Commons

### **THURSDAY, JUNE 23**

8:30 - 8:40 am  
  
8:40 - 10:10 am  
10:30 am - 12:00 pm  
1:30 - 3:30 pm  
1:30 - 3:30 pm  
4:00 - 6:00 pm  
4:00 - 6:00 pm  
7:30 - 9:00 pm  
9:00 - 11:00 pm  
9:00 - 11:00 pm

Presentation of the 2011 Genetics Society of America George Beadle Award to Joe Ecker, by Elliot Meyerowitz  
Plenary Session 1: Epigenetics/Small RNAs  
Plenary Session 2: Biotic Interactions/Biotic Stress  
Concurrent Session 1: Cell Biology  
Concurrent Session 2: Computational Biology  
Concurrent Session 3: Biochemistry/Metabolism  
Concurrent Session 4: New Technologies  
Workshops I a & b (Concurrent)  
Poster Session I (Odd Numbered Posters)  
Exhibitors

Union Theater  
Union Theater  
Union Theater  
Union Theater  
3650 Humanities  
Union Theater  
3650 Humanities  
See detailed program  
See detailed program  
Great Hall and Main Lounge

### **FRIDAY, JUNE 24**

8:30 - 10:00 am  
10:30 - 12:00 pm  
1:30 - 3:30 pm  
1:30 - 3:30 pm  
4:00 - 6:00 pm  
4:00 - 6:00 pm  
7:30 - 9:00 pm  
9:00 - 11:00 pm

Plenary Session 3: Stem Cells  
Plenary Session 4: Hormone Signaling  
Concurrent Session 5 Cell Walls and the Cuticle  
Concurrent Session 6: Light/Circadian Regulation  
Concurrent Session 7: Translational Plant Biology  
Concurrent Session 8: Development I: Organ and Cellular Polarity  
Workshops II a & b (Concurrent)  
Poster Session II (Even Numbered Posters)

Union Theater  
Union Theater  
3650 Humanities  
Union Theater  
3650 Humanities  
Union Theater  
See detailed program  
See detailed program

### **SATURDAY, JUNE 25**

6:45 - 7:45 am  
8:30 - 10:00 am  
10:30 am - 12:00 pm  
12:00 - 2:00 pm  
12:00 - 2:00 pm  
2:00 - 3:30 pm  
4:00 - 6:00 pm  
4:00 - 6:00 pm  
7:00 - 10:00 pm

5K Weed Stampede Fun Run  
Plenary Session 5: Natural Variation/Quantitative Genetics/Evolution  
Plenary Session 6: Systems Biology  
Poster Session III (Free for All)  
Exhibitors  
Workshops III a & b (Concurrent)  
Concurrent Session 9: Abiotic Stress Responses  
Concurrent Session 10: Development II: Cell Specification  
Conference Banquet

Union Theater Lobby  
Union Theater  
Union Theater  
See detailed program  
Great Hall and Main Lounge  
See detailed program  
3650 Humanities  
Union Theater  
The Sett@ Union South

**Note:** Members of the North American Arabidopsis Steering Committee (NAASC) are serving as the program committee for the 2011 meeting.

#### **NAASC Conference Co-Chairs**

**Mark Estelle, University of California, San Diego**  
**Jane Glazebrook, University of Minnesota**

#### **Lead Conference Organizer/NAASC Coordinator** **Joanna Friesner**

#### **NAASC Conference Organizing Committee Members**

**Scott Poethig, University of Pennsylvania**  
**George Haughn, University of British Columbia**  
**Xinnian Dong, Duke University**  
**Blake Meyers, University of Delaware**  
**Dominique Bergmann, Stanford University**  
**Wolf Frommer, Carnegie Institution for Science**

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# TABLE OF CONTENTS

---

Session Overview.....	ii
Sponsors .....	iv-viii
Exhibitors .....	ix-xiii
Program.....	xv–xxv
Workshop Schedule.....	xxvii– xxx
Abstract Listings	
Oral Abstract Listing (Abstracts 1 - 101).....	xxxi–xxxvi
Poster Abstract Listing (Abstracts 102 - 628).....	xxxvii–lxviii
Abstracts	
Keynote Lecture Abstracts .....	1–2
Plenary and Concurrent Abstracts.....	3–90
Workshop Abstracts .....	91–101
Poster Abstracts.....	102–628
Presenting Author Email List.....	Presenter Email 1–5
Author Index - All Authors .....	Author index 1–14
University Campus .....	Map 1
Memorial Union Floor Plan .....	Map 2

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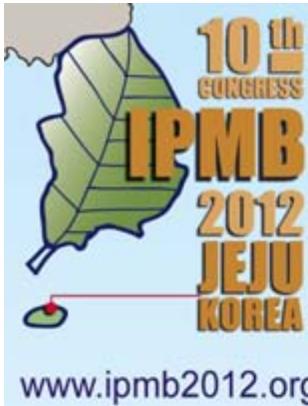
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[www.ipmb2012.org](http://www.ipmb2012.org)

# 10<sup>th</sup> International Congress on Plant Molecular Biology

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## Organizing Committee

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Booth 14



Booth 7

## Silver Exhibitors

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Booth 8



Booth 15

## Bronze Exhibitors

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Booth 17

**Data2Bio**

Booth 4

**Intavis**

Booth 16

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Booth 3

## Additional Exhibitors

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Booth 1

Gramene

Booth 11

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Geneva Scientific

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ABRC

Booth 13

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Booth 18

Qubit Systems

Booth 10

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Sensitivity	5 pg/μl
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Robotic interface capable	YES
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- \*Genetic regulations
- \*Circadian rhythms
- \*Regulation of plant growth
- \*Stress tolerance
- \*Abundance of ROS
- \**in planta* drug discovery

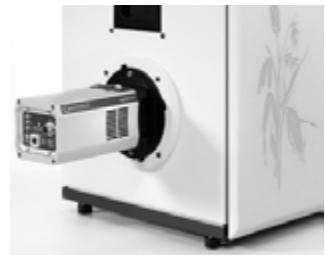


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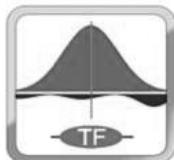
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##### Illumina GAIIX:

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- + \$200 / pairwise comparison

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#### Assembly



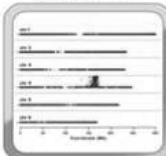
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## **NOTES**

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# PROGRAM

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## **Poster schedule**

All posters will remain up for the entire meeting and can be set up Thursday starting at 7:00 am. There will be three poster sessions, one Thursday evening, one Friday evening, and one Saturday during lunch. To determine when you should stand next to your poster, find your abstract in this book and note the new abstract number. The new number is your poster number, NOT the number it was assigned when you originally submitted. Posters are sequenced by topic and presenter. See the poster list below to determine which group contains your topic.

All posters with ODD numbers will be presented on Thursday evening.

All posters with EVEN numbers will be presented on Friday evening.

Saturday's midday poster session will be a "free-for-all" – plenty of time to look at all posters, or stand by your own if you need more time for discussion,

---

## **Wednesday, June 22, 2011**

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<b>1:00 pm – 7:00 pm</b>	<b>Registration</b>	<b>Main Lounge</b>
<b>6:00 – 7:30 pm</b>	<b>Keynote Address</b> Session chairs: <b>Jane Glazebrook (University of Minnesota) and Mark Estelle (University of California, San Diego)</b>	<b>Union Theater</b>
<b>6:00 pm</b>	<b>Sophien Kamoun, The Sainsbury Laboratory</b> <i>Suppression of plant immunity by Phytophthora effectors</i>	
<b>6:45 pm</b>	<b>Joanne Chory, The Salk Institute for Biological Studies and The Howard Hughes Medical Institute</b> <i>Using genetics and structural biology to dissect the molecular mechanisms of BR perception and signaling</i>	
<b>7:30 – 10:00 pm</b>	<b>Opening Reception</b>	<b>Tripp Commons</b>

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## **Thursday, June 23, 2011**

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<b>7:00 am – all day</b>	<b>Poster set-up</b> <b>Capital View/Langdon Room (4th floor, East and West)</b> Posters of orals (see abstract for indication if poster provided) (1-101) <b>Main Lounge (2nd floor, Central)</b> Abiotic Stress Responses (102 – 166) Biochemistry/Metabolism (167 – 195) Bioinformatics, Modeling, Systems Biology (196 – 211) <b>Tripp Commons (2nd floor, East)</b> Biotic Interactions/Biotic Stress (212 – 271) Cell Biology (272 – 339) Cell Walls and the Cuticle (340 – 355) Community Resources (356 – 366) Computational Biology (367 – 378)	<b>See locations below</b>
--------------------------	--	----------------------------

**Great Hall/Reception Room (4th floor, Central)**

- Development I: Organ and Cellular Polarity (379 – 415)
- Development II: Cell Specification (416 – 471)
- Epigenetic and Genetic Mechanisms (472 – 499, 628)
- Light/Circadian Regulation (500 – 533)
- Natural Variation/Quantitative genetics/Evolution (534 – 553)
- New Technologies (554 – 567)
- Other (568 – 572)
- Signal Transduction (573 – 622)
- Translational Plant Biology (623 – 627)

<b>7:00 am – 8:00 pm</b>	<b>Registration Continues</b>	<b>Annex Room</b>
<b>7:00 – 8:30 am</b>	<b>Breakfast</b>	<b>Inn Wisconsin</b>
<b>8:30 – 8:40 am</b>	<b>Award Presentation</b> <i>2011 GSA George Beadle Award to Joe Ecker by Elliot Meyerowitz</i>	<b>Union Theater</b>
<b>8:40 – 10:10 am</b>	<b>Plenary Session 1: Epigenetics/Small RNAs</b> <b>Blake Meyers, University of Delaware, Session Chair</b>	<b>Union Theater</b>
<b>8:40 am</b>	<b>Xuemei Chen, University of California, Riverside</b> <i>Biogenesis and functions of plant microRNAs</i>	
<b>9:10 am</b>	<b>Joseph Ecker, The Salk Institute</b> <i>Epigenomic Variation in Plants (and People)</i>	
<b>9:40 am</b>	<b>Rebecca Mosher, University of Arizona</b> <i>Imprinted expression of Pol IV-dependent siRNAs</i>	
<b>Session Sponsored by Genetics Society of America</b>		
 Genetics Society of America		
<b>10:10 – 10:30 am</b>	<b>Coffee Break</b>	<b>Union Theater Lobby</b>
<b>10:30 – 12:00 pm</b>	<b>Plenary Session 2: Biotic Interactions/Biotic Stress</b> <b>Xinnian Dong, Duke University, Session Chair</b>	<b>Union Theater</b>
<b>10:30 am</b>	<b>Xin Li, University of British Columbia</b> <i>Stability of Resistance proteins is controlled by SCF-mediated protein radation</i>	
<b>11:00 am</b>	<b>Paul Schulze-Lefert, Max Planck Institute for Plant Breeding Research</b> <i>From plant-pathogen interactions to plant-microbe communities</i>	
<b>11:30 am</b>	<b>Jeff Dangl, University of North Carolina at Chapel Hill</b> <i>Understanding plant-microbe interactions: Plant immune system function and rhizosphere metagenomics</i>	

**Session Sponsored by Monsanto**

<b>12:00 – 1:30 pm</b>	<b>Lunch</b>	<b>Inn Wisconsin</b>
<b>12:30 – 1:30 pm</b>	<b>Lunch Workshop: Epigenomics of Plants International Consortium (EPIC) - bring lunch to room Organizers: Doris Wagner and Blake Meyers</b>	<b>Old Madison</b>
<b>1:30 – 3:30 pm</b>	<b>Concurrent Session 1: Cell Biology</b>	<b>Union Theater</b>
<b>1:30 pm</b>	<b>Federica Brandizzi, Michigan State University, Session Chair <i>Morphological and Functional Identity of Organelles of the Early Plant Secretory Pathway</i></b>	
<b>1:55 pm</b>	<b>Roger Hangarter, Indiana University <i>Light-induced chloroplast movements in leaf cells</i></b>	
<b>2:20 pm</b>	<b>Michael Hothorn, Salk Institute for Biological Studies <i>Structural basis of brassinosteroid perception by a membrane receptor kinase</i></b>	
<b>2:34 pm</b>	<b>Hiroyasu Motose, Okayama University <i>NIMA-related Kinases Redundantly Regulate Directional Cell Expansion in Arabidopsis thaliana</i></b>	
<b>2:48 pm</b>	<b>Francisca Reyes, University of Wisconsin-Madison <i>The Role of SKIP3, a Novel Plant-Specific Endosomal Protein, in Plant Development and Brassinosteroid Signaling</i></b>	
<b>3:02 pm</b>	<b>Hyung-Taeg Cho, Seoul National University <i>Slow Trafficking of Arabidopsis ATP-Binding Cassette Protein Subfamily B4 Indicates Its Basal Auxin Efflux Function in the Plasma Membrane</i></b>	
<b>3:16 pm</b>	<b>Diane Bassham, Iowa State University <i>Degradation of the Endoplasmic Reticulum by Autophagy during ER stress in Plants</i></b>	

**Session Sponsored by Journal of Cell Science**



<b>1:30 – 3:30 pm</b>	<b>Concurrent Session 2: Computational Biology</b>	<b>3650 Humanities</b>
<b>1:30 pm</b>	<b>Veronica Grienesen, John Innes Centre, Session Chair <i>The Go-Between: Auxin as a mediator of cell-cell signaling</i></b>	
<b>1:55 pm</b>	<b>Przemyslaw Prusinkiewicz, University of Calgary <i>A computational model of Arabidopsis thaliana Leaf Margin Development</i></b>	
<b>2:20 pm</b>	<b>Justin Vaughn, University of Tennessee <i>Novel and Known Post-transcriptional Regulatory Sequences are Conserved across Plant Families</i></b>	
<b>2:34 pm</b>	<b>Shin-Han Shiu, Michigan State University <i>Cis-Regulatory Code of Stress Responsive Transcription in Arabidopsis thaliana</i></b>	

<b>2:48 pm</b>	<b>Pascal Braun, Center for Cancer Systems Biology at Dana Farber Cancer Institute</b> <i>Insights into Systems Organization, Network Evolution, and Pathogen Attack from a High-Quality <i>Arabidopsis</i> Interactome Network Map</i>	
<b>3:02 pm</b>	<b>Laura Helft, University of Wisconsin – Madison</b> <i>Repeat Conservation Mapping of Leucine-Rich Repeat Domains</i>	
<b>3:16 pm</b>	<b>Joshua Steffen, University of Utah</b> <i>Accurate Sequencing of 18 Genomes of <i>Arabidopsis thaliana</i> and Its Use in Imputing the Genome Sequences of Over 600 MAGIC Recombinant Inbred Lines</i>	
<b>3:30 – 4:00 pm</b>	<b>Coffee Break</b>	<b>3650 Humanities and Union Theater Lobby</b>
<b>4:00 – 6:00 pm</b>	<b>Concurrent Session 3: Biochemistry/Metabolism</b>	<b>Union Theater</b>
<b>4:00 pm</b>	<b>Mary Lou Guerinot, Dartmouth College, Session Chair</b> <i>Integrating metal uptake and distribution in plants</i>	
<b>4:25 pm</b>	<b>Yan Lu, Michigan State University</b> <i>The Role of a Zinc Finger Protein in Photosynthesis and Light Stress Tolerance</i>	
<b>4:50 pm</b>	<b>Ling Li, Iowa State University</b> <i>Uncovering Novel Signaling Interactions in Regulation of the Plant Metabolic Networks</i>	
<b>5:04 pm</b>	<b>Gregg Howe, Michigan State University</b> <i>Cytochrome P450 CYP94B3 Mediates Catabolism and Inactivation of Jasmonate</i>	
<b>5:18 pm</b>	<b>Anna Stepanova, North Carolina State University</b> <i>“Rooting” <i>YUCCA</i> Genes in the Auxin Biosynthetic Pathway</i>	
<b>5:32 pm</b>	<b>Cyril Zipfel, The Sainsbury Laboratory</b> <i>Mechanisms of BAK 1-Dependent Signaling</i>	
<b>5:46 pm</b>	<b>Stéphanie Arrivault, Max-Planck-Institute of Molecular Plant Physiology</b> <i>Photosynthetic Carbon Assimilation in <i>Arabidopsis thaliana</i></i>	

**Session Sponsored by Mendel Biotechnology**



<b>4:00 – 6:00 pm</b>	<b>Concurrent Session 4: New Technologies</b>	<b>3650 Humanities</b>
<b>4:00 pm</b>	<b>Simon Chan, University of California, Davis, Session Chair</b> <i>Haploid <i>Arabiodopsis thaliana</i>: Power Tools for Plant Genetics</i>	
<b>4:25 pm</b>	<b>Steven Briggs, University of California San Diego</b> <i>Proteome Dynamics Indicate That PAMP-Triggered and Effector-Triggered Signaling Converge Early</i>	

<b>4:50 pm</b>	<b>Magali Moreau, Boyce Thompson Institute for Plant Research</b> <i>Identifying protein-small molecule interactions using functional protein microarrays coupled with a photoactivated crosslinked ligand</i>
<b>5:04 pm</b>	<b>Bob Schmitz, Salk Institute for Biological Studies</b> <i>Base-Resolution Population Epigenomic Variation</i>
<b>5:18 pm</b>	<b>Mitch Sudkamp, Monsanto</b> <i>Exploring the <i>Arabidopsis</i> genome with single molecule PacBio sequencing</i>
<b>5:32 pm</b>	<b>Jose Alonso, North Carolina State University</b> <i>High-Throughput Recombineering and Its Applications for <i>Arabidopsis</i> Gene Function Characterization</i>
<b>5:46 pm</b>	<b>Siobhan Braybrook, Institute of Plant Science, University of Bern</b> <i>The Application of Atomic Force Microscopy as a Micro-Force Sensor: Probing the Mechanics of Living Plant Cell Walls During Development</i>

### Session Sponsored by Plant Methods Journal



<b>6:00 – 7:30 pm</b>	<b>Dinner</b>	<b>Inn Wisconsin</b>
<b>7:30 – 9:00 pm</b>	<b>Workshops I (concurrent)</b>	
	<b>1a: Gramene Database: A Resource For Plant Comparative Genomics and <i>Arabidopsis</i> Research</b> Organizers: Pankaj Jaiswal and Doreen Ware (See abstract #91)	<b>Union Theater</b>
	<b>1b: Integrative analysis of molecular profiling data</b> Organizers: Pierre Hilson and Katja Baerenfaller (See abstracts #92 – 95)	<b>3650 Humanities</b>
<b>9:00 – 11:00 pm</b>	<b>Poster Session 1</b>  Please present (stand by) your poster if your abstract in this book is <b>ODD</b> numbered  <b>Capital View/Langdon Room (4th floor, East and West)</b> Posters of orals (see abstract for indication if poster provided) (1-101)  <b>Main Lounge (2nd floor, Central)</b> Abiotic Stress Responses (102 – 166) Biochemistry/Metabolism (167 – 195) Bioinformatics, Modeling, Systems Biology (196 – 211)  <b>Tripp Commons (2nd floor, East)</b> Biotic Interactions/Biotic Stress (212 – 271) Cell Biology (272 – 339) Cell Walls and the Cuticle (340 – 355) Community Resources (356 – 366) Computational Biology (367 – 378)	<b>See locations below</b>

<b>Great Hall/Reception Room (4th floor, Central)</b>	
Development I: Organ and Cellular Polarity (379 – 415)	
Development II: Cell Specification (416 – 471)	
Epigenetic and Genetic Mechanisms (472 – 499, 628)	
Light/Circadian Regulation (500 – 533)	
Natural Variation/Quantitative genetics/Evolution (534 – 553)	
New Technologies (554 – 567)	
Other (568 – 572)	
Signal Transduction (573 – 622)	
Translational Plant Biology (623 – 627)	

<b>9:00 – 11:00 pm</b>	<b>Exhibitors</b>	<b>Great Hall and Main Lounge</b>
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## **Friday, June 24, 2011**

<b>7:00 – 8:30 am</b>	<b>Breakfast</b>	<b>Inn Wisconsin</b>
<b>8:30 – 10:00 am</b>	<b>Plenary Session 3: Stem Cells</b> Scott Poethig, University of Pennsylvania, Session Chair	<b>Union Theater</b>
<b>8:30 am</b>	<b>Elliot Meyerowitz, California Institute of Technology</b> <i>Arabidopsis Stem Cells in Development and Regeneration</i>	
<b>9:00 am</b>	<b>Sabrina Sabatini, University of Rome Sapienza</b> <i>SCARECROW Sustains Stem Cell Activity Inhibiting Cytokinin Dependent Cell Differentiation Input</i>	
<b>9:30 am</b>	<b>Ben Scheres, Utrecht University</b> <i>An integrated circuit for asymmetric cell division in Arabidopsis roots</i>	
<b>10:00 – 10:30 am</b>	<b>Coffee Break</b>	<b>Union Theater Lobby</b>
<b>10:30 – 12:00 pm</b>	<b>Plenary Session 4: Hormone Signaling</b> Mark Estelle, University of California, San Diego, Session Chair	<b>Union Theater</b>
<b>10:30 am</b>	<b>Ottoline Leyser, Sainsbury Laboratory University of Cambridge</b> <i>Hormonal Control of shoot branching</i>	
<b>11:00 am</b>	<b>Joseph Kieber, University of North Carolina</b> <i>The interaction of the cytokinin-regulated phosphorelay with other signals in Arabidopsis</i>	
<b>11:30 am</b>	<b>Ning Zheng, University of Washington</b> <i>Structural Mechanism of Jasmonate Perception</i>	
<b>12:00 – 1:30 pm</b>	<b>Lunch</b>	<b>Inn Wisconsin</b>
<b>12:10 – 1:30 pm</b>	<b>Lunch Workshop: MASC Subcommittee Workshop</b> - bring lunch to room Organizer: Joshua Heazlewood	<b>Old Madison</b>
<b>1:30 – 3:30 pm</b>	<b>Concurrent Session 5: Cell Walls and the Cuticle</b>	<b>3650 Humanities</b>
<b>1:30 pm</b>	<b>Ljerka Kunst, University of British Columbia, Session Chair</b> <i>CER7, a Core Subunit of the RNA-processing Exosome, has a Specific Role in Regulation of Cuticular Wax Deposition in Arabidopsis</i>	

1:55 pm	<b>Volker Bischoff, INRA</b> <i>The Role of Cell Wall Synthesis and Remodeling in Organ Growth</i>	
2:20 pm	<b>Katy Christiansen, Joint BioEnergy Institute</b> <i>A Functional Screen for Nucleotide Sugar Transporters</i>	
2:34 pm	<b>Catalin Voiniciuc, University of British Columbia</b> <i>The <i>Arabidopsis</i> FLYING SAUCERS Gene Encodes a Membrane Protein Required for Connections to the Cell Wall</i>	
2:48 pm	<b>Gerit Bethke, University of Minnesota</b> <i>The Effects of Plant Cell Wall Alterations on Plant Disease Susceptibility</i>	
3:02 pm	<b>Joshua Heazlewood, Lawrence Berkeley National Laboratory</b> <i>Subcellular Partitioning of Plant Cell Wall Biosynthesis in <i>Arabidopsis</i></i>	
3:16 pm	<b>Basil Nikolau, Iowa State University</b> <i>Combining Molecular Genetics and Mass-Spectrometry-Based High-Resolution Metabolite Imagine to Unravel the Surface Lipids of <i>Arabidopsis</i></i>	
1:30 – 3:30 pm	<b>Concurrent Session 6: Light/Circadian Regulation</b>	<b>Union Theater</b>
1:30 pm	<b>Stacey Harmer, University of California, Davis, Session Chair</b> <i>Identification of Plant Clock Genes Using Functional Genomics</i>	
1:55 pm	<b>Jose Pruneda-Paz, University of California, San Diego</b> <i>Uncovering clock transcriptional circuits by functional genomics</i>	
2:20 pm	<b>Jodi Stewart, University of Washington</b> <i>Growth Promoting Factors Have Distinct Effects on Seedling Growth Dynamics</i>	
2:34 pm	<b>Ling Zhu, University of Texas at Austin</b> <i>Antagonistic Regulation of Photomorphogenesis by Oppositely Acting bHLH Transcription Factors in <i>Arabidopsis</i></i>	
2:48 pm	<b>Dmitri Nusinow, University of California, San Diego</b> <i>A Circadian Complex is Critical for Growth Control in <i>Arabidopsis</i></i>	
3:02 pm	<b>Naeem Syed, University of Dundee</b> <i>Alternative Splicing Mediates Responses of the <i>Arabidopsis</i> Circadian Clock to Temperature Changes</i>	
3:16 pm	<b>Ming-Jung Liu, Academia Sinica</b> <i>Translational Control: a New Dimension in the Regulation of <i>Arabidopsis</i> Photomorphogenesis</i>	
3:30 – 4:00 pm	<b>Coffee Break</b>	<b>3650 Humanities and Union Theater Lobby</b>
4:00 – 6:00 pm	<b>Concurrent Session 7: Translational Plant Biology</b>	<b>3650 Humanities</b>
4:00 pm	<b>Zachary Lippman, Cold Spring Harbor Laboratory, Session Chair</b> <i>Single Gene Mutations Causing Heterosis in Tomato</i>	
4:25 pm	<b>Julia Bailey-Serres, Center for Plant Cell Biology/ UC Riverside</b> <i>Translation of submergence tolerance from the gene to the field using rice and <i>Arabidopsis</i></i>	

- 4:50 pm** **Devin O'Connor, University of California, Berkeley**  
*Localization of PIN1-Like Proteins in Grasses Suggests a Functional Specialization of Different PINs into 'Up-the-Gradient' and 'With-the-Flux' Modes of Auxin Transport*
- 5:04 pm** **Nubia Eloy, Ghent University, Belgium**  
*SAMBA- a new subunit of the Anaphase Promoting Complex (APC/C) with an essential role in plant growth and pollen development in *Arabidopsis**
- 5:18 pm** **Kenichi Tsuda, University of Minnesota**  
*Modeling of the Plant Hormone Signaling Network in MAMP-Induced Resistance*
- 5:32 pm** **Dirk Büssis, Max-Planck-Institute of Molecular Plant Physiology, Germany**  
*German Plant Research Goes BioEconomy*
- 5:46 pm** **Marie Navarro, University of Western Ontario**  
*Genomic Dissection of the Plant/pest Interaction: Transcriptome Analysis of *Arabidopsis* Response to *Tetranychus urticae* (Two Spotted Spider Mite) Feeding*

### Session Sponsored by International Plant Molecular Biology



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|-----------------------|---|----------------------|
| <b>4:00 – 6:00 pm</b> | <b>Concurrent Session 8: Development I: Organ and Cellular Polarity</b> | <b>Union Theater</b> |
|-----------------------|---|----------------------|
- 4:00 pm** **Jennifer Fletcher, Plant Gene Expression Center, Session Chair**  
*Regulation of *Arabidopsis* Gynoecium Polarity by *ULT* and *KAN* Family Proteins*
- 4:25 pm** **Niko Geldner, University of Lausanne**  
*The Endodermis- building a selective and polarized cellular barrier*
- 4:50 pm** **Ji-Young Lee, Boyce Thompson Institute for Plant Research**  
*An apical root growth program directed in the vascular stem cells*
- 5:04 pm** **Emanuele Scacchi, University of Lausanne, UNIL**  
*Spatio-Temporal Sequence of Cross-Regulatory Events in Root Meristem Growth*
- 5:18 pm** **Brenda Reinhart, Carnegie Institution, Stanford**  
*Expanding the Genetic Pathway for Polarity in *Arabidopsis* Leaf Development*
- 5:32 pm** **Monica Carabelli, Institute for Molecular Biology and Pathology, National Research Council**  
*HD-Zip II Transcription Factor Genes Control Adaxial-Abaxial Patterning in *Arabidopsis**
- 5:46 pm** **Sangho Jeong, University of Georgia**  
*Functional and informatic analyses of the *Arabidopsis* plastid*

<b>6:00 – 7:30 pm</b>	<b>Dinner</b>	<b>Inn Wisconsin</b>
<b>7:30 – 9:00 pm</b>	<b>Workshops II (Concurrent)</b>	
	<b>2a: TwitTAIR: Discover what's happening right now at TAIR!</b> Organizers: Philippe Lamesch, Eva Huala, and Donghui Li	<b>Union Theater</b>
	<b>2b: Receptor Ligands Regulating Plant Development</b> Organizers: Melinka Butenko, Rüdiger Simon, and Yvonne Stahl (See abstracts #96 – 98)	<b>3650 Humanities</b>
<b>9:00 – 11:00 pm</b>	<b>Poster Session II and Exhibitors</b> Please present (stand by) your poster if your abstract in this book is <b>EVEN</b> numbered	<b>See locations below</b>
	<b>Capital View/Langdon Room (4th floor, East and West)</b> Posters of orals (see abstract for indication if poster provided) (1-101)	
	<b>Main Lounge (2nd floor, Central)</b> Abiotic Stress Responses (102 – 166) Biochemistry/Metabolism (167 – 195) Bioinformatics, Modeling, Systems Biology (196 – 211)	
	<b>Tripp Commons (2nd floor, East)</b> Biotic Interactions/Biotic Stress (212 – 271) Cell Biology (272 – 339) Cell Walls and the Cuticle (340 – 355) Community Resources (356 – 366) Computational Biology (367 – 378)	
	<b>Great Hall/Reception Room (4th floor, Central)</b> Development I: Organ and Cellular Polarity (379 – 415) Development II: Cell Specification (416 – 471) Epigenetic and Genetic Mechanisms (472 – 499, 628) Light/Circadian Regulation (500 – 533) Natural Variation/Quantitative genetics/Evolution (534 – 553) New Technologies (554 – 567) Other (568 – 572) Signal Transduction (573 – 622) Translational Plant Biology (623 – 627)	

## Saturday, June 25, 2011

<b>6:45 – 7:45 am</b>	<b>Weed Stampede 5K Fun Run - Free!</b>	<b>Union Theater Lobby</b>
<b>7:00 – 8:30 am</b>	<b>Breakfast</b>	<b>Inn Wisconsin</b>
<b>8:30 – 10:00 am</b>	<b>Plenary Session 5: Natural Variation/Quantitative Genetics/Evolution</b> <b>George Haughn, University of British Columbia, Session Chair</b>	<b>Union Theater</b>
<b>8:30 am</b>	<b>Johanna Schmitt, Brown University</b> <i>Mapping local adaptation in Arabidopsis thaliana</i>	

<b>9:00 am</b>	<b>George Coupland, Max Planck Institute for Plant Breeding Research</b> <i>Using Arabidopsis relatives as sources of natural genetic variation in regulatory networks</i>	
<b>9:30 am</b>	<b>Cynthia Weinig, University of Wyoming</b> <i>Quantitative variation in the circadian clock and adaptation to heterogeneous settings</i>	
<b>10:00 – 10:30 am</b>	<b>Coffee Break</b>	<b>Union Theater Lobby</b>
<b>10:30 am – 12:00 pm</b>	<b>Plenary Session 6: Systems Biology</b> Nicholas Provart, University of Toronto, Session Chair	<b>Union Theater</b>
<b>10:30 am</b>	<b>Rodrigo Gutierrez, Pontificia Universidad Catolica de Chile</b> <i>Systems Biology to Dissect Nitrogen Regulatory Networks</i>	
<b>11:00 am</b>	<b>Siobhan Brady, University of California, Davis</b> <i>Mapping Spatiotemporal Gene Regulatory Networks in the Arabidopsis Root Stele</i>	
<b>11:30 am</b>	<b>Fumiaki Katagiri, University of Minnesota</b> <i>Properties and structure of the plant immune signaling</i>	
<b>12:00 – 2:00 pm</b>	<b>Lunch</b>	<b>Inn Wisconsin</b>
<b>12:00 – 2:00 pm</b>	<b>Poster Session III/Exhibitor Meeting</b> <b>Free-for-All: A time for further discussions</b> <b>Exhibitors – Great Hall and Main Lounge</b>	<b>See schedule for locations</b>
<b>2:00 – 3:30 pm</b>	<b>Workshops III (Concurrent)</b>  <b>3a: RNA-level gene regulation</b> <b>Organizer: Albrecht von Arnim</b> (See abstracts #99 – 101)	<b>Union Theater</b>
	 <b>3b: Hormone cross-talk: gene families and phenotypes</b> <b>Organizer: Mary Wildermuth</b>	<b>3650 Humanities</b>
<b>3:30 – 4:00 pm</b>	<b>Coffee Break</b>	<b>Union Theater Lobby</b>
<b>4:00 – 6:00 pm</b>	<b>Concurrent Session 9: Abiotic Stress Responses</b>	<b>3650 Humanities</b>
<b>4:00 pm</b>	<b>Julian Schroeder, University of California, San Diego, Session Chair</b> <i>Chemical Genetics Reveals Negative Regulation of Abscisic Acid Signaling by a Type III Effector Signaling Pathway</i>	
<b>4:25 pm</b>	<b>Kazuo Shinozaki, RIKEN Plant Science Center</b> <i>Regulatory gene network in stress responses to drought conditions</i>	
<b>4:50 pm</b>	<b>Jose Dinneny, Carnegie Institution, Stanford</b> <i>Moisture locally induces ABA biosynthesis to determine growth direction in Arabidopsis</i>	
<b>5:04 pm</b>	<b>Malia Dong, Michigan State University</b> <i>Clock Components CCA1 and LHY Regulate Expression of the CBF Cold Response Pathway and Freezing Tolerance in Arabidopsis</i>	
<b>5:18 pm</b>	<b>Jesse Woodson, The Salk Institute</b> <i>Heme Synthesis by Plastid Ferrochelatase I Regulates Nuclear Gene Expression</i>	

<b>5:32 pm</b>	<b>Terri Long, University of Illinois</b> <i>POPEYE, BRUTUS and Other Characters: Elucidating Molecular Mechanisms of the Iron Deficiency Response in Plants</i>
<b>5:46 pm</b>	<b>Won-Gyu Choi, University of Wisconsin, Madison</b> <i>The role of calcium signaling in the molecular response network to anaerobic stress in Arabidopsis</i>

**Session Sponsored by Agrisera**




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<b>4:00 – 6:00 pm</b>	<b>Concurrent Session 10: Development II: Cell Specification</b>	<b>Union Theater</b>
<b>4:00 pm</b>	<b>Dominique Bergmann, Stanford University, Session Chair</b> <i>Stomatal development: signaling fate and renewal</i>	
<b>4:25 pm</b>	<b>Dolf Weijers, Wageningen University</b> <i>Cell Specification and cell communication in embryonic root formation</i>	
<b>4:50 pm</b>	<b>Fabrice Besnard, ENS</b> <i>Cytokinin Inhibitory Fields control Phyllotaxis</i>	
<b>5:04 pm</b>	<b>Bastiaan Bergmann, New York University</b> <i>A Transcriptional Auxin Response Gradient in the <i>Arabidopsis</i> Root</i>	
<b>5:18 pm</b>	<b>Cara Winter, Duke University</b> <i>LEAFY Target Genes Reveal Floral Regulatory Logic, cis Motifs, and a Link to Biotic Stimulus Response</i>	
<b>5:32 pm</b>	<b>Michael Nodine, Whitehead Institute</b> <i>Contributions of the Maternal, Paternal, and Zygotic Genomes during Early Plant Embryogenesis</i>	
<b>5:46 pm</b>	<b>Ulrich Wenig, University of Erlangen, Germany</b> <i>RUG8, a Novel Player in Auxin-Dependent Stem Cell Specification and Meristem Patterning in <i>Arabidopsis</i> Roots</i>	

**Session Sponsored by ASPB**




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<b>7:00- 10:00 pm</b>	<b>Conference Banquet</b>	<b>The Sett @ Union South (See Map)</b>
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## **NOTES**

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# WORKSHOP SCHEDULE

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## Thursday, June 23: 12:30 – 1:30 pm

**Participants-** pick up lunch at 12:10 at the usual place and bring it to the workshop room

### Lunch Workshop: Epigenomics of Plants International Consortium (EPIC) workshop

**Room location:** Old Madison

Workshop Organizers: Doris Wagner (PI) and Blake Meyers (Steering Committee)

Reading the second code: mapping epigenomes to understand plant growth and adaptation to the environment. Interactions with the environment shape the plant body plan during development and control growth and survival responses of these sessile organisms via epigenetic modulation of genome accessibility. As a likely corollary of this is plants have a sophisticated epigenomic ‘toolkit’ and are leading the way in many areas of epigenomic research. Hence elucidation of the plant epigenome should be highly informative. An NSF funded Research Collaborative network (RCN) called EPIC has been formed to discuss how we as a community want to tackle this challenge. This workshop will introduce the EPIC RCN and will begin the discussion of the intellectual grand challenges, potentially transformative technologies, or other goals and tools needed to accomplish this mission.

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## Thursday, June 23: 7:30 – 9:00 pm

### 1a: Gramene Database: A Resource For Plant Comparative Genomics And Arabidopsis Research

**Room location:** Union Theater

Workshop Organizers: Pankaj Jaiswal and Doreen Ware

The Gramene database (<http://www.gramene.org>) is a curated, open-source, web-accessible resource for comparative genome analysis in the plants. It provides plant biologists with invaluable biological and genomic information on over a dozen species. Gramene's web interface provides information on genetic and physical maps, sequences, genes, proteins, genetic markers, mutants, QTLs, controlled vocabularies, literature citations, polymorphism data from genetic diversity projects, and metabolic pathways. These various types of information are integrated using visualization tools that enable the user to compare across different species and reference genomes.

This workshop will demonstrate the search and navigation of the available tools and data sets at Gramene and enable researchers working on Arabidopsis to use our tools to answer various biological and research questions for intra and inter-specific comparisons. Attendees will learn about the types of data available at Gramene, get tips on navigating the website, and use the database and tools to find data for their own projects. Attendees may also wish to bring their own data to work with in the workshop. Gramene is supported by a grant from the NSF (IOS: 0703908 Gramene: A Platform for Comparative Plant Genomics) and represents a collaborative effort between Cold Spring Harbor Laboratory, the Department of Plant Breeding and Genetics at Cornell University, the Department of Botany and Plant Pathology at Oregon State University, the Ensembl Genomes project, USDA-ARS, and various national and international projects dedicated to cereal genomics and genetics research. It is also partially supported by USDA and USDA-ARS.

#### Program

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|---------|--|
| 7:30 pm | <b>Doreen Ware</b> (Cold Spring Harbor Laboratory and USDA-ARS, Cornell University)- Gramene Database Workshop Introduction                                |
| 7:40 pm | <b>Josh Stein</b> (Cold Spring Harbor Laboratory)- Exploring the Plant Genomes (browse, search, upload personal data, analysis tools)- <b>Abstract #91</b> |
| 8:00 pm | <b>Josh Stein and Doreen Ware</b> - Phylogenetic analysis tools and targeted query tools using Biomart   |
| 8:15 pm | <b>Pankaj Jaiswal</b> (Oregon State University)- Comparative Pathways Networks and future directions   |
| 8:35 pm | <b>Doreen Ware</b> - Exploring the Plant's Genetic Diversity   |
| 8:50 pm | Q/A  |

### 1b: Integrative analysis of molecular profiling data

**Room location:** 3650 Humanities

Workshop Organizers: Pierre Hilson (VIB-Ghent University, Belgium) and Katja Baerenfaller (ETH Zurich, Switzerland)

The workshop will describe efforts towards the integration, combined analysis and interpretation of diverse molecular profiling datasets that characterize particular plant systems or processes. In this context, molecular profiles include transcript, protein,

metabolite and polymer profiles, as well as enzymatic activities and metabolic fluxes. The aim of the workshop is primarily to highlight the bottlenecks encountered in integration projects together with the practical solutions implemented to solve them. The novel solutions for integrative analyses comprise specific databasing strategies, the definition of standards and data types, statistical approaches, visualization platforms and modeling tools. The workshop will present recent achievements therein, illustrated by original results furthering our understanding of the biological processes under study. It is primarily addressed at researchers performing or planning integrative analyses and will leave room for questions and open discussion. The abstracts of the individual contributions can be found at [http://www.agron-omics.eu/index.php/resource\\_center/workshops/workshop-icar-2011](http://www.agron-omics.eu/index.php/resource_center/workshops/workshop-icar-2011).

### **Program**

7:30 pm	<b>Pierre Hilson</b> (VIB-Ghent University): Introduction
7:35 pm	<b>Sean Walsh</b> (ETH Zurich): A database approach to the integration, analysis and visualization of diverse molecular and phenotypic profiling data, <b>Abstract #92</b>
7:45 pm	<b>Katja Baerenfaller</b> (ETH Zurich): Integrating proteomics data: pep2pro, MASCP Gator and combined analyses with transcript data, <b>Abstract #93</b>
7:55 pm	<b>Rodrigo Gutierrez</b> (Universidad Catolica de Chile): Discriminative expression signatures in microarray data for functional network inference, <b>Abstract #94</b>
8:10 pm	<b>Jim Beynon</b> (University of Warwick): Linking plant transcriptional response networks triggered by biotic and abiotic stress
8:25 pm	<b>Nicholas J. Provart</b> (University of Toronto): Hypothesis Generation in Plant Biology Using Large Data Sets, <b>Abstract #95</b>
8:40 pm	<b>Eva Huala</b> (TAIR): Organizing categories, stumbling blocks and solutions for integration of molecular profiling data
8:55 pm	Discussion

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## **Friday, June 24: 12:10 – 1:30 pm**

**Participants-** pick up lunch at noon at the usual place and bring it to the workshop room

### **Lunch Workshop: MASC Subcommittee Workshop**

#### **Room location: Old Madison**

Workshop Organizer: Joshua Heazlewood, Lawrence Berkeley National Laboratory

The Multinational Arabidopsis Steering Committee (MASC) Subcommittees were established to facilitate communication in major focus areas of Arabidopsis research. A major objective of the subcommittees is to coordinate international research in the focus areas and to communicate information to the wider Arabidopsis research community.

The purpose of this workshop is to briefly highlight the current status of research, infrastructure and resources currently available to the Arabidopsis community in the subcommittee focus areas and to provide platform for input and feedback.

### **Program**

12:10 pm	<b>Joshua Heazlewood</b> : Introduction
12:15 pm	<b>Brian Dilkes</b> (Purdue University, USA): Natural Variation/Comparative Genomics
12:25 pm	<b>Joe Ecker</b> (Salk Institute, USA): ORFeomics
12:35 pm	<b>Katja Bärenfaller</b> (ETH Zurich, Switzerland): Proteomics
12:45 pm	<b>Basil Nikolau</b> (Iowa State University, USA): Metabolomics
12:55 pm	<b>Nick Provart</b> (University of Toronto, Canada): Bioinformatics
1:05 pm	<b>Joshua Heazlewood</b> (Lawrence Berkeley National Laboratory, USA): Phenomics
1:15 pm	<b>Rodrigo Gutierrez</b> (Pontificia Universidad Católica de Chile, Chile): Systems Biology
1:25 pm	Discussion (5 min)

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## **Friday, June 24: 7:30 – 9 pm**

### **2a: TwitTAIR: Discover what's happening right now at TAIR!**

#### **Room location: Union Theater**

Workshop Organizers: Philippe Lamesch, Eva Huala, Donghui Li (TAIR)

The Arabidopsis Information Resource (TAIR; <http://www.arabidopsis.org>) is a comprehensive web resource of Arabidopsis biology for plant scientists. TAIR curates and integrates information about genes, proteins, gene expression, mutant phenotypes, biological materials such as DNA and seed stocks, genetic markers, genetic and physical maps, biochemical pathways, genome organization, images of mutant plants, protein sub-cellular localizations, and publications. Gene product function data is updated

every two weeks from the latest published research literature and community data submissions. Gene structures are updated once a year using computational and manual methods as well as community submissions of new and updated genes. TAIR also provides extensive link-outs from our data pages to other *Arabidopsis* resources.

In the first part of this workshop we will focus on the new TAIR genome release TAIR10 as well as on some of the newer TAIR tools. In the second part, we will explain how to access the most up-to-date functional information provided by TAIR and how to use this information for the analysis of your own dataset. Finally, we will talk about TAIR's community annotation projects and the progress made in TAIR's journal collaboration efforts. During the last half hour of the workshop, we encourage TAIR users to ask questions about TAIR's tools and datasets and to make suggestions on how to improve TAIR to best serve the community.

#### **Program**

- 7:30 pm **Philippe Lamesch** (TAIR, Carnegie Institution for Science, Stanford, CA, USA): Gene structure curation at TAIR. TAIR10 genome release. Overview and demo of the newest analysis tools at TAIR.
- 7:55 pm **Donghui Li** (TAIR, Carnegie Institution for Science, Stanford, CA, USA): Gene function annotation at TAIR. How to access gene function information provided by TAIR.
- 8:20 pm **Donghui Li** (TAIR, Carnegie Institution for Science, Stanford, CA, USA): Community annotation. Coupling data submission with publication - TAIR's innovative journal collaboration effort (how it works, progress).
- 8:30 pm **Curator-user interactive session.** Discussions on TAIR's tools and datasets; suggestions for future improvement.

#### **2b: Receptor Ligands Regulating Plant Development**

##### **Room location: 3650 Humanities**

Workshop Organizers: Melinka Butenko (University of Oslo, Norway), Rüdiger Simon (Heinrich-Heine University Düsseldorf, Germany), Yvonne Stahl (Heinrich-Heine University Düsseldorf, Germany)

All multicellular organisms have evolved mechanisms to perceive and respond to extracellular chemical signals, including endogenous hormones and small peptides. In animals, peptide ligands represent a very important class of signaling molecules and perception is mediated by receptor tyrosine and serine/threonine kinases. Substantial evidence accumulated in the last decades show that plants also use receptor-like (RLK) serine/threonine and tyrosine proteins for intercellular communication. The *Arabidopsis* genome contains more than 400 genes encoding RLKs, there is however, scarce data on receptor-ligand systems where either genetic and/or biochemical evidence supports specific receptor-ligand interaction. Given that fundamental processes in plants are regulated by small peptide ligands it is important to promote further studies in this field. In this workshop we would like to highlight recent developments in receptor-ligand signaling, present new methodology developed to detect receptor-ligand interactions and direct attention to possible cross-talk between plant hormones and peptide ligand signaling.

#### **Program**

- 7.30 pm **Rüdiger Simon** (Heinrich-Heine University Düsseldorf, Germany): Welcome and introduction to receptor ligands regulating plant development
- 7.35 pm **Ive de Smet** (University of Nottingham, United Kingdom): Formative cell divisions in the *Arabidopsis* root - peptide hormones in control
- 7.50 pm **Melinka Butenko** (University of Oslo, Norway): Peptide ligands regulating cell separation in *Arabidopsis*
- 8 pm **Eugenia Russinova** (VIB Department of Plant Systems Biology, Gent, Belgium): Receptor endocytosis and brassinosteroid signaling in plants
- 8.15 pm **Jin Suk Lee** (University of Washington, USA): Receptor-Receptor and Ligand-Receptor Interactions Controlling Stomatal Patterning in *Arabidopsis* **Abstract #96**
- 8.30 pm **Ana Fernandez** (VIB Department of Plant Systems Biology, Gent, Belgium): Characterization of the GLV secretory peptides family **Abstract #97**
- 8.45 pm **Yvonne Stahl** (Heinrich-Heine University Düsseldorf, Germany): Ligand Receptor Interactions Involved in Stem Cell Maintenance Studied by Advanced Fluorescence Techniques **Abstract #98**
- 8.55 pm Discussion and Questions

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#### **Saturday, June 25: 2 – 3:30 pm**

#### **3a: RNA-level gene regulation**

##### **Room location: Union Theater**

Workshop Organizer: Albrecht von Arnim (University of Tennessee Knoxville, USA)

Much of gene regulation occurs at the level of RNA. Aside from 'transcription initiation' and 'RNA turnover by small RNAs', which are commonly considered, there exists a large number of additional molecular processes, such as polyadenylation, alternative

splicing, editing, translational control, and various modes of RNA degradation. Collectively, these processes are now attracting a considerable amount of attention by Arabidopsis researchers. Yet again, Arabidopsis is proving to be an excellent model system for their detailed investigation. This workshop will present emerging data on gene regulation at the RNA level, with an emphasis on agents other than small RNAs, which are being covered by another symposium at ICAR XXII.

### Program

- 2:00 pm **Albrecht von Arnim**: (University of Tennessee Knoxville) Welcome and introduction to RNA level gene regulation.  
2:00 pm **Dorothee Staiger** (University of Bielefeld, Germany): The RNA-binding Protein AtGRP7 - a Key Post-transcriptional Regulator at the Intersection between Biological Timing and Stress Responses **Abstract 99**  
2.15 pm **Yukio Kurihara** (Salk Institute for Biological Studies): Control of non-coding RNA processing **Abstract 100**  
2.30 pm **Matthew Willmann** (University of Pennsylvania): The use of high-throughput sequencing technologies to identify the substrates of RNA-dependent RNA polymerases in Arabidopsis  
2.45 pm **Ann Loraine** (University of North Carolina): Share and visualize RNA-Seq and other genome-scale data sets using Integrated Genome Browser  
3.00 pm **Anna Stepanova** (North Carolina State University): Translational regulation of hormonal responses in Arabidopsis  
3.15 pm **Reed Sorenson** (University of California, Riverside): mRNAs aggregate in AtUBP1C-granules during oxygen deprivation **Abstract 101**

### 3b: Hormone cross-talk: gene families and phenotypes

#### Room location: 3650 Humanities

Workshop Organizer: Mary Wildermuth (University of California, Berkeley, USA), [mwildermuth@berkeley.edu](mailto:mwildermuth@berkeley.edu)

In Arabidopsis, we observe extensive crosstalk between hormone signaling and response pathways. This cross-talk coordinates development, growth, flowering time, and (a) biotic stress responses. Crosstalk can occur at many levels including the modulation of active hormone levels, receptor and signaling pathways, and transcriptional regulators. The goal of this workshop is to coordinate the efforts of Arabidopsis researchers working on hormones and the processes mediated by those hormones to gain a more complete view of their integrated pathways of response.

The workshop consists of short hormone overviews, with examples given of specific gene families/family members mediating hormone crosstalk. In the moderated discussion, all attendees will help to (1) include (perhaps unpublished) phenotypic information associated with these genes in an integrated excel worksheet, to (2) prioritize key additional assays that should be performed for a gene or gene family, and (3) identify common themes associated with hormone crosstalk. I apologize that not all hormones could be highlighted due to time limitations.

### Program

- 2:00 pm **Mary Wildermuth** (PI), UC Berkeley: Introduction and Overview - Salicylic Acid  
2:15 pm **Julian Schroeder** (PI), UC San Diego: Overview – Abscisic acid  
2.25 pm **Javier Moreno** (Postdoc, Howe Lab), Michigan State University: Overview - Jasmonic Acid  
2.35 pm **Jose Alonso** (PI), North Carolina State University: Overview - Auxin and Ethylene  
2.50 pm **Yanhui Yin** (PI), Iowa State University: Overview – Brassinosteroids  
3:00 pm **Moderated Workshop Discussion**: Filling in the gaps in hormone cross-talk: Genes and Phenotypes

- 1 **Suppression of plant immunity by Phytophthora effectors**  
Sophien Kamoun
- 2 **Using genetics and structural biology to dissect the molecular mechanisms of BR perception and signaling**  
Chory, Joanne
- 3 **Biogenesis and functions of plant microRNAs**  
Yun Ju Kim, Lijuan Ji, Shengben Li, Xigang Liu, Rae Yumul, Xuemei Chen
- 4 **Epigenomic Variation in Plants (and People)**  
Joseph Ecker
- 5 **Imprinted expression of Pol IV-dependent siRNAs**  
Rebecca Mosher
- 6 **Stability of Resistance proteins is controlled by SCF-mediated protein degradation**  
Yu Cheng, Yingzhong Li, Shuai Huang, Yan Huang, Xinnian Dong, Yuelin Zhang, Xin Li
- 7 **From plant-pathogen interactions to plant-microbe communities**  
Davide Bulgarelli, Klaus Schläppi, Nahal Ahmadinejad, Emiel ver Loren Van Themaat, Matthias Rott, Paul Schulze-Lefert
- 8 **Understanding plant-microbe interactions: Plant immune system function and rhizosphere metagenomics**  
Jeff Dangl
- 9 **Morphological and Functional Identity of Organelles of the Early Plant Secretory Pathway**  
Federica Brandizzi
- 10 **Light-induced chloroplast movements in leaf cells**  
Roger Hangarter
- 11 **Structural basis of brassinosteroid perception by a membrane receptor kinase**  
Michael Hothorn, Youssef Belkadir, Marlene Dreux, Tsegaye Dabi, Joseph Noel, Ian Wilson, Joanne Chory
- 12 **NIMA-related Kinases Redundantly Regulate Directional Cell Expansion in *Arabidopsis thaliana***  
Hiroyasu Motose, Kaori Yoshimoto, Yuichiro Takahashi, Tatsuya Sakai, Taku Takahashi
- 13 **The Role of SKIP3, a Novel Plant-Specific Endosomal Protein, in Plant Development and Brassinosteroid Signaling**  
Francisca Reyes, Rafael Buono, Marisa Otegui
- 14 **Slow Trafficking of *Arabidopsis* ATP-Binding Cassette Protein Subfamily B4 Indicates Its Basal Auxin Efflux Function in the Plasma Membrane**  
Misuk Cho, Jiwon Lee, Minsoo Lee, Hyung-Taeg Cho
- 15 **Degradation of the Endoplasmic Reticulum by Autophagy during ER stress in Plants**  
Yimo Liu, Junmarie Soto Burgos, Diane Bassham
- 16 **The Go-Between: Auxin as a mediator of cell-cell signalling**  
Veronica Grienesen
- 17 **A Computational Model of *Arabidopsis thaliana* Leaf Margin Development**  
Przemyslaw Prusinkiewicz, Adam Runions, Gemma Bilsborough, Michalis Barkoulas, Miltos Tsiantis
- 18 **Novel and Known Post-transcriptional Regulatory Sequences are Conserved across Plant Families**  
Justin Vaughn, Bijoyita Roy, Albrecht von Arnim

**19 Cis-Regulatory Code of Stress Responsive Transcription in *Arabidopsis thaliana***

Cheng Zou, Kelian Sun, Joshua Mackaluso, Alexander Seddon, Rong Jin, Michael Thomashow, Shin-Han Shiu

**20 Insights into Systems Organization, Network Evolution, and Pathogen Attack from a High-Quality *Arabidopsis* Interactome Network Map**

Pascal Braun, Jim Beynon, Jeffery Dangl, Joseph Ecker, Marc Vidal, The Arabidopsis Interactome Mapping Consortium

**21 Repeat Conservation Mapping of Leucine-Rich Repeat Domains**

Laura Helft, Vignyan Reddy, Xiyang Chen, Teresa Koller, Rishabh Gupta, Andrew Bent

**22 Accurate Sequencing of 18 Genomes of *Arabidopsis thaliana* and Its Use in Imputing the Genome Sequences of Over 600 MAGIC Recombinant Inbred Lines**

Xiangchao Gan, Jonas Behr, Joshua Steffen, Katie Hildebrand, Lorraine Allchin, Leo Goodstadt, Oliver Stegle, Philipp Drewe, Rune Lyngsoe, Vipin Sreedharan, Edward Osborne, Chris Toomajian, Paula Kover, Gunnar Rätsch, Richard Clark, Richard Mott

**23 Integrating metal uptake and distribution in plants**

Mary Lou Guerinot

**24 The Role of a Zinc Finger Protein in Photosynthesis and Light Stress Tolerance**

Yan Lu, David Hall, Robert Last

**25 Uncovering Novel Signaling Interactions in Regulation of the Plant Metabolic Networks**

Ling Li, Marah Hoel, Eve Wurtele

**26 Cytochrome P450 CYP94B3 Mediates Catabolism and Inactivation of Jasmonate**

Gregg Howe, Abraham Koo

**27 "Rooting" *YUCCA* Genes in the Auxin Biosynthetic Pathway**

Anna Stepanova, Jeonga Yun, Jose Alonso

**28 Mechanisms of BAK1-Dependent Signalling**

Benjamin Schwessinger, Milena Roux, Freddy Boutrot, Catherine Albrecht, Vardis Ntoukakis, Yasuhiro Kadota, Cecile Segonzac, Man-ho Oh, Selena Gimenez-Ibanez, Jacqueline Monaghan, Frederikke Malinovsky, Jan Sklenar, John Rathjen, Delphine Chinchilla, Steven Huber, Alexandra Jones, Sacco de Vries, Cyril Zipfel

**29 Photosynthetic Carbon Assimilation in *Arabidopsis thaliana***

Stéphanie Arrivault, Marek Szecowka, Daniel Vosloh, Manuela Guenther, Alisdair Fernie, Mark Stitt

**30 Haploid *Arabidopsis thaliana*: Power Tools for Plant Genetics**

Simon Chan, Maruthachalam Ravi, Mohan Marimuthu, Sylvie Jolivet, Imran Siddiqi, Raphael Mercier

**31 Proteome Dynamics Indicate That PAMP-Triggered and Effector-Triggered Signaling Converge Early**

Chris van Schie, Tenai Eguren, Zhouxin Shen, Steven Briggs

**32 Identifying protein-small molecule interactions using functional protein microarrays coupled with a photoactivated crosslinked ligand**

Magali Moreau, Giulio Zampogna, Daniel Klessig, Sorina Popescu

**33 Base-Resolution Population Epigenomic Variation**

Bob Schmitz, Mark Urich, Mattia Pelizzola, Matthew Schultz, Mathew Lewsey, Joe Nery, Andrew Alix, Joseph Ecker

**34 Exploring the *Arabidopsis* genome with single molecule PacBio sequencing**

Mitchell Sudkamp, Xuefeng Zhou, Zhaolong Li, Randy Kerstetter, Wei Wu, Todd Michael

**35 High-Throughput Recombineering and Its Applications for *Arabidopsis* Gene Function Characterization**

Jose Alonso, Rongrong Zhou, Larissa Benavente, Anna Stepanova, Miguel Perez-Amador

- 36 The Application of Atomic Force Microscopy as a Micro-Force Sensor: Probing the Mechanics of Living Plant Cell Walls During Development**  
Siobhan Braybrook, Laurent Le Guillou, Emeric Bron, Cris Kuhlemeier, Herman Höfte, Alexis Peaucelle
- 37 Arabidopsis Stem Cells in Development and Regeneration**  
Elliot Meyerowitz
- 38 SCARECROW Sustains Stem Cell Activity Inhibiting Cytokinin Dependent Cell Differentiation Input**  
Laila Moubayidin, Di Mambro Riccardo, Pacifici Elena, Terpstra Inez, Perilli Serena, Dello Ioio Raffaele, Heidstra Renze, Costantino Paolo, and Sabrina Sabatini
- 39 An integrated circuit for asymmetric cell division in Arabidopsis roots**  
Ben Scheres
- 40 Hormonal control of shoot branching**  
Ottoline Leyser
- 41 The interaction of the cytokinin-regulated phosphorelay with other signals in Arabidopsis**  
Joseph Kieber
- 42 Structural Mechanism of Jasmonate Perception**  
Laura Sheard, Xu Tan, Haibin Mao, John Withers, Thomas Hinds, John Browse, Sheng Yang He, Gregg Howe, Ning Zheng
- 43 CER7, a Core Subunit of the RNA-processing Exosome, has a Specific Role in Regulation of Cuticular Wax Deposition in Arabidopsis**  
Ljerka Kunst, Tanya Hooker, Patricia Lam, Lifang Zhao
- 44 The Role of Cell Wall Synthesis and Remodelling in Organ Growth**  
Volker Bischoff, Herman Höfte
- 45 A Functional Screen for Nucleotide Sugar Transporters**  
Katy Christiansen, Jun Ito, Berit Ebert, Dominique Loque, Joshua Heazlewood
- 46 The Arabidopsis FLYING SAUCERS Gene Encodes a Membrane Protein Required for Connections to the Cell Wall**  
Catalin Voiniciuc, Gillian Dean, Jonathan Griffiths, George Haughn
- 47 The Effects of Plant Cell Wall Alterations on Plant Disease Susceptibility**  
Gerit Bethke, Le Nguyen, Rachael Grundman, Fumiaki Katagiri, Jane Glazebrook
- 48 Subcellular Partitioning of Plant Cell Wall Biosynthesis in Arabidopsis**  
Harriet Parsons, Jun Ito, Joshua Heazlewood
- 49 Combining Molecular Genetics and Mass-Spectrometry-Based High-Resolution Metabolite Imaging to Unravel the Surface Lipids of Arabidopsis**  
Basil Nikolau, Young-Jin Lee, Zhihong Song, Geng Ding, Daolin Cheng, Xiaobin Zheng, Ji Hyun Jun
- 50 Identification of Plant Clock Genes Using Functional Genomics**  
Stacey Harmer, Matthew Jones, Nozomu Takahashi, Polly Hsu, Reetika Rawat, Michael Covington, Luciano DiTacchio, Christopher Vollmers, Satchidananda Panda, Jacob Schwartz, Michelle Salemi, Brett Phinney
- 51 Uncovering clock transcriptional circuits by functional genomics**  
Jose Pruneda-Paz
- 52 Growth Promoting Factors Have Distinct Effects on Seedling Growth Dynamics**  
Jodi Stewart, Christopher Gee, Julin Maloof, Jennifer Nemhauser

- 53 Antagonistic Regulation of Photomorphogenesis by Oppositely Acting bHLH Transcription Factors in *Arabidopsis***  
Ling Zhu, Hui Shen, Jonathan Dang, Enamul Huq
- 54 A Circadian Complex is Critical for Growth Control in *Arabidopsis***  
Dmitri Nusinow, Anne Helfer, Elizabeth Hamilton, Jasmine King, Takato Imaizumi, Thomas Schultz, Eva Farre, Steve Kay
- 55 Alternative Splicing Mediates Responses of the *Arabidopsis* Circadian Clock to Temperature Changes**  
Naeem Syed, Allan James, Jacqueline Marshall, Gillian Nimmo, Gareth Jenkins, Paweł Herzyk, Hugh Nimmo, John Brown
- 56 Translational Control: a New Dimension in the Regulation of *Arabidopsis* Photomorphogenesis**  
Ming-Jung Liu, Szuhsien Wu, Ho-Ming Chen, Shu-Hsing Wu
- 57 Single Gene Mutations Causing Heterosis in Tomato**  
Ke Jiang, Uri Krieger, Soon-ju Park, Dani Zamir, Zachary Lippman
- 58 Translation of submergence tolerance from the gene to the field using rice and *Arabidopsis***  
Julia Bailey-Serres, Julián Peña-Castro, Seung Cho Lee, Takeshi Fukao
- 59 Localization of PIN1-Like Proteins in Grasses Suggests a Functional Specialization of Different PINs into 'Up-the-Gradient' and 'With-the-Flux' Modes of Auxin Transport**  
Devin O'Connor, Jennifer Bragg, John Vogel, Connie Lee, Sarah Hake
- 60 SAMBA- A new subunit of the Anaphase Promoting Complex (APC/C) with an essential role in plant growth and pollen development in *Arabidopsis***  
Nubia Eloy, Jelle Van Leene, Paulo Ferreira, Geert De Jaeger, Dirk Inze
- 61 Modeling of the Plant Hormone Signaling Network in MAMP-Induced Resistance**  
Kenichi Tsuda, Yungil Kim, Masanao Sato, Chad Myers, Jane Glazebrook, Fumiaki Katagiri
- 62 German Plant Research Goes BioEconomy**  
Dirk Büssis
- 63 Genomic Dissection of the Plant/Pest Interaction: Transcriptome Analysis of *Arabidopsis* Response to *Tetranychus urticae* (Two Spotted Spider Mite) Feeding**  
Marie Navarro, Gustavo Acevedo, Marc Cazaux, Johannes Mathieu, Marcus Schmid, Miodrag Grbic, Vojislava Grbic
- 64 Regulation of *Arabidopsis* Gynoecium Polarity by ULT and KAN Family Proteins**  
Jennifer Fletcher
- 65 The Endodermis - building a selective and polarised cellular barrier**  
Niko Geldner
- 66 An apical root growth program directed in the vascular stem cells**  
Jose Sebastian, Jing Zhou, Ji-Young Lee
- 67 Spatio-Temporal Sequence of Cross-Regulatory Events in Root Meristem Growth**  
Emanuele Scacchi, Paula Salinas, Bojan Gujas, Luca Santuari, Naden Krogan, Laura Ragni, Thomas Berleth, Christian Hardtke
- 68 Expanding the Genetic Pathway for Polarity in *Arabidopsis* Leaf Development**  
Brenda Reinhart, Tie Liu, Niki Newell, Tengbo Huang, Randall Kerstetter, M. Kathryn Barton
- 69 HD-Zip II Transcription Factor Genes Control Adaxial-Abaxial Patterning in *Arabidopsis* Leaf Morphogenesis**  
Monica Carabelli, Luana Turchi, Massimiliano Sassi, Marco Possenti, Valentino Ruzza, Carmen Melatti, Giorgio Morelli, Ida Ruberti

- 70 GRD and WOX Genes Cooperatively Promote Developmental Asymmetry in Arabidopsis Embryos**  
Sangho Jeong, Wolfgang Lukowitz
- 71 Mapping local adaptation in Arabidopsis thaliana**  
Johanna Schmitt, Amity Wilczek, Alexandre Fournier-Level, Arthur Korte, Martha Cooper, Magnus Nordborg
- 72 Using Arabidopsis relatives as sources of natural genetic variation in regulatory networks**  
George Coupland
- 73 Quantitative variation in the circadian clock and adaptation to heterogeneous settings**  
Weinig C, CE Edwards, MT Brock, MJ Rubin, BE Ewers, L Ping , CR McClung
- 74 Systems Biology to Dissect Nitrogen Regulatory Networks**  
Rodrigo Gutiérrez
- 75 Mapping Spatiotemporal Gene Regulatory Networks in the Arabidopsis Root Stele**  
Siobhan Brady, Mallorie Taylor-Teeple, Allison Gaudinier, Lifang Zhang, John Reece-Hoyes, Sebastian Ahnert, A. J. Marian Walhout, Doreen Ware
- 76 Properties and structure of the plant immune signaling**  
Kenichi Tsuda, Masanao Sato, Yungil Kim, Jane Glazebrook, Chad Myers and Fumiaki Katagiri
- 77 Chemical Genetics Reveals Negative Regulation of Abscisic Acid Signaling by a Type III Effector Signaling Pathway**  
Tae-Houn Kim, Felix Hauser, Tracy Ha, Shaowu Xue, Maik Böhmer, Noriyuki Nishimura, Katharine Hubbard, Nora Peine, Stephen Lee, Nadia Robert, Jane Parker, Julian Schroeder
- 78 Regulatory gene network in stress responses to drought conditions**  
Kazuo Shinozaki, Kazuko Yamaguchi-Shinozaki
- 79 Moisture locally induces ABA biosynthesis to determine growth direction in Arabidopsis roots**  
José Dinneny, Yun Bao
- 80 Clock Components CCA1 and LHY Regulate Expression of the CBF Cold Response Pathway and Freezing Tolerance in Arabidopsis**  
Malia Dong, Eva Farre, Michael Thomashow
- 81 Heme Synthesis By Plastid Ferrochelatase I Regulates Nuclear Gene Expression**  
Jesse Woodson, Juan Perez-Ruiz, Joanne Chory
- 82 POPEYE, BRUTUS and Other Characters: Elucidating Molecular Mechanisms of the Iron Deficiency Response in Plants**  
Durreshah Muhammad, Imrose Kauser, Lujaina Farooq, Ahmad Noweder, Terri Long
- 83 The role of calcium signaling in the molecular response network to anaerobic stress in Arabidopsis**  
WON-GYU CHOI
- 84 Stomatal development: signaling fate and renewal**  
Dominique Bergmann
- 85 Cell specification and cell communication in embryonic root formation**  
Dolf Weijers
- 86 Cytokinin Inhibitory Fields control Phyllotaxis**  
Fabrice Besnard, Yassin Refahi, Benjamin Marteaux, Valerie Morin, Pierre Chambrier, Jonathan Legrand, Geraldine Brunoud, Etienne Farcot, Coralie Cellier, Pradeep Das, Anthony Bishopp, Ykä Helariutta, Christophe Godin, Jan Traas, Yann Guédon, Teva Vernoux
- 87 A Transcriptional Auxin Response Gradient in the Arabidopsis Root**  
Bastiaan Bargmann, Gabriel Krouk, Tal Nawy, Idan Efroni, Kenneth Birnbaum

- 88 LEAFY Target Genes Reveal Floral Regulatory Logic, cis Motifs, and a Link to Biotic Stimulus Response**  
*Cara Winter, Ryan Austin, Servane Blanvillain-Baufume, Maxwell Reback, Marie Monniaux, Miin-Feng Wu, Yi Sang, Ayako Yamaguchi, Nobutoshi Yamaguchi, Jane Parker, Francois Parcy, Shane Jensen, Hongzhe Li, Doris Wagner*
- 89 Contributions of the Maternal, Paternal and Zygotic Genomes during Early Plant Embryogenesis**  
*Michael Nodine, David Bartel*
- 90 RUG8, a Novel Player in Auxin-Dependent Stem Cell Specification and Meristem Patterning in Arabidopsis Roots**  
*Ulrich Wenig, Stefan Meyer, Ruth Stadler, Norbert Sauer*
- 91 Gramene: It's Not Just For Grasses Anymore**  
*Joshua Stein, Ken Youens-Clark, Aaron Chuah, Genevieve DeClerck, Sharon Wei, William Spooner, Terry Casstevens, Jim Thomason, Jon Zhang, Charles Chen, AS Karthikeyan, Palitha Dharmawardhana, Marcela Monaco, Pankaj Jaiswal, Edward Buckler, Susan McCouch, Doreen Ware*
- 92 Integrating Diverse Data And Knowledge In A Large Collaborative Project**  
*Sean Walsh, Katja Baerenfaller, Matthias Hirsch-Hoffmann, Agron-omics Consortium Data Contributors, Pierre Hilson, Wilhelm Gruissem*
- 93 Integrating Proteomics Data: pep2pro, MASCP Gator and Combined Analyses with Transcript Data**  
*Katja Baerenfaller, Matthias Hirsch-Hoffmann, Sean Walsh, Lars Hennig, Daniel Stekhoven, Sacha Baginsky, Wilhelm Gruissem*
- 94 Discriminative Expression Signatures In Microarray Data For Functional Network Inference**  
*Tomas Puelma, Alvaro Soto, Rodrigo Gutiérrez*
- 95 Hypothesis Generation in Plant Biology Using Large Data Sets**  
*Nicholas J. Provart*
- 96 Receptor-Receptor and Ligand-Receptor Interactions Controlling Stomatal Patterning in Arabidopsis**  
*Jin Suk Lee, Takeshi Kuroha, Marketa Hnilova, Dmitriy Khatayevich, Jessica McAbee, Mehmet Sarikaya, Candan Tamerler, Keiko Torii*
- 97 Characterization Of The GLV Secretory Peptides Family**  
*Ana Fernandez, Andrzej Drozdzecki, Anh Nguyen, Kurt Hoogewijs, Rebecca De Clercq, Annemieke Madder, Pierre Hilson*
- 98 Ligand Receptor Interactions Involved in Stem Cell Maintenance Studied by Advanced Fluorescence Techniques**  
*Yvonne Stahl, Stephanie Grabowski, Claus A. M. Seidel, Rüdiger Simon*
- 99 The RNA-binding Protein AtGRP7 - a Key Post-transcriptional Regulator at the Intersection between Biological Timing and Stress Responses**  
*Dorothee Staiger, Martina Lummer, Tino Koester, Corinna Streitner, Christin Korneli, Matthias Wiedenluebbert, Fabian Humpert, Mark Schuettpelz, Markus Sauer, Craig Simpson, John Brown*
- 100 Control of Non-coding RNA Processing**  
*Yukio Kurihara, Ben Adamczyk, Motoaki Seki, Joseph Ecker*
- 101 mRNAs Aggregate In AtUBP1C-Granules During Oxygen Deprivation**  
*Reed Sorenson, Julia Bailey-Serres*

**102 Photosynthetic response of *Arabidopsis thaliana* to drought stress.**

Cyril Abadie, Jean-Michel Perault, Vincent Lebeurre, Rémi Lemoine, Jean-Philippe Biolley

**103 CPL1 is a Key Regulator of Iron Homeostasis in *Arabidopsis***

Emre Aksoy, Hisashi Koiba

**104 Expression and Localization of Metal-Responsive Proteins**

Shannon Alford, Steven Richardson

**105 A Genome-Wide Network Model Capturing Seed Germination Reveals Coordinated Regulation of Plant Cellular Phase Transitions**

George Bassel, Hui Lan, Enrico Glaab, Daniel Gibbs, Tanja Gerjets, Natalio Krasnogor, Anthony Bonner, Michael Holdsworth, Nicholas Provart

**106 Differential Impact of Lipoxygenase 2 and Jasmonates on Natural and Stress-induced Senescence in *Arabidopsis***

Martin Seltmann, Nadja Stingl, Markus Krischke, Martin Mueller, Susanne Berger

**107 Expression changes of *Arabidopsis isochorismate synthase 1* during development and abiotic stress**

Feng Yi Cao, Wolfgang Moeder, Kimberly Gao, William Urquhart, Darrell Desveaux, Keiko Yoshioka

**108 Differential Alternative Polyadenylation Of A Photosynthesis Related Gene *LHCB4.1* In *Arabidopsis* Mutant *oxt6***

JIE CHEN, QINGSHUN LI, XIAOHUI WU

**109 Exploring Metal Homeostasis Using Ionomics**

Heng-Hsuan Chu, Joe Morrissey, Ivan Baxter, Brett Lahner, David Salt, Mary Lou Guerinot

**110 Pause-and-Stop - The Effects of Osmotic Stress on Cell Proliferation During Early Leaf Development in *Arabidopsis***

Aleksandra Skirycz, Hannes Claeys, Stefanie De Bodt, Akira Oikawa, Shoko Shinoda, Megan Andriankaja, Katrien Maleux, Nubia Eloy, Frederik Cappens, Sang-Dong Yoo, Kazuki Saito, Dirk Inzé

**111 Control of cell death by metacaspases**

Nuria Coll, Petra Epple, Andrea Smidler, Charles Clover, Dominique Vercammen, Frank Van Breusegem, Jeff Dangl

**112 Transcription Regulatory Networks Involved in the Abiotic Stress Response of *Arabidopsis thaliana***

Inge De Clercq, Vanessa Vermeirissen, Thomas Van Parys, Yves Van de Peer, Frank Van Breusegem

**113 The *Arabidopsis* CALCINEURIN B-LIKE Protein Mediates Flower Development during Plant Growth in Saline Conditions**

Margaret Dietrich, Shea Monihan, Hugues Renault, Karen Schumaker

**114 Caesium enrichment is dependent on a conserved protein of the secretory system in yeast and plants**

Stephan Dräxl, Ulrike Kanter, Anton Schäffner

**115 Regulation of Stomatal Development by Carbon Dioxide**

Cawas Engenier, Honghong Hu, Amber Ries, Julian Schroeder

**116 A gene regulatory network based on RNA silencing control anthocyanin biosynthesis under high light**

Maïna Floris, Elodie Lanet, Christophe Robaglia

**117 Variation in Methyl Viologen Tolerance in *Arabidopsis* Accessions**

Miki Fujita, Yasunari Fujita, Satoshi Iuchi, Yuriko Kobayashi, Masatomo Kobayashi, Kazuko Yamaguchi-Shinozaki, Kazuo Shinozaki

- 118 Damaged DNA Binding Protein 1b (DDB1b) - DDB1a Interactions during *Arabidopsis* Development and Abiotic Stress Response**  
Ashwin Ganpudi, Dana Schroeder
- 119 Abiotic Stress Regulation of the At3G02400 Gene in *Arabidopsis thaliana***  
Will Gray III, David Chevalier
- 120 Investigation of SR45a Alternative Splicing Using Integrated Genome Browser**  
Alyssa Gullede, April Roberts, Ketan Patel, Hiral Vora, Vikram Bishnoi, Ann Loraine
- 121 Multiple Roles of WIN3 in Regulating Disease Resistance, Cell Death, and Flowering Time in *Arabidopsis***  
Guan-Feng Wang, Savanna Seabolt, Safae Hamdoun, Gina Ng, Jin Park, Hua Lu
- 122 How Do Environments Regulate Seed Quality?**  
Hanzi He, Leónie Bentsink, Henk Hilhorst
- 123 ATCSA-1 Is A Critical Factor For UV Tolerance In *Arabidopsis thaliana***  
Sascha Biedermann, Sutton Mooney, Hanjo Hellmann
- 124 Diversifying Selection in Abiotic Stress pathways in *Arabidopsis arenosa***  
Jesse Hollister, Kirsten Bomblies
- 125 Heat Induces the Splicing of bZIP60 messenger RNA by IRE1 in the Unfolded Protein Response in *Arabidopsis***  
Yan Deng, Sabrina Humbert, Jian-Xiang Liu, Renu Srivastava, Steven Rothstein, Stephen Howell
- 126 Influence of Abiotic Stress on Membrane Proteins Expression Levels in *Arabidopsis* Cell Culture**  
Radovan Hynek, Lucie Marsalova, Stepanka Kuckova, Peter Konik, Jiri Santrucek, Jan Martinec, Olga Valentova, Milan Kodicek
- 127 A Dynamic Stress Expression Map of the *Arabidopsis* root**  
Anjali Iyer-Pascuzzi, Terry Jackson, Hongchang Cui, Jalean Petricka, Wolfgang Busch, Hironaka Tsukagoshi, Philip Benfey
- 128 Functional and Evolutionary Analysis of Plant Adaptation to Salinity**  
David Jarvis, Javier Barrero-Gil, Karen Schumaker
- 129 Molecular And Genetic Characterization Of A Plastid-Specific Programmed Cell Death Pathway**  
Chanhong Kim, Keun Pyo Lee, Klaus Apel
- 130 Functional Roles of RNA-binding Proteins with RNA Chaperone Activity in Plant Response to Environmental Stresses**  
Minkyung Kim, Kyungjin Kwak, Hyunju Jung, Hunseung Kang
- 131 G x E GWAS: Detecting Gene-Environment Interaction on a Whole Genome Level**  
Arthur Korte, Bjarni Vilhjalmsson, Vincent Segura, Alex Platt, Magnus Nordborg
- 132 The Roles of NF-Y Transcription Factors in ABA Responses**  
Roderick Kumimoto, Chamindika Siriwardana, Krystal Gayler, Jan Risinger, Ben Holt III
- 133 Anti-insect capability of AtVSP is determined by its stability in the insect digestive canal**  
JIAJIN LEI
- 134 Calcium sensor proteins in the anaerobic response in *Arabidopsis***  
Ansul Lokdarshi, Won Gyu Choi, Daniel Roberts
- 135 Effects of abiotic stress on splicing patterns in *Arabidopsis thaliana***  
Ann Loraine, Alyssa Gullede, April Roberts, Ketan Patel

**136 Analysis of Malate Transporters Induced in Roots of Phosphorus Deficient or Aluminum Stressed*****Arabidopsis thaliana***Hayato Maruyama, Takayuki Sasaki, Soichi Kojima, Jun Wasaki**137 Comparative Functional Analysis of DREB2A and DREB2B in Arabidopsis**Junya Mizoi, Naomi Yasuda, Feng Qin, Kyonoshin Maruyama, Kazuo Shinozaki, Kazuko Yamaguchi-Shinozaki**138 Strand Specific Transcription in *Arabidopsis thaliana* Suspension Culture Cells Under High Salinity**Gaurav Moghe, Kelian Sun, Cheng Zou, Shin-Han Shiu**139 Post-transcriptional Regulation of the CBL10 Calcium Sensor is Critical for *Arabidopsis* Growth in Saline Conditions**Shea Monihan, YongSig Kim, Ramin Yadegari, Karen Schumaker**140 Three SnRK2 Protein Kinases Involved in ABA Signaling Function for the Control of Seed Dormancy and Abiotic Stress Tolerance in *Arabidopsis***Kazuo Nakashima, Yasunari Fujita, Kyonoshin Maruyama, Kazuo Shinozaki, Kazuko Yamaguchi-Shinozaki**141 The Study of the Structure and Function of *Arabidopsis* Gene *ICE2* Using Over Expresses Transgenic Lines**Maria Novokreshchenova**142 Roles of *Arabidopsis* Chloroplast Localized Molecular Chaperone Hsp90 in the Regulation of Plant Development and Abiotic Stresses**Sae-hong Oh, Bhavank Shah, Yao Wang, Rongmin Zhao**143 Functional Analysis of B-class Heat Shock Transcription Factors in *Arabidopsis***Naohiko Ohama, Takumi Yoshida, Junya Mizoi, Kazuo Shinozaki, Kazuko Yamaguchi-Shinozaki**144 SPL Transcription Factor Interacts With Activated Immune Receptors To Regulate Plant Immune Response**Meenu Padmanabhan, Shisong Ma, Savithramma Dinesh-Kumar**145 Development of transgenic near-isogenic rice lines harboring fungal resistance gene (OgPR1) from wild rice (*Oryza grandiglumis*)**Jung-Hun Pak**146 Generation of Transgenic Chinese Cabbage expressing *Arabidopsis* AVP1 H<sup>+</sup>-PPase**Meheo Park, Heeyeon Won, Jeungsul Han, Yulkyun Ahn, Jungho Kim, Hyeeun Lee, Myeongcheoul Cho**147 The Morphometric Landscape Of Root Architectural Plasticity**Daniela Ristova, Ulises Rosas, Gabriel Krouk, Kenneth Birnbaum, Gloria Coruzzi**148 Light signaling mediates cold acclimation response in *Arabidopsis***Rafael Catala, Joaquin Medina, Julio Salinas**149 Cytokinins Increase Flower Fertility and Fruit Set Under Non-Permissive High Temperatures**Ron Salzman, Lance Beem, Albert Liptay, Jerry Stoller**150 The HyPRP gene *EARLI1* has an auxiliary role for germinability and early seedling development under low temperature and salt stress conditions**Dan Xu, Xuan Huang, Zi-Qin Xu, Michael Schlappi**151 The *Arabidopsis* Homologues of the Yeast *rei1* and *reh1* Genes**Stefanie Schmidt, Joachim Kopka**152 Determining a possible role of EDR1 in autophagy**Irene Serrano, Yangnan Gu, Roger Innes

**153 The Role of Arabidopsis NF-YA Transcription Factors in Regulating Abscisic Acid Mediated Drought Responses**

Chamindika Siriwardana, Roderick Kumimoto, Ben Holt III

**154 A vacuole localized  $\beta$ -glucosidase contributes to drought tolerance in *Arabidopsis***

Pengtao Wang, Hao Liu, Hongjie Hua, Chun-Peng Song

**155 Arabidopsis DRGs: Ribosome Association, Interacting Partners, Association with Heat Stress Granules, and Possible Involvement in Translation Initiation**

Joel Stafstrom, Benjamin Nelson, Jennifer Kubic

**156 Engineering the Coenzyme Specificity and Redox Sensitivity of Two Stress-responsive Aldehyde Dehydrogenase Isozymes of *Arabidopsis thaliana***

Naim Stiti, Hans-Hubert Kirch, Dorothea Bartels

**157 MicroRNAs Associated with Environmental Stress in *Arabidopsis Thaliana***

Shawn Thatcher, Dong-Hoon Jeong, Brown Rebecca, Jixian Zhai, Blake Meyers, Pamela Green

**158 Arabidopsis Damage Associated Molecular Pattern Peptide 1 (AtPep1) and Its Receptors PEPR1 and PEPR2 are Involved in Osmotic Stress Response**

Mahmut Tör, Nicholas Holton

**159 Immunity-Related Members Of The DMR6 Family Of Oxidoreductases In Arabidopsis**

Guido Van den Ackerveken, Tieme Zeilmaker, Joyce Elberse, Nora Ludwig

**160 Unique drought signaling roles of the uncharacterized clade A protein phosphatase 2Cs HAI1, AIP1 and HAI3**

Bhaskara Badiger, Paul Verslues

**161 Cold Days and Warm Nights Induce Flowering by Enhancing *FT* and *SOC1* Expression in *Arabidopsis thaliana***

Micael Wendell, Sissel Torre, Jorunn Olsen

**162 The Model Plant *Arabidopsis thaliana* for Metabolomic and Proteomic Phenotyping**

Christiana Staudinger, Vlora Mehmeti, Wolfram Weckwerth, Stefanie Wienkoop

**163 COPPER AMINE OXIDASE 1 (CuAO1) in *Arabidopsis thaliana* is involved in nitric oxide biosynthesis and in abscisic acid mediated stress responses**

Rinukshi Wimalasekera, Corina Villar, Tahmina Begum, Günther Scherer

**164 Abstract Withdrawn**

**165 Arabidopsis splicing factor variant controls plant growth in response to nutrient conditions**

Takeshi Yoshizumi, Hiroaki Hongo, Takashi Kuromori, Youichi Kondou, Yoko Horii, Mika Kawashima, Tomoko Kuriyama, Yoko Imura, Asako Kamiya, Hiroaki Shimada, Yuichiro Watanabe, Minami Matsui

**166 AtPHO1 Expression In Guard Cells Influence The Response Of Stomata To Abscissic Acid**

Celine Zimmerli, Cecile Ribot, Yves Poirier

**167 GABA ( $\gamma$ -aminobutyric acid) Promotes Sporulation in the Necrotrophic Fungal Pathogen *Alternaria brassicicola***

Christopher Botanga, Angel Gray, Oliver Fiehn, Jane Glazebrook

**168 PHOSPHATIDIC ACID PHOSPHOHYDROLASE1 & 2 regulate phospholipid synthesis at the ER in *Arabidopsis***

Christian Craddock, Nicolette Adams, Peter Eastmond

**169 In vitro studies of RNA-Dependent RNA polymerases involved in RNA Silencing**

Anthony Devert, Nicolas Fabre, Bruno Canard, Christophe Robaglia, Patrice Crete

**170 Molecular Bases of Fe and Mn Transport: Key Transporters and Elemental Imaging**

Hannetz Roschzttardtz, Fanchon Divol, Rémy Cailliatte, Mathilde Séguéla, Daniel Couch, Stéphane Mari, Catherine Curie

**171 Dynamics of SCF<sup>TIR1/AFB</sup> Ubiquitin Ligase**

Kai-Ting Fan, Xiao-Yuan Yang, Adrian Hegeman, Jerry Cohen, William Gray

**172 Abstract Withdrawn****173 The LON2 Protease Contributes to Continued Matrix Protein Import into Peroxisomes**

Lisa Farmer, Matthew Lingard, Bonnie Bartel

**174 Determining Cross-Species Functionality of Riboswitches**

Zohaib Ghazi, Barbara Moffatt, Yingfu Li

**175 Molecular and Biochemical Study Toward Understanding the Cellular Signaling Mechanism of AtRALF1, a Ca<sup>2+</sup> Mobilizing Peptide Hormone**

Miyoshi Haruta, Michael Sussman

**176 A Role for Glyceraldehyde-3-phosphate Dehydrogenase in Plant Innate Immunity**

Elizabeth Henry, Jun Liu, Gitta Coaker

**177 Role of Transceptor CHL1 in Nitrate Sensing**

Cheng-Hsun Ho, Shan-Hua Lin, Yi-Fang Tsay

**178 Tryptophan Metabolism in Arabidopsis: a Model for Interaction between Primary and Secondary Metabolism**

Brad Hogan, Angus Wan, Scott Mottarella, La'Kesha Francis, Carolyn Crisp, Judith Bender, John Celenza

**179 Lumen Thiol Oxidase 1 (LTO1), a novel disulfide bond catalyst at the thylakoid membrane is required for photosynthesis**

Mohamed Karamoko, Sara Cline, Kevin Redding, Patrice Hamel

**180 Subunits of the Asymmetric Plastid ClpPR Protease Complex: Mutants, Stoichiometry, Evolution and Functional Implications**

Jitae Kim, Paul Dominic Olinares, Jerrold Davis, Verenice Rodriguez, Klaas van Wijk

**181 Transcriptional regulation of the iron deficiency response in *Arabidopsis thaliana***

Sun A Kim, Mary Lou Guerinot

**182 Molecular mechanisms of boric acid transport by NIP7;1, an anther-specific Nodulin Intrinsic Protein**

Tian Li, Won Gyu Choi, Ian Wallace, Jerome Baudry, Daniel Roberts

**183 Evaluation of IBA Transport in Arabidopsis Hypocotyls by Stable Isotope Labeling and GC-MS/MS**

Xing Liu, Gary Gardner, Jerry Cohen

**184 Gene Identification of Prephenate Aminotransferase Provides Novel Insights into Plant Phenylalanine Biosynthesis**

Hiroshi Maeda, Heejin Yoo, Natalia Dudareva

**185 Characterization of an Arabidopsis Aminotransferase Reveals Cross-talk Between Phenylalanine Biosynthesis and Auxin Homeostasis**

Michael Pieck, Jason Godfrey, Margaret Carbery, Youxi Yuan, Jennifer Normanly, John Celenza

**186 Characterization of Genetic Enhancers of the Auxin-Deficient Mutant *taa1***

Linda Robles, Jose Alonso, Anna Stepanova

**187 Adaptation of *Arabidopsis thaliana* to sustained nitrogen availability in the soil**

Armin Schlereth, Hendrik Tschoep, Vanessa Wahl, Yves Gibon, Mark Stitt

**188 A Role for START Lipid/Sterol Binding Domains in Transcription**

Kathrin Schrick, Sara Marlatt, Ligaya Roque, Henry Nguyen, Cuiwen He, Gitanjali Yadav

**189 Characterizing a Biosynthetic Module for the Formation of a Novel Pathogen-Induced Phytoalexin in Arabidopsis Roots**

Reza Sohrabi, Jung-Hyun Huh, Dorothea Tholl

**190 The bZIP Transcription Factor HY5 Modulates the Circadian Expression of the Monoterpene Synthase Gene QH6**

Tian-Hu Sun, Xiao Men, Kang Dong, Shan Lu

**191 Analysis of N-End Rule Pathway Components In Arabidopsis: PRT8 is a Novel E3 Ubiquitin Ligase That Targets Proteins With Aliphatic Hydrophobic Amino Terminal Residue**

Prabhavathi Talloji, Andreas Bachmair

**192 Dissecting the Complex Phenotype of MTN-deficient Mutants**

Ishari Waduwara-Jayabahu, Natasha Peer, Markus Wirtz, Rüdiger Hell, Yasmin Oppermann, Margret Sauter, Barbara Moffatt

**193 Studies On The Arabidopsis Fatty Acid Regulators By Coexpression Analysis**

Linlin Yin, Hongwei Xue

**194 Mutations in RIG2, a Membrane E3 Ubiquitin Ligase Which Interacts With GLUTAMINE DUMPER1, Suppress gdu1-1D Phenotype**

Shi Yu, Réjane Pratelli, Damian Guerra, Mark Wogulis, Judy Callis, Guillaume Pilot

**195 The Arabidopsis CYP724A1 Gene Encodes a Functional Brassinosteroid C-22 Hydroxylase**

Rujia Zhang, Youhui Deng, Xinjie Xia, Pedro SCF Rocha

**196 AthaMap-assisted Prediction of microRNA Targets**

Lorenz Bülow, Julio Bolívar, Reinhard Hehl

**197 Exploring the molecular network of glucosinolate biosynthesis using bioinformatic tools**

Yazhou Chen, Xiufeng Yan, Sixue Chen

**198 Investigation of Gravitropism Using Auxin Mutants and Multidimensional Data Modeling**

Misuk Cho, Nathan Miller, Edgar Spalding

**199 Enhanced Y1H assays to elucidate Arabidopsis gene regulatory networks**

Allison Gaudinier, Lifang Zhang, John Reece-Hoyes, Mallorie Taylor-Teeple, Albertha Walhout, Doreen Ware, Siobhan Brady

**200 PIN Mediated Auxin Redistribution During Phototropism In *Arabidopsis* Hypocotyls**

Tim Hohm, Christian Fankhauser, Sven Bergmann

**201 VirtualPlant: A Software Platform to Support Next-Generation Systems Biology Research**

Manpreet Kataria, Rodrigo Gutierrez, Gabriel Krouk, Rebecca Davidson, Tamara Tershakovec, Dennis Sasha, Gloria Coruzzi

**202 Identification of Promoter Motifs and Constitutive and Tissue-Specific Promoters in *Glycine Max* using a Data Mining Approach**

Kathleen Keating, Matthew Hudson

**203 Elucidating the Arabidopsis Auxin Response Using Smooth Curve Regression Analysis of a Mutant Time Course Transcriptomics Data Set**

Kim Kenobi

**204 Abstract Withdrawn**

**205 Leaf Senescence Database: a comprehensive resource for plant leaf senescence research**

Zhonghai Li, Xiaochuan Liu, Jinying Peng, Wenyang Li, Wenrong He, Jingchu Luo, Hongwei Guo

**206 An Incoherent Feed Forward Loop Defines Discrete Expression Patterns During Early *Arabidopsis thaliana* Trichome Development**

Kengo Morohashi, Erich Grotewold

**207 Share And Visualize Genome-Scale Data Sets Using Integrated Genome Browser**

David Norris, Hiral Vora, Alyssa Gulledge, Michael Lawrence, Lance Froehman, Ann Loraine

**208 Robustness of Crosstalk from Transcriptomics Data in *Arabidopsis thaliana***

Nooshin Omranian, Bernd Mueller Roeber, Zoran Nikoloski

**209 Expressolog Identification in Plant Species**

Rohan Patel, Hardeep Nahal, Robert Breit, Yani Chen, Nicholas Provart

**210 High-Throughput Systematic Genetic Interaction Analysis of MAPK Signaling in *Arabidopsis***

Shih-Heng Su, Nathan Miller, Patrick Krysan

**211 A Gene Regulatory Network for Vascular Development and Secondary Cell Wall Biosynthesis in the *Arabidopsis* Root**

Mallorie Taylor-Teeple, Sebastian Ahnert, Allison Gaudinier, Siobhan Brady

**212 Suppression of Defense Responses in Distantly Related Plants by Homologous RXLR Effectors**

Ryan Anderson, Devdutta Deb, Megan Casady, Rachel Fee, Brett Tyler, John McDowell

**213 Two-Component Elements Mediate Interactions Between Cytokinin and Salicylic Acid in Plant Immunity**

Cris Argueso, Fernando Ferreira, Petra Epple, Jennifer To, Claire Hutchison, G. Eric Schaller, Jeff Dangl, Joseph Kieber

**214 *Arabidopsis thaliana* and involved in the defense response against *Pseudomonas syringae***

Grace Armijo, Consuelo García, Aldo Seguel, Luis Leon, Paula Salinas, David Leiva, Loreto Holguigue

**215 Comparative Analysis of AvrB and AvrRpm1 Recognition in *Arabidopsis* and Soybean**

Tom Ashfield, Thomas Redditt, Andrew Russell, Ryan Kessens, Natalie Rodibaugh, Roger Innes

**216 Characterization of Candidate Programmed Cell Death Inducers in *Arabidopsis***

Shawn Bachan, Shisong Ma, Matthew Porto, Michael Snyder, S.P Dinesh-Kumar

**217 Investigating the Effector Complement of the *Arabidopsis* Obligate Biotrophic Pathogen, *Albugo laibachii Nc14***

Kate Bailey, Torsten Schultz-Larsen, Eric Kemen, Ariane Kemen, Alexandre Robert-Seilhan, Anastasia Gardiner, Jonathan Jones

**218 Abstract Withdrawn****219 UPR Signaling Pathway is Important in the Establishment of Defense Response in *Arabidopsis thaliana***

Francisca Blanco, Adrián Moreno, Ariel Orellana

**220 Proteic Signals Involved in Systemic Acquired Resistance (SAR) in Plants**

Heiko Breitenbach, Hakan Sarioglu, Thomas Colby, Lucia Jorda, Jane Parker, A. Corina Vlot

**221 Insect eggs suppress plant defense against herbivores in *Arabidopsis***

Friederike Bruessow, Caroline Gouhier-Darimont, Philippe Reymond

**222 The *Xanthomonas* Type III Effector XopD Targets the *Arabidopsis* Transcription Factor AtMYB30 to Suppress Plant Defence**

Joanne Canonne, Daniel Marino, Alain Jauneau, Cécile Pouzet, Christian Brière, Dominique Roby, Susana Rivas

**223 Investigating the Association Between Age-Related Resistance and the Transition to Flowering in *Arabidopsis thaliana***

Philip Carella, Marisa Melas, Daniel Wilson, Robin Cameron

**224 Impact of Increased Host Ploidy on the Sustained Growth and Reproduction of an Obligate Biotroph, the Powdery Mildew *Golovinomyces orontii***

Divya Chandran, Joshua Rickert, Mary Wildermuth

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

Ratnesh Chaturvedi, Barney Venables, Robby Petros, Larry Takemoto, Vamsi Nalam, Li Maoyin, Xuemin Wang, Jyoti Shah

**226 A Receptor-like Cytoplasmic Kinase Is Involved In The Activation of A Plant Innate Immune Receptor**

Jun Liu, James Elmore, Zuh-Jyh Lin, Gitta Coaker

**227 RESISTANCE TO FUSARIUM OXYSPORUM 3 (RFO3) is an S domain kinase**

Stephanie Cole, Andrew Diener

**228 An mRNA Export Component Plays a Role in Plant Immunity**

Oliver Dong, Hugo Germain, Xin Li

**229 Function of PBS1 Phosphorylation in PAMP Triggered Immunity**

Ullrich Dubiella, Roger Innes

**230 Quantitative Proteomics Reveals Dynamic Changes at the Plant Plasma Membrane during Immune Responses**

James Elmore, Jun Liu, Brett Phinney, Gitta Coaker

**231 A nuclear kinase is required for plant immunity against *Pseudomonas syringae* pv. *maculicola***

Zhengqing Fu, Rajinikanth Mohan, Shan Zhu, Xinnian Dong

**232 Genetic Approaches to Identify the *Arabidopsis* Virulence Targets of the Bacterial Pathogenicity Factor HopAM1**

Theresa Law, Meredith Horton, Derek Lundberg, Ajay Goel, Chiharu Akimoto-Tomiyama, Michael Iakovidis, Jeffery Dangl, Sarah Grant

**233 Nuclear localization of the bacterial effector AvrRps4 is required to induce resistance**

Katharina Heidrich, Lennart Wirthmueller, Jane Parker

**234 Complex Regulation of the R Gene SNC1 Revealed by *bon1* Enhancers and Suppressors**

Mingyue Gou, Zhilong Bao, Zhenying Shi, Donglei Yang, Jian Hua

**235 Characterization of hybrid necrosis in *Arabidopsis thaliana***

Ben Hunter, Kathryn Solórzano-Lowell, Kirsten Bomblies

**236 Proteomic Analysis of the Plant-Pathogen Interface**

Brenden Hurley, Mike Wilton, Yulu Liu, Corinna Felsensteiner, Stephane Angers, David Guttman, Darrell Desveaux

**237 The chs3-2D Mutation in the CHS3 LIM Domain Activates Constitutive Disease Resistance in *Arabidopsis***

Kaeli Johnson, Dongling Bi, Yan Huang, Zhaohai Zhu, Xin Li, Yuelin Zhang

**238 Functional Sites Of Leucine-Rich Repeats Of Pattern Recognition Receptors In The Plant Immune System**

Teresa Koller, Laura Helft, Andrew Bent

**239 Rowing up and down the MAMP-triggered calcium stream**

Mark Kwaaitaal, Rik Huisman, Jens Maintz, Anja Reinstädler, Ralph Panstruga

**240 AtCML9, a calmodulin-like protein, contributes to plant defence responses**

Louis-Jerome Leba, Cecilia Cheval, Christian Mazars, Benoit Ranty, Jean-Philippe Galaud, Didier Aldon

**241 Regulation of RNA Silencing and Hormone Responses by RAV/EDF Transcription Factors**

Mathew Lewsey, Anna Stepanova, Joseph Ecker

**242 Growth regulation in response to cross-kingdom communication**

Louisa Liberman, Philip Benfey

**243 Receptor-like Cytoplasmic Kinases in Plant Innate Immunity**

Zuh-Jyh Lin, Jun Liu, Gitta Coaker

**244 Molecular Characterization of *mlo*-based Powdery Mildew Resistance and the Role of Heterotrimeric G-Protein Signaling in *Arabidopsis* Defense**

Justine Lorek, Pawel Bednarek, Alan Jones, Ralph Panstruga

**245 *Arabidopsis MPL1 (MYZUS PERSICAЕ INDUCED LIPASE1)* Mediated Resistance Against Green Peach Aphid**

Joe Louis, Katarzyna-Lorenc Kukula, Vijay Singh, John Reese, Jyoti Shah

**246 Dissection of Membrane Trafficking in Plant Immunity**

Yi-Ju Lu, Susanne Salomon, Silke Robatzek

**247 Immunity-Related Members of the DMR6 Family of Oxidoreductases in *Arabidopsis***

Nora Ludwig, Joyce Elberse, Tieme Zeilmaker, Guido Van den Ackerveken

**248 Suppression of immunity in diverse plants by the conserved RXLR effectors Ha23 from *Hyaloperonospora arabidopsis* and Ps73 from *Phytophthora sojae***

Devdutta Deb, Theresa How-Yew-Kin, Ryan Anderson, Brett Tyler, John McDowell

**249 Dissecting DIR1 and DIR1-like Involvement During Systemic Acquired Resistance in *Arabidopsis* and Cucumber**

Marisa Melas, Jennifer Faubert, Marc Champigny, Heather Shearer, Philip Carella, Robin Cameron

**250 Auxin plays multiple roles in promoting susceptibility to *Pseudomonas syringae***

Andrew Mutka, Stephen Fawley, Eve Mellgren, Tiffany Tsao, Barbara Kunkel

**251 Abstract Withdrawn****252 Involvement of ER body in the Strategy for Environmental Adaptation of *Arabidopsis thaliana*.**

KIMI Ogasawara, Noriyuki Hatsugai, Mikio Nishimura

**253 Functional Characterization of RNA-binding Proteins and MicroRNAs as Posttranscriptional Regulators in Plant Defense Responses**

Youngju Park, HwaJung Lee, Hunseung Kang

**254 Metabolic Incompatibility between *Arabidopsis* and the biotrophic pathogen *Hyaloperonospora arabidopsis***

Johannes Stuttmann, Hans-Michael Hubberten, Rainer Hoefgen, Jane Parker

**255 The Roles of the CC and LRR Domains of the RPS5 NB-LRR Protein in Pathogen Recognition**

Dong Qi, Roger Innes

**256 JMJ27, an *Arabidopsis* JmjC Domain-Containing H3K9 Histone Demethylase Is Required for Defense Against *Pseudomonas Syringae***

Aditya Dutta, Julie Caruana, Ramesh Raina

**257 Conservation of RIN4 function in Arabidopsis and Soybean**

Thomas Redditt, Tom Ashfield, Andrew Russell, Natalie Rodibaugh, Roger Innes

**258 Arabidopsis ABP30.6, a Novel Actin-Bundling Protein, Contribute to Resistance to *Botrytis cinerea***

Yuan Li, Lihui Zhang, Ting Zhang, Guoqin Liu, Dongtao Ren

**259 A Tale of Two Effectors From *Albugo laibachii* Nc14**

Torsten Schultz-Larsen, Eric Kemen, Kate Bailey, Ariane Kemen, Anastasia Gardiner, Jonathan Jones

**260 The bHLH transcription factors MYC2, MYC3 and MYC4 regulate glucosinolates biosynthesis in *A.thaliana***

Fabian Schweizer, Philippe Reymond

**261 DNA Repair Proteins Are Directly Involved in Regulation of Gene Expression during Plant Immune Response**

Junqi Song, Wendy Durrant, Shui Wang, Shunping Yan, EK Han Tan, Xinnian Dong

**262 AtVDAC1 Regulates Defense Response Against Bacterial Pathogen.**

Chika Tateda, Kanako Watanabe, Tomonobu Kusano, Yoshihiro Takahashi

**263 Investigating the Transcriptional Regulation of RRS1 Genes in Response to Biotic and Abiotic Stresses.**

Dominique Trémousaygue, Jérôme Novacki, Patrick Dabos, Céline Remblières, Binbin Zhou, Yves Marco

**264 Three members of the CBP60 family of proteins are differentially involved in salicylic acid mediated defences in Arabidopsis**

William Truman, Kenichi Tsuda, Suma Sreekanta, You Lu, Lin Wang, Jane Glazebrook

**265 SGT1 and chloroplast-generated ROS: The new players in coronatine-induced chlorosis and *Pseudomonas syringae* pv. *tomato* disease associated cell death**

Srinivasa Uppalapati, Yasuhiro Ishiga, Choong-Min Ryu, Tamding Wangdi, Takako Ishiga, Kirankumar Mysore

**266 Plant SUMO Paralogs Are Negative Regulators Of The Innate Immune Response**

Harrold Van den Burg

**267 Quantitative PhosphoProteomic Analysis of R-gene Mediated Immune Signaling.**

Chris van Schie, Zhouxin Shen, Steven Briggs

**268 Regulation of Salicylic Acid Signaling and Response by the GH3 acy adenylase PBS3**

Mary Wildermuth, Rachel Okrent, Sharon Marr, Diane Burgess, Alyssa Wong, Megan Casey

**269 Identification of mobile defense-inducing signals in plants**

Finni Wittek, Thomas Hoffmann, David Mackey, Jane Parker, Wilfried Schwab, A. Corina Vlot

**270 DNA Damage Response Potentiates Immune Response In Plants**

Shunping Yan, Wendy Durrant, Wei Wang, Junqi Song, Xinnian Dong

**271 A Signaling Cascade Activated by *Pseudomonas syringae* Through Abscisic Acid And Jasmonic Acid Signaling Suppresses Salicylic Acid Accumulation**

Xiao-yu Zheng, Natalie Spivey, Weiqing Zeng, Sheng Yang He, Xinnian Dong

**272 The Role of the Conserved Protein, CACTIN, in Arabidopsis. An Exciting, Essential, and Unique Eukaryotic Gene.**

Katherine Baldwin, Patrick Masson

**273 AGD1, a Class 1 ARF-GAP that Localizes to Punctate Bodies of the Endomembrane System, Regulates Multiple Components of Root Hair Growth in Arabidopsis**

Cheolmin Yoo, Satoshi Naramoto, J. Alan Sparks, Hiroo Fukuda, Elison Blancaflor

**274 Dissecting The Requirement For Plant RanGAP1 Subcellular Targeting And GAP Activity For Its Cellular And Developmental Functions**  
Joanna Boruc, Thushani Rodrigo-Peiris, Iris Meier

**275 GT-2 Family Transcription Factors Regulate Cell Growth in *Arabidopsis***  
Christian Breuer, Ayako Kawamura, Keiko Sugimoto

**276 Identification of Nuclear Genes Encoding Chloroplast-Localized Proteins Required for Embryo Development in *Arabidopsis***  
Nicole Bryant, Johnny Lloyd, Colleen Sweeney, Fumiyoji Myouga, David Meinke

**277 Abstract Withdrawn**

**278 SWEET Sugar Transporters For Cellular Efflux Highjacked For Nutrition Of Pathogens**  
Li-Qing Chen, Bi-Huei Hou, Sylvie Lalonde, Mara Hartung, Xiao-Qing Qu, Jung-Gun Kim, William Underwood, Ginny Antony, Frank White, Shauna Somerville, Mary Beth Mudgett, Wolf Frommer

**279 The Localization of APYRASE1/2 and Their Roles in Regulating of Growth and Development**  
Tsan Yu Chiu, Stanley Roux

**280 Membrane Bound Regulators of Actin Depolymerizing Factor (ADF)**  
Katrina Cuddy, Paris Grey, David Oppenheimer

**281 Dissecting the pathway of delivering tail-anchored proteins to the plant outer nuclear envelope**  
Mintu Desai, Iris Meier

**282 AtCenp-E2, a Kinesin-7, Plays a Conserved Role in Mitosis and is Important for Meiosis Progression**  
Kristophe Diaz, Denise Butler, Akielia Mayers, Adán Colón-Carmona

**283 secRFP, as a Powerful Marker for Plant Secretory Pathway Mutant Screening**  
Wenyan Du, Federica Brandizzi

**284 Early Signaling in Plant Immunity**  
Tenai Eguren, Zhouxin Shen, Steve Briggs, Earl Kang, Michelle Lee

**285 Four amino acids guide the assembly or disassembly of *Arabidopsis* histone H3.3-containing nucleosomes**  
Leilei Shi, Jing Wang, Fang Hong, David Spector, Yuda Fang

**286 Characterisation Of The ER Accessory Protein AXR4**  
Alison Ferguson, Ranjan Swarup

**287 Identification of a Novel Endosome Associated Protein that Promotes Movement of the SHORT-ROOT Transcription Factor**  
Koji Koizumi, Shuang Wu, Kimberly Gallagher

**288 *Arabidopsis* ARCP Protein DDH1/CSI1 Is A Novel Microtubule-associated Protein Which Is Required For Microtubule Stability**  
Hong-Bo Gao, Yu Mei, Hong-Wei Xue

**289 Calcium signaling during the *Arabidopsis* gravitropic response**  
Won-Gyu Choi, Gabriele Monshausen, Simon Gilroy

**290 Developmental Traits Contributing to Heterosis in *Arabidopsis* Hybrids Between C24, Landsberg *erecta*, and Columbia Accessions.**  
Michael Groszmann, Ian Greaves, Amanda Huen, Yingjie Yu, Mark Talbot, Maria Alonso-Peral, Jean Finnegan, William Peacock, Elizabeth Dennis

**291 Defining The Role of Endomembrane Trafficking in EDR1-KEG Controlled Programmed Cell Death**  
Yangnan Gu, Roger Innes

- 292 Coordination of Phosphatidylinositol-4-OH kinase PI4K $\beta$ 1 and Phosphatidylinostol-4-phosphate Phosphatase RHD4 Activities During *Arabidopsis* Root Hair Growth**  
Feng Guo, Erik Nielsen
- 293 Tonoplast Membrane Protein Mislocalizes to the ER In *impaired traffic to tonoplast* Mutants**  
Sang Won Han, Maria Rodriguez-Welsh, Jiameng Zheng, Marcela Rojas-Pierce
- 294 Transgenic polyglutamine proteins show length-dependent aggregation in *Arabidopsis***  
David Nash, Benjamin Harrison
- 295 MSL2 and MSL3 Provide a Functional Link Between Membrane Stretch and Chloroplast Division**  
Margaret Wilson, Gregory Jensen, Elizabeth Haswell
- 296 Assessing the Function of Matrix Attachment Region-Binding Filament-Like Protein (MFP1) in *Arabidopsis thaliana***  
Amanda Havighorst, Annkatrin Rose
- 297 Activity of the MCM Complex in the Endosperm of *Arabidopsis***  
Rowan Herridge, Robert Day, Richard Macknight
- 298 Abstract Withdrawn**
- 299 An insight into the function of the HUA2 gene family**  
Preetam Janakirama, Sathya Sheela Jali, Uday Sajja, Vojislava Grbic
- 300 Proteomics dissection of the *Arabidopsis thaliana* vacuolar proteome. New insights into the composition and molecular mass of protein complexes of plant vacuole**  
Nolwenn Jarno, Florent Villiers, Yohan Coute, Jérôme Garin, Christophe Bruley, Jacques Bourguignon, Michel Jaquinod
- 301 Capping Protein: a Membrane-Associated Actin-Binding Protein in *Arabidopsis***  
Jose Jimenez-Lopez, Xia Wang, Shanjin Huang, Christopher Staiger
- 302 Cell Biology of the trans-acting siRNA pathway in plants**  
Virginie Jouannet, Martin Crespi, Alexis Maizel
- 303 An *Arabidopsis* ABCA Family Gene Important for Seed Storage Lipid**  
Sangwoo Kim, Yasuyo Yamaoka, Hirofumi Ono, Donghwan Shim, Jae-Ung Hwang, Ikuo Nishida, Youngsook Lee
- 304 Testing for Interaction between the Exocyst Complex and Myosin XI Family Members in Cell Expansion**  
Amy Klocko, Valerian Dolja, John Fowler
- 305 DAYSLEEPER, An Essential Domesticated Transposase in *Arabidopsis***  
Marijn Knip, B de Pater, P Hooykaas
- 306 Reactive Oxygen Species Facilitate Lateral Root Emergence in *Arabidopsis***  
Daniel Lewis, Gloria Muday
- 307 The ATG1/ATG13 Protein Kinase Complex is Both a Regulator and A Target of Autophagic Recycling in *Arabidopsis***  
Faoliang Li, Anongpat Suttangkakul, Taijoon Chung, Richard Vierstra
- 308 An N-Glycan-Dependent Endoplasmic Reticulum-Associated Degradation System in *Arabidopsis***  
Wei Su, Zhi Hong, Yidan Liu, Yang Xia, Jianming Li
- 309 The NEV and AGD6 ARF-GAPs Redundantly Control Plant Development.**  
Christian Burr, Iris Chen, Sara Orlowski, Mark Daniels, Sarah Liljegren

**310 AtMAP65-1 and AtMAP65-2 Positively Regulate Axial Cell Growth in Etiolated *Arabidopsis* Hypocotyls**  
Jessica Lucas, Stephanie Courtney, Matt Hassfurder, Sonia Dhingra, Adam Bryant, Sidney Shaw

**311 RNS2, a Conserved Member of the RNase T2 Family, Is Necessary For rRNA Decay in Plants**  
Melissa Hillwig, Anthony Contento, Diane Bassham, Gustavo MacIntosh

**312 Sensitivity in Flowering Time Regulation by Coupling Noncoding Transcript Splicing and Chromatin Silencing in *FLC* repression**  
Sebastian Marquardt, Fuquan Liu, Caroline Dean

**313 Dissecting the pathway of delivering tail-anchored proteins to the plant outer nuclear envelope**  
Mintu Desai, Iris Meier

**314 The Heterodimeric Enzyme that Modifies the Wobble Position of Cytosolic tRNAs is Required for Seed Development in *Arabidopsis* and is a Member of a Diverse Family of Zinc-Dependent Deaminases**  
Rosanna Muralla, Rita Miller, David Meinke

**315 Abstract Withdrawn**

**316 Biotic Stress Induces the Unfolded Protein Response (UPR) Through the Unconventional Splicing of bZIP60 mRNA Mediated by IRE1 in *Arabidopsis thaliana***  
Adrian Moreno, Francisca Blanco, Ignacio Moreno, Ariel Orellana

**317 The Reticulon-like Proteins BT1 and BT2 Regulate the Intracellular Trafficking and Activity of the FLS2 Membrane Receptor**  
Hyoung Yool Lee, Christopher Bowen, George Popescu, Hong Gu Kang, Naohiro Kato, Sorina Popescu

**318 Ethylene Signaling from the Endoplasmic Reticulum to the Nucleus Mediated by EIN2**  
Hong Qiao, Joseph Ecker

**319 Division Plane Orientation in Plant Cells**  
Carolyn Rasmussen, Brian Sun, Tianying Su, Laurie Smith

**320 Investigating the Secretory Pathway: From Imaging to Gene**  
Luciana Renna, Federica Brandizzi

**321 Expression and localization divergence in the evolution of the Filament-like protein 4 (FLIP4) family in *Arabidopsis thaliana***  
Colby Richardson, Dominic Balcon, Annkatrin Rose

**322 Chemical Genetics Uncovers Inhibitors of a Golgi-independent Pathway for Tonoplast Membrane Proteins**  
Efraín Rivera Serrano, María Rodríguez-Welsh, Glenn Hicks, Natasha Raikhel, Marcela Rojas-Pierce

**323 Tracing the endocytic route and signalling of BR receptor-ligand complex**  
Eugenia Russinova, Niloufer Irani, Simone Di Rubbo, Evelien Mylle, Joanna Pizon, Jos Van den Begin, Jaroslava Hniličková, Miroslav Sisa, Anna-Mária Szatmári, Josep Vilarrasa Blasi, Daniël Van Damme, Ladislav Kohout, Miroslav Strnad, Karin Schumacher, Ana Caño-Delgado, Annemieke Madder, Jiří Friml

**324 Investigating the Role of Polarized Vesicle Secretion in Early Pollen Pistil Interactions in the Brassicaceae**  
Darya Safavian, Daphne Goring

**325 Monitoring dynamic changes in ER  $\text{Ca}^{2+}$  levels using the FRET-based  $\text{Ca}^{2+}$  sensor D1ER**  
Han-Wei Shih, Gabriele Monshausen

**326 ESCRT proteins are required for starch turnover**  
Christoph Spitzer, Marisa Otegui

**327 ADF4 is Important for Actin Turnover in the Cortical Array of Hypocotyl Epidermal Cells**

Jessica Henty, Laurent Blanchoin, Brad Day, Christopher Staiger

**328 Integrity of the early secretory organelles in plant cells**

Giovanni Stefano, Federica Brandizzi

**329 The Exocyst Complex in Cytokinesis of the Plant Cell**

Matyas Fendrych, Lukas Synek, Michal Hala, Tamara Pecenкова, Hana Toupalova, Rex Cole, John Fowler, Viktor Zarsky

**330 The Dynamics of Actin Filament Arrays is required for Vacuolar Fusion of Guard Cells during Stomatal Opening in *Arabidopsis***

Xue-Chen Wang, Fei Ren, Li-Juan Li, Xin-Qi Gao, Peng-Cheng Wei

**331 Functional Analysis of B1-type Cyclins during Plant Growth and Stress Response**

Annika Weimer, Manoj Kumar, Petra Bulankova, Farshad Roodbarkelari, Anne-Catherine Schmit, Peter Doerner, Karel Riha, Arp Schnittger

**332 The ER-Localized TWD1 Immunophilin Is Necessary for Localization of Multidrug Resistance-Like Proteins Required for Polar Auxin Transport in *Arabidopsis* Roots**

Guosheng Wu, Marisa Otegui, Edgar Spalding

**333 Metabolic Sugar Signal Promotes *Arabidopsis* Meristem G2 to M Transition**

Anna Skylar, Frances Sung, Xuelin Wu

**334 An Intragenic Mutation Restores the Function of a Defective Brassinosteroid Receptor on the Membrane in *Arabidopsis***

Yang Xia, Jianming Li

**335 The importance of PHOSPHATIDYL SERINE SYNTHASE1 in microspore development of *Arabidopsis thaliana***

Yasuyo Yamaoka, Junya Mizoi, Yuki Fujiki, Ikuo Nishida

**336 SYN3 Is Required For Chromosome Synapsis and Condensation during Meiosis in *Arabidopsis***

Li Yuan, Xiaohui Yang, Christopher Makaroff

**337 Functions for tethering complex exocyst in *Arabidopsis* exocytosis and PM recycling**

Viktor Zarsky, Matyas Fendrych, Tamara Pecenкова, Ivan Kulich, Edita Drdova - Jankova, Lukas Synek, Michal Hala, Rex Cole, John Fowler

**338 The Exocyst: A Vital Role In The Pollen-Stigma Interactions In *Arabidopsis thaliana***

Yara Zayed, Laura Chapman, Daphne Goring

**339 Formation and function of a ROP signaling scaffold at specialized domains of the Endoplasmic Reticulum**

Chunhua Zhang, Robert Stahelin, Simeon Kotchoni, Lacey Samuels, Daniel Szymanski

**340 Induction and Development of Tracheary Elements in In Vitro Cultures of *Arabidopsis***

Anika Benske, Jenny Bolivar, Tanya Falbel, Sara Patterson

**341 Abstract Withdrawn**

**342 Root Hair-Specific EXPANSIN A7 Is Required for Root Hair Elongation in *Arabidopsis***

Changfa Lin, Hee-Seung Choi, Hyung-Taeg Cho

**343 Two ABC Transporters Which Deposit Steryl Glucoside on Pollen Coat Are Important for Pollen Fitness**

Hyunju Choi, Yu-Young Kim, Kiyoshi Ohyama, Toshiya Muranaka, Youngsook Lee

**344 Characterization of MUM ENHANCER 4, a gene required for mucilage production in *Arabidopsis thaliana***Uday Divi, Andrej Arsovski, Tamara Western**345 The *Arabidopsis* Deficient in Cutin Ferulate (DCF) Encodes a Transferase Required for Ferulylation of ω-Hydroxy Fatty Acids in Cutin Polymers**Carsten Rautengarten, Berit Ebert, Mario Quellet, Edward Baidoo, Jay Keasling, Henrik Scheller**346 Identification and Analysis of Seed Coat Epidermal-Specific Promoter in *Arabidopsis thaliana* and *Brassica napus***Elahe Esfandiari, Zhaoqing Jin, Ashraf Abdeen, Jonathan Griffiths, Tamara Western, George Haughn**347 Re-examining the Role of Apoplastic Calcium in Cell Wall Modification**Matthew Gillham, Charlotte Jordans, Simon Conn, Isabel Moller, Andreas Schreiber, Rachel Burton, Brent Kaiser, Stephen Tyerman, Roger Leigh**348 The FEI2 RLK/SOS5 Pathway Regulates the Synthesis of Cellulose in *Arabidopsis* Seed Coat Mucilage Via CESAs**Smadar Harpaz-Saad, Heather McFarlane, Uday Divi, Tamara Western, Joseph Kieber**349 Identification and Characterization of Candidate Genes involved in Secondary Cell Wall Formation**Sathya Jali, Julian Verdonk, Christine Ondzighi, Cynthia Cass, Hannelz Roschttardtz, Gary Baisa, Patrick Masson, Marisa Otegui, John Sedbrook, Sebastian Bednarek**350 REDUCED WALL ACETYLATION Leads To Impairment In Cuticle, Trichome Cell Death And Enhanced Resistance Against *Botrytis cinerea***Majse Nafisi, Daniele Silvestro, Meike Burow, Helle Martens, Maria Hansen, Henrik Scheller, Yumiko Sakuragi**351 The Interconversion of UDP-Arabinopyranose and UDP-Arabinofuranose is Indispensable for Plant Development in *Arabidopsis thaliana***Carsten Rautengarten, Berit Ebert, Thomas Herter, Christopher Petzold, Tadashi Ishii, Aindrila Mukhopadhyay, Bjoern Usadel, Henrik Scheller**352 Three-Dimensional Architecture of *Arabidopsis* Cell Walls at Molecular Resolution as Revealed by Electron Tomography**Purbasha Sarkar, Elena Bosneaga, Manfred Auer**353 The Role of the FEI Receptor Like-Kinases in Regulating Plant Cell Wall Function**Blaire Steinwand, Joseph Kieber**354 A Screen of *Arabidopsis* Insertion Lines Identifies Candidate Cell Wall Digestibility Genes**Carl-Erik Tornqvist, Nick Santoro, Shane Cantu, Cliff Foster, Tanya Falbel, Jenny Bolivar, Jonathan Walton, Sara Patterson**355 CFL1, A WW Domain Protein, Regulates Cuticle Development by affecting the Activity of a Class IV Homeodomain Transcription Factor**Renhong Wu, Shuai Li, Shan He, Friedrich Wäßmann, Caihong Yu, Genji Qin, Lukas Schreiber, Li-Jia Qu, Hongya Gu**356 ABRC: A Central Hub for *Arabidopsis* Teaching Resources**Jelena Brkljacic, Emma Knee, Debbie Crist, Nicholas Holomuzki, James Mann, Ren Leaflight, Christopher Bartos, Luz Rivero, Randy Scholl, Erich Grotewold**357 The iPlant Collaborative**Victoria Bryan**358 A Mutant in Every Gene? Genome Coverage by ABRC Resources**Emma Knee, Jelena Brkljacic, Debbie Crist, Christopher Bartos, Luz Rivero, Randy Scholl, Erich Grotewold

**359 Report on Plant Resource Project in RIKEN BRC**

Masatomo Kobayashi, Hiroshi Abe, Satoshi Iuchi, Toshihiro Kobayashi

**360 An Equation For A Vibrant Database: Curators + Journals + Community = Success**

Donghui Li, Tanya Berardini, Raymond Chetty, Bob Muller, Eva Huala

**361 A Comprehensive Dataset of Genes with a Loss-of-Function Mutant Phenotype in *Arabidopsis thaliana***

Johnny Lloyd, David Meinke

**362 An international bioinformatics infrastructure to serve the Arabidopsis community**

Blake Meyers

**363 Effects of Sterilization Methods on Germination of Arabidopsis Seeds**

Luz Rivero, Nicholas Holomuzki, Garrett Posey, Jelena Brkljacic, Emma Knee, Deborah Crist, Randy Scholl, Erich Grotewold

**364 A Gateway to Elucidating Protein Function in Arabidopsis**

The Arabidopsis Interactome Mapping Consortium

**365 Navigating NCBI resources for Plant Genomics**

Anjana Vatsan, Vyacheslav Chetvernin, William Klimke, Sergey Resenchuk, Brian Smith-White, Igor Tolstoy, Deanna Church, Donna Maglott, Tatiana Tatusova

**366 Content Advances and New Developments in the T-DNA Insertion Allele Collection GABI-Kat**

Gunnar Huep, Nils Kleinboelting, Cordelia Bolle, Prisca Vielhoefer, Heinz Saedler, Dario Leister, Bernd Weisshaar

**367 A Deep Plant Alignment Database Integrated with Proteomic Data Constructed Using New Sequence Clustering and Alignment Algorithms**

Andrew Carroll

**368 Integration of Systems Biology and Genetic Approaches indicates that the COP9 Signalosome is an ancient regulator of the DNA damage response**

Osnat Atias, Yair Halimi, Shaul Pollack, Claus Schwechheimer, Benny Chor, Daniel Chamovitz

**369 Comprehensive Phylogenetic Analysis of the F-Box Gene Superfamily in Plants Reveals Divergent Evolutionary Histories Indicative of Genomic Drift**

Zhihua Hua, Cheng Zou, Shin-Han Shiu, Richard Vierstra

**370 Comprehensive Phylogenetic Analysis of the F-Box Gene Superfamily in Plants Reveals Divergent Evolutionary Histories Indicative of Genomic Drift**

Zhihua Hua, Cheng Zou, Shin-Han Shiu, Richard Vierstra

**371 From EST to RNAseq: A New Strategy to Annotate the Arabidopsis Genome for TAIR10**

Philippe Lamesch, David Swarbreck, Eva Huala

**372 A Reduction in 24nt Small RNA in Arabidopsis Hybrids May Contribute to Hybrid Vigor**

Ying Li, Kranthi Varala, Matthew Hudson

**373 A Data Model of Root Gravitropism**

Nathan Miller, Tessa Durham Brooks, Misuk Cho, Edgar Spalding

**374 Quantitation of Cellular Dynamics in Growing Arabidopsis Roots with Light Sheet Microscopy**

Giovanni Sena, Zak Frentz, Kenneth Birnbaum, Stanislas Leibler

**375 Extensive Genomic and Transcriptomic Variation in the 19 Founders of the Arabidopsis MAGIC Lines**

Joshua Steffen, Jonas Behr, Philipp Drewe, Katie Hillebrand, Paula Kover, Rune Lyngsoe, Richard Mott, Edward Osborne, Gunnar Rätsch, Sebastian Schultheiss, Vipin Sreedharan, Oliver Stegle, Chris Toomajian, Gan Xiangchao, Richard Clark

**376 Novel and Known Post-transcriptional Regulatory Sequences are Conserved across Plant Families**Justin Vaughn, Bijoyita Roy, Albrecht von Arnim**377 Construction of A Novel Conceptual Coexpression Network for Biological Knowledge Discovery**Hairong Wei**378 Construction and Validation of a Brassinosteroid Gene Regulatory Network (GRN) in the Control of Plant Growth and Development in *Arabidopsis thaliana***Huaxun Ye, Lei Li, Jaroslaw Zola, Maneesha Aluru, Honqing Guo, Sarah Anderson, Peng Liu, Steve Rodermel, Srinivas Aluru, Yanhai Yin**379 Hydro-patterning: Moisture induced Polarity in Lateral Root Initiation**Pooja Aggarwal, Jose Dinneny**380 ABSCISIC ACID INSENSITIVE 4 (ABI4) Mediates ABA and Cytokinin Inhibition of Lateral Root Formation by Reducing Auxin Polar Transport**Doron Shkolnik-Inbar, Dudy Bar-Zvi**381 The roles of SHORT INTERNODES/STYLISH during leaf vein development in *Arabidopsis thaliana***Tammy Baylis, Izabela Cierlik, Eva Sundberg, Jim Mattsson**382 The Molecular Basis of Natural Variation in Root Development**Wolfgang Busch, Mónica Meijón Vidal, Radka Uhliřová**383 *Arabidopsis thaliana* Small Auxin-Up RNA 63 (SAUR63) functions in plant growth by regulating basipetal auxin transport**Keun Chae, Cameron Issacs, Paul Reeves, Greg Maloney, Esther Park, Gloria Muday, Jason Reed**384 ATHB12, a homeodomain-leucine zipper (HD-Zip) class I protein, controls the stem elongation through the regulation of GA20ox1 level.**Yoon-Sun Hur, Sunghan Kim, Choong-Ill Cheon**385 Meristematic Growth Zones are Altered in Exocyst Mutants to Affect *Arabidopsis* Root Growth**Rex Cole, John Fowler**386 ER-localized PIN8 Modulates Cell And Plant Development by Regulating Intracellular Auxin Homeostasis**Cristina Dal Bosco, Alexander Dovzhenko, Nina Woerner, Xing Liu, Tatiana Resch, Jan Hegermann, Margitta Eismann, Alisher Touraev, Erwin Heberle-Bors, Ivan Paponov, Benedetto Ruperti, Jerry Cohen, Klaus Palme**387 Differential Root Growth Is Regulated By Auxin-Mediated Interaction of PIN2 And The Cell Wall**Alexander Dovzhenko, Francesco Pinosa, Henrik Skibbe, Michael Melzer, Hans Burkhardt, Olaf Ronneberger, Klaus Palme**388 Altered Auxin Transport and Gravitropic Response in the *scd1* Mutant**Tanya Falbel, Jonathan Isley, Cassie Mattox, Gloria Muday**389 Characterization of second-site enhancer mutations of the auxin-resistant4 mutations**Lawrence Hobbie, Phillip Berges, Pauline Gould, Dan Guo, Dipika Jadav, Achala Jayasena, Evan Lanz, Wenqian Liu, Wendy Podany, Patricia Raimondi, Jeff Runkel, Sumeet Sandhu, Isiah Washington**390 The *Arabidopsis OSR1* Regulates Organ Growth and Final Organ Size in Orchestration with ARGOS and ARL**Guangping Feng, Zhixiang Qin, Jingzhou Yan, Xiaoran Zhang, Yuxin Hu**391 RABBIT EARS Regulates microRNA164 Genes in *Arabidopsis* Sepal and Petal Development**Tengbo Huang, Vivian Irish

**392 Analyses of the effect of exogenous polyamines on the stem growth of *Arabidopsis thaliana***

Jun-ichi Kakehi, Wurina Tong, Kaori Yoshimoto, Hiroyasu Motose, Masaru Niitsu, Taku Takahashi

**393 The *Arabidopsis* NGATHA transcription factors act as negative regulators of cell proliferation in lateral organ growth**

Byung Ha Lee, So Hyun Kwon, Min Jeong Lee, Jeong Hoe Kim

**394 An apical root growth program directed in the vascular stem cells**

Jose Sebastian, Jing Zhou, Ji-Young Lee

**395 PHYTOCHROME INTERACTING FACTOR 4 regulates auxin biosynthesis at high temperature**

Keara Franklin, Sang Ho Lee, Dhaval Patel, Chen Gu, Angela Spartz, Songqing Ye, Peng Yu, Gordon Breen, Vinod Kumar, Jerry Cohen, Philip Wigge, William Gray

**396 Lateral Root Development Associated with a Polyadenylation Factor in *Arabidopsis***

Man Liu, Xiaohui Wu, Arthur Hunt, Qingshun Li

**397 Class II HD-ZIP Proteins Are Oppositely Regulated by Ad/abaxial Regulators and Control Meristem Size, Leaf Blade Development and Proliferation of Stem Tissue**

Tie Liu, Nicole Newell, Brenda Reinhart, Tengbo Huang, Randy Kerstetter, M. Kathryn Barton

**398 NIMA-related Kinases Redundantly Regulate Directional Cell Expansion in *Arabidopsis thaliana***

Hiroyasu Motose, Kaori Yoshimoto, Yuichiro Takahashi, Tatsuya Sakai, Taku Takahashi

**399 UNHINGED Controls Leaf Vein Pattern in *Arabidopsis***

Shankar Pahari, Ryan Cormack, Michael Blackshaw, Alyssa Clarke, Elizabeth Schultz

**400 PLA- I γ1 and PLA- I γ2 Proteins Are Required for Shoot Apical Meristem Development and Leaf Polarity in *Arabidopsis***

Jong-Yoon Park, Mijin Oh, Ilha Lee

**401 A Role for CSLD3 During Cell Wall Synthesis in Apical Plasma Membranes of Tip-Growing Root Hair Cells**

Sungjin Park, Amy Szumlanski, Fangwei Gu, Feng Guo, Erik Nielsen

**402 Imprinted Expression of Polarizing Genes in the Seed Endosperm is Subject to Natural Variation**

Brittany Pope, Jenkin Chan, Rina Ishii, Jonathan Fitz Gerald

**403 Control of Stomatal Polarity by a Peripherally-Localized LRR Receptor-Like Kinase**

Sandra Keerthisinghe, Jeannette Nadeau, Jessica Lucas, Tsuyoshi Nakagawa, Fred Sack

**404 LAZY1 and ARG1 define two genetic pathways of gravitropism in inflorescence**

Shu Sasaki, Atsuko Sato, Masaaki Watahiki, Kotaro Yamamoto

**405 How to Grow Straight? *tortifolia 2*, an α-Tubulin Point Mutation Links Helical Expansion of Single Cells and Torsional Organ Growth**

Henrik Buschmann, Malay Das, Dierk Niessing, Clive Lloyd, Tony Schaeffner

**406 JAGGED LATERAL ORGANS controls auxin signalling and organ development**

Madlen Rast, Rüdiger Simon

**407 STIMPY Modulates Cytokinin Signaling in Meristem Development in *Arabidopsis thaliana***

Anna Skylar, Xuelin Wu

**408 Linking the genetics and biochemistry of ROP signaling cascades to the mechanics of leaf epidermal morphogenesis**

Chunhua Zhang, John Mason, John Roesel, Eileen Mallory, David Umulis, Dan Szymanski

**409 Plastid Signal Is Involved In The Dynamic Regulation of FIL Expression Pattern**

Toshiaki Tameshige, Maki Kondo, Keiro Watanabe, Koichi Toyokura, Ryuji Tsugeki, Kiyoshi Tatemashu, Mikio Nishimura, Kiyotaka Okada

**410 AKIN10 and FUSCA3 Interact to Control Lateral Organ Development and Phase Transitions in Arabidopsis**

Allen Tsai, Sonia Gazzarrini

**411 The MADS-domain Factors AGL15, AGL18, AGL24 and SVP Act Redundantly to Prevent Premature FT Expression and Leaf Curling**

Chieh-Ting Wang, Donna Fernandez

**412 Polyadenylation Factor PCFS4 and Arabidopsis Development**

Denghui Xing, Qingshun Li

**413 MACCHI-BOU 2 involved in bract suppression in *Arabidopsis thaliana***

Ryo Yonehara, Masahiko Furutani, Masao Tasaka

**414 Control of Multiple Organ Development by the miR160-regulated Auxin Signaling**

Xiaodong Liu, Jian Huang, Yao Wang, Kanhai Khanna, Zhixin Xie, Heather Owen, Dazhong Zhao

**415 Function and Phylogeny of Cytokinin Response Factors**

Paul Zwack, Dana Gerken, Leslie Goertzen, Aaron Rashotte

**416 A set of mutants defective in Caspary Strip formation**

Julien Alassimone, Niko Geldner

**417 Functional analysis of regulators of STM in Arabidopsis**

Jose Antonio Aguilar Martinez, Neelima Sinha

**418 A Forward Genetic Approach to Identify Paternal Effects on Early Embryogenesis in *Arabidopsis thaliana***

Yashodar Babu, Agnes Henschen, Martin Bayer

**419 FUN Is Where It's At: RNA Profiling Of The *Arabidopsis* Funiculus**

Mark Belmonte, Sara Kost, Ryan Kirkbride, Julie Pelletier, Robert Goldberg, Edward Yeung, John Harada

**420 DORNRÖSCHEN-LIKE (DRNL) Activity Marks *Arabidopsis* Floral Organ Founder Cells, Precedes Auxin Response Maxima and Initiates Organogenic Competence.**

John Chandler, Ingo Seeliger, Bianca Jacobs, Melanie Cole, Petra Comelli, Wolfgang Werr

**421 Genome-wide Direct Target Analysis Reveals A Role For SHORT-ROOT In Root Vascular Patterning Through Cytokinin Signaling**

Hongchang Cui, Yueling Hao

**422 Peptide Hormones During Root Development And Branching In *Arabidopsis***

Bert De Rybel, Ianto Roberts, Dominique Audenaert, Gieljan De Rop, Kun Yue, Elisabeth Williams, Zhefeng Lin, Maria Njo, Tom Beeckman, Ive De Smet

**423 amiGO RBR is a useful tool for network dissection**

Sara Diaz-Trivino, Alfredo Cruz-Ramirez, Yujuan Du, Ikram Blilou, Hongtao Zhang, Yuchen Long, Ben Scheres

**424 A Putative Leucine Zipper Protein Essential for the Activation of *FLC* and Delay of Flowering Time by *FRI***

Lei Ding, Sang Yeol Kim, Scott Michaels

**425 An Analysis of Vascular Phenotypes in ADP-RIBOSYLATION FACTOR A1 Mutant Cotyledons**

Jessica Erickson, Elizabeth Schultz

**426 A Chemical Genomics Approach to Identify Enhancers and Repressors of Somatic Embryogenesis in Arabidopsis**

Martijn Fiers, Tom Stekelenburg, Sean Cutler, Kim Boutilier

**427 Long-distance Regulation of Cambium Activity**

Javier Agusti, Stefanie Suer, Silvia Herold, Martina Schwarz, Pablo Sanchez, Karin Ljung, Tobias Sieberer, Elizabeth Dun, Philip Brewer, Christine Beveridge, Eva Maria Sehr, Thomas Greb

**428 *TFL1* Controls Flowering Transition and Vesicle Transport in *Arabidopsis***

Yu Mei, Desmond Bradley, Yoshie Hanzawa

**429 Finding Meristemoid-Specific Genes: Fuel for Stomatal Development**

Robin Horst, Kylee Peterson, Lynn Pillitteri, Keiko Torii

**430 Signalling Components of BABY BOOM–induced Somatic Embryogenesis**

Anneke Horstman, Hiroyuki Fukuoka, Mieke Weemen, Gerco Angenent, Richard Immink, Kim Boutilier

**431 The AP2/ERF Transcription Factor WIND1 Controls Cell Dedifferentiation in *Arabidopsis***

Akira Iwase, Masaru Ohme-Takagi, Keiko Sugimoto

**432 The COP9 signalosome regulates cell division rates and root meristem function in *Arabidopsis* embryos and seedlings**

Nahill Matari, Laila Moubayidin, Sabrina Sabatini, Giovanna Serino, Pablo Jenik

**433 Developmental Profiling of Gene Activity in *Arabidopsis* Seed Compartments Identifies Significant Differentiation in Endosperm Domain Identities**

Ryan Kirkbride, Mark Belmonte, Julie Pelletier, Meryl Hashimoto, Anhthu Bui, Brandon Le, Robert Goldberg, John Harada

**434 *AINTEGUMENTA-LIKE6 (AIL6)* regulates cellular differentiation in flowers**

Beth Krizek, Marcie Eaddy

**435 A *WUSCHEL*-like Gene Controls Stem Secondary Growth in Trees**

Jeanette Nilsson, Melis Kucukoglu, Bo Zheng, Göran Sandberg, Ove Nilsson

**436 Dynamic Regulation of H3K27 Trimethylation during *Arabidopsis* Differentiation**

Marcel Lafos, Phillip Kroll, Mareike Hohenstatt, Daniel Schubert

**437 Interplay of GRAS Transcription Factors in the *Arabidopsis* Shoot System**

Mi-Hyun Lee, Jun Lim

**438 Misexpressed CPC Affects the Cell Fate Specification in the *Arabidopsis* Root Epidermis**

Yeon Hee Kang, Sang-Kee Song, John Schiefelbein, Myeong Min Lee

**439 Gating of sperm entry to the Central Cell during double fertilization is mediated by *GLAUCE***

Yehoram Leshem, Cameron Johnson, Samuel Wuest, Quy Ngo, Ueli Grossniklaus, Venkatesan Sundaresan

**440 Genome-wide Analysis of SCL3-responsive Transcriptome in the *Arabidopsis* Root**

Jun Lim, Shin Ae Lee, Kwang Suk Chang, Jung-Ok Heo

**441 HYL1 mediates patterning of the *Arabidopsis* root stem cell niche by regulating PLETHORA**

Jinxin Liu, Yuke He

**442 Detection of Transcriptome Landscape in *Arabidopsis* Male Meioocytes Using High-throughput Sequencing**

Pingli Lu, Hongxing Yang, Yingxiang Wang, Hong Ma

**443 Exploring Two Ethylene Biosynthetic Enzymes as Potential Targets of *Arabidopsis* RING E3 Ligase, XBAT32, During Lateral Root Production.**

Wendy Lyzenga, Sophia Stone

**444 C2H2 Factors Regulate Cell Identity and Asymmetric Divisions in the *Arabidopsis* Root**Miguel Moreno-Risueno, Rosangela Sozzani, Jalean Petricka, Philip Benfey**445 SCARECROW Sustains Stem Cell Activity Inhibiting Cytokinin Dependent Cell Differentiation Input**Laila Moubayidin, Di Mambro Riccardo, Pacifici Elena, Terpstra Inez, Perilli Serena, Dello Ioio Raffaele, Heidstra Renze, Costantino Paolo, and Sabrina Sabatini**446 Three AIL/PLT Transcription Factors Function Together in Regulating Shoot Apical Meristem Activity**Janaki Mudunkothge, Beth Krizek**447 Dissecting Receptor Function in Shoot Stem Cell Maintentance in Living Meristematic Tissue**Zachary Nimchuk, Paul Tarr, Carolyn Ohno, Vijay Chickarmane, Qu Xiang, Elliot Meyerowitz**448 Abstract Withdrawn****449 The role of CORYNE in root development.**Helge Pallakies, Rüdiger Simon**450 Local Auxin Biosynthesis Is A Key Step In The Patterning Of The *Arabidopsis* Female Gametophyte**Aneesh Panoli, Monica Alandete-saez, Yunde Zhao, Venkatesan Sundaresan**451 RETINOBLASTOMA-RELATED Protein and Cytokinin Interaction during Root Meristem Cell****Differentiation**Serena Perilli, Jose Manuel Perez-Perez, LLavata Peris C, Polverari L, Moubayidin L, Di Mambro R, Costantino P, Scheres B, and Sabatini S**452 Capturing the Dynamics of Stomatal Cell Specification in Growing Leaves**Sarah Robinson, Pierre Barbier de Reuille, Dominique Bergmann, Przemyslaw Prusinkiewicz, Enrico Coen**453 Endoreduplication Represses Small Cell Identity**Adrienne Roeder, Elliot Meyerowitz**454 What Makes a Root Hair? Integrated Transcriptomic and Proteomic Analysis of *Arabidopsis* Trichoblasts**Ping Lan, Wenseng Li, Ya-Yun Liao, Simonetta Santi, Wolfgang Schmidt**455 A Novel Semi-dominant Allele of MONOPTEROS Reveals Pleiotropic Functions for MONOPTEROS during Plant Development**Jasmine Garrett, Miranda Meents, Hongwei Hou, Kamran Kaviani, James Meservy, Michael Blackshaw, LeeAnna Tavernini, Danielle Styranko, Elizabeth Schultz**456 Phenotypic Analysis of an Embryo-Aborted Mutant in *Arabidopsis***Jiao Shi, Jingjing Liu, Li-Jia Qu**457 Molecular Genetic Analysis of *Arabidopsis TSO1*, a Regulator of Cell Proliferation and Differentiation During Flower Development**Paja Sijacic, Charles Hawkins, Zhongchi Liu**458 Characterizing LEAFY Transcriptional Complexes in *Arabidopsis thaliana***Nirodhini Siriwardana, Matthew Tegowski, Rebecca Lamb**459 Radial patterning in the *Arabidopsis* root: transcriptional effect of SHR at cell-type resolution**Rosangela Sozzani, Miguel Moreno-Risueno, Jaimie Van Norman, Wolfgang Busch, Siobhan Brady, Philip Benfey**460 The Folypolyglutamate Synthetase Plastidial Isoform is Required for Postembryonic Root Development in *Arabidopsis***Ayinash Srivastava, Perla Ramos-Parra, Mohamed Bedair, Ana Robledo-Hernández, Yuhong Tang, Lloyd Sumner, Rocío Díaz de la Garza, Elison Blancaflor

461 Abstract Withdrawn

**462 RUL3, a Novel Regulator of Auxin-dependent Root Patterning and Differentiation**

Christine Uehlken, Ruth Stadler, Norbert Sauer

**463 Nuclear Size Matters; The Role of Chromatin Organization in Seed Maturation, Dormancy and Germination in *Arabidopsis thaliana***

Martijn van Zanten, Christian Zöll, Wim Soppe

**464 The GATA-type Transcription Factors HAN-LIKE1 and HAN-LIKE2 are Required for Apical-Basal Pattern Formation During *Arabidopsis thaliana* Embryogenesis**

Matthew Volny, Wolfgang Lukowitz

**465 Trehalose 6-phosphate acts at the shoot apex to induce flowering in *Arabidopsis thaliana***

Vanessa Wahl, Jathish Ponnu, Armin Schlereth, Stéphanie Arrivault, Annika Franke, Regina Feil, John Lunn, Markus Schmid, Mark Stitt

**466 Identification of Targets of the *Arabidopsis* B3 Domain Protein FUSCA3**

Fangfang Wang, Sharyn Perry

**467 RUG3 is a New Mediator of Auxin Response During Specific Developmental Processes**

Magdalena Weingartner, Benjamin Weller, Norbert Sauer

**468 The Vegetative Transcriptome of *Arabidopsis thaliana***

Matthew Willmann, Yeonjong Koo, Kevin McCormick, Blake Meyers, R. Scott Poethig

**469 Mutations in the GW-Protein SUO Reveal a Developmental Function for MiRNA-mediated Translational Repression in *Arabidopsis***

Li Yang, R. Scott Poethig

**470 A Gene Encoding an Auxin Receptor TIR1 is a Direct Target of the MADS-domain Protein AGL15 and Impacts on *Arabidopsis* Somatic Embryogenesis**

Qiaolin Zheng, Yumei Zheng, Whitney Burnie, Sharyn Perry

**471 Mobile Transcription Factors AHL3/4 Regulate Xylem Development**

Jing Zhou, Ji-Young Lee

**472 XAP5 CIRCADIAN TIME KEEPER (XCT): A Global Player in Plant Growth, Development, and Stress Signaling?**

Shajahan Anver, Assen Roguev, Nevan Krogan, Stacey Harmer

**473 Poly(A) in the 5' Untranslated Regions of a Large Family of *Arabidopsis* mRNA Suggests a Broad Role for Cap Independent Translation in Plant Stress Responses**

Raymond Moore, Kim Mogen, Scott Ballantyne

**474 High Resolution Profiling of Small RNAs in *Arabidopsis thaliana* Roots**

Natalie Breakfield, Philip Benfey

**475 Histone Methylation Associated with Changes in Gene Expression During Senescence**

Judy Brusslan, Ana Rus-Alvarez, Judd Rice, Michael Hitchler, Matteo Pellegrini

**476 Transcriptome Profiling Indicates The Existence Of Post-Transcriptional Control In Response To Abscisic Acid And Glucose In *Arabidopsis thaliana***

Gustavo Duarte, Cleverson Matiolli, Delphine Gey, Sandra Pelletier, Jean-Pierre Renou, Renato Vicentini, Michel Vincentz

**477 Intraspecific *Arabidopsis* Hybrids Have Altered Levels Of sRNA and DNA Methylation**

Ian Greaves, Michael Groszmann, Zayed Albertyn, Emily Ying, Jennifer Taylor, William Peacock, Elizabeth Dennis

**478 The Histone Acetyltransferase GCN5 Affects Trichome Patterning**Amy Hark, Ashley Kendig, Jenna Kotak, Elizabeth McCain**479 An siRNA Pathway Controls Transposition in Plants Subjected to Stress**Hidetaka Ito, Hervé Gaubert, Etienne Bucher, Marie Mirouze, Isabelle Vaillant, Jerzy Paszkowski**480 The AtJmj12 encoding JmjC Domain-Containing Protein Represses the Expression of FLC in Arabidopsis**Young-Min Jeong, Jae-Young Yun, Richard Amasino, Yoo-Sun Noh**481 Arabidopsis RbAp46/48-Like Proteins Associate with a Histone Deacetylase to Act Redundantly in Chromatin Silencing**Danhua Jiang, Xiaofeng Gu, Wannian Yang, Yannick Jacob, Scott Michaels, Yuehui He**482 Cytokine pathway plays as cross node of IKU genetic controlling and epigenetic regulation in endosperm growth**Jing LI, Li Hui TAN, Xin NIE, Frederic BERGER**483 Interactions Between TOPLESS and Histone-Modifying Enzymes**Rhiannon Macrae, Jeffrey Long**484 Decapping Proteins Are Involved in miRNA Pathway in Arabidopsis thaliana**Kazuki Motomura, Naoyoshi Kumakura, Atsushi Takeda, Yuichiro Watanabe**485 Cytosolic Electron Transfer Component-like Protein Deficiency Impaired Expression of Imprinted Gene FWA in the Endosperm**Miyuki Nakamura, Yuki Kinoshita, Tetsu Kinoshita**486 Initiation and Maintenance of Epigenetic Transposable Element Silencing in Arabidopsis**Saivageethi Nuthikattu, Andrea McCue, Jennifer Bosse, Christopher DeFraia, R. Keith Slotkin**487 Genomes, Transcriptomes, Methylomes and smRNAomes of the Arabidopsis Accessions Col-0, Cvi-0 and Ler-1**Ronan O'Malley, Robert Schmitz, Ryan Lister, Jarrod Chapman, Issac Ho, Jason Affourtit, Zhoutao Chen, Brian Desany, Jim Knight, Daniel Rokhsar, Michael Egholm, Timothy Harkins, Joseph Ecker**488 Divergent Roles For the Two Poll-like Organelle DNA Polymerases of *Arabidopsis thaliana***Jean-Sébastien Parent, Etienne Lepage, Normand Brisson**489 Genetic Analysis of *Arabidopsis phyA'* Epiallele – A case of Transcriptional Silencing Associated with Exonic Methylation**Vibha Srivastava, Gulab Rangani**490 Characterization of the microRNA miR396 regulatory network in plants.**Ramiro Rodriguez, Juan Debernardi, Martin Mecchia, Javier Palatnik**491 Abstract Withdrawn****492 In Association with AGAMOUS or Polycomb Group Proteins, KNU Promoter Status Determines the Timing of Arabidopsis Floral Stem Cell Termination**Bo Sun, Zemiao He, Toshiro Ito**493 Characterization of a Novel Mutation in the PHD Finger Implicates HSI2 in Chromatin-mediated Epigenetic Repression of Seed-specific Genes in *Arabidopsis* Seedlings**Vijaykumar Veerappan, Jing Wang, Huazhong Shi, Randy Allen**494 The use of high-throughput sequencing technologies to identify the substrates of RNA-dependent RNA polymerases in *Arabidopsis*.**Matthew Willmann, Qi Zheng, Ying Chen, Isabelle Dragomir, Fan Li, Brian Gregory

- 495 An Arabidopsis AT-hook motif protein is required for silencing of transposable elements and the developmental transition to flowering**  
*Yifeng Xu, YiZhong Wang, XiaoFeng Gu, Bo Sun, Kian-Hong Ng, Yuehui He, Toshiro Ito*
- 496 Multiple cis-elements Regulate The Vernalization-induced Expression of VERNALIZATION INSENSITIVE 3**  
*Jihyeon Yu, Jinwoo Shin, Ilha Lee*
- 497 ARABIDOPSIS TRITHORAX-RELATED3/SET DOMAIN GROUP2 Is Required for Repression of Arabidopsis thaliana Flowering in Non-Inductive Photoperiods and in Non-Vernalized Winter Annuals**  
*Jae-Young Yun, Yosuke Tamada, Ye Eun Kang, Richard Amasino*
- 498 Characterization of the DAWDLE Gene in Arabidopsis**  
*Seyit Yuzauk, David Chevalier*
- 499 Extensive Gene Regulatory Networks Utilizing Trans-Acting siRNAs in Legumes**  
*Jixian Zhai, Dong-Hoon Jeong, Emanuele Paoli, Sunhee Park, Benjamin Rosen, Yupeng Li, Alvaro González, Zhe Yan, Scott Jacksonc, Gary Stacey, Doug Cook, Pamela Green, Janine Sherrier, Blake Meyers*
- 500 Towards Defining Roles for the *Arabidopsis* GOLDEN2-LIKE genes, AtGLK1 and AtGLK2, in the Regulation of Core Circadian Clock Components and Physiological Outputs**  
*R Brandon Celaya, Candace Webb, Steve Knowles, Sheen Lu, Jose Pruneda-Paz, Steve Kay, Elaine Tobin*
- 501 COP1-Mediated Degradation of BBX22/LZF1 Optimizes Seedling Development in Arabidopsis**  
*Chiung-swey Chang, Julin Maloof, Shu-Hsing Wu*
- 502 The LRB1 and LRB2 Pair of Arabidopsis BTB E3s Modify Red Light Signaling by Regulating Phytochrome Levels**  
*Matthew Christians, Derek Gingerich, Brandon Blaisdel, Richard Vierstra*
- 503 Automated Analysis of Hypocotyl Growth Dynamics During Shade Avoidance in Arabidopsis**  
*Benjamin Cole, Steve Kay, Joanne Chory*
- 504 Investigation Of DNA-Protein Interactions At The Promoter Of The Circadian Clock Gene LHY In *Arabidopsis thaliana***  
*Sian Davies, Claire Hill, Mark Spensley, Emma Picot, Isabelle Carré*
- 505 Phytochromes Regulate HEMERA Accumulation Via Direct Interaction**  
*Rafaelo Galvao, Meng Chen*
- 506 Cell Autonomous and Cell-Type Specific Circadian Rhythms in Arabidopsis**  
*Miriam Hassidim, Esther Yakir, Rachel Green*
- 507 Assessment of Arabidopsis Phytochrome phyABCDE Null Mutant**  
*Wei Hu, Keara Franklin, Robert Sharrock, Clark Lagarias*
- 508 ESD6/HOS1 Participates In The Control Of Photoperiodic Flowering In Arabidopsis Negatively Regulating CONSTANS Abundance**  
*Ana Lazaro, Federico Federico Valverde, Manuel Pineiro, Jose Jarillo*
- 509 Gulliver2 Suppresses the Dwarfism of the brassinosteroid insensitive1-5 mutant**  
*Bokyung kim, Sunghwa Choe*
- 510 Phytochrome-Regulated Arabidopsis BPG2 Binds to Plastid Ribosomal RNAs and Regulates Ribosomal RNA Processing**  
*Byung-Hoon Kim, Przemyslaw Malec, Albrecht von Arnim*

- 511 Bimolecular Fluorescence Complementation Studies Support An *in vivo* Interaction Between The F-BOX Protein COLD TEMPERATURE GERMINATING10 And PHYTOCHROME INTERACTING FACTOR 1**  
Santosh Kumar, Nihar Nayak, Kathleen Martin, Kim Schafermeyer, Taylor Lloyd, Randy Dinkins, Michael Goodin, Bruce Downie
- 512 An Approach To Identify PHYTOCHROME INTERACTING FACTOR1 (PIF1) Interacting Proteins From *Arabidopsis* Seeds**  
Rekha Kushwaha, Santosh Kumar, Bruce Downie
- 513 Coordinated Transcriptional Regulation Underlying the Circadian Clock in *Arabidopsis***  
Gang Li, Hamad Siddiqui, Yibo Teng, Rongcheng Lin, Xiang-yuan Wan, Jigang Li, On-Sun Lau, Xinhao Ouyang, Mingqiu Dai, Jianmin Wan, Paul Devlin, Xing Wang Deng, Haiyang Wang
- 514 PIL1: A Negative Regulator of the Shade Avoidance Transcriptional Network of Plants**  
Lin Li, Robert Schmitz, Kazumasa Nito, Benjamin Cole, Li Lin, Samuel Hazen, Joseph Ecker, Joanne Chory
- 515 Rapid, Organ-Specific Transcriptional Responses to Light Regulate Photomorphogenic Development in Dicot Seedlings**  
Matthew Hudson, Ying Li
- 516 Utilization of ChIP-Seq to Identify PSEUDO RESPONSE REGULATOR 7 Transcriptional Targets in the *Arabidopsis* Circadian Clock**  
Tiffany Liu
- 517 Differential Expression Of PIF1-Targeted Genes In Various PIF1 And CTG10 Mutants**  
Taylor Lloyd, Santosh Kumar, Bruce Downie
- 518 Towards the modelling of the shade avoidance response of the *A. thaliana* hypocotyl**  
Séverine Lorrain, Micha Hersch, Sven Bergmann, Christian Fankhauser
- 519 A Transcription Factor Overexpression Screen for Novel Regulators of the *Arabidopsis* Circadian Clock**  
Jeffrey Nelson, Steve Kay
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Yun Zhou, Wei Li, Xiaojun Kang, Min Ni
- 521 Arabidopsis shade avoidance strategy is temperature-dependent and involves the receptor-like kinase ERECTA**  
Dhaval Patel, Keara Franklin
- 522 Screening of Mutants in the BBX24/STO Light Signaling Pathway**  
Annalisa Rizza, Qing Xu, Gunther Neuhaus, Marta Rodríguez-Franco
- 523 Tuning of circadian period by micronutrients availability in *Arabidopsis***  
Patrice Salomé, Michele Oliva, Ute Krämer, Detlef Weigel
- 524 PRMT5, a Piece Connecting the Circadian Clock and Alternative Splicing**  
Sabrina Sanchez, Petrillo Ezequiel, Xu Zhang, Matias Ruggnone, Carlos Hernando, Micaela Godoy Herz, Craig Simpson, John Brown, Justin Borevitz, Paloma Mas, Alberto Kornblith, Marcelo Yanovsky
- 525 Regulation of Flowering Time by a bHLH Transcription Factor in *Arabidopsis***  
Nidhi Sharma, Enamul Huq
- 526 FHY3 and FAR1 mediate clock adaptation to the light environment in *Arabidopsis thaliana***  
Hamad Siddiqui, Gang Li, Safina Khan, Haiyang Wang, Paul Devlin

**527 A Genetic Screen Identifying Mutations Which Suppress or Enhance the Phenotype of a Red Light Hypersensitive Mutant**

Gavin Sunde, Timothy Lauer, Matthew Christians, Richard Viestra, Derek Gingerich

**528 Genetic Dissection of Early Phytochrome Signaling Mechanisms**

Elise Van Buskirk, He Wang, Meina Li, Rafaelo Galvao, Tao Ma, Detlef Weigel, Meng Chen

**529 LIGHT-REGULATED WD 1 and PSEUDO-RESPONSE REGULATOR 9 Form a Positive Feedback Regulatory Loop in the *Arabidopsis* Circadian Clock**

Ying Wang, Jing-Fen Wu, Norihito Nakamichi, Hong-Gil Nam, Shu-Hsing Wu

**530 Circadian Clock-Regulated Phosphate Transporter PHT4;1 Plays an Important Role in *Arabidopsis* Defense**

Guoying Wang, Jiangli Shi, Gina Ng, Stephanie Battle, Chong Zhang, Hua Lu

**531 The conserved aspartate residue is important for both phytochrome photoconversion and photomorphogenesis in *Arabidopsis thaliana***

Junrui Zhang, Richard Vierstra

**532 Changes in Plant Physiology and Development Induced by Green Light**

Tingting Zhang, Stefanie Maruhnich, Kevin Folta

**533 VAS1 Negatively Modulates Auxin Biosynthesis to Inhibit the Shade Avoidance Responses in Plants**

zuyu Zheng, Yongxia Guo, Karin Ljung, Joseph Noel, Joanne Chory

**534 Combining Nested Association Mapping and Correlated Genome Associations to a Quantitative Trait Network to Unravel the Genetic Basis of Fitness in *Arabidopsis thaliana***

Benjamin Brachi, Zhang Qingrun, Nathalie Faure, Cedric Glorieux, Adeline Vazquez, Hubert Guillaume, Magnus Nordborg, Joy Bergelson, Fabrice Roux

**535 Identification of key genes underlying quantitative resistance to *Xanthomonas campestris* in *Arabidopsis thaliana* by Genome Wide Association mapping**

Marilyne Debieu, Anne Genissel, Cedric Glorieux, Nathalie Faure, Carine Chauveau, Joy Bergelson, Dominique Roby, Fabrice Roux

**536 Implementation of Large-Scale QTL Studies of the Seedling Root Gravitropic Response Using Scanner Technology and Automated Image Processing in an Undergraduate Setting**

Grant Dewey, Autumn Longo, Sarah Merithew, Devon Niewohner, Halie Smith, Tessa Durham Brooks

**537 Combining Nested Association Mapping and ecology-phenotype relationships to identify ecological and evolutionary forces acting on vegetative growth in *Arabidopsis thaliana***

Nathalie Faure, Benjamin Brachi, Romain Villoutreix, Qingrun Zhang, Aude Darracq, Joël Cuguen, Joy Bergelson, Magnus Nordborg, Fabrice Roux

**538 An Adaptive Model for Parental Genomic Imprinting**

Maria Cartagena, Rui Lui, Jenkin Chan, Jonathan Fitz Gerald

**539 Ribosome Number is Negatively related to Biomass Accumulation in *Arabidopsis* in a Stable Diurnal Growth Regime**

Hirofumi Ishihara, Eva-Theresa Pyl, Alexander Ivakov, Maria Piques, Waltraud Schulze, Ronan Sulpice, Mark Stitt

**540 HSP90-facilitated Divergence of Transcription Factors in Brassinosteroid Signaling**

Jennifer Lachowiec, Jennifer Nemhauser, Christine Queitsch

**541 Genetic Mapping Of Broad Resistance To Downy Mildew In *Arabidopsis* C24**

Dmitry Lapin, Rhonda Meyer, Guido van den Ackerveken

**542 Binding Site Divergence Between a Pair of Recently Duplicated AP2 Transcription Factors in Two *Arabidopsis* Species**  
Melissa Lehti-Shiu, Kelian Sun, Cheng Zou, Shin-Han Shiu

**543 Automated Measurements of Root Gravitropism Add the Time Domain to Quantitative Trait Loci Analysis**  
Candace Moore, Logan Johnson, Miron Livny, Edgar Spalding

**544 Genetic Analysis of Seed Longevity In *Arabidopsis thaliana***  
Thu-Phuong Nguyen, Leónie Bentsink

**545 Ovule Patterning and Development in *Arabidopsis***  
Dunia Pino Del Carpio, Cornelia Gieseler, Rüdiger Simon

**546 Structure/Function Analysis of the NF-YB family in *Arabidopsis***  
Jan Rissing, Rod Kumimoto, Ben Holt

**547 Molecular Evolution and Selection Patterns of Plant F-Box Proteins with C-Terminal Kelch Repeats**  
Nadine Schumann, Aura Navarro-Quezada, Kristian Ullrich, Carsten Kuhl, Marcel Quint

**548 Towards genome-wide association genetics to identify loci involved in responses to Potyviruses in plants**  
Valerie Schurdi-Levraud, Patrick Cosson, Zofia Nehr, Melodie Caballero, Fabrice Roux, Frederic Revers

**549 Tetraploidy in natural populations of *Arabidopsis thaliana* is a transient character state**  
Elisabeth Svedin, Tena Graham, Brian Dilkes

**550 Incidence and Pattern of Nonrandom Mating in *Arabidopsis thaliana* Across the Genetic Spectrum**  
Ann Carlson, Sara Crawford, Robert Swanson

**551 Intragenic Tandem Repeats Confer Phenotypic Variability**  
Soledad Undurraga, Matthieu Legendre, Christine Queitsch

**552 The scale of adaptation in *Arabidopsis thaliana*: identifying the ecological factors that act on phenology.**  
Romain Villoutreix, Benjamin Brachi, Nathalie Faure, Nina Hautekeete, Yves Piquot, Dominique Roby, Joël Cuguen, Fabrice Roux

**553 *AaTFL1* Regulates Multiple Aspects of Perennial Flowering in *Arabis alpina***  
Renhou Wang, Maria Albani, Coral Vincent, Sara Berganza, Ming Luan, Yan Bai, Christiane Kiefer, Rosa Castillo, Bao Liu, George Coupland

**554 The Cyber-Language of Flowers: PhytoCognito as Fusion of Omic Data Analysis and Natural Language Processing of the *Arabidopsis* Literature**  
Amir Assadi, Noah Larsen

**555 Targeted protein aggregation in *Arabidopsis thaliana* plants as a tool to specifically knock-out protein function**  
Camilla Betti, Silvie Coutuer, Jie Xu, Isabelle Van Houtte, Dirk Inze, Frederic Rousseau, Joost Schymkowitz, Eugenia Russinova

**556 Temporal EIN3 transcription factor binding reveals role of protein-DNA interactions in hormone crosstalk**  
Katherine Chang, Hai Li, Gary Hon, Mattia Pelizzola, Mark Urich, Robert Schmitz, Paul Kuo, Joseph Nery, Hong Qiao, Trey Ideker, Joseph Ecker

**557 Abstract Withdrawn**

- 558 High Throughput Automated Imaging and Machine Learning Methods for Quantifying Variation of Morphological Traits of *Arabidopsis Thaliana* Roots and Shoots**  
*Hesam Dashti, Hesam Dashti, James Driver, Amir Assadi*
- 559 Plant Methods: an Independent Open Access Journal for Technological Innovation in the Plant Sciences**  
*Brian Forde, Mike Roberts*
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*Guillaume Queval, Rudy Vanderhaeghen, Leen Vermeersch, Pierre Hilson*
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*Susan Bush, Patrick Krysan*
- 562 PCR from Plant Tissue without DNA Extraction**  
*Maiju Kyllainen, Pia Kuusisto, Pak Yang Chum*
- 563 Determining Degradation and Synthesis Rates of *Arabidopsis* Proteins Using the Kinetics of  $^{15}\text{N}$  Labeling of 2D Gel Separated Proteins**  
*Lei Li, Clark Nelson, Cory Solheim, James Whelan, A. Harvey Millar*
- 564 CRES-T is a novel gene silencing system, useful for functional analysis of transcription factors and manipulation of plant traits**  
*Miho Ikeda, Masaru Nakata, Tomomi Mito, Kyoko Matsui, Nobutaka Mitsuda, Masaru Ohme-Takagi*
- 565 Artificial Chimeric Repressors Can Increase Seed Oil Content and Plant Biomass**  
*Chikara Ohto, Madoka Yonekura, Nobuhiko Muramoto, Satoshi Kondo, Tomoko Tanaka, Kyoko Matsui, Tomotsugu Koyama, Nobutaka Mitsuda, Masaru Ohme-Takagi, Norihiro Mitsukawa*
- 566 High-resolution imaging of statolith dynamics under hypergravity conditions by using a new centrifuge microscope**  
*Masatsugu Toyota, Masao Tasaka, Miyo Morita*
- 567 Teaching Tools in Plant Biology: A New, Award-Winning, On-Line Educational Resource Published by *The Plant Cell***  
*Mary Williams*
- 568 A Role of LATERAL ORGAN BOUNDARIES DOMAIN 37, 38, and 39 in a Subset of ABA Signaling in *Arabidopsis***  
*Chuloh Cho, Jungmook Kim*
- 569 Suppression of Cell Death of *mod1* by Reducing ROS through Disturbing Mitochondrial Complex I**  
*Jian Wu, Yuefeng Sun, Yonghong Wang, Jiayang Li*
- 570 Comprehensive study of plant transcription factors**  
*Nobutaka Mitsuda, Youichi Kondou, Tomotsugu Koyama, Kyoko Matsui, Takeshi Yoshizumi, Miho Ikeda, Yuko Takiguchi, Shinobu Takada, Miki Fujita, Kazuo Shinozaki, Norihiro Ohtsubo, Minami Matsui, Masaru Ohme-Takagi*
- 571 Unraveling Gene Regulatory Network in *Arabidopsis thaliana***  
*Mohammad Amin Omidbakhshfard, Bernd Mueller-Roeber*
- 572 A Mitochondria Localized PPR Protein Is Required For Embryogenesis In *Arabidopsis***  
*Shan He, Jingjing Liu, Li-Jia Qu*
- 573 NO CATALASE ACTIVITY 1 (NCA1) is an ancient post-transcriptional regulator of plant catalase activity**  
*Thomas Hackenberg, Trine Juul, Aija Auzina, Anna Malolepszy, Kåre Nielsen, Frank van Breusegem, Jan-Elo Jorgensen, Stig Andersen*

**574 Sugar Induced Expression of the PAP1 Gene in *Arabidopsis thaliana***Bettina Broeckling, Blaire Steinwand, Daniel Bush**575 Signaling pathways Controlling Cell Wall Loosening and Cell Separation During Floral Abscission**Melinka Butenko, Chun-Lin Shi, Bin Liu, Michelle Leslie, Sara Patterson, Sarah Liljegren, Reidunn Aalen**576 Genetic dissection of GA regulated root growth**Ester Cancho, Susana Ubeda-Tomás, Malcolm Bennett**577 Abolishing of bZIP Factor Dependent Gene Expression through Phosphorylation-mimicking Serine Mutations in the DNA-binding Domain**Tobias Kirchler, Sebastian Briesemeister, Oliver Kohlbacher, Klaus Harter, Christina Chaban**578 Application of a FRET-based Calmodulin Sensor to imaging Calmodulin-Dependent Signaling in *Arabidopsis***Alexandra Chanoca, Simon Gilroy**579 Phosphobinding Domain in Plants**David Chevalier**580 Regulation of auxin response in the *Arabidopsis* hypocotyl**Goh Choe, Mark Estelle**581 Identification and Characterization of a Novel JA Signaling Component, JAH2**KwiMi Chung, Agnes Demianski, Inez Oh, Barbara Kunkel**582 Auxin Response: How Does ABP1 Signal to Induce Cell Expansion?**Nathan Deed, Karine David**583 Comparison of ABA-, glucose- and sucrose-mediated gene expression profiles between *Arabidopsis* and rice: insights into ancestral angiosperm signaling pathways**Luiz Del-Bem, Renato Vicentini, Michel Vincentz**584 Monitoring reactive oxygen species in the auxin-induced oxidative burst of *Arabidopsis thaliana* roots**Desiree den Os, Gabriele Monshausen**585 Genetic Identification of Factors Involved in GLV Secretory Peptide Perception**Sarieh Ghorbani, Andrzej Drozdzecki, Pierre Hilson**586 Candidate GPCR Signaling Systems Integrate Multiple Environmental Signals in G-protein Dependent and Independent Pathways**Timothy Gookin, Xiaofen Jin, Rui-Sheng Wang, Sixue Chen, Sarah Assmann**587 The AFB4 Auxin Receptor is a Negative Regulator of Auxin Signaling in *Arabidopsis* Seedlings**Katie Greenham, Cristina Castillejo, Colleen Doherty, Steve Kay, Mark Estelle**588 Abstract Withdrawn****589 RIC7 Negatively Regulates Stomatal Movements via Interaction with Exo70B1**Daewoong Hong, Jae-Ung Hwang, Byeong Wook Jeon, Eun-Jung Lee, Soo Young Kim, Youngsook Lee**590 Characterization of <> in Auxin Signaling may Reveal Novel Functions of the COP9 Signalosome**He Huang, Marcel Quint, William Gray**591 The Plant U-box/ARM E3 ligases as potential signalling proteins for S-Domain Receptor Kinases**Emily Indriolo, Pirashaanthy Tharmapalan, Daphne Goring

**592 A Soluble ABC Protein AtNAP9/AtABC120 Might Play Important Functions for Early Seedling Development Regulated by Abscisic Acid and Light**

Jun-Young Jin, Sehoon Kwon, Soo Young Kim, Enrico Martinoia, Youngsook Lee

**593 Patatin-Related Phospholipases A In Auxin Signal Transduction: Mis-Regulation Of Early Auxin-Induced Genes In pPLA Knockouts**

Corinna Labusch, Maria Shishova, Yunus Effendi, Günther Scherer

**594 Characterization of *gig1* (*glucose insensitive growth 1*) Reveals the Involvement of the Plastidic Copper Transporter PAA1 in Sugar-mediated Interorganellar Communication**

Shin Ae Lee, Jun Lim

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**596 Inferring the signaling network of ORE1: a molecular and functional approach to a leaf senescence regulatory pathway**

Lilian Matallana-Ramirez, Bernd Mueller-Roeber, Salma Balazadeh

**597 Mutations in the Cytokinin Signaling Pathway Can Alter Auxin Signaling**

Dennis Mathews, McKenzie Shaw, Eric Schaller

**598 Silver Ions Block Ethylene Responses In Arabidopsis Predominantly Through The ETR1 Ethylene Receptor**

Brittany McDaniel, Brad Binder

**599 Function Of The N-end Rule Pathway Of targeted Proteolysis In The Regulation Of Abscisic Acid Insensitive 5 (ABI5) During Seed Germination in *Arabidopsis thaliana***

Nurulhikma Md Isa, Daniel Gibbs, Michael Holdsworth

**600 Modulation of Jasmonate Signaling Through Production of JAZ Splice Variants**

Javier Moreno, Gregg Howe

**601 Surface potentials in wounded *Arabidopsis* leaves**

Seyed Ali Mousavi, Stephan kellenberger, Edward Farmer

**602 Quantitative Analysis of Abscisic Acid Signalling in *Arabidopsis***

Eric Nam, Akira Endo, Peter McCourt

**603 Structural and Functional Characterization of DAWDLE (DDL) in *Arabidopsis thaliana*.**

Lakshmi Narayanan, David Chevalier

**604 GID1-dependent GA signaling can stimulate germination of the GA-insensitive mutant *sly1* in the absence of DELLA degradation**

Sven Nelson, Tohru Ariizumi, Shinjiro Yamaguchi, Camille Steber

**605 Transcriptional Profiling of the Floral Organ Abscission Mutant *hae-3 hsl2-3* by RNA-Seq**

Chad Niederhuth, John Walker

**606 The Role of the Cytokinin Response Factors (CRFs) in Cytokinin Signaling and Plant Development**

Tracy Raines, Joseph Kieber

**607 A Transcriptional Cascade Regulates Cytokinin Signaling in *Arabidopsis***

Yi-Hsuan Chiang, Hyo Jung Kim, Kristine Hill, Rebecca Argyros, Dennis Mathews, Joseph Kieber, G. Eric Schaller

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Laura Sheard, Xu Tan, Haibin Mao, Tom Hinds, Sheng Yang He, John Browse, Gregg Howe, Jose Rizo-Rey, Ning Zheng

**609 Identification of the Ligand Binding Site in a Non-Island-Type Leucine-Rich Repeat Receptor Kinase**

Hidefumi Shinohara, Yuji Moriyama, Yoshikatsu Matsubayashi

**610 Chemical Screen Uncovers Link Between One-Carbon Metabolism and Sucrose Signaling in Arabidopsis**

Michael Stokes, Abhishek Chattopadhyay, Olivia Wilkins, Malcolm Campbell

**611 The TOC Complex May Mediate the Plastid Localization of a Gravity Signal Transducer in Arabidopsis**

Allison Strohm, Patrick Masson

**612 AtPP2CF1 Encodes a Functional *Arabidopsis* PP2C Which Belongs to Group E**

Hiroki Sugimoto, Satoshi Kondo, Nobuhiko Muramoto, Tomoko Tanaka, Etsuko Hattori, Ken'ichi Ogawa, Norihiro Mitsukawa, Chikara Ohto

**613 *Arabidopsis ASA1* Is Important for Jasmonate-Mediated Regulation of Auxin Biosynthesis and Transport during Lateral Root Formation**

Jiaqiang Sun, Yingxiu Xu, Songqing Ye, Hongling Jiang, Qian Chen, Fang Liu, Wenkun Zhou, Rong Chen, Xugang Li, Olaf Tietz, Xiaoyan Wu, Jerry Cohen, Klaus Palme, Chuanyou Li

**614 Over Expression of Fatty Acid Amide Hydrolase in *Arabidopsis thaliana* Alters Flowering Time Under Short Day Conditions Through its Hydrolytic Activity**

Neal Teaster, Kent Chapman, Elison Blancaflor

**615 Two Plant Glutamate-Like Receptors from *Arabidopsis* are Expressed in Root Phloem Tissue and are Involved Lateral Root Initiation**

Eric Vincill, Arielle Clarin, Anthony Bieck, Edgar Spalding

**616 Identification and characterization of suppressors of the *tir1-1* auxin signaling defect**

Jonathan Wenger, Kai-ting Fan, Marcel Quint, William Gray

**617 Trehalose-6-phosphate and sucrose signalling in *Arabidopsis thaliana***

Umesh Yadav, Regina Feil, Mark Stitt, John Lunn

**618 Abstract Withdrawn****619 Determining the function and subcellular localization of the *Arabidopsis* LAZY1 protein**

Takeshi Yoshihara, Edgar Spalding, Moritoshi Iino

**620 Type-A response regulators are required for proper root apical meristem function through the post-transcriptional regulation of the PIN auxin efflux carriers**

Wenjing Zhang, Jennifer To, Chia-Yi Cheng, G. Eric Schaller, Joseph Kieber

**621 *Arabidopsis* Tyrosylprotein Sulfotransferase Acts in the Auxin/PLETHORA Pathway in Regulating Postembryonic Maintenance of the Root Stem Cell Niche**

Wenkun Zhou, Lirong Wei, Jian Xu, Qingzhe Zhai, Hongling Jiang, Rong Chen, Qian Chen, Jiaqiang Sun, Jinfang Chu, Lihuang Zhu, Chun-Ming Liu, Chuanyou Li

**622 IQD22, a negative regulator of GA response, plays a role in the regulatory network among the GA, calcium and auxin pathways**

Xin Zhou, Tai-ping Sun

**623 Abstract Withdrawn****624 Genetic Dissection of the Plant/Pest Interaction: Mapping of *Arabidopsis* Resistance Gene to *Tetranychus urticae* (Two Spotted Spider Mite) Feeding**

Marc Cazaux, Cherise Poo, Marie Navarro, Richard Clark, Miodrag Grbic, Vogislava Grbic

**625 From *Arabidopsis* to *Camelina*: Translating Our Understanding of Trichome Development**

Kevin Dorn, David Marks

**626 High-Level Expression of a Set of Six Thermostable Cell Wall-Degrading Enzymes in Tobacco Chloroplasts: A First Step Towards Development of the Auto-Saccharification System of Bioenergy Crops**

Yoichi Nakahira, Yasuhiro Kashima

**627 Evaluation of *Arabidopsis thaliana* as an Experimental Host for *Xylella fastidiosa***

Elizabeth Rogers

**628 Targeting Mechanisms of the H3K4 Tri-Methyltransferase SET DOMAIN GROUP Protein 2 in *Arabidopsis***

Li Wang, Xiaoyu Zhang

**1 Suppression of plant immunity by Phytophthora effectors***Sophien Kamoun***The Sainsbury Laboratory, Norwich Research Park, NR4 7UH United Kingdom**

To enable parasitism and symbiosis, plant-associated organisms intimately interact with plant cells often through specialized cellular structures. For example, biotrophic fungal and oomycete pathogens form haustoria that invaginate host cell plasma membrane to deliver pathogenicity effectors and acquire nutrients. In response and to restrict pathogen colonization, the attacked plant cell undergoes significant cellular reorganization involving organelle relocation, polarized secretion of anti-microbial molecules, and cell wall reinforcements around contact sites. Plant pathogens, such as the oomycete *Phytophthora infestans*, deploy an arsenal of pathogenicity effector proteins to counteract plant immune responses. However, the extent to which plant pathogen effectors interfere with defense-related focal secretion is poorly known. VRblb2 is one of several hundred RXLR-type proteins, a major class of host-translocated effectors in *P. infestans* and other *Phytophthora* species. Similar to other RXLR effectors, AVRblb2 is a modular protein with the N-terminal half, comprised of a signal peptide and the RXLR domain, involved in trafficking to host cell cytoplasm and the C-terminal region carrying the biochemical effector activities. Members of the AVRblb2 family are recognized inside plant cells by the broad-spectrum resistance protein Rpi-blb2 of the wild potato *Solanum bulbocastanum*. However, the primary activity of AVRblb2 and other RXLR effectors is to promote virulence and the precise modes of action and host targets of these effectors remain largely unknown. We show that AVRblb2 focally accumulates inside plant cells and promotes virulence by interfering with the execution of polarized host defenses. AVRblb2 significantly enhances susceptibility of host plants to *P. infestans* by targeting the host papain-like cysteine protease C14 and specifically preventing its secretion into the apoplast. Plants altered in C14 expression were significantly affected in susceptibility to *P. infestans* in a manner consistent with a positive role of C14 in plant immunity. Our findings point to a novel counter-defense strategy that plant pathogens utilize to neutralize secreted host defense proteases. Effectors, such as AVRblb2, can be used as molecular probes to dissect focal immune responses at pathogen penetration sites.

**2 Using genetics and structural biology to dissect the molecular mechanisms of BR perception and signaling***Joanne Chory***Plant Biology Laboratory, The Salk Institute for Biological Studies and The Howard Hughes Medical Institute, La Jolla, CA 92037 USA**

Growth involves the coordination of cell division and expansion, which is the result of developmental programs initiated by plant hormones in response to environmental cues, such as light and temperature. Brassinosteroids (BRs) are a class of hormones that are essential for normal plant growth and development. In the absence of BR production or perception, plants exhibit an extreme dwarf stature, which has been attributed to defects in cell expansion and division. During the past decade, the BR receptor has been characterized and key players in both the BR signaling and biosynthesis pathways have been identified. In this talk, I will summarize the state of the field, discuss our most recent results in dissecting the activation mechanism of the BR receptor, and present data that shows that BRs act locally to promote cell expansion, with the major target tissue being the epidermis. The interactions of BRs with other plant hormones that affect extension growth and vascular development will also be explored.

This work was funded by the NSF, NIH and the Howard Hughes Medical Institute.

### 3 Biogenesis and functions of plant microRNAs

*Yun Ju Kim, Lijuan Ji, Shengben Li, Xigang Liu, Rae Yumul, Xuemei Chen*

**University of California, Riverside, California, USA**

We have been studying the mechanisms underlying the metabolism of microRNAs (miRNAs) in *Arabidopsis*. The steady-state levels of miRNAs are determined by the balance between miRNA biogenesis and miRNA degradation. miRNA biogenesis is a multi-step process that includes transcription of *MIR* genes, processing of miRNA precursors, modification of miRNA/miRNA\* duplexes, and effector binding of mature miRNAs. miRNA degradation in vivo requires the SDN family of small RNA exonucleases. Our recent studies on key players in some of these miRNA metabolic processes will be discussed.

miRNAs play key roles in plant development. We have uncovered the functions of two argonaute proteins, which are effectors of miRNAs, and two miRNAs in the regulation of floral stem cells. This work will be discussed to highlight the importance of miRNAs in plant development and the complexities in miRNA-argonaute interactions.

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### 4 Epigenomic Variation in Plants (and People)

*Joseph Ecker*

**The Salk Institute, La Jolla**

Traditionally, phenotype is defined by a combination of genetic and environmental interactions. Missing from this equation is an understanding of the impact that epigenetic variation has on phenotype. Consequently, we are sequencing the genomes, DNA methylomes and transcriptomes of hundreds of *Arabidopsis thaliana* accessions and tens of human cell types to better understand the interplay and impact of genetic, and epigenetic variation on phenotype. Recent results of these studies will be discussed.

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### 5 Imprinted expression of Pol IV-dependent siRNAs

*Rebecca Mosher*

**The School of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA**

Most eukaryotes produce a complex population of small RNA mediators of gene silencing including microRNAs, trans-acting siRNAs, piwi-associated RNAs, and heterochromatic siRNAs. In *Arabidopsis thaliana* the most abundant class of small RNAs are produced through the action of the plant specific DNA-dependent RNA polymerase IV (Pol IV) from thousands of discreet genomic loci. Pol IV-dependent (p4)-siRNAs catalyze transcriptionally-repressive DNA methylation on transposable elements and other repetitive sequences, but are also produced from unique genomic regions, including some genes, making the biological roleof p4-siRNAs unclear.

P4-siRNAs display two expression patterns – type II p4-siRNAs are produced throughout the plant, while type I are restricted to gametophytes and the developing endosperm, where expression is imprinted and maternal-specific. Prior to fertilization maternal DNA is demethylated at regions producing p4-siRNAs, suggesting that loss of DNA methylation is required for maternal-specific p4-siRNA production. The role of DNA methylation and other chromatin modifications in the expression of p4-siRNAs will be discussed,

**6 Stability of Resistance proteins is controlled by SCF-mediated protein degradation**

*Yu Cheng<sup>1</sup>, Yingzhong Li<sup>2</sup>, Shuai Huang<sup>1</sup>, Yan Huang<sup>1</sup>, Xinnian Dong<sup>3</sup>, Yuelin Zhang<sup>2</sup>, Xin Li<sup>1</sup>*

<sup>1</sup>UBC, Vancouver, <sup>2</sup>NIBS, Beijing, <sup>3</sup>Duke University, Durham

Resistance proteins play a central role in recognizing pathogens and initiating downstream defense cascades in plants. Most R proteins contain the evolutionarily conserved nucleotide binding (NB) and leucine-rich repeat (LRR) domains. They are structurally related to the nucleotide-binding domain and LRR-containing (NLR) proteins which function as immune receptors in animals. Under the uninfected state, R protein activities have to be strictly controlled to prevent autoimmunity. Over-accumulation of NB-LRR R proteins leads to auto-activation of immune responses. It is unclear how R protein levels are regulated in plants. We found that the F-box protein CPR1 in Arabidopsis targets NB-LRR R proteins such as SNC1 and RPS2 for degradation, suggesting that SCF-mediated stability control of plant R proteins plays an important role in regulating R protein levels and preventing autoimmunity.

**7 From plant-pathogen interactions to plant-microbe communities**

*Davide Bulgarelli, Klaus Schläppi, Nahal Ahmadinejad, Emiel ver Loren Van Themaat, Matthias Rott, Paul Schulze-Lefert*

**Dept. Plant-Microbe Interactions, Max-Planck-Institute for Plant Breeding Research, Cologne, D-50829, Germany**

Soil microbial communities represent an outstanding reservoir of potential probiotic and plant protective associations. However, the genetic relationships between plants and the microbes inhabiting the rhizosphere (the fraction of soil directly influenced by the plant) or the root interior (endorhizosphere) are largely unknown. Also, a systematic survey of the taxonomic composition of the root microflora and its physiological functions is poorly understood. To fill this gap we have developed culture-independent methods to study the root-associated microflora of *Arabidopsis thaliana* and *A. thaliana* relatives grown in natural soils. We used contrasting soils, different *A. thaliana* accessions and *Cardamine hirsuta* to address the following questions: (i) Does the plant shape its rhizosphere microbial community and, if so, is this influenced by the plant genotype? (ii) Does the soil type contribute to the structure of the rhizosphere microbial community? Using a 454 pyrosequencing-based approach we identified Bacteroidetes, Proteobacteria, Chloroflexi and Actinobacteria as major bacterial phyla co-colonizing *A. thaliana* or *C. hirsuta* roots. Whilst these soil bacteria become highly enriched in roots, the bacterial communities in the rhizosphere (soil in direct contact with roots) showed only a weak taxonomic differentiation compared to bulk soil. The 454 pyrosequencing-deduced taxonomic composition of the root-associated microflora was validated by FISH-based direct visualization of individual bacteria on the root surface, providing evidence for a spatial substructure of the bacterial community. I will report our attempts to obtain insights into potential functions of the root microflora.

**8 Understanding plant-microbe interactions: Plant immune system function and rhizosphere metagenomics**

*Jeff Dangl*

**Dept. of Biology and Carolina Center for Genome Sciences University of North Carolina at Chapel Hill**

Our overall goal is to understand how particular pathogen virulence effector proteins have evolved to manipulate plant signaling machinery and function as virulence factors. We further want to know how these manipulations of host defense machinery are recognized by the NB-LRR receptors of the plant immune system. Our rationale is that by understanding how a collection of pathogen virulence factors act inside the host cell, we will better understand the normal, defense relevant function of their targets. We also are interested in expanding the experimental tool kit beyond binary plant-pathogen interactions and have begun to deploy second generation DNA sequencing technologies to characterize the *Arabidopsis* rhizosphere microbiome.

**9 Morphological and Functional Identity of Organelles of the Early Plant Secretory Pathway***Federica Brandizzi***Michigan State University-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA**

A fundamental question in eukaryotic cell biology is how cells maintain efficient compartmentalization and control the delivery and integration of biomolecules into specialized organelles. In our lab, we address these questions using the plant secretory pathway as a model. This pathway is vital to the inner workings of the cell and for communicating with the external environment during growth and in response to stress; it consists of several organelles that synthesize, shuttle, and store a large part of the cell's proteins, lipids, and sugars. In plants, the activities of the endoplasmic reticulum (ER) and Golgi apparatus, the initial organelles of the secretory pathway, are also fundamental for the synthesis and deposition of the building blocks of energy-rich compartments such as the cell wall and storage vacuole. Both organelles have unique architecture and functions, which are maintained despite exchange of membranes and luminal contents with other organelles. Efforts in our lab focus on identifying the mechanisms governing the morphology and function of the ER and the Golgi and defining the extent to which their architecture influences their function. To address these questions we have carried out forward genetic screens based on confocal microscopy analyses of *Arabidopsis* seedlings expressing ER and Golgi markers. The screens are designed to identify mutants with aberrant distribution of the ER and Golgi markers compared to non mutagenized seedlings. Through the characterization of these mutants we are identifying novel genes and mutations that uncover new information on the mechanisms for the integrity of the ER and the Golgi with respect to other organelles, cytoskeleton and flow of biosynthetic cargo. Our most recent findings will be presented in this talk.

In particular, we are interested in understanding how the Golgi establishes and maintains its integrity, and the role of this organelle in the synthesis and deposition of the cell wall. We also aim to establish factors that dictate the characteristic architecture of the ER (this renewal), as well as to define the role of the ER in coordinating genome responses during stress (Project 2.3.3). Understanding the mechanisms underlying the morphological and functional integrity of the Golgi and the ER will not only help answer fundamental questions in cell biology, but will also provide tools to exploit the largely unused potential of the secretory pathway to convert fixed carbon to high-value products such as biofuels and proteins for use in industry.

**10 Light-induced chloroplast movements in leaf cells***Roger Hangarter***Indiana University, Bloomington, (IN), USA**

Light-dependent chloroplast movement in photosynthetic cells optimizes photosynthetic light absorption. When the fluence rate of light is high, chloroplasts migrate to the anticlinal walls, parallel to the incident light. This repositioning of chloroplasts is stimulated by blue light perceived via the plasma membrane-associated phototropin photoreceptors. Recent work suggests that chloroplast movement is driven by actin re-organization at the plasma membrane rather than myosin-based translocations. A number of proteins important for normal chloroplast movements have recently been identified and light-dependent changes in the dynamics of chloroplast-associated actin dynamics have been observed. We will present results for a recently identified protein called THRUMIN1, which serves as a critical link between photoreceptor activity at the plasma membrane and the regulation of cortical actin dynamics. Our data indicate that THRUMIN1 is involved in bundling of actin filaments at the plasma membrane in response to blue light perceived by the phototropin photoreceptors. The light-dependent actin bundling mediated by THRUMIN1 may in turn dynamically remodel actin filament arrays nucleated by CHUP1 at the chloroplast outer envelope to drive chloroplast movements.

**11 Structural basis of brassinosteroid perception by a membrane receptor kinase***Michael Hothorn<sup>1</sup>, Youssef Belkhadir<sup>1</sup>, Marlene Dreux<sup>2</sup>, Tsegaye Dabi<sup>1</sup>, Joseph Noel<sup>1</sup>, Ian Wilson<sup>2</sup>, Joanne Chory<sup>1</sup>*<sup>1</sup>**Salk Institute, La Jolla, CA, USA, <sup>2</sup>The Scripps Research Institute, La Jolla, CA, USA**

Polyhydroxylated steroids are regulators of body shape and size in higher organisms. In metazoans intracellular receptors recognize these molecules. Plants however perceive steroids at membranes, using the membrane-integral receptor kinase BRI1. The crystal structure of the BRI1 ectodomain reveals a superhelix of 25 twisted leucine-rich repeats (LRRs), an architecture that is strikingly different from the assembly of LRRs in animal Toll-like receptors. A 70 amino-acid island domain between LRRs 21 and 22 folds back into the interior of the superhelix to create a surface pocket for binding the plant hormone brassinolide. Known loss- and gain-of-function mutations closely map to the hormone-binding site and validate our structural models. Importantly, steroid binding to BRI1 generates a docking platform for a smaller co-receptor kinase that is essential for activation of the brassinosteroid signaling pathway. Our findings have novel mechanistic implications for diverse hormone, developmental and innate immunity signaling pathways in plants that rely on similar receptors.

*Note: Also a poster presentation.*

**12 NIMA-related Kinases Redundantly Regulate Directional Cell Expansion in *Arabidopsis thaliana****Hiroyasu Motose<sup>1</sup>, Kaori Yoshimoto<sup>1</sup>, Yuichiro Takahashi<sup>1</sup>, Tatsuya Sakai<sup>2</sup>, Taku Takahashi<sup>1</sup>*<sup>1</sup>**Division of Bioscience, Graduate School of Natural Science & Technology, Okayama University, <sup>2</sup>Graduate School of Science & Technology, Niigata University**

NIMA-related kinases (NEKs) are a family of Ser/Thr protein kinases in eukaryotes. In fungi and animal cells, NEKs regulate several mitotic events including G2/M transition, centrosome separation, and spindle formation. To elucidate plant NEK functions, we analyze seven NEK members encoded in the genome of *Arabidopsis thaliana*. The promoter GUS analysis shows that NEKs are expressed in

specific tissues including apical meristems, vascular system, and stomatal guard cells. All of the seven NEKs tagged with GFP colocalize with NEK6 and microtubules. NEK6 interacts with NEK4 and NEK5, phosphorylates tubulin and armadillo-repeat containing kinesin 1 (ARK1), and regulates epidermal cell expansion through suppression of excessive microtubule stabilization. Triple mutant analysis indicates that NEK1, NEK2, and NEK3 regulate directional cell expansion in root epidermal cells. To identify signaling component downstream of NEKs, we isolate several proteins interacting with NEK6 by using immunoprecipitation and yeast two-hybrid analysis. These results suggest that plant NEKs interact with each other and redundantly regulate directional cell expansion. The functional redundancy and diversification of plant NEKs will be discussed.

*Note: Also a poster presentation.*

### 13 The Role of SKIP3, a Novel Plant-Specific Endosomal Protein, in Plant Development and Brassinosteroid Signaling

*Francisca Reyes, Rafael Buono, Marisa Otegui  
UW Madison, WI, USA*

Endosomes play an important function in the sorting of plasma membrane proteins that are internalized by endocytosis. In endosomes, those internalized plasma membrane proteins that have been targeted by degradation by the addition of ubiquitin moieties, are sorted by the ESCRTs (Endosomal Sorting Complex Required for Transport) and the ATPase Vps4p/SKD1 (Suppressor of K<sup>+</sup> transport growth defect 1) into endosomal intraluminal vesicles for their subsequent degradation in the vacuolar lumen. We have identified a plant-specific protein called SKIP3 (SKD1-interacting protein 3) that has a strong positive effect on the *in vitro* ATPase activity of both *Arabidopsis* SKD1 and yeast Vps4p. Interestingly, although *SKIP3* is not present in yeast, the expression of *Arabidopsis SKIP3* in yeast mutant cells lacking Vta1p, an unrelated positive modulator of Vps4p activity, partially rescues the mutant endosomal trafficking defects. To analyze the function of SKIP3 in plants, we created *skip3* knock down lines by expressing artificial microRNAs (amiRNA) targeted to the *SKIP3* transcript. These plants show severe developmental alterations such as dwarfism, impaired organogenesis, and reduced fertility. At the cellular level, the *skip3* plants exhibit limited cell expansion. Abnormal transcript levels of brassinosteroid (BR) responsive genes indicate that at least partially, the developmental defects in the *skip3* amRNA plants are due to defective BR signaling. Interestingly, SKIP3 seems to have originated within the plant lineage by the rearrangement of protein domains found in other ESCRT-related proteins.

*Note: Also a poster presentation.*

### 14 Slow Trafficking of *Arabidopsis* ATP-Binding Cassette Protein Subfamily B4 Indicates Its Basal Auxin Efflux Function in the Plasma Membrane

*Misuk Cho<sup>1</sup>, Jiwon Lee<sup>2</sup>, Minsoo Lee<sup>1</sup>, Hyung-Taeg Cho<sup>1</sup>  
<sup>1</sup>Seoul National University, Seoul, Korea, <sup>2</sup>Korea Basic Science Institute, Daejeon, Korea*

Intracellular trafficking of auxin transporters has been implicated in diverse developmental processes in plants. While the dynamic trafficking pathways of PINFORMED (PIN) auxin efflux proteins have been studied extensively, the trafficking of ATP-binding cassette protein subfamily B proteins (ABCBS, another group of auxin efflux carriers) has not yet been characterized. In this study, we address the trafficking characteristics of ABCB4 in *Arabidopsis thaliana* root epidermal cells. Together with the FM4-64 tracer, ABCB4 was endocytosed into the endosome at a slow rate and this process was facilitated by brefeldin A (BFA). On the other hand, the internal aggregation of ABCB4 mediated by BFA remained unaltered in both the BFA-resistant version of GNOM and *gnl1* mutants, which suggests that ABCB4 and PINs use different trafficking pathways. Fluorescence recovery after photo-bleaching analysis showed that ABCB4 is strongly retained in the plasma membrane (PM) and only a minor portion of the ABCB4 in the PM comes through the recycling route. In addition, ABCB4 exhibited a much slower rate of degradation trafficking to the vacuole than PIN2. Collectively, these data suggest that ABCB4 is native to the PM and likely to function in basal auxin efflux. These findings are in direct contrast to PINs, which are subject to dynamic controls.

### 15 Degradation of the Endoplasmic Reticulum by Autophagy during ER stress in Plants

*Yimo Liu, Junmarie Soto Burgos, Diane Bassham  
Iowa State University, Ames, IA, USA*

Autophagy is activated in response to multiple abiotic stresses in plants, including salt, drought, nutrient and oxidative stress. This activation is required for plant tolerance of these stress conditions, either for recycling of nutrients or for clearance of damaged and misfolded proteins. The endoplasmic reticulum (ER) is a central organelle of protein folding and maturation in the secretory pathway. Accumulation of unfolded or misfolded proteins causes stress and activates the unfolded protein response (UPR), leading to upregulation of expression of ER chaperones and activation of quality control pathways. In some organisms, ER stress induces autophagy; here we show that in the model plant *Arabidopsis thaliana* autophagy is activated during ER stress and functions in the transport of ER fragments to the vacuole for degradation. The ER stress-inducers tunicamycin and dithiothreitol both caused rapid activation of autophagy in *Arabidopsis* plants, as demonstrated by increased production of autophagosomes and increased expression of the autophagy-related gene *AtATG18a*. A soluble ER marker was shown to localize to autophagosomes under these conditions and to accumulate in the vacuole upon inhibition of vacuolar proteases, demonstrating that portions of the ER are delivered to the vacuole by autophagy during ER stress. Analysis of the regulation of autophagy in *Arabidopsis* during ER stress is underway.

*Note: Also a poster presentation.*

**16 The Go-Between: Auxin as a mediator of cell-cell signalling**Veronica Grienesen**John Innes Centre, UK**

To unravel how molecular and cellular properties bring forth developmental phenomena at the tissue level, multi-level modelling approaches can be fundamental. Our studies on auxin transport have been demonstrating how precise localisation of PINs within a tissue context can underly the formation of inter- and intra-cellular auxin gradients and robustly guide plant development, such as Arabidopsis root growth and lateral root initiation. However, what coordinates the cellular polarity required for the correct localisation of the PINs on cells? To address this fundamental question, it is enlightening to zoom in further and link sub-cellular spatial dynamics of certain polarity proteins (ROPs) to auxin gradients. An intricate paradigm system in which the full complexity of these interactions comes together is the development of pavement cells (PCs); these cells grow into complex forms that resemble pieces from a jigsaw puzzle, generating interdigitating patterns with the neighbouring cells. Our modelling efforts show how auxin can act as a go-between and trigger, through small GTPases (ROPs), the spontaneous emergence of intra-cellular polarity, independent of pre-patterns or localised polarising signals. Furthermore, due to the generated intercellular auxin gradients, cell-cell communication arises enabling PCs to coordinate their polarities and interlock. This constitutes a novel mechanism for cell polarity coordination through auxin, based on known molecular interactions and in close agreement with experiments.

**17 A Computational Model of *Arabidopsis thaliana* Leaf Margin Development**Przemyslaw Prusinkiewicz<sup>1</sup>, Adam Runions<sup>1</sup>, Gemma Bilsborough<sup>2</sup>, Michalis Barkoulas<sup>2</sup>, Miltos Tsiantis<sup>2</sup>**<sup>1</sup>University of Calgary, Calgary, AB, Canada, <sup>2</sup>University of Oxford, Oxford, UK**

An understanding of how biological forms are generated is a key challenge in developmental biology. Through a combination of developmental genetics and computational modeling we have gained an understanding of the regulatory mechanisms that control the development of *Arabidopsis* leaf margin. At the heart of the model is a feedback loop between transport of the growth-promoting hormone auxin by the efflux carrier PINFORMED1 (PIN1) and polar localization of PIN1 by auxin. This mechanism operates in the presence of CUP-SHAPED COTYLEDON2 (CUC2) protein, which enables the reorientation of PIN1. Auxin represses CUC2 expression, which yields an interspersed pattern of PIN1 convergence points and CUC2 activity. This pattern controls local growth rates at the margin, producing serrations at sites of high auxin activity and indentations at sites of high CUC2 expression. The model captures the developmental sequence and form of wild type *Arabidopsis* leaves as well as *pin1* and *cuc2* mutants, leaves overexpressing CUC2 locally or uniformly, and leaves resulting from auxin application. Furthermore, it explains the basipetal progression of serration formation as a consequence of the spatio-temporal distribution of growth rates within the leaf blade, and highlights the role of CUC2 as a factor stabilizing positions and form of serrations. From a broader perspective, the model provides an example of complex morphogenetic processes coupling molecular-level patterning with growth.

Reference: G. Bilsborough, A. Runions, M. Barkoulas, H. Jenkins, A. Hasson, C. Galinha, P. Laufs, A. Hay, P. Prusinkiewicz, and M. Tsiantis (2011): Model for the regulation of *Arabidopsis thaliana* leaf margin development, *PNAS* **108**(8): 3424-3429.

**18 Novel and Known Post-transcriptional Regulatory Sequences are Conserved across Plant Families**Justin Vaughn, Bijoyita Roy, Albrecht von Arnim**University of Tennessee, Knoxville, TN, USA**

Gene regulatory regimes at the post-transcriptional level operate via mRNA sequence motifs. For example, ~30% of mRNAs contain at least one small open reading frame (uORF) upstream of the major ORF. Here we present results from a comparative transcriptome study between *Arabidopsis* and five other families of dicot plants aimed at examining uORF conservation specifically and UTR conservation in general. We identified several hundred conserved RNA motifs of 5-30 nucleotides in length. Within the 5' UTR, purine-rich motifs were overrepresented. In contrast, in the 3' UTR more complex motifs were common, some of which are probable target sites for RNA binding proteins or miRNAs, and some of which may serve as sites for subcellular localization of mRNAs. These data have implications for the RNA regulon concept. Surprisingly, AUG was the most conserved triplet in the 5' UTR in all plant lineages evaluated. Given that conserved-peptide uORFs are rare, a large proportion of the associated uORFs must function in a peptide-independent fashion, whereas many others may evolve neutrally. Previous research has established that components of the basal translation machinery, such as subunits of eukaryotic initiation factor 3 and of the 60S ribosomal subunit, compensate for the inhibitory effect of certain uORFs. A computational model of the translation initiation process was implemented to show how eIF3 may contribute to the competence of the translation machinery for re-initiation. Supported by DOE DE-FG02-96ER20223 and NSF DBI-0820047.

**19 Cis-Regulatory Code of Stress Responsive Transcription in *Arabidopsis thaliana***Cheng Zou, Kelian Sun, Joshua Mackaluso, Alexander Seddon, Rong Jin, Michael Thomashow, Shin-Han Shiu**Michigan State University, East Lansing, MI, USA**

Environmental stress leads to dramatic transcriptional re-programming that is central to plant survival. At the *cis*-regulation level, substantial knowledge has accumulated on how a few plant *cis*-regulatory elements (CREs) function in stress regulation but many more CREs remain to be discovered. In addition, the plant stress *cis*-regulatory code, i.e. how CREs work independently and/or in concert to specify stress responsive expression, is mostly unknown. Based on gene expression patterns under various stress conditions, we identified a large number of putative CREs (pCREs) in *Arabidopsis thaliana* with multiple characteristics of authentic *cis*-elements. Interestingly, pCREs implicated in biotic and abiotic responses belong to two distinct pCRE superfamilies. Using these pCREs, we uncovered regulatory

rules based on pCRE presence/absence with machine learning methods. These pCRE presence/absence rules allow significantly better stress response predictions than those based on known *cis*-elements. In addition, regulatory rules based on pCRE combinatorial relationships out-perform rules based solely on pCRE presence/absence, highlighting the importance of combinatorial controls in stress responsive transcription. Furthermore, instead of a few master regulatory rules for each stress condition, many rules were discovered, and each appears to control only a small subset of stress responsive genes. As a proof of concept, we demonstrated experimentally the necessity of a novel pCRE, in combination with the ABA responsive elements, in controlling salt induced gene expression. Our findings provide a large number of potential CREs and cis-regulatory interactions relevant to plant stress response. Thus, this study contributes significantly to a better understanding of plant stress cis-regulatory logic and provides prioritized targets for further experimentation.

*Note: Also a poster presentation.*

## 20 Insights into Systems Organization, Network Evolution, and Pathogen Attack from a High-Quality *Arabidopsis* Interactome Network Map

*Pascal Braun<sup>1</sup>, Jim Beynon<sup>2</sup>, Jeffery Dangl<sup>3</sup>, Joseph Ecker<sup>4</sup>, Marc Vidal<sup>5</sup>, The Arabidopsis Interactome Mapping Consortium<sup>1</sup>*

<sup>1</sup>Center for Cancer Systems Biology (CCSB) at Dana-Farber Cancer Institute, Boston, MA, <sup>2</sup>University of Warwick, Warwick, UK, <sup>3</sup>University of North Carolina, Chapel Hill, NC, <sup>4</sup>The Salk Institute for Biological Studies, La Jolla, CA, <sup>5</sup>Center for Cancer Systems Biology (CCSB) at Dana-Farber Cancer Institute, Boston, MA

Elucidating mechanisms of life requires analysis of whole systems and understanding the complex interplay of the individual components. Proteins control and mediate the majority of biological activities and interactions among proteins play a decisive role in the dynamic modulation of cellular behavior. Protein-protein interactions are essential constituents of all cells and interactome analysis is an important component in the quest for a systems level understanding of living systems.

We recently completed mapping of the first binary interactome network for the reference plant *Arabidopsis thaliana*. We identify 6,200 high-quality interactions among 2,700 proteins and thus double the number of experimentally supported physical protein-protein interactions. Using tools of graph theory we identify biologically relevant network communities from which a picture of the overall interactome network organization starts to emerge. Combination of interaction and comparative genomics data yielded insights into network evolution, and biological inspection resulted in many hypotheses for unknown proteins and revealed unexpected connectivity between previously studied components of phytohormone signaling pathways.

Using this map we investigate how bacterial and fungal pathogens perturb their host's network. We found that pathogen effectors from these evolutionary distant pathogens converge on network hubs, which appear "guarded" by resistance proteins, and which we show to be functionally important for the host's immune responses.

Together, we show how high-quality protein interactome network maps provide us with tools for elucidating complex biological systems.

## 21 Repeat Conservation Mapping of Leucine-Rich Repeat Domains

*Laura Helft<sup>1</sup>, Vignyan Reddy<sup>1</sup>, Xiyang Chen<sup>2</sup>, Teresa Koller<sup>1</sup>, Rishabh Gupta<sup>1</sup>, Andrew Bent<sup>1</sup>*

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Leucine-rich repeats (LRRs) are a protein-ligand interaction domain found in hundreds of *Arabidopsis* receptors whose roles include hormone perception, ubiquitination, and pathogen detection. Because LRR domains can be hundreds of amino acids long, it is of interest to computationally predict functionally significant regions prior to *in vivo* analyses. We have developed Repeat Conservation Mapping (RCM), a method that identifies, among homologous LRR domains, spatial regions of conservation and divergence on the surface of LRR domains. RCM utilizes the common properties of LRR three-dimensional structures to predict solvent-exposed versus buried residues, as well as spatially proximal amino acids. RCM then scores the conservation of locations on the predicted surface of the LRR domain. The method detects some of the same sites as other computational methods such as positive selection (Ka/Ks) analysis, optimal docking analysis, and Consurf, but also identifies novel functionally important sites. RCM correctly predicted the ligand binding regions of a number of solved LRR-ligand crystal structures. The algorithm further found locations that are important for receptor function on the surface of two LRR-kinase immune receptors in *Arabidopsis*, FLAGELLIN-SENSING 2 (FLS2) and EF-TU RECEPTOR (EFR). *In vivo* studies of mutated FLS2 and EFR receptors validated the functional significance of the regions identified by RCM. Based on the results of *in vivo* studies and RCM, we are now working to alter the specificity of FLS2 in order to permit recognition of non-immunogenic flagellin peptides. The RCM method should be modifiable for use with other repetitive domains, such as ankyrin repeats. The LRR-specific implementation of RCM is available online at <http://www.bentlab.russell.wisc.edu/main/main.php>.

*Note: Also a poster presentation.*

**22 Accurate Sequencing of 18 Genomes of *Arabidopsis thaliana* and Its Use in Imputing the Genome Sequences of Over 600 MAGIC Recombinant Inbred Lines**

Xiangchao Gan<sup>1</sup>, Jonas Behr<sup>2</sup>, Joshua Steffen<sup>3</sup>, Katie Hildebrand<sup>4</sup>, Lorraine Allchin<sup>1</sup>, Leo Goodstadt<sup>1</sup>, Oliver Stegle<sup>2</sup>, Philipp Dreyer<sup>2</sup>, Rune Lyngsøe<sup>1</sup>, Vipin Sreedharan<sup>2</sup>, Edward Osborne<sup>3</sup>, Chris Toomajian<sup>4</sup>, Paula Kover<sup>5</sup>, Gunnar Rätsch<sup>2</sup>, Richard Clark<sup>3</sup>, Richard Mott<sup>1</sup>

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The MAGIC genetic reference panel of recombinant inbred lines (Kover et al 2009, PLoS Genet) is descended from 19 founder accessions. We have sequenced the genomes of 18, (the 19<sup>th</sup> being the reference Col-0) using Illumina short reads and assembled them using a novel algorithm IMR/DENOM that integrates iterative alignment with denovo assembly. The resulting assemblies have an error rate of about 1 SNP per 10kb,in the 80% non-repetitive fraction of the genome.

The genome of each MAGIC line is a mosaic of the founders, so in order to impute the MAGIC genomes from the 19 founders, we re-sequenced over 500 MAGIC lines at very low coverage, in barcoded pools of 96 samples, giving about 300,000 usable randomly positioned SNPs per line, from which we could determine the mosaic breakpoints with an accuracy of about 1kb. Using the resulting imputed genomes, it is possible to test genotype-phenotype association between any phenotype measured on the MAGIC lines and the actual causal variants. We have also generated RNAseq data for some of these lines and show that it is possible to deduce the mosaic structure from these as well.

We present the genome assembly pipeline we used to assemble the MAGIC progenitor sequences and to generate the imputed genomes, and present initial results on the association of phenotypes to variants.

**23 Integrating metal uptake and distribution in plants**Mary Lou Guerinot**Department of Biological Sciences, Dartmouth College, Hanover, NH 03755**

Metal homeostasis is an equilibrium between metal uptake and metal efflux, making metal transporters key players in controlling cellular metal content. Our long-term goal is to understand how plants acquire metal micronutrients from the soil and distribute them while protecting themselves from the potential damage metals can cause to living tissues. Our lab has previously identified the major Fe transporter responsible for Fe uptake from the soil, IRT1, a founding member of the ZIP family of metal transporters. We have identified an upstream regulator of IRT1 called *uri* that controls most of the iron-regulated genes in Arabidopsis. We have also identified a mutant that accumulates iron due to constitutive expression of the iron deficiency response genes, including IRT1. We have been continuing our studies on localization of metals using synchrotron X-ray fluorescence spectroscopy, allowing us to understand the role of various genes in metal distribution.

**24 The Role of a Zinc Finger Protein in Photosynthesis and Light Stress Tolerance**Yan Lu, David Hall, Robert Last**Michigan State University**

To understand the functions of plastid-targeted proteins, including those regulate photosynthesis and light stress tolerance, ~5000 *Arabidopsis thaliana* T-DNA lines were analyzed with morphological, chemical and physiological assays. Approximately 300 mutants were identified with photosynthetic defects. For example, two mutants of a zinc finger protein (*Low Quantum Yield of PSII 1, LQY1*) were found to have a reduced PSII electron transport rate after high-light treatment (Lu et al., 2011). The mutants dissipate more excess light energy via non-photochemical quenching than wild type, and they accumulate more reactive oxygen species under high light. Analysis of thylakoid membrane protein complexes showed that a significant proportion of LQY1 protein binds to PSII core monomer and CP43-less PSII monomer (a marker of ongoing PSII repair and reassembly). The amount of monomer-bound LQY1 protein increases upon high-light treatment. Consistent with a role of LQY1 protein in PSII repair and reassembly, the mutants have less PSII-light harvesting complex II supercomplex than wild type after high-light treatment. Recombinant LQY1 protein can break and reform the disulfide bonds of substrate proteins. LQY1 is more abundant in stroma-exposed thylakoids, where key steps of PSII repair and reassembly take place. Absence of LQY1 protein accelerates turnover and synthesis of PSII reaction center protein D1. These results suggest that LQY1 protein may be involved in maintaining PSII activity in high light by regulating repair and reassembly of PSII complexes. LQY1 homologs are found in diverse land plants, but not in cyanobacteria or algae, which may reflect the plants' adaptation to excess light stress during the transition to land. This example illustrates that the large-scale genome data can be used to identify novel genes controlling photosynthesis and light stress tolerance.

**References**

Lu, Y., Hall, D.A., and Last, R.L. (2011). The role of a small zinc finger-containing membrane protein in photosynthesis and photoprotection in Arabidopsis. *Plant Cell*, In press.

**25 Uncovering Novel Signaling Interactions in Regulation of the Plant Metabolic Networks**Ling Li, Marah Hoel, Eve Wurtele**Iowa State University, Ames, (Iowa), US**

Understanding of how plant composition is regulated has been elusive. The factors that regulate metabolism are key to utilization of crops for improved plant composition and production of novel constituents, yet little is known concerning the mechanisms controlling how much carbon flows to oil, starch, protein and other constituents. Recently we identified a regulatory function in starch metabolism for Arabidopsis locus At3g30720 (*QOS*); transgenic lines with up- or down-regulated *QOS* expression have a normal appearance but an altered starch content (Li et al., 2009), and a transcriptome with shifts in the accumulation of specific transcripts. *QOS* is among the approximately 5-20% of gene models in eukaryotic genomes that encode proteins that lack sequence homology with any known motifs (POFs, proteins with obscure features) and also are species-specific (i.e., in the case of *QOS*, the primary sequence is recognizable in Arabidopsis ecotypes, but not identifiable in any other sequenced species, including the closely related (*Brassica napus*). Introduction of the Arabidopsis-specific *QOS* gene to soybean results in decreased seed oil and carbohydrate, and increased seed protein. Thus, this species-specific POF can affect composition in a non-native species. Iterative mutant generation and transcriptomics/metabolite profiling in Arabidopsis reveal several other POFs as candidates in regulation of starch metabolism. Taken together, the data indicate *QOS* as a novel regulator of plant composition, and begin to reveal the skeleton of a previously undefined network in which *QOS* and other POFs participate.

*Note: Also a poster presentation.*

**26 Cytochrome P450 CYP94B3 Mediates Catabolism and Inactivation of Jasmonate**Gregg Howe, Abraham Koo**Michigan State University**

The phytohormone jasmonoyl-L-isoleucine (JA-Ile) signals through the COI1-JAZ coreceptor complex to control key aspects of plant growth, development, and immune function. Despite detailed knowledge of the JA-Ile biosynthetic pathway, little is known about the genetic basis of JA-Ile catabolism and inactivation. We used coexpression analysis to identify a wound-inducible cytochrome P450 (CYP94B3) that performs a key step in JA-Ile catabolism in Arabidopsis. Metabolite analysis of wounded leaves showed that loss of

CYP94B3 function in *cyp94b3* mutants causes hyperaccumulation of JA-Ile and concomitant reduction in 12-hydroxy-JA-Ile (12OH-JA-Ile) content, whereas overexpression of this enzyme results in severe depletion of JA-Ile and corresponding changes in 12OH-JA-Ile levels. *In vitro* studies showed that CYP94B3 has JA-Ile 12-hydroxylase activity, and that 12OH-JA-Ile is less effective than JA-Ile in promoting the formation of COI1-JAZ receptor complexes. CYP94B3-overexpressing plants displayed phenotypes indicative of JA-Ile deficiency, including defects in male fertility, resistance to jasmonate-induced growth inhibition, and susceptibility to insect attack. Increased accumulation of JA-Ile in wounded *cyp94b3* leaves was associated with enhanced expression of a subset of JA-responsive genes. We conclude that CYP94B3 exerts negative feedback control on JA-Ile levels as a mechanism for attenuating jasmonate responses. These findings reveal a new class of enzymes active in JA metabolism, and provide new insight into the function of a conserved P450 family whose physiological role in plants was previously unknown.

*Note: Also a poster presentation.*

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## 27 "Rooting" YUCCA Genes in the Auxin Biosynthetic Pathway

*Anna Stepanova, Jeonga Yun, Jose Alonso*

**North Carolina State University, Raleigh, NC, USA**

Auxin is a key growth regulator in plants that controls nearly every aspect of plant's life cycle, from embryo development to fruit ripening and organ senescence. In the past years the mechanisms of auxin transport, perception, signaling and response have been studied in great detail. In contrast, the deciphering of auxin biosynthesis has lagged behind, in part due to the existence of multiple routes of auxin production in plants and the high level of gene function redundancy at multiple steps of the pathway. To date, only three gene families have been unequivocally implicated in auxin biosynthesis in *Arabidopsis*: tryptophan aminotransferases *TAA1/TARs*, cytochrome P450s *CYP79B2/B3*, and flavin monooxygenases *YUCs*. While the former two families have been conclusively shown to function in the indole-3-acetaldoxime (IAOx) and indole-3-pyruvic acid (IPA) routes of auxin production, respectively, the point of action of the *YUC* gene family is currently unknown.

To investigate where in the auxin biosynthetic pathway *YUCs* work with respect to *TAA1/TARs* and *CYP79B2/B3*, we took a genetic approach. Our data indicate that the function of the *TAA1/TAR* family is required for auxin production via *YUCs*. In contrast, *YUC*-mediated auxin biosynthesis does not depend on functional *CYP79B2/B3*. Our studies position *YUCs* parallel to the IAOx route in the IPA branch of auxin biosynthesis, presumably downstream of *TAA1* and *TARs*.

*Note: Also a poster presentation.*

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## 28 Mechanisms of BAK1-Dependent Signalling

*Benjamin Schwessinger<sup>1</sup>, Milena Roux<sup>1,7</sup>, Freddy Boutrot<sup>1</sup>, Catherine Albrecht<sup>2</sup>, Vardis Ntoukakis<sup>1</sup>, Yasuhiro Kadota<sup>1</sup>, Cecile Segonzac<sup>1</sup>, Man-ho Oh<sup>3</sup>, Selena Gimenez-Ibanez<sup>1,5</sup>, Jacqueline Monaghan<sup>1</sup>, Frederike Malinovsky<sup>1</sup>, Jan Sklenar<sup>1</sup>, John Rathjen<sup>1,6</sup>, Delphine Chinchilla<sup>4</sup>, Steven Huber<sup>3</sup>, Alexandra Jones<sup>1</sup>, Sacco de Vries<sup>2</sup>, Cyril Zipfel<sup>1</sup>*

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The regulatory leucine-rich repeat (LRR) receptor-like kinase BAK1 plays an important role in brassinosteroid (BR) responses, innate immunity and cell death control. BAK1 forms ligand-induced hetero-oligomers with the LRR receptor-kinases (RKs) BRI1 that perceives BR, as well as with the LRR-RKs FLS2 and EFR acting as pattern-recognition receptors for the bacterial pathogen-associated molecular patterns flagellin (flg22) and EF-Tu (elf18), respectively. It is currently unclear if and how BAK1 can provide signalling specificity in the different pathways it is involved in.

BAK1 is an active RD kinase and has been suggested to function as a signal enhancer for BRI1. The definition of the phosphorylation events in the BRI1-BAK1 complex permitted a better understanding of the early molecular mechanisms governing BR signalling. Interestingly, no phosphosites for FLS2 or EFR have been identified so far. Given that the mode of regulation of non-RD kinases (such as FLS2 and EFR) is different to that of RD kinases, it is unclear whether the BRI1-BAK1 model can be generalized to non-RD kinases. We have recently revealed clear biochemical differences between the FLS2/EFR-BAK1 complex(es) and the paradigmatic BRI1-BAK1 model. In addition, we showed that the function of BAK1 can be uncoupled mechanistically and that BAK1 differentially regulates these pathways in a phosphorylation-dependent manner. We will present novel results on the phosphorylation events and ligand-dependent receptor complexes governing early BAK1-dependent signalling, as well as evidence for trade-offs between BAK1-dependent pathways.

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## 29 Photosynthetic Carbon Assimilation in *Arabidopsis thaliana*

*Stéphanie Arrivault, Marek Szecowka, Daniel Vosloh, Manuela Guenther, Alisdair Fernie, Mark Stitt*

**Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany**

Understanding the regulation and integration of CO<sub>2</sub> fixation with end-product synthesis and other pathways is one of the major challenges in plant biochemistry. In this perspective, our goal was to accurately quantify intermediates involved in the Calvin-Benson cycle (Arrivault *et al.*, 2009). However, to understand the process of carbon assimilation, it is important to determine the temporal kinetics of carbon flow into central carbon metabolism. This can be achieved by supplying stable isotopes, and using MS-based methods to monitor the flow of the heavy isotope into various metabolites. For this purpose, we used newly adapted LC-MS/MS and GC-MS methods that allow the quantification of all the isotopomers of a total of 40 metabolites involved in the Calvin-Benson cycle, photorespiration and starch

and sucrose synthesis. Arabidopsis rosettes were supplied with  $^{13}\text{CO}_2$  and harvested at various time intervals to determine the degree and pattern of labelling of intermediates. Multivariate analysis showed that label rapidly appeared in the intermediates of the Calvin-Benson cycle, although even after 1 hour of supplying  $^{13}\text{CO}_2$ , partially labelled isotopomers were still present. Most likely this is due to internal recycling of  $^{12}\text{C}$  derived from photorespiratory intermediates, which are present as large pools with slow turn-over time. To test this hypothesis, labelling kinetics will be determined at a lower  $\text{O}_2$  concentration to reduce photorespiration. We are also using non-aqueous fractionation to determine the subcellular distribution of the isotopomers in order to resolve the carbon fluxes in the chloroplast ( $\text{CO}_2$  fixation and starch synthesis) and cytosol (sucrose synthesis).

*Note: Also a poster presentation.*

**30 Haploid *Arabidopsis thaliana*: Power Tools for Plant Genetics**

Simon Chan<sup>1</sup>, Maruthachalam Ravi<sup>1</sup>, Mohan Marimuthu<sup>1</sup>, Sylvie Jolivet<sup>2</sup>, Imran Siddiqi<sup>3</sup>, Raphael Mercier<sup>2</sup>

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Creating true-breeding homozygotes (e.g. recombinant inbred lines or RILs) from a heterozygous F1 typically involves many generations of inbreeding. To accelerate this process, plant breeders produce haploid plants from a heterozygous parent, then convert them into fertile diploids that are homozygous for every locus in the genome. *Arabidopsis thaliana* haploids can now be made through a simple genetic cross. When a cenh3 GFP-tailswap mutant with altered centromeres is crossed to wild type, mutant chromosomes are lost after fertilization. Up to 50% of viable progeny are haploids produced by complete genome elimination, and we have introduced dominant markers into cenh3 GFP-tailswap to facilitate their selection. Haploid *Arabidopsis* plants convert into fertile diploids spontaneously. Each haploid yields >50 fertile diploid seeds through random chromosome segregation during meiosis. Haploid genetics has many applications: 1) New RIL sets can be made in only two generations. 2) Multiple mutant construction: it is feasible to homozygose 8 unlinked mutations in a single generation. 3) Gametophyte lethal mutations can be studied in a haploid plant. 4) Any nuclear genome can be combined with the cytoplasmic genomes of choice. 5) Tetraploid *Arabidopsis* can be converted into diploids to facilitate genetic manipulations. Lastly, we are using the principle of centromere-mediated genome elimination to engineer clonal reproduction (synthetic apomixis) in *Arabidopsis*. Crossing a mutant with diploid gametes (*spo11 rec8 osd1*, or *MiMe*) to a mutant with altered centromeres yielded up to 34% clonal progeny with the same heterozygous genotype as their *MiMe* parent. Thus, clonal reproduction in an *Arabidopsis* cross can be created by manipulating four conserved genes. This result raises hope that apomixis can eventually be engineered in crops, allowing vigorous hybrids to be propagated through seed.

**31 Proteome Dynamics Indicate That PAMP-Triggered and Effector-Triggered Signaling Converge Early**

Chris van Schie, Tenai Eggen, Zhouxin Shen, Steven Briggs

**University of California San Diego, La Jolla, California, USA**

PAMP-triggered immunity (PTI) is mediated by the interaction of pattern recognition receptors (PRRs) on the plant cell surface with ubiquitous pathogen molecules (PAMPs) such as bacterial flagellin (flg22). Effector-triggered immunity (ETI) is mediated by cytoplasmic R proteins (RPM1) that are activated when a pathogen effector (AvrRpm1) disrupts the R protein complex. The outline of a signal transduction pathway - from PRRs through MAP kinases to transcription factors – has emerged to explain PTI, but R protein signaling remains enigmatic. ETI and PTI both confer resistance to infection; the associated changes in the transcriptome are highly similar suggesting that they are the result of common signaling pathways. We will present evidence that flg22 and RPM1 induce an overlapping set of the same signals (changes at specific sites of protein phosphorylation). Many of these changes occur in plasma membrane and cytoplasmic proteins soon after exposure to pathogen molecules indicating that PTI and ETI activate the same signaling pathways. Additional evidence for shared signaling is provided by our finding that a histone demethylase, whose phosphorylation is triggered by either RPM1 or BTH treatment, is necessary for flg22 signaling and for normal BTH responses.

**32 Identifying protein-small molecule interactions using functional protein microarrays coupled with a photoactivated crosslinked ligand**

Magali Moreau, Giulio Zampogna, Daniel Klessig, Sorina Popescu

**Boyce Thompson Institute for Plant Research, Ithaca, NY, USA**

Determining the molecular targets of small molecules is challenging due to our limited ability to detect interactions that are often transient and involve partners in low abundance. Functional protein microarrays (FPMs) have become increasingly useful in the study of protein-protein interactions in a proteome-wide context, and one promising direction involves the co-application of microarray technologies to screening with small molecule ligands. We are exploiting *Arabidopsis* FPMs to screen for proteins that bind to salicylic acid (SA), a phytohormone that affects a number of plant physiological processes but is most recognized as a key player in disease resistance. Our FPMs contain over 5000 *Arabidopsis* proteins transiently expressed in and purified from the model plant *Nicotiana benthamiana*. To date, only a few direct protein targets of SA have been identified in plants using classical methods; however, the combined use of an FPM and a photoactivatable SA analog has allowed us to identify several additional *Arabidopsis* proteins as potential SA binding proteins (SABPs). For several of our candidate SABPs, SA binding has also been confirmed using multiple independent assays. We will discuss the biological significance of SA binding to our candidate SABPs and explain how our methodology is broadly applicable to identifying other small molecules targets and to better understanding protein function and cellular signaling networks.

**33 Base-Resolution Population Epigenomic Variation**

Bob Schmitz, Mark Urich, Mattia Pelizzola, Matthew Schultz, Mathew Lewsey, Joe Nery, Andrew Alix, Joseph Ecker

**The Salk Institute for Biological Studies (CA), USA**

Recent advances in genomic technologies have enabled the scientific community to generate sequence information for hundreds of species. Moreover, methods for linking genotype to phenotype using genome-wide association mapping are continuously improving. As a result of these two endeavors, the first landmark studies using this association-mapping technique from an entire range of organisms have surfaced. While remarkable progress is being made in identifying some causal variants, many remain elusive. One possible source of naturally occurring causal variants that is currently being overlooked are epigenetic alleles (epialleles). Epialleles often contain changes in their DNA methylation status. Very few epialleles have been identified across kingdoms, which is likely a result of the difficulty in their identification. Consequently, we have sequenced the genomes, DNA methylomes and transcriptomes of 150 *Arabidopsis thaliana* accessions to better understand the interplay and impact of genetic, epigenetic and transcriptional variation on phenotypic variation. In this population, we have identified over 9000 differentially methylated regions (DMRs) with each accession containing approximately

5100 each. Most of these DMRs aligned to transposons and repetitive elements, but each accession contained 270 DMRs overlapping with genes (epialleles). Hierarchical clustering of DMRs detected between these accessions revealed extensive epigenetic variation within the population that broadly grouped into two classes: 1) DMRs overlapping with transposons and 2) DMRs overlapping with genes. DMRs overlapping with transposons occur at a higher allele frequency in the population compared to DMRs overlapping with genes. In fact, 45 percent of epialleles appear to resemble rare variants as they occur at an allele frequency of 5 percent in the population. Genes required for plant defense and protein degradation was overrepresented for the presence of epialleles compared to other gene families. Our future goals include determining the basis for these epialleles and their impact on phenotypic variation in this population.

*Note: Also a poster presentation.*

### 34 Exploring the *Arabidopsis* genome with single molecule PacBio sequencing

*Mitchell Sudkamp, Xuefeng Zhou, Zhaolong Li, Randy Kerstetter, Wei Wu, Todd Michael  
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Pacific Biosciences (PacBio) single molecule, long read, high throughput sequencing holds the promise of uncovering a new level of plant genome structure. Long single molecule reads enable structural variation de-convolution, haplotype phasing, ortholog mapping, and copy number estimation, all of which currently pose significant challenges in large, repetitive, polyploid, and economically important plant genomes. In addition, the "real-time" nature of the PacBio sequencing technology enables direct detection of DNA modifications, such as methylation. However, plant DNA is often highly modified due to both biological as well as physical parameters, which potentially could interfere with library fidelity and base calling of the PacBio sequencing platform. We utilized the golden standard plant genome *Arabidopsis thaliana* to test out various DNA prep methods and sequencing parameters to establish the utility of the long single molecule sequencing in plant genome assembly. We are currently developing hybrid assembly techniques that leverage the throughput of second generation sequencing technologies and the contiguity of the PacBio single molecule reads.

### 35 High-Throughput Recombineering and Its Applications for *Arabidopsis* Gene Function Characterization

*Jose Alonso<sup>1</sup>, Rongrong Zhou<sup>1</sup>, Larissa Benavente<sup>1</sup>, Anna Stepanova<sup>1</sup>, Miguel Perez-Amador<sup>2</sup>  
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Expression profiling of genes of interest at cellular resolution and the sub-cellular localization of the corresponding proteins are information-reach approaches aimed at addressing gene function. These types of studies require the visualization of the endogenous gene products with specific antibodies or the generation of whole-gene translational fusions with reporter genes, e.g. fluorescent proteins (FPs). To facilitate construction of such translational fusions and to ensure that all regulatory sequences are present, we have adapted a bacterial homologous recombination system (recombineering) to insert FPs in the genes of interest contained in transformation-ready bacterial artificial chromosomes (TACs). This approach has several advantages compared to other classical strategies. First, the researcher does not have to guess what the regulatory regions of a gene are, since tens of thousands of base-pairs flanking the gene of interest can be included in the construct. Second, because the genes of interest are not amplified by PCR, there is practically no limit to the size of a gene to be tagged. Third, there are no restrictions on the location where the FP can be inserted since the position is determined by sequence homology with the recombination primers. To examine the feasibility of using this recombineering-based tagging system at a whole-genome scale, two sets of 96 *Arabidopsis* genes were selected and 189 sequence-verified constructs were obtained. Our results indicate that the recombineering procedure is extremely efficient and suitable for a genome-wide endeavor. Some of the possible applications, such as the potential of the recombineering technology for studying transcriptional networks, will be discussed.

*Note: Also a poster presentation.*

### 36 The Application of Atomic Force Microscopy as a Micro-Force Sensor: Probing the Mechanics of Living Plant Cell Walls During Development

*Siobhan Braybrook<sup>1</sup>, Laurent Le Guillou<sup>2</sup>, Emeric Bron<sup>3</sup>, Cris Kuhlemeier<sup>1</sup>, Herman Höfte<sup>4</sup>, Alexis Peaucelle<sup>3</sup>  
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The development of any shape or form in plants is bounded by the cell wall. Here, we focus on the biomechanical information provided by the wall, how it controls shape changes and cell growth, and the upstream cues leading to mechanical changes in the wall. We have applied Atomic Force Microscopy (AFM) as a micro-force sensor to probe the material properties of the cell wall of living plant cells and tissues, a new and exciting application in plants. We developed methods to examine the elasticity and viscoelasticity of plant cell walls *in planta* at cell and tissue levels, and in single cell systems; this information was then correlated with plasticity/growth. The elastic behavior of walls is presented as an apparent Young's modulus. The viscoelastic behavior of walls is presented as a bulk elasticity constant and a time relaxation constant. Our techniques have been applied to the shoot apical meristems of plants from throughout the plant kingdom (dicots, monocots, gymnosperms, bryophytes) in order to investigate the mechanical basis of organogenesis. Surprisingly, all meristems examined exhibit similar elasticity despite their variable geometries and wall compositions. In *Arabidopsis*, we examined how changes in pectin structure and auxin application can alter wall elasticity, and correlated this with organ formation. We have also applied these methods to tobacco BY-2 cells in order to examine the underlying basis of wall mechanical changes in a simple system. The information obtained via these methods provides insight into plant cell growth and shape development on a microscopic scale and allows for in depth analysis of cell wall structure as it relates to the mechanics of growth.

*Note: Also a poster presentation.*

**37 Arabidopsis Stem Cells in Development and Regeneration***Elliot Meyerowitz***California Institute of Technology, Pasadena, CA, USA**

Plant pluripotent stem cells are found in meristems. In the shoot apical meristem, a stem cell niche, the maintenance of appropriate numbers of stem cells and positioning of varied types of stem cells relies on a series of different modes of cell-cell communication. Our laboratory concentrates on signaling via peptides such as CLAVATA3, through the small molecules auxin and cytokinins, and through mechanical signals that cells in the meristem impose on each other. We are attempting to develop hypotheses, represented as computational models, to predictively model cell-cell interactions in the shoot apical meristem. We have models that represent primordium formation due to interactions of auxin and mechanical stress, and that represent the partitioning of the meristem into different regions of gene expression due to responses to cytokinins.

It is generally thought that all living plant cells, even those outside of meristems, are totipotent, that is, that they can lead to regeneration of new plants if properly isolated and cultured. Regeneration from cultured organ explants proceeds through an intermediate of callus tissue, thought to form by dedifferentiation of cells. We also study the de novo generation of shoot apical meristems in callus tissue, and the generation of callus from organ explants. Our work in this area indicates that callus forms from a pre-existing population of perivascular adult stem cells, and that callus cells are not dedifferentiated, rather being cell types characteristic of lateral root primordia, organized spatially as they are in roots.

Thus, multiple approaches to plant stem cells are leading to predictive models of stem cell niche organization, and to surprises in regard to regeneration.

**38 SCARECROW Sustains Stem Cell Activity Inhibiting Cytokinin Dependent Cell Differentiation Input**

*Laila Moubayidin<sup>1</sup>, Di Mambro Riccardo<sup>1</sup>, Pacifici Elena<sup>1</sup>, Terpstra Inez<sup>2</sup>, Perilli Serena<sup>1</sup>, Dello Ioio Raffaele<sup>1</sup>, Heidstra Renze<sup>2</sup>, Costantino Paolo<sup>1</sup>, and Sabrina Sabatini<sup>1</sup>*

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Understanding the molecular mechanisms through which plant meristems are maintained is a central question in developmental biology. In the root of *Arabidopsis thaliana*, stem cells in the apical region of the meristem self-renew and produce daughter cells that differentiate in the distal meristem transition zone. To ensure root growth, the rate of cell differentiation must equal the rate of generation of new cells. Cell differentiation takes place in the transition zone that is localized in the distal part of the root meristem, but must be synchronized and balanced with division of the stem cells that are localized in the apical part of the meristem. We have previously shown that maintenance of the *Arabidopsis* root meristem size - and consequently root growth - is controlled by the interaction between two hormones at the meristem transition zone: cytokinins, which promote cell differentiation, and auxin, which promotes cell division, but it is still unknown how the cytokinin/auxin interaction maintains a balance between cell differentiation at the transition zone and cell division in the stem cell niche. Here we show that SCARECROW (SCR) maintains stem cell activity repressing cytokinin-mediated differentiation input in the stem cell niche through down-regulation of the cytokinin-responsive transcriptional regulator ARR1 thus controlling root meristem size.

**39 An integrated circuit for asymmetric cell division in *Arabidopsis* roots***Ben Sheres***Department of Biology, Utrecht University, Padualaan 8, 3584 CH, NL**

Stem cells and their daughters in the root display specific asymmetric divisions at fixed locations. We investigate how such divisions are spatially regulated. The SHORTROOT-SCARECROW transcription factor pathway plays a role in patterning the quiescent center and cortex/endodermis stem cells and provides mitotic potential to the stem cell daughters that form the proximal meristem. This activity involves the conserved RETINOBLASTOMA-RELATED (RBR) pocket protein, and we have established molecular links between the RBR pathway and SCARECROW action that form a feedback control system. In addition, RBR activity is modulated by auxin abundance, itself regulated through an intercellular distribution system, and by cell cycle progression. Formal analysis of this feedback circuit indicates that it acts as a bistable switch that ensures the occurrence of an asymmetric division at fixed positions. Our work illustrates how formative divisions that shape plant tissues can be robustly positioned by dynamic regulatory circuits that combine intracellular and extracellular loops.

**40 Hormonal control of shoot branching***Ottoline Leyser***Sainsbury Laboratory, University of Cambridge**

Plants adapt their form according to the environmental conditions in which they are growing. This developmental plasticity requires the integration of multiple inputs into ongoing developmental programmes. We are using the regulation of shoot branching as a model system to investigate the mechanisms underlying developmental plasticity. Shoot branching is regulated by an environmentally sensitive network of systemically moving hormonal signals, providing a rich source of information that can be locally interpreted to determine branching behaviour. At the heart of this network is the auxin transport system. All active shoot apices export auxin into the polar auxin transport stream, which transports it rootward. Thus ultimately all shoot apices are in communication through their export of auxin into shared auxin transport paths to the root. Competition between apices for these transport paths can explain a range of phenomena in shoot branching, such as apical dominance. Furthermore, the modulation of the degree of competition, either locally or systemically, by environmental inputs can explain developmental plasticity in shoot branching. For example, strigolactones are transported from root to shoot and their production is up regulated when nutrient availability is low. Current evidence suggests that they act by reducing auxin transporter accumulation in the polar transport stream, increasing competition between branches for access to auxin transport routes in the main stem.

**41 The interaction of the cytokinin-regulated phosphorelay with other signals in Arabidopsis***Joseph Kieber***Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280**

Cytokinins, N<sup>6</sup>-substituted adenine derivatives, have been implicated a wide variety of plant growth and development processes. A basic framework for cytokinin signal transduction has emerged that is similar to two-component phosphorelays, which rely on the transfer of phosphates between alternating histidine and aspartic acid residues. Cytokinins are perceived by a family of histidine kinase receptors (AHKs), which, following binding of cytokinin, transfer a phosphoryl group to the histidine phosphotransfer proteins (AHPs), which in turn donate the phosphate to the response regulators proteins (ARRs), thereby regulating their activity. These elements are partially functionally redundant in mediating the response to cytokinin and in various roles in regulating plant growth and development. We have analyzed the function of these two-component elements using several approaches, including the generation and analysis of multiple mutant lines. We have characterized their function in multiple plant processes, including roles in the development of the female gametophyte and in the response to pathogens. For the former, we find that cytokinin is required in the sporophytic tissue to generate a signal required for early gametophytic development. For pathogen responses, we find that cytokinin is important in determining the amplitude of immunity responses, at least in part through an increase in SA biosynthesis. Our results also suggest a negative effect of salicylic acid on cytokinin signaling. Finally, we have also examined the interaction of the cytokinin response pathway with auxin transport and with ethylene biosynthesis. We find that cytokinin alters the expression of the PIN efflux carriers, primarily through a post-translational mechanism. This leads to an altered distribution of auxin in a multiple type-A ARR mutant, which in turn leads to a perturbation in the pattern of cell division in the root tip.

**42 Structural Mechanism of Jasmonate Perception**

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Jasmonates (JAs) are a family of plant hormones that regulate plant growth, development, and responses to stress. The F-boxprotein CORONATINE-INSENSITIVE 1 (COI1) mediates JA signaling by promoting hormone-dependent ubiquitination and degradation of transcriptional repressor JAZ proteins. Despite its importance, the mechanism of JA perception remains unclear. Here we present structural and pharmacological data to show that the true JA receptor is a complex of both COI1 and JAZ. COI1 contains an open pocket that recognizes the bioactive hormone, (3R,7S)-jasmonoyl-L-isoleucine (JA-Ile), with high specificity. High-affinity hormone binding requires a bipartite JAZ degron sequence consisting of a conserved  $\alpha$ -helix for COI1 docking and a loop region to trap the hormone in its binding pocket. In addition, we identify a third critical component of the JA co-receptor complex, inositol pentakisphosphate, which interacts with both COI1 and JAZ adjacent to the ligand. Our results unravel the mechanism of JA perception and highlight the ability of F-box proteins to evolve as multi-component signaling hubs.

**43 CER7, a Core Subunit of the RNA-processing Exosome, has a Specific Role in Regulation of Cuticular Wax Deposition in Arabidopsis***Ljerka Kunst, Tanya Hooker, Patricia Lam, Lifang Zhao***Department of Botany, University of British Columbia, Vancouver, Canada**

The outer epidermal cell walls of plant shoots are covered with a cuticle composed of cutin polymer matrix embedded with waxes. The cuticle serves as a barrier protecting plants from desiccation, UV-light, pathogens, and insects. We are using genetic analyses to unravel the regulation of cuticular wax synthesis and deposition in *Arabidopsis*. Recently, we have discovered a novel wax regulatory pathway, which involves the CER7 ribonuclease, a core subunit of the exosome homologous to yeast RRP45p. CER7 and its close homologue RRP45a are ubiquitously expressed in the plant and together are required for viability. However mutations in CER7 alone result in a wax deficiency phenotype likely due to a functional specialization of CER7 in *Arabidopsis*. We hypothesized that CER7 ribonuclease controls wax production by degrading an mRNA specifying a repressor of *CER3*, a key wax biosynthetic gene. In the absence of this repressor, *CER3* is expressed, leading to wax deposition. A prediction of this model is that inactivation of the repressor would bypass the requirement of CER7 in wax biosynthesis. To identify the putative repressor, and gain insights into CER7-mediated regulation of cuticular wax biosynthesis, we carried out a screen for suppressors of *cer7*. Cloning and preliminary characterization of several genes mutated in these suppressors revealed that they play roles in RNA silencing.

**44 The Role of Cell Wall Synthesis and Remodelling in Organ Growth***Volker Bischoff, Herman Höfte***Institut Jean-Pierre Bourgin, UMR1318 INRA/AgroParisTech Versailles, France**

In this presentation, I will address the impact of the regulation of cellulose synthesis on plant growth at different scales. (1) Synthesis of the glucan chains, assembly into (2) elementary microfibrils and (3) higher order microfibrils, (4) orientation in the cell wall and in (5) different cell walls of the same cell and (6) the role of the orientation in different cell layers in the control of growth anisotropy at the organ level. In this context, I will discuss new findings on the organization of the cellulose synthase complex and the role of the microtubules in the targeted insertion of the complexes into the plasma membrane and retrieval from the membrane as well as the regulation of the interaction of the complexes with cortical microtubules. Finally, I will discuss new insights into the role of different cell layers in the control of the directionality of organ growth.

**45 A Functional Screen for Nucleotide Sugar Transporters***Katy Christiansen, Jun Ito, Berit Ebert, Dominique Loque, Joshua Heazlewood***Joint BioEnergy Institute, Berkeley, CA, USA**

Plant cell walls are an important source of carbon in the effort to develop renewable biofuels. To better understand the mechanisms of cell wall biosynthesis, we have developed a functional screen for nucleotide sugar transporters (NSTs) from *Arabidopsis thaliana*. Most nucleotide sugars are synthesized in the cytosol, but incorporation into polysaccharides destined for the cell wall occurs in the Golgi apparatus. Our screen utilizes a vesicle secretion mutant in *S. cerevisiae* to express nucleotide sugar interconverting enzymes and NSTs from *Arabidopsis*. Candidates were identified by abundance and location using proteomic techniques. We have successfully expressed the *Arabidopsis* UDP-Glucose Dehydrogenase, UGD2, in *S. cerevisiae*, allowing for interconversion of endogenous UDP-Glucose to UDP-Glucuronic acid. This result demonstrates that it is possible to engineer *Arabidopsis* nucleotide sugar interconverting pathways into yeast. We are validating the screen by expressing GONST1 in vesicles to assay for the transport of endogenous GDP-Mannose. To determine the presence of various nucleotide sugars, we have developed a method for detection of nucleotide sugars in yeast metabolite preps using LC MS/MS and a ZIC-HILIC column. By combining expression of candidate proteins in yeast and detection by MS, we can quickly and sensitively assay *Arabidopsis* NSTs for functional activity.

**46 The *Arabidopsis FLYING SAUCERS* Gene Encodes a Membrane Protein Required for Connections to the Cell Wall***Catalin Voiniciuc, Gillian Dean, Jonathan Griffiths, George Haughn***Department of Botany, University of British Columbia, Vancouver, Canada**

The genetic analysis of mutants defective in seed coat development facilitates the discovery of genes involved in cell wall biogenesis. The *Arabidopsis thaliana* seed coat epidermis is a dispensable cell layer that secretes large amounts of pectinaceous mucilage forming donut-shaped pockets between the primary cell wall and the plasma membrane. The epidermal cells then synthesize a volcano-shaped secondary wall, which protrudes through the center of the mucilage pocket where it connects to the primary wall. Hydration of mature seeds triggers the rapid expansion of pectins, which ruptures the outer tangential primary wall from the radial wall, and forms a mucilage halo around the seed. Although large fragments of tangential wall remain attached to the columella after mucilage extrusion, very little is known about what mediates this specific attachment.

My research focuses on *flying saucers* (*fly*), a unique *Arabidopsis thaliana* mutant which is characterized by the presence of discs at the periphery of extruded mucilage. Preliminary evidence suggests that *fly* discs are primary cell walls which have lifted off the columella, and are attached to mucilage which fails to expand properly upon hydration. Using positional cloning and sequence analysis, I found that *FLY* encodes a putative Zinc Finger transmembrane protein targeted for secretion. I hypothesize that *FLY* is a plasma membrane protein that anchors the primary cell wall. Functional characterization of the *FLY* protein should provide insight into the molecular machinery that mediates cell wall-plasma membrane attachment in plants.

*Note: Also a poster presentation.*

**47 The Effects of Plant Cell Wall Alterations on Plant Disease Susceptibility***Gerit Bethke, Le Nguyen, Rachael Grundman, Fumiaki Katagiri, Jane Glazebrook***University of Minnesota, St. Paul, MN, US**

Plant cell walls constitute an early line of defense against pathogen attack. Dr. Nick Carpita and co-workers at Purdue University have screened a large set of *Arabidopsis* T-DNA insertion lines mutated in genes likely to be involved in cell wall biosynthesis for alterations in cell wall composition using Fourier transform infrared microspectroscopy (<http://cellwall.genomics.purdue.edu>). We collected mutants from this collection and other studies, for a total of 92 lines. We have screened them with pathogens with different lifestyles. To date, we have finished the screen with *Pseudomonas syringae* pv. *maculicola*, a moderately virulent bacterial pathogen with a hemi-biotrophic lifestyle, and two fungal necrotrophs, *Botrytis cinerea* and *Alternaria brassicicola*.

Mutations in 31 out of 92 genes, including those affecting nucleotide-sugar interconversion, phenylpropanoid synthesis, carbohydrate polymerization and pectin methylesterification, showed modestly enhanced bacterial growth. Four mutants showed enhanced resistance (*gae5*, *pmr4*, *pmr5*, and *pmr6*). To further test the roles of these genes in resistance to *Pseudomonas* we collected additional lines with allelic mutations in the genes of interest. So far we found 5 cases in which two alleles of the same gene caused phenotypes similar to the original observations (*uxs4*, *gae6*, *cslE1*, *4cl4* and *4cl-like7*). Furthermore, we started combining related mutations. Most cell wall biosynthetic genes are members of large gene families so this approach might overcome functional redundancies among members of the same gene family. Currently, we think that pectin may play an important role since several mutants that showed changes in pathogen growth were affected in the biosynthesis or modification of pectin or showed changes in pectin content.

For *Botrytis* and *Alternaria* we have screened the same collection of T-DNA insertion lines and for each pathogen we found two mutations that cause differences in fungal growth. We have ordered additional lines for allelic mutations in these genes and started to analyze them.

*Note: Also a poster presentation.*

**48 Subcellular Partitioning of Plant Cell Wall Biosynthesis in *Arabidopsis****Harriet Parsons, Jun Ito, Joshua Heazlewood***Lawrence Berkeley National Laboratory, Berkeley, (CA), USA**

The plant cell wall is comprised of a variety of complex sugar polymers including cellulose, hemicellulose and pectin. These diverse sugars represent a considerable carbon source for the production of compounds such as biofuel by engineered microorganisms. The process by which these complex sugars are synthesized and incorporated into the cell wall is poorly understood. A significant proportion of cell wall polysaccharides are synthesized within the plant Golgi apparatus from cytosol derived nucleotide sugars. We recently enriched and characterized the cytosol of *Arabidopsis* using proteomics and reproducibly identified several thousand proteins. Employing computational approaches and exploiting other subcellular proteomic studies we developed a curated list of over 1,300 proteins from this sub-compartment. In conjunction we have developed a isolation and purification procedure for the plant Golgi apparatus that employs and orthogonal approach utilizing density centrifugation followed by charge based separation by Free Flow Electrophoresis (FFE). Proteomic characterization of Golgi purified fractions from *Arabidopsis* cell culture FFE separation indicates the method is suitable for isolation of this organelle from plants. We have reproducibly identified around 430 proteins from these fractions which included over 50 glycosyl transferases from multiple gene families) whose roles are likely involved in matrix polysaccharide biosynthesis. Defining these complex subcellular compartments for the first time in plants has enabled the identification of experimental targets involved in plant cell wall biosynthesis. These include Golgi localized nucleotide sugar transporters, principle cytosolic and Golgi enzymes involved in nucleotide sugar biosynthesis and Golgi localized glycosyl transferases. We are currently utilizing functional genomics and analytical approaches to characterize nucleotide sugar biosynthesis, transport and matrix polysaccharide biosynthesis in *Arabidopsis*.

*Note: Also a poster presentation.*

**49 Combining Molecular Genetics and Mass-Spectrometry-Based High-Resolution Metabolite Imaging to Unravel the Surface Lipids of *Arabidopsis****Basil Nikolau, Young-Jin Lee, Zhihong Song, Geng Ding, Daolin Cheng, Xiaobin Zheng, Ji Hyun Jun***Iowa State University, Ames, IA, USA**

The cuticle is a complex and unique mixture of lipids that covers the very outer surface of all aerial organs of terrestrial plants. This lipid-coat functions as a water-barrier and a protective layer against the environmental stresses. Despite the identification and characterization of many mutants that affect the deposition of the cuticle (called *eceriferum* (*cer*) mutants), there are many unanswered questions concerning the regulation of the metabolism that generates this protective barrier. Because the cuticle is the product of a single cell layer of the plant, and it is unidirectionally secreted to the surface, addressing these questions requires the combined application of new technologies to investigate the distribution of cuticle metabolites at the cellular and subcellular levels, combined with the molecular and biochemical characterization of the isolated *CER* genes. We have developed high spatial resolution techniques that use mass spectrometry to image epicuticular lipids of the surfaces of *Arabidopsis thaliana*. This technology has the ability to image the distribution of metabolites to a resolution of ~12 µm, which is less than the size of a typical plant cell. We combine this new technology with biochemical and genetic characterizations of *Arabidopsis cer* mutants to provide a more accurate annotation of *cer* gene functions.

*Note: Also a poster presentation.*

**50 Identification of Plant Clock Genes Using Functional Genomics**

*Stacey Harmer<sup>1</sup>, Matthew Jones<sup>1</sup>, Nozomu Takahashi<sup>1</sup>, Polly Hsu<sup>1</sup>, Reetika Rawat<sup>1</sup>, Michael Covington<sup>1</sup>, Luciano DiTacchio<sup>2</sup>, Christopher Vollmers<sup>2</sup>, Satchidananda Panda<sup>2</sup>, Jacob Schwartz<sup>1</sup>, Michelle Salemi<sup>1</sup>, Brett Phinney<sup>1</sup>*

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Circadian rhythms are found in most eukaryotes and some prokaryotes and are generated by an endogenous oscillator or clock. A functional circadian clock provides an adaptive advantage, presumably by allowing organisms to anticipate regular changes in the environment. Basic features of the circadian system are shared across eukaryotes, but most clock components are not conserved across higher taxa. A combination of genetic, genomic, and modeling approaches has led to rapid progress in determining the molecular nature of the circadian oscillator in the model plant *Arabidopsis thaliana*. As in metazoa, multiple interlinked transcriptional feedback loops influenced by a variety of post-translational regulatory mechanisms comprise the plant circadian clock. But despite these advances, there are conspicuous gaps in our understanding of the molecular clockwork in higher plants. To help fill these gaps, we have taken functional genomic approaches to identify new clock genes and place them within the clock mechanism. Using mass spectrometry, we identified a novel transcription factor that binds specifically to a circadian cis-regulatory motif. We show that this factor, *RVE8*, forms a negative feedback loop with another clock protein, *PRR5*. We have also used a data mining approach to identify genes co-regulated with the well-known clock gene *TOC1*. We found that one such gene, *JMJD5*, acts in parallel with *TOC1* to promote expression of morning-phased clock genes. Surprisingly, we found that the human ortholog of *JMJD5* acts within the human circadian clock and that the plant and human genes have conserved cellular functions.

**51 Uncovering clock transcriptional circuits by functional genomics**

*Jose Pruneda-Paz*

**USCD**

Extensive clock-regulated transcriptional networks control almost every biological process in plants. Clock controlled physiological responses are coupled with daily oscillations in environmental conditions resulting in enhanced fitness and growth vigor. While identification of clock components and their associated molecular interaction has established the basic network architecture of plant clocks, the input mechanisms that set the pace of the clock remain elusive. Likely the redundant nature of clock circuits has limited our ability to identify the transcriptional mechanisms by which environmental signals, such as light and temperature, regulate the expression of clock components. To uncover direct regulators of a key clock component (*CCA1*), we recently established an alternative functional genomics approach in which the yeast-one hybrid system was used to screen a collection of clock regulated transcription factors for their binding to the *CCA1* promoter. This strategy was instrumental for the discovery of a novel clock component (CHE) that directly regulates the *CCA1* promoter activity. We have now expanded this collection to include all predicted *Arabidopsis* transcriptional regulator, and improved the yeast one-hybrid procedure to establish high-throughput automated screens. Using this larger collection we identified a bHLH transcription factor (TCF) that negatively regulates *CCA1*. Both the clock phenotypes at high temperatures and the heat-induced expression of TCF, indicate that is involved in temperature compensation, a mechanism by which clocks maintain a similar pace within a range of physiological temperatures. Our results suggest that TCF provides a molecular link for the regulation of *CCA1* expression by temperature..

**52 Growth Promoting Factors Have Distinct Effects on Seedling Growth Dynamics**

*Jodi Stewart<sup>1</sup>, Christopher Gee<sup>1</sup>, Julin Maloof<sup>2</sup>, Jennifer Nemhauser<sup>1</sup>*

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Early in development, plants must respond to light cues with appropriate growth responses to ensure their survival. During photomorphogenesis, the seedling growth program is converged upon by light signals, endogenous hormone flux and metabolic changes. We use a variety of approaches to elucidate the role of exogenous sucrose in modifying *Arabidopsis* seedling growth dynamics. In addition to its known effects on germination, high-resolution temporal analysis revealed that sucrose could extend the number of days plants exhibited rapid hypocotyl elongation, leading to dramatic increases in ultimate seedling height. In addition, sucrose changed the timing of daily growth maxima, demonstrating that diel growth dynamics are more plastic than previously suspected. Sucrose-dependent growth promotion required function of multiple phytochrome-interacting factors (PIFs), and overexpression of *PIF5* led to growth dynamics similar to plants exposed to sucrose. Consistent with this result, sucrose was found to increase levels of PIF5 protein. PIFs have well-established roles as integrators of response to light levels, time of day and phytohormone signaling. Our findings strongly suggest that carbon availability acts as an additional key modifier of the known photomorphogenetic signaling network. In addition to the effects of sucrose on later phases of photomorphogenesis, our ongoing research is revealing unexpected relationships between hormones during the earliest phases of growth.

*Note: Also a poster presentation.*

**53 Antagonistic Regulation of Photomorphogenesis by Oppositely Acting bHLH Transcription Factors in *Arabidopsis***

*Ling Zhu, Hui Shen, Jonathan Dang, Enamul Huq*

**University of Texas at Austin, Austin, TX, US**

The Phytochrome Interacting Factors (PIFs), a small group of bHLH transcription factors repress photomorphogenesis both in the dark and light. Light signals perceived by the phytochrome family of photoreceptors induce rapid degradation of PIFs to promote photomorphogenesis. Here we show that HECATE proteins, another small group of bHLH proteins antagonistically regulate PIF1 function to promote photomorphogenesis. Both HEC1 and HEC2 heterodimerize with PIF1 in yeast-two-hybrid assays as well as *in vitro* and *in vivo* co-immunoprecipitation assays. Promoter:GUS and GFP fusion proteins showed that *PIF1* and *HEC* genes are co-expressed in the same tissues and the proteins are co-localized in the nucleus. RNA interference-mediated downregulation of *HEC1* or *HEC2* or *hec1*

mutant induces hyposensitivity to light-induced seed germination and chlorophyll accumulation, two hallmark processes oppositely regulated by PIF1. By contrast, constitutive overexpression of *HEC2* induces seed germination after FR light exposure and increased chlorophyll accumulation compared to wild type. The seed germination phenotypes of *HEC2* overexpression lines are GA-dependent as the overexpression lines in *gal* mutant background failed to germinate. In addition, the seed germination phenotype of *hec1* or *hec* RNAi lines or *hec1* mutant is eliminated in the *pif1* background, suggesting that *pif1* is epistatic to *hec* functions. Taken together, these data suggest the HECATE proteins promote photomorphogenesis by negatively regulating the function of PIF1 and possibly other PIFs in *Arabidopsis*. **Keywords:** *Arabidopsis* /bHLH transcription factor/ homo/hetero-dimerization/photomorphogenesis.

**Note:** Also a poster presentation.

#### 54 A Circadian Complex is Critical for Growth Control in *Arabidopsis*

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The circadian clock in plants is required for adaptive responses to daily and seasonal changes in environmental conditions. Hypocotyl growth in *Arabidopsis thaliana* seedlings is controlled by both light and the circadian clock to consolidate the phase of cell elongation to the end of the night under diurnal cycles. However, the molecular mechanism by which the circadian oscillator gates growth is unclear. Here we identify a protein complex containing *EARLY FLOWERING 3* (*ELF3*), *EARLY FLOWERING 4* (*ELF4*) and the MYB-domain containing transcription factor *LUX ARRHYTHMO* (*LUX*) that directly participates in the regulation of plant growth. *ELF3* is both necessary and sufficient to form a complex between *ELF4* and *LUX*. Formation of the *ELF4-ELF3-LUX* complex is diurnally regulated, and both the individual proteins and the complex peak during the early evening. *ELF3*, *ELF4*, and *LUX* are required for the proper expression pattern of the growth promoting transcription factors *PHYTOCHROME-INTERACTING FACTOR 4* (*PIF4*) and *PHYTOCHROME-INTERACTING FACTOR 5* (*PIF5*) under diurnal conditions. Mutation in any of the three complex members results in elevated *PIF4* and *PIF5* levels, leading to inappropriate hypocotyl elongation. *LUX* binds to the promoters of *PIF4* and *PIF5* and recruits the *ELF4-ELF3-LUX* Evening Complex (EC) to the *PIF4* and *PIF5* promoters *in vivo*. Mutations in *PIF4* and/or *PIF5* are epistatic to loss of the EC, suggesting that regulation of *PIF4* and *PIF5* is a critical function of the *ELF4-ELF3-LUX* complex. Therefore, the EC underlies the molecular circadian gating of hypocotyl growth in the early evening. **Note:** Also a poster presentation.

#### 55 Alternative Splicing Mediates Responses of the *Arabidopsis* Circadian Clock to Temperature Changes

Naeem Syed<sup>1</sup>, Allan James<sup>2</sup>, Jacqueline Marshall<sup>1</sup>, Gillian Nimmo<sup>2</sup>, Gareth Jenkins<sup>2</sup>, Paweł Herzyk<sup>2</sup>, Hugh Nimmo<sup>2</sup>, John Brown<sup>3</sup>

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Circadian clocks play a crucial role in regulating physiology and behaviour to anticipate predictable environmental changes. How sessile organisms, such as plants, respond to seasonal and shorter term fluctuations in temperature is not clear. To better understand this we have used a high resolution RT-PCR system to firstly characterise alternative splicing in *Arabidopsis* clock components and secondly to examine the contribution of alternative splicing both in plants undergoing temperature transitions and in plants acclimated to different steady state temperatures. This revealed extensive alternative splicing in clock genes and dynamic changes in alternatively spliced transcripts, some of which are temperature-dependent and contribute markedly to changes in clock gene expression in temperature transitions by production of non-functional transcripts and/or induction of nonsense-mediated decay. Interestingly, temperature-associated alternative splicing during transitions had opposite effects on pairs of partially redundant clock components (*LHY/CCA1*; *PRR7/PRR9*; *PRR3/PRR5*) implying functional differences between them. Therefore, temperature associated alternative splicing is an additional mechanism involved in the control and operation of the plant circadian clock. **Note:** Also a poster presentation.

#### 56 Translational Control: a New Dimension in the Regulation of *Arabidopsis* Photomorphogenesis

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The environmental "light" plays a vital role in regulating the plant growth and development. Transcriptomic profilings were widely used to examine how light regulates the changes of mRNA populations at a genome-wide scale. However, it remains unclear if translational regulation represents a new dimension of gene expression regulation in response to the light signal. Through a transcriptomic comparison of steady-state and polysome-bound mRNAs, we revealed an increased translational efficiency in de-etiolating *Arabidopsis* seedlings. Over 3,500 genes are subjected to translational regulation whereas only about 770 genes have increased mRNA abundances in response to the light signal. This result suggests a stronger impact of translational control over transcriptomic changes during photomorphogenesis. Genes encoding ribosomal protein are preferentially regulated at the translational level, possibly contributing to the enhancement of translation efficiency as observed. We also uncovered mRNAs regulated at the translational level share characteristics of longer half-lives and shorter cDNA length. Cis-elements enriched in the 5'untranslated regions of mRNAs preferentially regulated at the translational level were identified. The positive impact of the cis-element in translation has been experimentally confirmed. Taken together, our study revealed a previously neglected aspect of gene expression regulation during *Arabidopsis* photomorphogenesis. The molecular signatures associated with mRNAs regulated at the translational level also offer new directions to perform mechanistic studies of light-triggered translational enhancement in *Arabidopsis*. **Note:** Also a poster presentation.

**57 Single Gene Mutations Causing Heterosis in Tomato**Ke Jiang<sup>1</sup>, Uri Krieger<sup>2</sup>, Soon-ju Park<sup>1</sup>, Dani Zamir<sup>2</sup>, Zachary Lippman<sup>1</sup><sup>1</sup>Cold Spring Harbor Laboratory, <sup>2</sup>Hebrew University, Faculty of Agriculture

Despite countless studies in multiple systems, the genetic and molecular underpinnings for how hybrid progeny outperform their parental inbreds (heterosis) have not been resolved. We have found that single gene mutations in the heterozygous state can cause overdominance for yield. By taking advantage of a large 'mutant library' in an isogenic cultivated tomato background, we screened 70 heterozygous mutants for yield traits, which exposed six candidate heterosis loci, and two underlying genes have been identified. The strongest heterotic effect originates from the gene *SINGLE FLOWER TRUSS* (*SFT*), which encodes the flowering hormone florigen and exhibits a 60% increase in yield when loss-of-function mutations, irrespective of allele, are in the heterozygous condition. A detailed phenotypic analysis has revealed that *sft*/*+* heterosis can be traced to a simple reduction in gene dosage, which ultimately causes cumulative pleiotropic effects on inflorescence production and flower number throughout the life of the plant. More recently, we have found that the inflorescence branching gene *COMPOUND INFLORESCENCE* (*S/WOX9*) increases yield by producing 20-40% branched (bifurcated) inflorescences in mutant heterozygotes, also through a dosage effect. One conclusion from these findings is that genes regulating the floral transition and inflorescence architecture impact reproductive capacity and yield in a dosage-dependent manner. We have therefore begun using quantitative transcriptomics to link heterosis genes with expression changes in specific developmental pathways and larger transcriptional networks. By taking advantage of the large and easily exposed nature of tomato meristems, we have captured and compared transcriptome dynamics from 17 temporally defined stages of meristem maturation from three genotypes to reveal when and how heterosis genes are acting.

**58 Translation of submergence tolerance from the gene to the field using rice and Arabidopsis**

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The genetic dissection of abiotic stress tolerance in model and crop species can aid the production of food in marginalized areas. When tolerance is enhanced by one or few loci within the target species then marker-assisted breeding can be used to transfer key genes to widely grown varieties. An example of this is the identification of a haplotype of the *SUBMERGENCE1* (*SUB1*) locus of an East Indian landrace of rice, successfully used in recent years to provide submergence tolerance to highly productive varieties. This accomplishment required precise mapping of *SUB1A*, encoding an ethylene responsive transcription factor. Mechanistic analysis found that the submergence-specific expression of *SUB1A* results in constrained consumption of available carbohydrates, limiting the leaf/stem cell elongation growth typically promoted by flooding of aerial organs that result from ethylene-induced enhancement of GA responsiveness. *SUB1A* is sufficient to confer submergence tolerance in transgenic rice, when ectopically expressed under the control of a near-constitutive promoter, however its near-constitutive expression in Arabidopsis results in pleiotropic phenotypes that do not include submergence tolerance. Nevertheless, careful examination of the effect of heterologous *SUB1A* expression in Arabidopsis led to the finding that in both Arabidopsis and rice *SUB1A* alters regulation of genes associated with ABA responsiveness and flowering. In rice, this ABA responsiveness translates to better survival of dehydration stress. In Arabidopsis, the delayed flowering phenotype of *SUB1A*-expressing lines correlates with inhibition of mRNAs encoding *FLOWERING LOCUS T* and *CONSTANS*. *SUB1A* overexpression in rice similarly limits expression of the orthologs of these genes, revealing that delayed flowering is a component of the quiescence associated with submergence tolerance. Thus, abiotic stress responses and the associated metabolic and developmental remodeling can be conserved across species, increasing the utility of model plants in the translation of tolerance to crops. Funded by NIFA 2008-35100-04528.

**59 Localization of PIN1-Like Proteins in Grasses Suggests a Functional Specialization of Different PINs into 'Up-the-Gradient' and 'With-the-Flux' Modes of Auxin Transport**Devin O'Connor<sup>1</sup>, Jennifer Bragg<sup>2</sup>, John Vogel<sup>2</sup>, Connie Lee<sup>1</sup>, Sarah Hake<sup>1,2</sup><sup>1</sup>UC Berkeley PGEc, Berkeley, (CA), USA, <sup>2</sup>USDA-ARS, Albany, (CA), USA

Cell-to-cell auxin transport provides positional cues on which many developmental processes depend. Current models suggest that in aerial tissues the *Arabidopsis* PINFORMED1 (AtPIN1) auxin efflux carrier has two concurrent functions: 1) concentrating auxin "up-the-gradient" in the L1 during maxima formation and later while stabilizing existing auxin traces, and 2) transporting auxin "with-the-flux" in the internal tissues after maxima formation and during vein patterning (1). We used full-length fluorescent-protein fusion constructs under their native promoters to localize the three closest AtPIN1 homologs in the model grass *Brachypodium distachyon*, here termed BdPIN1a, BdPIN1b and Sister-of-PIN1 (BdSoPIN1). BdPIN1a and BdPIN1b expression was almost entirely restricted to internal tissues. Cellular localization of BdPIN1a was primarily basal in the meristem, incipient mid-vein, and during subsequent vein patterning, suggesting "with-the-flux" auxin transport. In contrast, BdSoPIN1 expression was highest in the meristem L1 and was only detected internally adjacent to L1 maxima and later during leaf vein patterning. Cellular localization of BdSoPIN1 was primarily oriented toward auxin maxima both internally and in the L1, suggesting BdSoPIN1 function is restricted to "up-the-gradient" creation or stabilization of auxin maxima. These data suggest a spatial and functional specialization of the grass PIN1-like proteins. We present a model for their action in both lateral organ initiation and leaf vein patterning.

1. E. M. Bayer et al., Genes Dev 23, 373-384 (2009).

**Note:** Also a poster presentation.

## 60 SAMBA- A new subunit of the Anaphase Promoting Complex (APC/C) with an essential role in plant growth and pollen development in *Arabidopsis*

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The largest E3 ubiquitin-ligase complex, known as Anaphase Promoting Complex/Cyclosome (APC/C) mediates the proteolysis of cell cycle regulators, such as CYCLIN B and SECURIN that are essential for sister-chromatid separation and exit from mitosis. Despite its importance, the role of APC/C in plant cells and the regulation of its activity during cell division remains poorly understood.

Using Tandem Affinity Purification (TAP) of protein complexes, we identified a novel plant protein, SAMBA (encoded by AT1G32310), that plays a key role in organ size control in the model plant *Arabidopsis thaliana*. SAMBA was found to interact with the Anaphase Promoting Complex (APC), suggesting that it modulates the control of the metaphase to anaphase transition of the cell cycle, and thus, in effect cell proliferation.

To clarify the role of SAMBA during plant development we analyzed loss-of-function and the gain-of-function mutants. Our result shows that down regulation of SAMBA significantly enhances the size and mass of all analyzed plant organs including seeds, roots, and leaves. A cell based kinematic analysis of leaf growth revealed that *samba* mutants have a faster cell cycle explaining the enhanced growth properties. Preliminary data suggest that SAMBA is involved in the degradation of A-type cyclins. Besides its effect on plant growth, *samba* mutants hampered male gametophyte development.

*Note: Also a poster presentation.*

## 61 Modeling of the Plant Hormone Signaling Network in MAMP-Induced Resistance

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Pattern-Triggered Immunity (PTI) is a key mode of plant immunity against pathogens. PTI is triggered by recognition of conserved microbial features called Microbe-Associated Molecular Patterns (MAMPs). Plant hormone signaling sectors defined by jasmonic acid, ethylene and salicylic acid play major roles in PTI and they interact with each other in a complex manner. To get more insight into roles of the hormone signaling sectors, we constructed an *Arabidopsis* quadruple mutant (*dde2/ein2/pad4/sid2*). We reported that the network defined by the four genes, *DDE2*, *EIN2*, *PAD4* and *SID2*, accounts for most of PTI against *Pseudomonas syringae* triggered by a MAMP, flg22 (Tsuda et al., PLoS Genetics 5: e1000772 2009). We estimated the effects of the wild-type genes and their interactions on immunity using the data from all combinatorial (single, double, triple, and quadruple) mutants. This signaling allocation analysis revealed quantitative contributions of each single signaling sector and genetic relationships among the signaling sectors. However, mechanistic relationships between the signaling sectors remain undefined. In order to build a mechanistic model for the network controlled by the four signaling sectors in PTI, we measured MAMP-induced resistance in all the combinatorial mutants as well as wild type using three different MAMPs (flg22, elf18, chitosan) as inputs and two different pathogens (*P. syringae* pv. *tomato* DC3000, *P. syringae* pv. *maculicola* ES4326) as outputs. We are currently building semi-dynamic mechanistic network models regarding the four signaling sectors in MAMP-induced resistance using Bayesian network and non-linear regression approaches.

*Note: Also a poster presentation.*

## 62 German Plant Research Goes BioEconomy

Dirk Büssis

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The German Ministry of Education and Research (BMBF) has recently announced the new Research Strategy 2030. At the centre of this research strategy is the BioEconomy, the transition away from an oil based economy towards a bio based economy. In the first six years the Ministry will fund programs within this research strategy with 2.4 bio. Euros. Programs will combine research activities in the fields of white biotechnology, animal breeding, soil and climate research as well as plant research. The research strategy integrates the whole value added chain from primary production to the consumer.

Plant research is an important cornerstone of the Research Strategy 2030. Based on the successful research program GABI (Genome Analysis in the Plant Biologic System) the new program Plant Biotechnology for the Future is set to start during 2011. Like the GABI program it is built on strong public private partnerships. In two modules research is carried out towards a bio based economy. In the module "transfer" potential applications will be transferred from model plants into crop species. In the module "products" applied research in crop species will lead to the development of new plants for future applications.

The German Ministry of Education and Research funds two more important programs in plants research. In the PLANT Knowledge Based BioEconomy (PLANT-KBBE) program international co operations are being funded. Starting from a core of trilateral co operations between German, French and Spanish groups, multilateral consortia have formed including partners from other European countries and even Canada. A new initiative is the German Plant Phenotyping Network (DPPN) that will concentrate on developing applied phenotyping methods for the BioEconomy.

Finally, the initiative "excellence clusters in agronomy" funded by the BMBF is running for two years already. In large consortia a close co operation between plant breeders, animal breeders, basic researchers, bio informatitians and private enterprises has been established. German plants research is well set up developing towards knowledge based bio economy.

**63 Genomic Dissection of the Plant/Pest Interaction: Transcriptome Analysis of *Arabidopsis* Response to *Tetranychus urticae* (Two Spotted Spider Mite) Feeding**

*Marie Navarro<sup>1</sup>, Gustavo Acevedo<sup>1</sup>, Marc Cazaux<sup>1</sup>, Johannes Mathieu<sup>2</sup>, Marcus Schmid<sup>2</sup>, Miodrag Grbic<sup>1</sup>, Vojislava Grbic<sup>1</sup>*

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The Two Spotted Spider Mite *Tetranychus urticae* (TSSM) is a cosmopolitan, polyphagous pest feeding on more than 1100 plants species, including 150 of economic interest. *T. urticae* is notorious for its ability to rapidly develop cross-resistance to different pesticides, increasing the difficulty to control this pest. Despite this agricultural importance, very little is known about the molecular and genetic components underlying the interaction between a plant and TSSM.

Taking advantage of the feeding of TSSM on *Arabidopsis*, and the recently sequenced genome of TSSM ([www.jgi.doe.gov/sequencing/why/50028.html](http://www.jgi.doe.gov/sequencing/why/50028.html)), our group is establishing *Arabidopsis*/TSSM interaction as a novel system for understanding plant-pest interactions. We performed a screen of *Arabidopsis* accessions based on damages induced by TSSM, and have used two accessions with the most contrasting phenotypes for a microarray analysis of the *Arabidopsis* response to TSSM feeding. This analyse reveals a large overlap between genes induced in both tolerant and susceptible strains with qualitative differences in the plant transcriptome. A major difference between tolerant and susceptible accessions includes a time shifts of the transcriptome expression. The comparison between *Arabidopsis* response to TSSM and other pests revealed that TSSM elicits a specific response of defence pathways and secondary metabolites that has similarities to the response to pathogens. In parallel, transcriptome analysis of the TSSM responses to feeding on *Arabidopsis* revealed induction of many detoxifying enzymes, providing an opportunity to study the response of both plant and pest upon their interaction.

**Note:** Also a poster presentation.

**64 Regulation of *Arabidopsis* Gynoecium Polarity by ULT and KAN Family Proteins***Jennifer Fletcher***Plant Gene Expression Center, Albany, CA, USA**

The angiosperm gynoecium is a highly complex structure that is essential for reproduction and seed dispersal. Gynoecium development involves the patterning of the tissues along the apical-basal, adaxial-abaxial and medial-lateral axes. In *Arabidopsis*, organ polarity establishment requires the activities of hormones and various families of transcription factors, but only a few epigenetic regulators of organ polarity have been defined. Work in my lab has determined that the SAND domain protein ULTRAPETALA1 (ULT1) functions as a trithorax Group factor that counteracts Polycomb Group chromatin-mediated repression of target gene transcription during flower development. Here I shall describe a role for *ULT1* and the related *ULT2* gene in controlling asymmetric gynoecium patterning. I'll also discuss results showing that the ULT proteins and members of the KANADI (KAN) family of transcription factors act coordinately to regulate polarity along the apical-basal axis and antagonistically to regulate polarity along the adaxial-abaxial axis.

**65 The Endodermis - building a selective and polarised cellular barrier***Niko Geldner***University of Lausanne, Lausanne, Switzerland**

I will report on our investigations into the molecular mechanisms of endodermal differentiation in *Arabidopsis*. The endodermis is a cell layer present in all higher plants. Its defining feature is the presence of particular, belt-like cell wall modifications, called Casparyan Strips (CS), present in the longitudinal, median plane of the cell. CSs fulfill roles similar to animal tight and adherens junction and the endodermis is thought to be of central importance for plant nutrition and stress resistance. We have established markers that highlight two separate polar domains, divided by a median domain at the CS, termed CSD(1). In addition, we have discovered the first proteins that mark the CSD and have demonstrated that these "CASPs" are important for correct deposition of Casparyan Strips(2). CASPs apparently form polymers within the membrane that provide a platform for the localisation of cell wall biosynthetic enzymes, necessary for CS formation. The CASPs represent an entirely novel way to generate an epithelial diffusion barrier in eukaryotic cells. Using forward and reverse genetic approaches, we have identified a number of additional players that are necessary for CASP localisation or formation of Casparyan Strip. These new players include a receptor-like kinase, necessary for correct accumulation of CASPs, as well as enzymes involved in the localised polymerisation of the lignin-like Casparyan Strips. This now presents us with several entry points for a mechanistic dissection of the formation of this particular, plant-specific cellular barrier. Besides that, our specific mutants now provide the unprecedented opportunity to test the many assumptions about the role of Casparyan Strips in plants.

(1) Alassimone J, Naseer S, **Geldner N**. A developmental framework for endodermal differentiation and polarity. *Proc Natl Acad Sci U S A*. 2010, Mar 16;107(11):5214-9.

(2) Roppolo D, De Rybel B, Dénervaud Tendon V, Pfister A, Alassimone J, Vermeer JEM, Yamazaki M, Stierhof YD, Beeckman T, **Geldner N**. A novel protein family mediates Casparyan Stripformation in the endodermis. *Nature*, 2011, *in press*.

**66 An apical root growth program directed in the vascular stem cells***Jose Sebastian<sup>1</sup>, Jing Zhou<sup>2</sup>, Ji-Young Lee<sup>1,2</sup>***<sup>1</sup>Boyce Thompson Institute for Plant Research, Ithaca, NY, USA, <sup>2</sup>Cornell University, Ithaca, NY, USA**

*Arabidopsis* primary root develops from the root apical meristem (RAM). The RAM harbors the stem cell niche that consists of pluripotent stem cells encircling few (2-4) mitotically inactive cells, called quiescent centre (QC). The QC maintains the stem cell niche and prevents stem cells from premature differentiation, thereby ensures root growth.

Over the years, several studies enhanced our knowledge about the genetic networks and other signaling modules that govern the development and function of RAM. One of them is the pathway directed by *SHORT ROOT* (*SHR*) and *SCARECROW* (*SCR*). When this pathway is perturbed, the apical root growth is significantly retarded. This has been thought to be resulted from the loss of QC identity. Recently we and colleagues reported the bidirectional cell signaling process that involves the movement of *SHR* and microRNA (miR) 165/6. This mechanism ensures a proper spatial distribution of HD-ZIP III transcription factors.

We found that *SHR-SCR-miR165/6* regulation is important for the apical root growth and that *PHABULOSA* (*PHB*) plays a critical role as a downstream regulator. In *phb shr* and *phb scr* mutants, the roots grew significantly longer than in *shr* and *scr*. This root growth recovery was not resulted from the restoration of QC identify. *PHB* was found to regulate the apical root growth in the procambium, a part of the proximal meristem of the root stem cell niche. A high dose of *PHB* expressed in the root procambium inhibited the apical root growth. Furthermore, it affected the QC identity. Interestingly, the *SHR-PHB* pathway seems to regulate the apical root growth via the crosstalk with the cytokinin signaling cascade. This intricate regulatory program points vascular stem cells as an important place in the RAM activity.

**67 Spatio-Temporal Sequence of Cross-Regulatory Events in Root Meristem Growth***Emanuele Scacchi<sup>2</sup>, Paula Salinas<sup>2</sup>, Bojan Gujas<sup>2</sup>, Luca Santuari<sup>2</sup>, Naden Krogan<sup>1</sup>, Laura Ragni<sup>2</sup>, Thomas Berleth<sup>1</sup>, Christian Hardtke<sup>2</sup>***<sup>1</sup>University of Toronto, Toronto, Canada , <sup>2</sup>University of Lausanne, Lausanne, Switzerland**

A central question in developmental biology is how multicellular organisms coordinate cell division and differentiation to determine organ size. In *Arabidopsis* roots, this balance is controlled by cytokinin-induced expression of SHORT HYPOCOTYL 2 (SHY2) in the so-called transition zone of the meristem, where SHY2 negatively regulates auxin response factors (ARFs) by protein-protein interaction.

The resulting down-regulation of PIN-FORMED (PIN) auxin efflux carriers is considered the key event in promoting differentiation of meristematic cells. Here we show that this regulation involves additional, intermediary factors and is spatio-temporally constrained. We found that the described cytokinin-auxin crosstalk antagonizes BREVIS RADIX (BRX) activity in the developing protophloem. BRX is an auxin-responsive target of the prototypical ARF MONOPTEROS (MP), a key promoter of vascular development, and transiently enhances PIN3 expression to promote meristem growth in young roots. At later stages, cytokinin induction of SHY2 in the vascular transition zone restricts BRX expression to down-regulate PIN3 and thus limit meristem growth. Interestingly, proper SHY2 expression requires BRX, which could reflect feedback on the auxin responsiveness of SHY2 because BRX protein can directly interact with MP, likely acting as a cofactor. Thus, cross-regulatory antagonism between BRX and SHY2 could determine ARF activity in the protophloem. Our data suggest a model in which the regulatory interactions favor BRX expression in the early proximal meristem and SHY2 prevails because of supplementary cytokinin induction in the later distal meristem. The complex equilibrium of this regulatory module might represent a universal switch in the transition toward differentiation in various developmental contexts.

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**68 Expanding the Genetic Pathway for Polarity in *Arabidopsis* Leaf Development**

*Brenda Reinhart<sup>1</sup>, Tie Liu<sup>1</sup>, Niki Newell<sup>1</sup>, Tengbo Huang<sup>2</sup>, Randall Kerstetter<sup>2</sup>, M. Kathryn Barton<sup>2</sup>*

<sup>1</sup>**Carnegie Institution, Stanford, CA, USA, <sup>2</sup>Waksman Institute, Rutgers University, Piscataway, NJ, USA**

We are investigating how the pluripotent cells of the *Arabidopsis* meristem are patterned into complex organs by combining genetic research with transcriptional profiling to identify gene networks acting during differentiation. Adaxial and abaxial fate determinants act antagonistically to pattern the top and bottom halves of the leaf blade. Therefore, we reasoned that by identifying genes oppositely regulated by adaxial and abaxial fate determinants, we would find good candidates for genes involved in leaf development. A dexamethasone (DEX) inducible form of the adaxial fate determinant REV, an HD-Zip class III transcription factor, was created by fusion to the glucocorticoid receptor (GR-REV). To identify targets of REV, microarray analysis was used to determine changes in gene expression at various times after DEX induction of GR-REV transgenic plants. We also performed a similar experiment with an abaxial fate determinant, KANADI1 (KAN1). Using 2-way ANOVA analysis to look for interactions between genotype and time, we identified 24 genes oppositely regulated by REV and KAN ( $p$ -value  $<0.01$ ). 12 were up-regulated by REV and down-regulated by KAN, and the other 12 had the opposite pattern of regulation.

Consistent with previous reports suggesting that REV is a positive regulator of transcription while KAN is a negative regulator, the genes up-regulated by REV and down-regulated by KAN were the most likely to be verified by quantitative RT-PCR (8/11 tested) and to be direct targets as judged by cycloheximide treatment of the samples. Our list of target genes includes transcription factors, components of signaling pathways, and potential effectors of cell shape changes. We are currently determining their possible roles in *Arabidopsis* development.

*Note: Also a poster presentation.*

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**69 HD-Zip II Transcription Factor Genes Control Adaxial-Abaxial Patterning in *Arabidopsis* Leaf Morphogenesis**

*Monica Carabelli<sup>1</sup>, Luana Turchi<sup>1</sup>, Massimiliano Sassi<sup>1</sup>, Marco Possenti<sup>2</sup>, Valentino Ruzza<sup>1</sup>, Carmen Melatti<sup>1</sup>, Giorgio Morelli<sup>2</sup>, Ida Ruberti<sup>1</sup>*

<sup>1</sup>**Institute of Molecular Biology and Pathology, National Research Council, Rome, Italy, <sup>2</sup>National Research Institute for Food and Nutrition, Rome, Italy**

The *Arabidopsis* genome encodes for 10 Homeodomain-Leucine Zipper II (HD-Zip II) proteins. It has been previously shown that *ATHB2*, *HAT1*, *HAT2* (HD-Zip II  $\delta$  subfamily), *HAT3* and *ATHB4* ( $\gamma$  subfamily) are induced by changes in the Red/Far Red ratio of the light environment. However, these genes are also tightly regulated during plant development with both distinct and overlapping patterns (Ciarbelli *et al.*, Plant Mol. Biol. 2008, 68: 465-78). In order to understand the role of the light-regulated HD-Zip II genes in plant development, we have analyzed single and multiple mutants within  $\delta$  and  $\gamma$  subfamilies. Here we present the phenotype of mutants in the *HAT3* and *ATHB4* genes. Young seedlings show a gradual loss of cotyledon and leaf expansion, up to completely radialized organs. The pattern of vascular development is also profoundly altered, in a manner that is tightly linked to lamina expansion. Fully radialized leaves lack procambial cells whereas trumpet shaped leaves show hyperproliferation of phloem with respect to xylem, a feature that is found in the vasculature of abaxialized leaves. *In situ* and GUS/GFP reporter analyses of the  $\gamma$  subfamily genes show that they are expressed in the adaxial side of cotyledons and leaves. Taken together, these data demonstrate that *HAT3* and *ATHB4* are required to specify adaxial identity in leaf morphogenesis. We are currently analyzing the molecular and genetic relationships between the  $\gamma$  HD-Zip II genes and members of the HD-Zip III family genes, such as *PHB*, *PHV* and *REV*, key determinants of adaxial leaf identity.

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**70 *GRD* and *WOX* Genes Cooperatively Promote Developmental Asymmetry in *Arabidopsis* Embryos**

*Sangho Jeong, Wolfgang Lukowitz*

**University of Georgia**

The basic body plan of plants is established in the embryo. Upon fertilization, the former egg cell undergoes a series of dynamic intracellular re-organizations, resulting in a highly polarized zygote. The zygote then divides asymmetrically to produce daughter cells with fundamentally different developmental fates: the apical cell produces the proembryo while the basal cell develops into the mostly extra-embryonic suspensor. This fascinating process, from fertilization to asymmetric first division, takes about 4-8 hours in *Arabidopsis* and sets the coordinates of the apical-basal axis.

Establishment of apical and basal fates is known to depend on the *YODA* MAP kinase cascade and *WOX* homeodomain transcription factors. Here we report characterization of the *GROUNDED* (*GRD*) gene which encodes small nuclear protein of the RWP–RK family. Mutations in *GRD* cause anatomical defects consistent with a loss of developmental asymmetry. Furthermore, suspensor-specific *WOX8* expression disappears while proembryo-specific *ZWILLE* expression expands in the mutants, suggesting that the basal fates are lost. Molecular and genetic studies indicate that *GRD* facilitates *YDA*-dependent signaling to promote the basal fates. Interestingly, *grd* embryos are sensitive to the dosage of the *WOX* genes, and *grd;wox8;wox9* triple mutants arrest as zygotes or 1-cell embryos without apparent polarity. These results indicate that *GRD* acts cooperatively with *WOX* proteins to establish embryonic polarity in the first division. A recent report that the *WRKY2* transcription factor affects the asymmetric first division as well as *WOX8* expression lends further support to the importance of transcriptional regulation for zygote development. Further studies on the interactions of these factors are in progress.

**71 Mapping local adaptation in *Arabidopsis thaliana***

*Johanna Schmitt*<sup>1</sup>, *Amity Wilczek*<sup>2</sup>, *Alexandre Fournier-Level*<sup>1</sup>, *Arthur Korte*<sup>3</sup>, *Martha Cooper*<sup>1</sup>, *Magnus Nordborg*<sup>3</sup>

<sup>1</sup>Brown University, <sup>2</sup>Deep Springs College, <sup>3</sup>Gregor Mendel Institute

Adaptation to local environments has been observed experimentally in many organisms, and will be critical for species persistence in the face of rapid environmental change. However, the genetic mechanisms underlying local adaptation are still largely unexplored. We tested for local adaptation to climate by growing a set of *Arabidopsis thaliana* ecotypes across the species native climate range in common garden field experiments in Finland, England, Germany, and Spain. Genotypes originating in climates similar to the site of planting had high relative fitness in each site, providing direct evidence for adaptation to climate in this model species. However, genotypes originating in climates historically warmer than the site of planting had higher relative fitness than native genotypes in every site, evidence of lagging adaptation in response to changing climate. A genome-wide association study of survival and lifetime reproduction revealed that the genetic basis of local adaptation differs among regions. Effect sizes of the SNPs associated with fitness were weakly correlated across field sites, and only 12 of the 797 most strongly associated SNPs for each trait were common across sites. Thus distinct environment-specific loci contributed to fitness variation in each field site. Moreover, the molecular functions under- or overrepresented among genes linked to associated SNPs were also different across traits and sites. Alleles conferring higher fitness within each site were distributed significantly closer to that site than genomic controls in all sites except Finland, providing a geographic signature of local selection. Alleles of strongly fitness-associated SNPs in each site also exhibited higher levels of climate specialization than genomic controls.

**72 Using *Arabidopsis* relatives as sources of natural genetic variation in regulatory networks**

*George Coupland*

Max Planck Institute for Plant Breeding, Cologne, Germany

We are interested in the molecular mechanisms underlying the floral transition. Use of molecular-genetic approaches in *A. thaliana*, including natural-genetic variation, has identified six pathways that control flowering in response to endogenous signals or environmental cues, such as vernalization and day length. A limitation of these methods is that *A. thaliana* does not exhibit important flowering behaviors found elsewhere in the plant Kingdom. In particular, *A. thaliana* is an annual species and does not show the perennial behaviour that dominates in many plant communities. We are using *Arabidopsis* relatives to analyze flowering traits not shown by *A. thaliana*. This approach has the advantage of being able to use the mechanistic information from *A. thaliana* to easily identify orthologues, exploit the small genomes of many relatives and to study the traits in the evolutionary context of the phylogeny of the Brassicaceae family. As a perennial relative, we have used *Arabis alpina*. This species lives for many years, flowers each year and most accessions absolutely require exposure to winter temperatures for flowering. We have sequenced the genome of *A. alpina* (as part of an international consortium) and shown how specific transcription factors involved in temperature response are differently regulated between annual *A. thaliana* and perennial *A. alpina*, and that this contributes to the ability of the perennial species to flower each year. Evolution of the regulation of these genes involves changes in chromatin regulation and in gene structure. We are exploring natural variation in perennial flowering in *Arabis alpina* and how vernalization response intersects with the age of the shoot to influence flowering time. This analysis provides an example of how using relatives allows the use of regulatory networks originally defined in *A. thaliana* to analyze phenotypic traits not shown by the model species

**73 Quantitative variation in the circadian clock and adaptation to heterogeneous settings**

*Weinig C*<sup>1</sup>, *CE Edwards*<sup>1</sup>, *MT Brock*<sup>1</sup>, *MJ Rubin*<sup>1</sup>, *BE Ewers*<sup>1</sup>, *L Ping*<sup>2</sup>, *CR McClung*<sup>2</sup>

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The circadian clock is taxonomically ubiquitous, suggesting a central role in adaptation; yet no studies have demonstrated the adaptive significance of the clock in ecologically relevant field settings. We raised mutants, natural accessions, and RILs of *Arabidopsis thaliana* in the field as well as RILs of *Brassica rapa* to test a) the relationship between variation in the clock and in plant performance and b) the sensitivity of the clock to seasonally varying environmental inputs.

We observed that short- and long-period mutants have lower fruit set, an important determinant of fitness, than the cognate wild type in spring seasonal settings. These patterns of fruit set were validated in growth-chamber experiments manipulating exogenous T-cycles. Observed differences in fruit set were partly attributable to variation in meristem deployment, i.e., short-period mutants not only produced more fruit in short T-cycles but also had more branches relative to wild-type and long-period genotypes.

In a panel of natural accessions and RILs, we observed that circadian phase was likewise associated with variation in fruit set and branching in a spring seasonal setting among plants grown at low-density. At high-density, circadian phase was associated with variation in internode elongation and plant size. The role of the clock in shade-avoidance was validated in a growth-chamber experiment, e.g., short-period mutants elongated more in response to foliar-shade cues under short T-cycles than did long-period mutants.

Because physiological processes vary diurnally, we were interested in if the circadian clock may partly regulate the expression of physiological traits. In RILs of *B. rapa*, circadian period was correlated with photosynthesis and gas exchange. Preliminary transgenic rescue experiments suggest that *GIGANTEA* may underlie one QTL that jointly affects circadian period, photosynthesis, leaf mass/area, and deltaC<sup>13</sup>.

**74 Systems Biology to Dissect Nitrogen Regulatory Networks**Rodrigo Gutiérrez**FONDAP Center for Genome Regulation. Millennium Nucleus for Plant Functional Genomics. Department of Molecular Genetics and Microbiology. Pontificia Universidad Católica de Chile. Santiago, Chile.**

Genomics approaches have provided us with thousands of genes whose expression is modulated in response to various nitrogen (N) metabolite/nutrient treatments in Arabidopsis. Recently, systems approaches have been utilized to identify discrete molecular networks that plants utilize to adapt metabolic, cellular and developmental processes to changing N availability. The challenge now is to understand the molecular mechanisms underlying N-regulation of gene networks and bridge the gap between N-sensing, signaling and downstream physiological and developmental changes. We used systems approaches to identify gene regulatory networks involved in N responses in Arabidopsis roots. Using next generation sequencing technologies and bioinformatics analysis of the sequence data, we identified nitrate-regulated microRNAs and other sRNAs in Arabidopsis roots. Detailed analysis of one nitrate-responsive miR393/AFB3 regulatory module, revealed a type I incoherent feed-forward loop that is induced by nitrate and repressed by N forms produced by nitrate reduction and assimilation. To understand the functional role of this nitrate regulatory module for plant development, we analyzed root system architecture changes in response to nitrate treatments in *afb3* mutant plants and in miR393 overexpressor plants. Our results indicate that this microRNA/TARGET pair is a novel N-responsive regulatory module that controls root system architecture in response to external and internal N availability in Arabidopsis. Integrated genomics, network bioinformatics and molecular genetics efforts identified some of the regulatory networks downstream of the miR393/AFB3 regulatory module leading to changes in root development.

**75 Mapping Spatiotemporal Gene Regulatory Networks in the Arabidopsis Root Stele**Siobhan Brady<sup>1</sup>, Mallorie Taylor-Teeple<sup>1</sup>, Allison Gaudinier<sup>1</sup>, Lifang Zhang<sup>2</sup>, John Reece-Hoyes<sup>3</sup>, Sebastian Ahnert<sup>4</sup>, A. J. Marian Walhout<sup>3</sup>, Doreen Ware<sup>2</sup>**<sup>1</sup>University of California, Davis, <sup>2</sup>Cold Spring Harbor Laboratory, <sup>3</sup>UMass Medical School, <sup>4</sup>University of Cambridge**

Arabidopsis root development provides a remarkably tractable system to delineate tissue-specific, developmental gene regulatory networks and to study their functionality in a complex multicellular model system overdevelopmental time. Tightly controlled gene expression within tissues is a hallmark of multicellular development and is accomplished by transcription factors (TFs) and microRNAs. We present an automated, enhanced yeast one hybrid (eY1H) assay using a tissue-specific TF resource to comprehensively map gene regulatory networks in the Arabidopsis root stele. These gene regulatory networks are robust and highly combinatorial in nature. Using these methods and computational modeling, we have additionally modeled a gene regulatory network that regulates distinct transcriptional events in developmental time. Distinct regulatory modules were identified that temporally drive the expression of genes involved in xylem specification and in the subsequent synthesis of secondary cell wall metabolites associated with xylem differentiation.

**76 Properties and structure of the plant immune signaling**Kenichi Tsuda<sup>1</sup>, Masanao Sato<sup>1\*</sup>, Yungil Kim<sup>2</sup>, Jane Glazebrook<sup>1</sup>, Chad Myers<sup>2</sup> and Fumiaki Katagiri<sup>1</sup>**<sup>1</sup>Department of Plant Biology and <sup>2</sup>Department of Computer Science and Engineering, Microbial and Plant Genomics Institute, University of Minnesota, MN, USA.**

The plant immune signaling network is different from other plant signaling networks because pathogens not only initiate signaling events but also interfere with plant signaling. Microbial pathogens also evolve much faster than plants. Therefore, the plant immune signaling network must have properties that allow it to withstand perturbations from a wide variety of pathogens without heavily relying on evolutionary adaptation. Unnecessary immune responses carry negative impacts on plant fitness, further constraining possible network properties. Pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) are two modes of plant immunity. PTI is initiated by recognizing molecular patterns common among related microbes, including pathogens and benign microbes. Pathogens well-adapted to a host plant deliver effectors into the plant cell that interfere with PTI signaling and negate PTI. Plants may have receptors that recognize some of the pathogen effectors and trigger ETI, resulting in immunity. We demonstrated that at least some cases of PTI and ETI extensively share the signaling machinery and that what distinguishes PTI and ETI is the way the common signaling network operates. There is synergy among signaling sectors in PTI and compensation in ETI. The latter explains the robustness of ETI. In ETI compensation does not result from simple redundancy among sectors. We found that negative regulatory relationships between different signaling sectors are very common. Such prevalent negative regulatory relationships suggest that only part of the signaling network is highly activated at a given time. This likely reduces negative impacts of immune responses. If the primary sectors are perturbed by effectors, some other sectors may be released from suppression and provide back-up immunity, resulting in robustness of immunity against network perturbations by pathogen effectors.

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## 77 Chemical Genetics Reveals Negative Regulation of Abscisic Acid Signaling by a Type III Effector Signaling Pathway

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Studies have shown that interference in resistance responses can occur when plants are exposed to both abiotic stress and pathogen attack. Initial abiotic stress can interfere with pathogen resistance. Conversely, initial plant immune signaling may interrupt subsequent ABA signal transduction. However, at which level initial immune signaling interferes with ABA signal transduction remains to be investigated. By screening a 9,600 compound chemical library, we identified a small molecule, DFPM, that blocks ABA-dependent gene expression and also inhibits ABA-induced stomatal closure. Transcriptome analyses show that DFPM also stimulates expression of plant disease responsive genes. Major early regulators of disease responses are required for DFPM-inhibition of ABA signal transduction, whereas downstream salicylic acid signaling mechanisms were not required for interference with ABA signaling. Furthermore, the level at which pathogen-induced signaling disrupts ABA signaling was step-wise analyzed and characterized starting from ABA receptors to downstream events. Our findings present evidence that activation of type III effector plant disease signaling rapidly disrupts ABA signal transduction and an R gene and the pathogen signaling mechanisms required for this response and the ABA signaling level at which this occurs will be presented, illuminating how an initial biotic stress pathway can interfere with ABA signaling. This research was supported by an NIH grant (J.I.S.)

## 78 Regulatory gene network in stress responses to drought conditions

Kazuo Shinozaki<sup>1,2</sup>, Kazuko Yamaguchi-Shinozaki<sup>1,3</sup>

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Plants respond and tolerate water deficit conditions through molecular as well as physiological processes. Drought stress induces a variety of genes at transcriptional level. Their gene products function in stress tolerance and response. There are several different regulatory systems in stress-responsive gene expression; one group is ABA-dependent and the other ABA-independent. In one of the ABA-independent pathways, a cis-acting element (DRE/CRT) and its binding proteins, DREB1/CBF and DREB2, are important cis- and trans-acting elements in stress-responsive gene expression, respectively. DREB2 is also involved in heat stress response and regulates many HSPs. In the ABA-dependent pathways, bZIP transcription factors (AREB/ABF) function as major regulatory factors after the accumulation of endogenous ABA. SnRK2 PKase phosphorylates and activates AREB/ABF, and is regulated by PP2C and PYR/RCARABA receptor. We have identified key enzymes in ABA biosynthesis (NCED3) and metabolism (CYP707A3), and analyzed signal transduction pathways upstream of the AREB transcription factors in drought stress response. We will discuss complex regulatory networks in the early process of drought stress response, especially important roles of SnRK2. ABA is predominantly produced in vascular tissues, but acts in distant guard cell responses. We analyzed ABA transport system and showed that one of ATP-binding cassette (ABC) transporter genes, AtABCG25, encodes a protein that is responsible for ABA transport and responses in Arabidopsis. AtABCG25 exhibited ATP-dependent ABA transport. The active control of multi-cellular ABA responses to environmental stresses among plant cells will be discussed.

Umezawa et al. PNAS 106:17588, 2009, Miyazono et al. Nature 462:609 2009, Kuromori et al. PNAS 107:2361, 2010, Hirayama and Shinozaki Plant J 61:1041, 2010.

## 79 Moisture locally induces ABA biosynthesis to determine growth direction in Arabidopsis roots

José Dinneny<sup>1,3</sup>, Yun Bao<sup>2</sup>

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Plants have evolved to survive in a heterogeneous environment. Soil, in particular, is a highly complex matrix, in which roots must navigate through to find optimal supplies of nutrients and water. Never-the-less, our understanding of how roots sense and interpret local environmental stimuli is poor. We have identified a GFP-reporter line, *PG1*, which shows an asymmetric expression pattern at the tip of the Arabidopsis root. We have determined that *PG1* expression is repressed by local contact of root cap cells with moisture, while its expression is induced in cells exposed to air. Thus, *PG1* is the first local indicator of moisture content in the environment.

GFP expression in the *PG1* reporter is driven by a promoter cloned from the *NCED2* gene, which encodes an enzyme performing the rate-limiting step in ABA biosynthesis. Using a simplified assay to measure aspects of the hydrotropism response, we have found that *nced2* mutants have reduced affinity for moist conditions and grow into the air more frequently than wild type. Thus, local induction of *NCED2* expression in the root cap maintains growth towards moist conditions.

We have utilized the *PG1* reporter along with Fluorescence Activated Cell Sorting (FACS) to profile global transcriptional changes associated with cells in direct contact with air. These data confirm that *NCED2* has enriched expression in cells of the root directly exposed to air and has also allowed us to identify several other pathways whose activity is locally regulated by moisture. In summary, our results show that the root can sense moisture at the local level and that this plays an important role in guiding many biological processes including growth.

*Note: Also a poster presentation.*

**80 Clock Components CCA1 and LHY Regulate Expression of the CBF Cold Response Pathway and Freezing Tolerance in Arabidopsis**

Malia Dong, Eva Farre, Michael Thomashow  
**Michigan State University, USA**

Many plants increase in freezing tolerance in response to low non-freezing temperature, a phenomenon known as cold acclimation. In *Arabidopsis*, cold acclimation is associated with the induction or repression of over a thousand genes. The *CBF* cold response pathway has a central role in this process. Within minutes of transfer to low temperature, genes encoding three closely related transcription factors, *CBF1, 2* and *3*, are induced and alter expression of more than one hundred target genes, which impart freezing tolerance. Previous studies have shown that *CBF1, CBF2* and *CBF3* are subject to circadian regulation and that their cold induction is gated by the circadian clock. In plants carrying mutations in the central clock components CCA1 and LHY, cold induction of *CBF1, 2* and *3* was greatly impaired and circadian regulation of *CBF1* and *CBF3* was essentially eliminated; circadian regulation of *CBF2* continued, although with significantly reduced amplitude. Circadian regulation and cold induction of the *CBF* regulon genes were also greatly diminished in plants carrying the *cca1-11/lhy-21* double mutation. Chromatin-immunoprecipitation experiments indicated that regulation by these clock components is direct. Furthermore, the *cca1-11/lhy-21* double mutation resulted in impaired freezing tolerance in both non-acclimated and cold-acclimated plants. These results indicate that circadian clock components CCA1 and LHY contribute to plant freezing tolerance through regulation of the *CBF* cold response pathway.

*Note: Also a poster presentation.*

**81 Heme Synthesis By Plastid Ferrochelatase I Regulates Nuclear Gene Expression**

Jesse Woodson, Juan Perez-Ruiz, Joanne Chory  
**The Salk Institute**

Chloroplast signals regulate hundreds of nuclear genes during development and in response to stress, but little is known of the signals or signal transduction mechanisms of plastid-to-nucleus (retrograde) signaling. In *Arabidopsis thaliana*, genetic studies using Norflurazon (NF), an inhibitor of carotenoid biosynthesis, have identified five *GUN* (genomes uncoupled) genes, implicating the tetrapyrrole pathway as a source of a retrograde signal. Loss-of-function of any of these *GUN* genes leads to increased expression of photosynthesis-associated nuclear genes (PhANGs) when chloroplast development has been blocked by NF. Here we present a new *Arabidopsis* gain-of-function mutant, *gun6-ID*, with a similar phenotype. The *gun6-ID* mutant overexpresses the conserved plastid ferrochelatase 1 (FC1, heme synthase). Genetic and biochemical experiments demonstrate that increased flux through the heme branch of the plastid tetrapyrrole biosynthetic pathway increases PhANG expression. The second conserved plant ferrochelatase, FC2, co-localizes with FC1, but FC2 activity is unable to increase PhANG expression in undeveloped plastids. These data suggest a model where heme, specifically produced by FC1, may be used as a retrograde signal to coordinate PhANG expression with chloroplast development.

*Note: Also a poster presentation.*

**82 POPEYE, BRUTUS and Other Characters: Elucidating Molecular Mechanisms of the Iron Deficiency Response in Plants**

Durreshah Muhammad, Imrose Kauser, Lujaina Farooq, Ahmad Noweder, Terri Long  
**University of Illinois at Chicago, Chicago, IL, USA**

Iron is a critical component of many essential biological processes in plants. Understanding how *Arabidopsis* roots respond to low iron conditions could lead to the generation of plants with increased iron content and/or tolerance to poor soils. Previously, we described how the bHLH transcription factor, POPEYE (PYE) directly regulates the expression of gene targets involved in diverse process including oxidative stress response, transcriptional regulation, and iron homeostasis (Long et al, 2010. Plant Cell). A screen of PYE targets reveals several with altered response to iron deficiency that have not previously been shown to play a role in iron homeostasis. We describe the function of these PYE targets and discuss how PYE-mediated regulation of these targets contributes to the iron deficiency response.

In addition to transcriptional targets, we also found that PYE interacts with several closely related PYE homologs, including ILR3. ILR3 controls auxin-mediated iron homeostasis (Rampey et al, 2006 Genetics), although it has not previously been examined under conditions of iron deficiency. Notably, ILR3 also interacts with BTS, a putative iron binding E3 ligase that appears to negatively control response to iron deficiency. Analysis of the responses of *ilr3*, *pye-1*, and *bts-1* mutants to iron deprivation supports the idea that these three proteins may form a regulatory complex that senses and controls response to low iron content within plants roots.

*Note: Also a poster presentation.*

**83 The role of calcium signaling in the molecular response network to anaerobic stress in *Arabidopsis***

WON-GYU CHOI  
**The University of Wisconsin, Madison, WI, USA**

Plants growing in flooded soils show a complex set of molecular responses during adaptation to these conditions. However, anaerobic stress can still lead to a severe reduction in yield resulting in major crop losses. Despite this impact on agriculture, cellular signals responsible for triggering the molecular response to flooding remain poorly understood. Cytoplasmic  $\text{Ca}^{2+}$  changes have been tentatively linked to anaerobic stress. We observed hypoxia-induced  $\text{Ca}^{2+}$  changes in *Arabidopsis* roots using real-time  $\text{Ca}^{2+}$  imaging with the confocal microscope. Within seconds following hypoxia treatment, we observed cell-type specific changes in  $\text{Ca}^{2+}$  in the columella cells of the root cap; these changes were blocked by  $\text{La}^{3+}$  but not by ruthenium red. In order to understand the molecular network responding to this

Ca<sup>2+</sup> change, we searched available transcriptional databases for root-expressed Ca<sup>2+</sup>-responsive/related proteins showing upregulation under hypoxia. Those genes showing upregulation include 16 of the *CMLs* and a range of other Ca<sup>2+</sup> transporters such *AC1* and *CAX2*, and putative signaling components such as *CPK2, 23, 28* and *32*. Q-PCR on RNA isolated from the apical ~300 µm of the hypoxic root shows that, as expected, *ADH1* was highly upregulated and this induction was inhibited by blocking the Ca<sup>2+</sup> increase with La<sup>3+</sup>. A similar pattern was seen with *CML38*, however, *CML39* (a closely related *CML*) was induced to higher levels upon the La<sup>3+</sup> treatment, as was *CPK2, 23, 28, 32* and *AC1*. *CAX2* transcription was unaffected by blocking the Ca<sup>2+</sup> increase. These results indicate that Ca<sup>2+</sup> may act as a rapid cellular signal to elicit the flooding response and also trigger a complex rewiring of a transcriptional network of genes encoding Ca<sup>2+</sup>-responsive/related proteins that interpret the Ca<sup>2+</sup> signal. This work is funded by NASA and NSF.

**Note:** *Also a poster presentation.*

**84 Stomatal development: signaling fate and renewal**Dominique Bergmann**Biology Department, Stanford University, Stanford, CA 94305**

See full abstract 629.

**85 Cell specification and cell communication in embryonic root formation**Dolf Weijers**Laboratory of Biochemistry, Wageningen University, Wageningen, the Netherlands**

Plant growth and development is controlled through the activity of stem cells within specialized niches, the meristems. These meristems are first established in the early embryo, when the organism consists of few cells. The work in my group aims at understanding the mechanisms underlying the initiation of the root meristem in the early embryo.

Root initiation is first manifested by the specification of an extra-embryonic suspensor cell as the hypophysis, the future quiescent center. This cell specification event is triggered by signals from the adjacent embryonic cells. The auxin-dependent transcription factor AUXIN RESPONSE FACTOR5 / MONOPTEROS (ARF5/MP) is a critical regulator of hypophysis specification, and acts in the embryonic lineage to promote cell-cell communication. We have recently identified a number of direct MP target genes, and among these found a small mobile bHLH transcription factor that acts as a novel intracellular signal that mediates MP-dependent root formation.

MP also promotes transport of auxin to the hypophysis. However, the response machinery in this cell is not known. In a systematic effort to define auxin-dependent patterning steps and ARF gene expression patterns, we have found a novel auxin response module that operates in the extra-embryonic suspensor to promote hypophysis specification, but also to prevent transformation to embryonic cell fate. Hence, auxin triggers several different responses in the early embryo, depending on the cellular context. I will present our progress in identifying the gene networks and pathways that are controlled by auxin in the different cell types of the embryo during root meristem initiation.

**86 Cytokinin Inhibitory Fields control Phyllotaxis**

*Fabrice Besnard<sup>1</sup>, Yassin Refahi<sup>2</sup>, Benjamin Marteaux<sup>1</sup>, Valerie Morin<sup>1</sup>, Pierre Chambrier<sup>1</sup>, Jonathan Legrand<sup>1</sup>, Geraldine Brunoud<sup>1</sup>, Etienne Farcot<sup>2</sup>, Coralie Cellier<sup>1</sup>, Pradeep Das<sup>1</sup>, Anthony Bishop<sup>3</sup>, Ykä Helariutta<sup>3</sup>, Christophe Godin<sup>2</sup>, Jan Traas<sup>1</sup>, Yann Guédon<sup>2</sup>, Teva Vernoux<sup>1</sup>*

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Phyllotaxis, the geometric arrangement of organs on a plant stem, is a striking example of developmental pattern formation. It emerges from very precise spatio-temporal patterns of organ initiation at the shoot apical meristem (SAM). The divergence angle between two initiation sites fixes the position of organ around the stem, and periodicity of organ formation leads to their apico-basal distribution along the stem. Most of models for phyllotaxis propose the existence of inhibitory fields produced by existing organs, which collectively determine the site of new organ initiation where the sum of the inhibitory effects is the lowest. Recent molecular data indicate that polar transport of the plant hormone auxin could create in the SAM auxin depletion zones acting as inhibitory fields. However direct evidence for the existence of these fields in the SAM is still missing and inhibitory fields have never been observed so far in plants.

Using a reverse genetic approach we have identified the *Arabidopsis* Phosphotransfer Protein 6 (AHP6), a negative regulator of cytokinin signaling, as a regulator of phyllotaxis. We show that intercellular movement of AHP6 generates inhibitory fields of cytokinin signaling in the shoot apex and also controls phyllotaxis. By combining an original and extensive analysis of phyllotaxis with live imaging in wild-type and *ahp6* mutant plants, we demonstrate that the spatial distribution of AHP6 imposes a temporal sequence on organ initiation. We have thus identified a previously unrecognized mechanism by which an inhibitory field controls specifically the periodicity of organ initiation at the shoot apex. We propose a new model for phyllotaxis based on two hormonal inhibitory fields, one auxin-related sufficient for positioning the initia and another linked to cytokinin, required for a precise temporal organogenetic sequence. More generally, this work gives interesting perspectives to understand how space and time can be coupled during patterning processes occurring in growing structure like meristems.

*Note: Also a poster presentation.*

**87 A Transcriptional Auxin Response Gradient in the *Arabidopsis* Root**Bastiaan Bargmann, Gabriel Krouk, Tal Navy, Idan Efroni, Kenneth Birnbaum**New York University, New York, NY, USA**

Auxin has been shown to play critical roles in numerous aspects of plant development, including cell division, cell expansion, cell fate determination, cell maturation and meristem maintenance. This versatile phytohormone is asymmetrically and dynamically distributed by means of active polarized transport. In the root apical meristem, an auxin concentration gradient is understood to emanate from a maximum in the stem cell niche. However, the biological relevance or a tangible interpretation of such a gradient is not unequivocal. Nor is it clear to what extent responses to auxin influence (and are influenced by) cellular identity.

Here, we conduct a high resolution analysis of transcriptional responses to auxin treatment in four different cell populations of the root, making use of tissue-specific GFP marker lines and Fluorescence Activated Cell Sorting. This study presents a contextual view of auxin responses and provides substantiation of its spatial distribution. Ordering of auxin-affected genes by their induction kinetics reveals a gradient of gene expression responses in the proximal-distal axis of the root, such that the expression of genes induced by auxin

is generally enriched in the meristematic zone compared to the elongation and differentiation zones and vice versa for auxin-repressed genes. This approach exposes a remarkably sizeable scope of influence on gene expression along the longitudinal axis of the root.

Tissue-specific analysis further highlights the effects of auxin on transcriptional cell identity and maturation, particularly in the stele, where genes characteristic for developing xylem are induced by auxin and markers for mature xylem are repressed. Additionally, we study the effect of auxin signal deprivation on meristem maintenance and cell differentiation by using inducible expression of gain-of-function Aux/IAA repressors.

*Note: Also a poster presentation.*

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## 88 LEAFY Target Genes Reveal Floral Regulatory Logic, *cis* Motifs, and a Link to Biotic Stimulus Response

*Cara Winter<sup>1</sup>, Ryan Austin<sup>2</sup>, Servane Blanvillain-Baufume<sup>3</sup>, Maxwell Reback<sup>4</sup>, Marie Monniaux<sup>5</sup>, Miin-Feng Wu<sup>4</sup>, Yi Sang<sup>4</sup>,*

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The transition from vegetative growth to flower formation is critical for the survival of flowering plants. The plant-specific transcription factor LEAFY (LFY) has central, evolutionarily conserved roles in this process, both in the formation of the first flower and later in floral patterning. We performed genome-wide binding and expression studies to elucidate the molecular mechanisms by which LFY executes these roles. Our study reveals that LFY directs an elaborate regulatory network in control of floral homeotic gene expression. LFY also controls the expression of genes that regulate the response to external stimuli in *Arabidopsis*. Thus, our findings support a key role for LFY in the coordination of reproductive stage development and disease response programs in plants that may ensure optimal allocation of plant resources for reproductive fitness. Finally, motif analyses reveal a possible mechanism for stage-specific LFY recruitment and suggest a role for LFY in overcoming polycomb repression.

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## 89 Contributions of the Maternal, Paternal and Zygotic Genomes during Early Plant Embryogenesis

*Michael Nodine, David Bartel*

**Whitehead Institute, Cambridge, MA, USA**

In animals, maternally-derived gene products control early embryonic development prior to the activation of the zygotic genome. However, it has been unclear whether a prolonged period of maternal control also occurs in plant embryos. To investigate this issue directly, we crossed different *Arabidopsis thaliana* accessions and performed transcriptome profiling on hybrid embryos at the 1-cell/2-cell, 8-cell and ~32-cell stages using mRNA-Seq. Single-nucleotide polymorphisms within the mRNA-Seq tags revealed maternal and paternal genome contributions to more than 7000 transcripts. For the vast majority of genes, the maternally and paternally derived loci contributed approximately equal amounts of transcripts – even in the 1-cell/2-cell stages. Thus in contrast to previously proposed models, our results indicate that both parental genomes are active very early during *Arabidopsis* embryogenesis.

Although most transcripts were biallelic, we identified >100 transcripts that were derived predominantly or exclusively from the maternal locus and >100 transcripts preferentially or exclusively derived from the paternal locus. These parent-of-origin-dependent transcripts encode proteins with annotated functions including transcriptional regulation, RNA binding, chromatin remodeling, signal transduction and hormone metabolism. We are currently testing whether these transcripts are inherited from the gametes or derived from imprinted loci. Since only a few examples of inherited transcripts or gene imprinting in plant embryos have been reported, our identification of hundreds of parent-of-origin-dependent transcripts suggests that one or both of these mechanisms play an important role in shaping the early embryonic transcriptome.

*Note: Also a poster presentation.*

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## 90 RUG8, a Novel Player in Auxin-Dependent Stem Cell Specification and Meristem Patterning in *Arabidopsis* Roots

*Ulrich Wenig, Stefan Meyer, Ruth Stadler, Norbert Sauer*

**University of Erlangen, Germany**

A screen for putative plasmodesmata mutants, based on p*AiSUC2*:GFP plants, revealed a mutation in a previously unknown gene that was named *REDUCED UNLOADING OF GFP8* (*RUG8*), based on the observed phenotype. *RUG8* encodes a putative, plant specific transcription factor, which is involved in stem cell specification and meristem patterning processes in the *Arabidopsis* root. Loss of this gene leads to an altered appearance of the quiescent center (QC), premature differentiated stem cells and premature elongated meristematic cells. All affected cells show *RUG8* expression in wild type plants, which was analyzed in plants expressing a p*RUG8*:*RUG8-GFP* construct. In line with the phenotypic differences, the mutant plants failed to form an auxin maximum in the QC and showed a shift of *PINFORMED1* (*PIN1*) expression to the columellar region. Our working hypothesis is that the ectopic *PIN1* expression causes the observed loss of the auxin maximum in the QC, which in turn leads to altered expression of other auxin regulated genes. This includes *WUSCHEL-RELATED HOMEOBOX5* (*WOX5*) and the members of the *PLETHORA* (*PLT*) gene family. In addition, *rug8* mutants show patchy expression of *SCARECROW* (*SCR*), which leads to random, mostly periclinal cell divisions or supernumerary cell files in the ground tissue. These results indicate, that *RUG8* is a novel, so far uncharacterized key-regulator of the meristem and an important factor for the auxin distribution in the root.

**91 Gramene: It's Not Just For Grasses Anymore**

*Joshua Stein<sup>1</sup>, Ken Youens-Clark<sup>1</sup>, Aaron Chuah<sup>1</sup>, Genevieve DeClerck<sup>2</sup>, Sharon Wei<sup>1</sup>, William Spooner<sup>1</sup>, Terry Casstevens<sup>2</sup>, Jim Thomason<sup>1</sup>, Jon Zhang<sup>2</sup>, Charles Chen<sup>2</sup>, AS Karthikeyan<sup>2</sup>, Palitha Dharmawardhana<sup>3</sup>, Marcela Monaco<sup>1</sup>, Pankaj Jaiswal<sup>3</sup>, Edward Buckler<sup>4,5</sup>, Susan McCouch<sup>2</sup>, Doreen Ware<sup>1,5</sup>*

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Gramene (<http://www.gramene.org>) is a curated resource for comparative functional genomics in crops and model plant species. Its strength derives from the integration of genome annotation and functional data with the use of ontologies and by applying a phylogenetic framework for genome comparison. In addition to *Arabidopsis thaliana* and *A. lyrata*, we host genome browsers for eight other species spanning a broad taxonomic range. Navigation and visualization tools promote interspecies browsing and the simultaneous display of multiple species based on whole genome alignments. Gramene additionally provides comparative information in the form of browsable phylogenetic trees and the assignment of orthology and paralogy relationships. Gramene's genetic diversity database holds genotype, phenotype, and germplasm data for the major population studies in *Arabidopsis*. Tools for searching and viewing SNP data include the display of diversity data in the context of functional impacts on gene structure. Platforms for conducting genome wide association studies (GWAS) currently employ external analysis tools Tassel and Flapjack. Gramene is supported by grants NSF DEB-0723510, NSF IOS-0703908, and USDA CRIS 1907-21000-030.

**92 Integrating Diverse Data And Knowledge In A Large Collaborative Project**

*Sean Walsh<sup>1</sup>, Katja Baerenfaller<sup>1</sup>, Matthias Hirsch-Hoffmann<sup>1</sup>, Agron-omics Consortium Data Contributors<sup>3,2</sup>, Pierre Hilson<sup>4</sup>, Wilhelm Gruissem<sup>1</sup>*

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A substantial part of the Agron-omics consortium resource is devoted to profiling the *Arabidopsis* leaf in a number of environmental conditions at four developmental- and two diurnal- time-points during growth. A diverse range of quantitative molecular and phenotypic data is generated in this and other sub-projects. Integrating and interrogating this information is especially challenging when contributing partners are geographically dispersed. Vital to our analytical pipeline is a database integration that exploits standard and advanced features of the MySQL database engine (e.g. stored routines) and tools. This implementation is utilized for the processes of data capture, validation, documentation, the tracking of provenance, for certain mathematical-, statistical- and structural data transformations, for integration with R/Matlab and for storing visualization routines. In addition, the system provides access controlled user workspaces and the ability to run high performance queries across multiple/high volume datasets. Novel datasets also require the integration of pre-existing knowledge and consequently a range of molecular annotations and classifications are included. Since the database engine and tools are freely available, the data and code can be simply and rapidly replicated for community dissemination and/or extension. This development provides a useful template for a computational platform that has analytical value during a project and beyond.

**93 Integrating Proteomics Data: pep2pro, MASCP Gator and Combined Analyses with Transcript Data**

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Mass spectrometry-based proteomics has now become an important tool for obtaining qualitative and quantitative protein information. Data analysis of the proteomics data usually requires integration of search results from different experiments and search algorithms. The results are then often combined with other proteome data or compared with additional datasets like transcript data. To accomplish the integration of proteomics data at these different levels specialised tools have been built that are presented here.

pep2pro is a comprehensive proteome analysis database that offers solutions to the various challenges of developing a proteome data analysis database. Its capacity for the integration and analysis of large proteome databases was demonstrated by creating the organ-specific *Arabidopsis thaliana* dataset containing 14'522 identified proteins based on more than 2.6 million spectra that can be accessed at [www.pep2pro.ethz.ch](http://www.pep2pro.ethz.ch). The datasets in this database have been aggregated with other large-scale *Arabidopsis* proteome datasets in the MASCP Gator aggregation portal (<http://gator.masc-proteomics.org/>), which has been built as a proteomics community effort lead by Joshua Heazlewood. For combined analyses of the Agron-omics quantitative proteomics data on leaf 6 with additional datasets, the final proteomics result data that had been processed in pep2pro have been integrated into the AgroRDB. Querying this database followed by statistical analyses allows effective data analysis and reveals new insights into the regulation of protein and transcript levels.

**94 Discriminative Expression Signatures In Microarray Data For Functional Network Inference**

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DNA microarray technology is currently the most widely used approach for profiling gene expression changes in model organisms. There are thousands of publicly available microarray data, which provides information on the expression of thousands of genes under many experimental conditions. This tremendous resource can be used to understand gene expression and to predict new properties of *Arabidopsis* genes.

In this work we present a novel machine learning method, designed specifically to integrate large microarray datasets and predict gene functional networks for a biological processes of interest. By using the existing knowledge available in Gene Ontology, our method is trained to find expression signatures: expression patterns found in subsets of experiments that discriminate the biological process of interest. The method then uses these signatures to predict new genes linked to the process and form a functional network. In contrast to state-of-the-art classification algorithms such as support vector machines (SVMs) or coexpression networks, our method exposes the data that is useful to make functional predictions for specific processes. Cross-validation and a year-2008 rollback analysis showed that our method performs better than co-expression networks and similar to SVMs for most biological processes. Hence, the proposed method has the discriminative power of supervised methods like SVMs, but unlike them, it provides valuable additional information that help biologists understand the biological process and guide future experiments.

We applied our method to identify new components of the nitrogen response in *A. thaliana*. We integrated the output of the method with existing large interaction datasets as a functional network using VirtualPlant ([www.virtualplant.org](http://www.virtualplant.org)) and proposed novel regulatory networks involved in the nitrate response that were validated experimentally.

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**95 Hypothesis Generation in Plant Biology Using Large Data Sets**

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We have developed tools, available as part of the Bio-Array Resource at <http://bar.utoronto.ca>, for exploring large data sets from plants, to allow deeper insights into biological questions and to help guide lab-based research. An emerging theme in plant biology is that interactions, be they regulatory or protein-protein, create networks. In the former instance, coexpression networks can provide more robust support for inferred biological involvement than simple coexpression analyses alone. Coexpression networks developed using publicly-available gene expression data sets from dormant and germinating seeds have provided high-quality candidates for genes involved in regulating these two important processes (joint work with George Bassel - Division of Plant & Crop Sciences, University of Nottingham; and Hui Lan and Anthony Bonner - Department of Computer Science, University of Toronto). In the latter instance, the complex cellular functions of an organism frequently rely on physical interactions between proteins. A map of all protein-protein interactions, an interactome, is thus an invaluable tool. An interactome for *Arabidopsis thaliana* predicted from interacting orthologs in 7 organisms will be presented (joint work with Matt Geisler and Jane Geisler-Lee - Southern Illinois University Carbondale). These predictions can aid researchers by extending known complexes and pathways with candidate proteins. Finally, methods for integrating networks of coexpression, protein-protein interaction, and of other high-throughput data, can provide additional levels of support for novel function identification. An algorithm for doing so, called GeneMANIA, will be presented and discussed (joint work with Quaid Morris - CCBR, University of Toronto). [nicholas.provart@utoronto.ca](mailto:nicholas.provart@utoronto.ca), <http://www.csb.utoronto.ca/faculty/provart-nicholas> BRADY AND PROVART. (2009). Web-Queryable Large-Scale Data Sets for Hypothesis Generation in Plant Biology. *The Plant Cell* 21:1034-1051.

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**96 Receptor-Receptor and Ligand-Receptor Interactions Controlling Stomatal Patterning in *Arabidopsis***

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*Tamerler, Keiko Torii*

**University of Washington**

Asymmetric cell division is crucial for generating diversity during development in all multicellular organisms. In plants, stomatal development offers an excellent model to study asymmetric division due to its simple cell types and accessibility. Genetic studies in *Arabidopsis* suggest that putative signaling ligands, EPIDERMAL PATTERING FACTOR1 (EPF1) and EPF2, emitted from stomatal precursor cells are perceived by putative cell-surface receptors, TOO MANY MOUTHS (TMM) and ERECTA-family receptor kinases (RKs) of the neighboring cells to enforce proper stomatal patterning. Interestingly, EPF2 appears to limit the number of cells that undergo lineage entry. EPF1, on the other hand, primarily regulates orientation of spacing divisions. Although putative ligands and receptors in stoma development are now identified, their mode of action in controlling different developmental processes is not known. Biochemical evidence of these molecules acting as ligand-receptor pairs is lacking. Thus, we performed genetic and biochemical approaches to detect receptor-receptor and ligand-receptor interactions controlling proper stomatal patterning and differentiation. We have obtained double transgenic *Arabidopsis* lines expressing epitope-tagged receptors and performed co-immunoprecipitation assays to test dimerization of ERECTA-family RKs and TMM in vivo. Furthermore, we successfully produced recombinant, bioactive EPF1 and EPF2 peptides that inhibit stomatal differentiation when applied to *Arabidopsis* leaves. Using these bioactive EPF peptides will allow us to test the direct ligand-receptor association. Such studies will provide new insight into how specific ligand-receptor combinations may correctly regulate cell patterning and differentiation.

**97 Characterization Of The GLV Secretory Peptides Family**

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Reverse genetics screen conducted in *Arabidopsis thaliana* lead to the identification of a family of plant secreted peptides. We named the corresponding genes GOLVEN (waves in Dutch) because corresponding gain-of-function mutants have an altered root waving phenotype, resulting in root curling. GOLVEN (abbreviated as GLV) peptides share the same tripartite structure as other known plant peptides, including CLV3/CLE, but without primary sequence similarity. Interestingly *Arabidopsis* roots treated with synthetic peptides containing the GLV motif, conserved at the carboxyl-terminal end, show similar phenotype as the gain-of-function mutants. All GLV peptide treatments changed the direction of growth of the root in a concentration-dependent manner, although some differences were observed between different peptides. Promoter-reporter fusion lines were generated for all 11 *Arabidopsis* GLV genes to investigate their transcription pattern. Taken together, GLV genes were expressed in almost all plant organs, however in very specific cells, and as early as embryogenesis. In addition to altered tropic responses, specific glv mutants also show defects in root length, root meristem size and lateral root architecture. The peculiar GLV transcription patterns and the observed GLV-related phenotypes suggest that GLV signalling is involved in multiple developmental processes, some of which associated with the phytohormone auxin.

**98 Ligand Receptor Interactions Involved in Stem Cell Maintenance Studied by Advanced Fluorescence Techniques**

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Plants possess at the tip of the shoot and the root meristems that harbor pluripotent stem cells from which all cells of the plant body derive. Intercellular signaling processes mediated by small peptide ligands and their respective receptors play important roles in the necessary dynamic but also tight regulation of the transition from stem cell fate to differentiation. Stem cells in the *Arabidopsis* shoot apical meristem express and secrete the CLAVATA3 (CLV3) peptide and this signal is transmitted by the membrane localized leucine rich repeat receptor kinase CLAVATA1 (CLV1) and CLV2/CRN (CORYNE) to the subjacent organizing cells, which express the homeodomain transcription factor WUSCHEL (WUS). This signaling eventually leads to a negative feedback loop adjusting stem cell homeostasis in the shoot. Recently we discovered that a similar regulation, consisting of the CLV3-related peptide CLE40, the membrane localized receptor kinase ARABIDOPSIS CRINKLY 4 (ACR4) and the WUS-homolog WOX5, also exists in the distal root meristem. We are currently studying ligand-receptor interactions and putative complex formations involved in plant stem cell regulation. We are analyzing the dynamics of these processes in living cells, especially upon binding of the peptidaceous ligands to their respective receptors. We are utilizing experimental setups using fluorescently labeled receptors and ligands for these analyses using fluorescence correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP), fluorescence resonance energy transfer (FRET) and fluorescent lifetime imaging (FLIM) techniques.

**99 The RNA-binding Protein AtGRP7 - a Key Post-transcriptional Regulator at the Intersection between Biological Timing and Stress Responses**

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The circadian clock causes a considerable fraction of the genome to be rhythmically expressed. In recent years, RNA-based regulation emerges as an important control mechanism in the circadian system. The clock-regulated RNA-binding protein AtGRP7 negatively autoregulates by alternative splicing (AS) and NMD. Global transcript profiling unveils that AtGRP7 influences steady-state abundance of a suite of rhythmic transcripts in a phase-dependent manner, suggesting it acts as a slave oscillator downstream of the clock that contributes to configuring the circadian transcriptome. Furthermore, stress-responsive transcripts are prevalent among the target genes. To investigate a general influence of AtGRP7 on AS we have employed a high resolution RT PCR panel (Simpson et al (2008) Plant J 53, 1035) to measure changes in AS patterns in plants mis-expressing AtGRP7 and have identified different types of AS events that are affected by AtGRP7. To determine which transcripts are bound by AtGRP7 in vivo we perform RNA immunoprecipitation of transgenic plants expressing epitope-tagged AtGRP7. To monitor intracellular trafficking of AtGRP7 we have designed a codon-optimized synthetic variant of the reversible photoswitchable fluorescent reporter protein DRONPA. Using FRAP and photoactivation we show that an AtGRP7-DRONPA-s fusion protein shuttles between the nucleus and cytoplasm, in line with a role in splicing and additional roles in RNA processing in the cytoplasm.

**100 Control of Non-coding RNA Processing**

*Yukio Kurihara<sup>3</sup>, Ben Adamczyk<sup>1</sup>, Motoaki Seki<sup>2</sup>, Joseph Ecker<sup>3</sup>*

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Eukaryotes possess several RNA surveillance mechanisms that control transcript turnover. One key pathway is nonsense-mediated decay (NMD). NMD eliminates aberrant mRNAs that contain a premature termination codon, and in plants, the NMD pathway can also lead to degradation of mRNAs with relatively longer 3'UTRs. We have found that most mRNA-like noncoding RNAs have features of NMD substrates, and we have reported that many of these are targeted by the NMD mechanism. This was evidenced by tiling array assays using Arabidopsis plants with mutations in UP-FRAMESHIFT proteins, which are essential factors for normal NMD function.

To identify whether exoribonucleases, XRN2-3, are involved in NMD-induced RNA degradation, tiling array analyses were carried out in mutant backgrounds including *xrn2*, *xrn3*, *xrn4* and *fry1* (*FRY1* is a regulator of XRN activity). Although we could not identify an NMD-related exoribonuclease, we discovered that in *fry1* there were greater than one hundred new transcripts that extended the 3'end of specific mRNAs; some of these were also observed in *xrn3*. The length of most 3' RNA extensions was < 500 nt. In order to further characterize such 3'extensions, we carried out strand-specific RNA-Seq (Lister et al. 2008, Cell 133:523-536) in *fry1*, *xrn3*, *xrn2 xrn3*, *xrn2 xrn4* and *xrn3 xrn4*. We identified thousands of 3'extensions that were upregulated in *fry1* mutant and hundreds that were also upregulated in genotypes that possess the *xrn3-3* mutation; a partial loss-of-function allele with reduced activity of XRN3. The increased accumulation of all 3'extensions was confirmed by quantitative RT-PCR in *xrn3* as well as *fry1*. In addition, we found that mutations in *XRN2* and *XRN4* have little effect of 3'extensions, likely acting redundantly with *XRN3*. These observations reveal that XRN3 is the main activity required to eliminate 3'extensions which arise from thousands of transcripts.

**101 mRNAs Aggregate In AtUBP1C-Granules During Oxygen Deprivation**

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*Arabidopsis thaliana* seedlings respond to a severe decline in cellular oxygen availability through regulation of gene expression at both transcriptional and post-transcriptional levels. Low oxygen stress quickly constrains ATP synthesis. Plants respond by reducing energy consumption, manifested, in part, through reduced synthesis of proteins. However mRNA translation continues for transcripts that facilitate metabolic and other essential acclimation responses. This reconfiguration of the translome involves selective restriction in translational initiation of over 65% of the cellular mRNAs within 2 h of stress, which is rapidly reversed upon re-aeration. We hypothesize that a pool of translationally inactive mRNA is transiently sequestered. In mammals, processing bodies, germ granules, and stress granules (SG) have been characterized as complexes of mRNA turnover and sequestration, and their constituent proteins linked to numerous developmental and stress response processes. Such mRNP complexes and their role in regulating individual mRNAs are not well described in plant cells. We have characterized *AtUBP1C*, a putative *Arabidopsis* ortholog of the mammalian SG protein gene, TIA1. Conservation of protein domain architecture suggests that *AtUBP1C*, along with its two paralogues *AtUBP1A* and *AtUBP1B*, may have conserved functions. We have found that cytosolic AtUBP1C-GFP dynamically and reversibly relocates into large (0.2-2μm) cytoplasmic granules in response to low oxygen. Polyadenylated RNAs are present in UBP1C-granules and their formation is dependent on mRNA release from polysomes. The decreased survival of the *ubp1c-1* loss-of-function mutant of low oxygen stress and carbohydrate starvation indicates an important role of AtUBP1C during an energy crisis. *ubp1c-1* and *AtUBP1C*-targeted artificial miRNA knock-down mutants display similar pleiotropic phenotypes at the seed, developing seedling, and mature plant stages. Immunopurification of epitope-tagged AtUBP1C is underway to investigate the specificity and function of UBP1C in mRNA sequestration during oxygen deprivation. Sponsored by NSF grants IOS-0750811 and IGERT DGE-0504249.

**102 Photosynthetic response of *Arabidopsis thaliana* to drought stress**

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Global warming will expose plants to increasing drought periods that will limit photosynthesis and therefore productivity. Using an open infra-red gas analysis system that allow fluorescence measurements (CIRAS-2, PPSystems) we investigated the photosynthetic response of individual leaves of Col-0 *A. thaliana* submitted to a 12 days-long drought stress.

Before the stress, Col-0 plants were grown 46 days after emergence in a growth chamber under controlled conditions so that mature leaves could be sampled using the standard CIRAS-2 leaf chamber (2,5 cm<sup>2</sup>). Measurements (4 plants and 3 leaves per plant) were done 0, 3, 6, 9, 12 days after the last watering.

As compared to the control, neither gas exchanges nor fluorescence parameters were modified during the first 6 days of the stress. After 9 days, stomatal conductance ( $g_s$ ) was more decreased (-60%) than net assimilation ( $A_N$ , -30%). Substomatal CO<sub>2</sub> concentration (C<sub>i</sub>) and A<sub>N</sub>/C<sub>i</sub> curves pointed out a decrease of the assimilation under CO<sub>2</sub> saturated concentration (A<sub>sat</sub>), suggesting a possible metabolic limitation in addition of the g<sub>s</sub> limitation. Fluorescence data reveal that the PSII maximum quantum yield (F<sub>v</sub>/F<sub>m</sub>) and the photochemical quenching (qP) were not yet affected.

Twelve days after the last watering, C<sub>i</sub> increased while g<sub>s</sub>, A<sub>N</sub> and A<sub>sat</sub> were drastically reduced. This indicated that the metabolic limitation could explain the reduction of CO<sub>2</sub> assimilation. Concomitantly, a drop in F<sub>v</sub>/F<sub>m</sub> together with a slight decrease of the photochemical quenching (qP) were recorded. The reduction in A<sub>N</sub>/qP ratio suggested that ATP and NADPH,H<sup>+</sup> were no more used for CO<sub>2</sub> uptake but might be consumed by other metabolic processes.

In conclusion, our results show that the photosynthetic adaptation of *A. Thaliana* to drought may vary with the extent of stress.

**103 CPL1 is a Key Regulator of Iron Homeostasis in *Arabidopsis***

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CPL1 is a phosphatase homologous to yeast/animal RNA polymerase II C-terminal domain (pol II CTD) phosphatases. CPL1 was previously identified as a negative regulator of stress-responsive gene expression, and could dephosphorylate the pol II CTD *in vitro*. Microarray analyses of *cpl1* mutant in comparison to wild type C24 plants revealed significant increase in transcript abundance involved in the iron homeostasis in *Arabidopsis*. Understanding the molecular mechanisms of iron homeostasis in plants is important for the correction of nutritional disorders in both plants and humans. Relative expression levels of iron-regulated genes (*IRT1*, *FRO2*) and transcription factors (*FIT1*, *bHLH38*, *bHLH39*, *bHLH100*, and *bHLH101*) were higher in multiple *cpl1* mutants in iron sufficient conditions. The genes were induced significantly faster and to higher levels in *cpl1* under iron deficient conditions. Promoter-LUC analysis confirmed that the upregulation in *cpl1* occurred at the transcriptional level. FRO enzyme activity was significantly higher in *cpl1* mutants both under iron sufficient and deficient conditions. However, iron deficiency did not trigger IRT1 protein hyperaccumulation in *cpl1* roots indicating that *cpl1* did not override post-translational regulation of IRT1 protein. Compared to the wild type, root growth of *cpl1* seedling was less affected under iron deficiency, and its response to cadmium was altered. Two downstream genes (*IRT1* and *FRO2*) of iron acquisition pathway were suppressed in *fit1* single and *cpl1 fit1* double mutants. These results indicate that CPL1 is a negative regulator of iron deficiency signaling upstream of FIT1.

**104 Expression and Localization of Metal-Responsive Proteins**

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Plants are sessile organisms, and cannot flee from an onslaught of deleterious offenders in their environment. They have developed fascinating mechanisms for resisting damage from predators, parasites, inclement weather, and toxins. Our research is focused on understanding how plants respond to external stimuli at the molecular level. Specifically we are interested in the ability of plants to take up metals from the environment and maintain vigor, rather than suffer from metal toxicity. Our interest stems from recent research and field applications of plants used in phytoremediation, a bioremediation process that uses plants to remove contaminants in the environment. Metal contamination is a well-documented problem for industrialized countries. To progress the science of how plants can be better engineered for phytoremediation, we are studying the basic molecular biology of plant response to metals. We are therefore examining the subcellular localization of proteins involved in metal response and expression analysis of the associated genes in *Arabidopsis*. We are generating stably transformed plants with protein:GFP fusions of proteins involved in selenium and cadmium response and promoter:GUS fusions of the respective gene promoters. We plan to examine the expression of these metal-responsive genes under varying physiological conditions to elucidate more precise responses to environmental stimuli. This research is being performed as a part of required undergraduate research for biochemistry majors at Presbyterian College. The research will allow the students involved to achieve the following goals: 1) Gain knowledge of RNA and DNA manipulation in lab setting; 2) Understand and carry out the process of cloning; 3) Explore possible applications of creating transgenic organisms; and 4) Learn how to document research activities for future dissemination. Our findings will further the knowledge of the molecular basis of plant responses to metals and educate future scientists in the field of plant molecular biology and biochemistry.

## 105 A Genome-Wide Network Model Capturing Seed Germination Reveals Coordinated Regulation of Plant Cellular Phase Transitions

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Seed germination is a complex trait of key ecological and agronomic significance. Few genetic factors regulating germination have been identified, and the means by which their concerted action controls this developmental process remains largely unknown. Using publicly available gene expression data from *Arabidopsis thaliana* we generated a condition-dependent network model of global transcriptional interactions (SeedNet) that shows evidence of evolutionary conservation in flowering plants. The topology of the SeedNet graph reflects the biological process, including two state-dependent sets of interactions associated with dormancy or germination. SeedNet highlights interactions between known regulators of this process and predicts the germination-associated function of uncharacterized hub nodes connected to them with 50% accuracy. An intermediate transition region between the dormancy and germination subdomains is enriched with genes involved in cellular phase transitions. We demonstrate a novel role for the phase transition regulators *SERRATE* and *EARLY FLOWERING IN SHORT DAYS* from this region in seed germination, indicating conserved mechanisms control transitions in cell identity in plants. The SeedNet dormancy region is strongly associated with vegetative abiotic stress response genes. These data suggest that seed dormancy, an adaptive trait that arose evolutionarily late, evolved by co-opting existing genetic pathways regulating cellular phase transition and abiotic stress. SeedNet is available as a community resource (<http://vseed.nottingham.ac.uk>) to aid dissection of this complex trait and gene function in diverse processes.

## 106 Differential Impact of Lipoxygenase 2 and Jasmonates on Natural and Stress-induced Senescence in *Arabidopsis*

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Jasmonic acid and related oxylipins are controversially discussed to be involved in regulating the initiation and progression of leaf senescence. To this end we analysed profiles of free and esterified oxylipins during natural senescence and upon induction of senescence-like phenotypes by dark treatment and flotation on sorbitol in *Arabidopsis thaliana*. Jasmonic acid and free 12-oxo-phytodienoic acid increased during all three processes with the strongest increase of jasmonic acid after dark treatment. Arabidopsides content did only increase considerably in response to sorbitol treatment. Mono- and digalactosyldiacylglycerols decreased during these treatments and aging. Lipoxygenase 2-RNAi plants were generated which produce basal levels of jasmonic acid and 12-oxo-phytodienoic acid but do not exhibit accumulation during natural senescence or upon stress treatment. Chlorophyll loss during aging and upon dark incubation was not altered in these lines suggesting that LOX2-derived oxylipins are not involved in these processes. In contrast, lipoxygenase2-RNAi lines and the allene oxide synthase deficient mutant were less sensitive to sorbitol than the wild type indicating that oxylipins contribute to the response to osmotic stress.

## 107 Expression changes of *Arabidopsis isochorismate synthase 1* during development and abiotic stress

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The importance of salicylic acid (SA) in plant immunity has long been recognized. In addition, recent studies have also suggested a role for SA in abiotic stress responses. It has been reported that there are two distinct SA biosynthetic pathways; the benzoate pathway that involves phenylalanine ammonia lyase, and a pathway that requires isochorismate synthase (ICS). The latter pathway appears to be the major contributor to SA accumulation during pathogen infection. We investigated the regulation of *ICS1* expression in response to four different types of abiotic stress using transgenic *Arabidopsis* plants expressing an *ICS1* promoter-β-glucuronidase (GUS) reporter gene. As expected, the *AtICS1::GUS* gene was up-regulated upon application of SA and its biological analog benzo (1,2,3) thiadiazole-7-carbothionic acid S-methyl ester (BTH). The effect of low temperature, dehydration, low humidity, and salinity stress on *ICS1::GUS* expression was examined and it was found that salt stress induced *ICS1::GUS* expression. Up-regulation of *ICS1* and accumulation of SA in response to salt stress was confirmed by quantitative real-time PCR and gas chromatography-mass spectrometry, respectively. Moreover, abscisic acid was capable of suppressing SA-induced *ICS1::GUS* expression. GUS histochemical staining revealed tissue- and developmental stage-specific expression of GUS driven by the *ICS1* promoter. The role of the *ICS1*-dependent SA biosynthetic pathway in abiotic stress responses will be discussed.

## 108 Differential Alternative Polyadenylation Of A Photosynthesis Related Gene *LHCB4.1* In *Arabidopsis*

**Mutant oxt6**

*JIE CHEN, QINGSHUN LI, XIAOHUI WU*

**MIAMI UNIVERSITY, OXFORD(OH), US**

Messenger RNA (mRNA) polyadenylation is a crucial step in most eukaryotic gene expression. A poly(A) site marks the end of the genome-encoded transcript, and the poly(A) tail is required for regulation of mRNA stability, exportation to cytoplasm, and efficient translation initiation. Alternative PolyAdenylation (APA) at exons, introns, 5'UTR, or 3'UTR would result in varieties of transcripts, and/or consequently variety of proteins being generated. Recent analysis of large data sets using poly(A) tag sequencing indicated approximately 70% of *Arabidopsis* genes having more than one unique poly(A) site. On the other hand, many genes have been found to

use APA significantly differently in wild-type and the *oxt6* mutant in which the gene (At1g30460) encoding cleavage and polyadenylation specificity factor (CPSF30) is interrupted (Zhang et al 2008, *Plos One*). One such gene, encoding light harvesting complex photosystem II, *LHCB4.1*, was found to use its first APA site more frequently in wt than that in the *oxt6* mutant; whereas the second and third APA sites are used less frequently in wt than those of *oxt6* mutant. Under low temperature conditions, however, the APA site utility was switched when comparing wt and *oxt6*. Thus, we hypothesize that *oxt6* might cause some biological and molecular consequences under low temperature by affecting APA of this and other genes. In this research, 3'RACE and sequencing analysis were used to confirm APA sites of *LHCB4.1*. Quantitative real time PCR was used to examine abundance of transcripts of different lengths. The different usage of APA in *LHCB4.1* transcript in wt and *oxt6* under different temperature and light conditions are then used to test the hypothesis. Further testing results will be presented.

### 109 Exploring Metal Homeostasis Using Ionomics

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Plants are a major source of iron for human nutrition. Unfortunately, iron is frequently a limiting nutrient for plant growth with low iron availability caused by high soil pH being one of the broadest ranging abiotic stresses in agriculture. Around 30% of the arable fields are too alkaline for optimal crop production. In order to solve the problem of iron deficiency, it is desirable to breed plants that have increased iron in those parts that are consumed by humans, and that are tolerant of high pH soil. To do this, we must first understand the molecular basis of iron uptake, transport, and storage in plants. Two fast neutron mutants, 131:61 and 118:65, with decreased and increased iron accumulation in seeds respectively, were identified using ionomics, which analyzes shoots and seeds of ecotypes and mutants via high throughput ion profiling. 131:61 exhibits interveinal chlorosis, and cannot set seeds when grown on alkaline soil. Despite the iron deficiency phenotype, 131:61 has the same concentration of iron in its shoots as wild type plants, suggesting a defect in iron mobilization rather than in iron uptake. The mutation in 131:61 has been mapped to a 160 kb deletion and I am determining which gene(s) in the deletion region are responsible for the iron-related phenotype. In contrast, 118:65 is able to thrive and has a higher iron concentration in its seeds than wild type when plants are grown on alkaline soil. 118:65 exhibits strong rhizosphere acidification that may explain the ability to thrive on alkaline soil. 118:65 also grows better on high pH plates and I am using this phenotype to map this mutation. Hopefully, the genes we identified will be helpful for improving iron content in crop seeds and/or tolerance to iron limitation.

### 110 Pause-and-Stop - The Effects of Osmotic Stress on Cell Proliferation During Early Leaf Development in *Arabidopsis*

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Despite its importance for agriculture, environmental stress-induced growth inhibition, which is responsible for significant yield reductions, is only poorly understood. Here we investigated the molecular mechanisms underlying cell cycle inhibition in young proliferating leaves of the model plant *Arabidopsis thaliana* when subjected to mild osmotic stress. A detailed cellular analysis demonstrated that as soon as osmotic stress is sensed, cell cycle progression rapidly arrests, but cells are kept in an ambivalent state allowing a quick recovery ("pause"). Remarkably, cell cycle arrest coincides with an increase in 1-aminocyclopropane-1-carboxylate (ACC) levels and the activation of ethylene signaling. Careful study showed that ethylene acts on cell cycle progression via inhibition of cyclin-dependent kinase A activity independently of EIN3 transcriptional control. When the stress persists, cells exit the mitotic cell cycle and initiate the differentiation process ("stop"), reflected by early endoreduplication onset, in a process independent of ethylene, mediated by DELLA proteins. Nonetheless, the potential to partially recover the reduced cell numbers remains thanks to the activity of meristemoids. Together, these data present a novel conceptual framework to understand how environmental stress reduces plant growth.

### 111 Control of cell death by metacaspases

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Programmed cell death is essential for plant survival and development. Ten years ago metacaspases were proposed by homology modeling to be distant homologues of animal caspases. The genome of *Arabidopsis thaliana* encodes nine putative metacaspases (AtMCs), which can be classified into two groups: type I metacaspases (AtMC1, AtMC2 and AtMC3) contain an LSD1-like zinc finger prodomain, whereas type II metacaspases (AtMC4-9) lack any prodomain. Metacaspases are thought to be positive regulators of cell death, while the zinc finger protein LSD1 has been shown to negatively regulate cell death and disease resistance. Our data demonstrate that two type I metacaspases, AtMC1 and AtMC2, antagonistically control cell death in *Arabidopsis*. AtMC1 acts as a positive regulator of cell death requiring caspase-like catalytic residues for its function. Activation of AtMC1 is complex and developmentally regulated. AtMC1 is required for the cell death that accompanies successful innate immune responses mediated by intracellular NB-LRR receptor proteins. The regulatory function of both AtMC1 and AtMC2 is enhanced by removal of the putative prodomain similar to the activation mechanism of some animal caspases. The inhibitory function of AtMC2 does not require classic cysteine catalytic residues. This is reminiscent of the

mode of action of animal caspase-12, which negatively regulates caspase-1, dampening the inflammatory response to bacterial infection. Caspase-12 also inhibits NOD-like receptor-mediated innate immunity, in line with our observation that AtMC2 inhibits the analogous plant innate immune receptors. Our data suggest an ancient link between cell death control and innate immune receptor function.

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**112 Transcription Regulatory Networks Involved in the Abiotic Stress Response of *Arabidopsis thaliana***

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Plant stress responses are complex and organized by fine-tuned regulation through complex regulatory networks. Although many large-scale gene expression studies of different stress responses are available, it remains challenging to infer the underlying transcription regulatory networks. In this study, we used the network inference algorithm LeMoNe to predict regulatory modules and their condition-dependent regulators from a large-scale compendium of abiotic stress-related gene expression data. Our obtained networks consist of 381 modules of co-expressed genes containing a total of 6710 differentially expressed genes and 1082 predicted regulators. Through GO enrichment analysis, comparison with protein-protein interactions and other biological data, we show that the inferred networks contain functionally coherent co-expression modules with regulators that relate to similar biological processes as the module genes. For example, modules involved in important abiotic stress response processes like protective pigments biosynthesis pathways, endoplasmic reticulum (ER) unfolded protein response, cold and heat acclimation, and nutrient deficiency responses were found. Furthermore, cis-regulatory element enrichment analysis and comparison of the predicted regulatory interactions with large-scale gene expression data from regulator perturbation experiments indicate that LeMoNe infers a biologically relevant regulation program. In conclusion, through reverse-engineering of abiotic stress-related gene expression data, we obtained transcription regulatory networks that can provide further insight into the abiotic stress response of plants.

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**113 The Arabidopsis CALCINEURIN B-LIKE Protein Mediates Flower Development during Plant Growth in Saline Conditions**

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It is estimated that, by 2050, agricultural productivity must increase by 70% to feed the projected nine billion people worldwide. An increase in productivity of this magnitude will require cultivation of crops on marginal soils where the build-up of salt (soil salinity) is often a major challenge. Therefore, identifying the pathways that enable plant growth in saline environments is a priority for plant biology. The CALCINEURIN B-LIKE (CBL10) protein is required for the vegetative growth of *Arabidopsis* in saline conditions and we provide evidence that it is also critical for reproductive growth during exposure to salt. The *CBL10* transcript is expressed throughout flower development in the filaments of the stamen and the style and transmitting tract of the pistil. When wild type *Arabidopsis* and the *cbl10* mutant were grown in hydroponic culture and treated with 10 mM NaCl at the start of bolting, wild type developed fully fertile siliques while the mutant had shortened siliques with reduced seed set. Reduced fertility was specific for NaCl as the *cbl10* mutant treated with 10 mM KCl developed fully fertile siliques. Preliminary analysis of the NaCl-induced phenotype in the mutant suggests that stamen elongation and anther dehiscence are reduced and that growth of wild type pollen tubes through the transmitting tract of mutant pistils is blocked. Experiments are underway to determine the minimal NaCl concentration that affects flower development, to characterize the flowering phenotype in more detail, and to determine if CBL10 regulates flower development through the SALT-OVERLY-SENSITIVE pathway.

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**114 Caesium enrichment is dependent on a conserved protein of the secretory system in yeast and plants**

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The non-essential caesium (Cs<sup>+</sup>) is released mainly by accidents in nuclear power plants in form of the radioactive isotope <sup>137</sup>Cs. High physico-chemical similarity between hydrated Cs<sup>+</sup> and potassium (K<sup>+</sup>) ions is responsible for the uptake into the cell and the toxic effects Cs<sup>+</sup> causes in many organisms. In plants, micromolar concentrations reduce growth and evoke salt stress symptoms. Vacuolar disposal is expected to be part of a detoxification mechanism, but it remains unknown whether proteins with specific influence on Cs<sup>+</sup> enrichment exist.

In *Saccharomyces cerevisiae*, Heuck et al. (2009) described several mutants, which only affect Cs<sup>+</sup>-accumulation at constant levels of K<sup>+</sup>. A homology search attempted to mirror these yeast genes in *Arabidopsis thaliana*. Although this included the transfer from a unicellular to a complex organism, one *A. thaliana* T-DNA insertion line reflected the phenotype of the according yeast mutant showing a specific reduction in Cs<sup>+</sup> content. The gene encodes a protein of the secretory system, the v-SNARE SEC22 (*S. cerevisiae* YLR268W and *A. thaliana* AT1G11890). Constitutive expression of *AtSEC22* can partially complement the yeast mutant, pointing towards a conserved mechanism. Kinetics in the yeast mutant indicate that extrusion or vacuolar sequestration, but not the uptake of Cs<sup>+</sup> is affected. To understand the mechanistic background of this regulation, we apply protein-protein interaction studies, as well as an inspection of the vacuolar deposit in these mutants.

Since Cs<sup>+</sup>-accumulation is specifically hindered, mutant lines for *SEC22* might be applicable for landuse in contaminated areas. Homologous proteins are present in crop plants like maize or rice.

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Heuck et al. (2009) Genome-wide analysis of caesium and strontium accumulation in *Saccharomyces cerevisiae*. Yeast, 27: 817–835.

### **115 Regulation of Stomatal Development by Carbon Dioxide**

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Atmospheric carbon dioxide (CO<sub>2</sub>) levels are capable of modulating stomatal development and little is known about how signals are perceived and transduced in this pathway. To date, only one mutant (*hic*; Nature 408, 2000) has been reported to show a de-regulation of CO<sub>2</sub>-controlled stomatal development. Since stomata are major mediators of gas and water flux between the plant and the environment, in light of the rapidly changing global climate, there is a need for plant biologists to identify and understand the key mechanisms that mediate the perception and relay of signals originating from abiotic environmental stimuli to regulate stomatal development. We have previously isolated *Arabidopsis thaliana* carbonic anhydrase mutants which show an impaired stomatal response to shifts in CO<sub>2</sub> levels. These plants also show, relative to wild type plants, an increased stomatal density at ambient CO<sub>2</sub> levels. Here we report on the putative control mechanisms at play in the stomatal development differences present in these mutants. We have characterized the temporal progression of stomatal development in this mutant using cell lineage specific markers and confocal microscopy. Cell-type specific spatio-temporally targeted complementation studies using heterologous carbonic anhydrase expression in our mutants indicates that the CO<sub>2</sub> control of stomatal density functions non-cell autonomously and points to several mechanisms/genes in this pathway. In an attempt to capture these cell-cell signaling candidates, we are conducting CO<sub>2</sub>-dependent transcriptome analyses. We have also isolated several candidates as part of a screen for enhancers and suppressors of our mutant phenotype.

### **116 A gene regulatory network based on RNA silencing control anthocyanin biosynthesis under high light**

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RNA silencing regulates gene expression among eukaryotes through the action of small RNA (19-25nt). Under high light stress, plant accumulates anthocyanins that are thought to protect against the damage caused by reactive oxygen species. We found that *Arabidopsis* mutants affected in posttranscriptional RNA silencing pathways are impaired in anthocyanin biosynthesis under high light stress. A systematic analysis of RNA silencing targets in the anthocyanin biosynthesis pathway show that microRNA are at the heart of a complex genetic regulatory network acting positively and negatively to tune anthocyanin biosynthesis under high light stress.

### **117 Variation in Methyl Viologen Tolerance in *Arabidopsis* Accessions**

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Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. These stresses often cause accumulation of reactive oxygen species (ROS), leading to the development of oxidative stresses. To understand the crosstalk in biotic and abiotic stress signaling, we focused on analysis of oxidative stress response. Methyl viologen (MV) is a herbicide that catalyzes the formation of superoxide free radical, a major ROS. We found an ecotype Nos-d to show resistance to methyl viologen. The Nos-d also showed tolerance to osmotic stress, but not to salinity or stress related hormones. Nos-d also showed tolerance to another oxidative stress-related chemicals, but the difference between Nos-d and Col was very slight. To isolate another MV-tolerant accessions, MV tolerance of *Arabidopsis* accessions distributed from RIKEN BRC was evaluated. Several MV tolerant accessions were identified. Analysis using Nos-d x Col F2 population suggested that the resistant phenotype is linked to a single locus. Mapping of the locus of MV tolerance is now in progress to understand the tolerance mechanism.

### **118 Damaged DNA Binding Protein 1b (DDB1b) - DDB1a Interactions during *Arabidopsis* Development and Abiotic Stress Response**

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Damaged DNA Binding protein 1 (DDB1) was initially discovered as a component of the human nucleotide excision repair pathway. Subsequently, it has been found to function as a substrate adaptor protein of CULLIN4 based E3 ubiquitin ligases, thereby mediating proteosomal degradation of target proteins via WD40 repeat specific interactions. The *Arabidopsis* genome encodes two conserved DDB1 homologs redundant in function – DDB1a and DDB1b. While null alleles of *DDB1a* result in no obvious developmental phenotypes, null alleles of *DDB1b* appear lethal. In this study, we characterize a viable *DDB1b* allele and genetic interactions with *DDB1a* during development and in response to various abiotic stresses as well as describing the lethal *ddb1b ddb1a* double mutant.

**119 Abiotic Stress Regulation of the At3G02400 Gene in *Arabidopsis thaliana***Will Gray III, David Chevalier**Mississippi State University, Starkville, MS, United States of America**

Plants are continuously exposed to environmental stresses that cause damage at the cellular level. DNA damage is one of the most important damages because it affects the carrier of the genetic information. These DNA damages induce many responses within plants by triggering signaling pathways. These signaling pathways can induce the expression of specific genes in order to identify the agents causing these damages, the types of DNA damages and repair mechanisms. We have identified a gene from *Arabidopsis thaliana* whose expression is induced by the genotoxic agent Mitomycin C. This gene, At3G02400, contains a Forkhead Associated (FHA) domain. FHA domain is a phosphobinding domain present in proteins from bacteria, animals, humans and plants with important cellular functions such as cell cycle regulation and DNA repair. The involvement of several genes with an FHA domain in DNA repair in both animals and bacteria and the induced expression of At3G02400 by Mitomycin C suggest that this gene is involved in DNA repair in plants. We are testing this hypothesis by studying the regulation of its expression and identifying the function of this gene. In order to study the expression of this gene, we are using a reporter line and bio-computing tools. To identify the function of the gene, we have isolated two T-DNA mutants and are screening TILLING mutants. The outcomes of this particular study will introduce new knowledge about DNA repair, while adding key insights about the function of the At3G02400 gene. In addition to this, the learning gained from this research will potentially impact human medical research on human disease in order to fine treatments and cures.

**120 Investigation of SR45a Alternative Splicing Using Integrated Genome Browser**Alyssa Gulledge, April Roberts, Ketan Patel, Hiral Vora, Vikram Bishnoi, Ann Loraine**UNC Charlotte, Charlotte**

RNA-Seq experiments have the potential to transform our thinking about RNA splicing, its differential regulation under environmental or biotic stresses, and many other aspects of RNA metabolism and regulation. However, RNA-Seq experiments produce massive data sets; a single lane from an Illumina GAIIX sequencer can produce tens of millions of high-quality "reads". The sheer size of these data sets means that discovering novel and biologically significant patterns requires software that can support visual exploration, as well as quantitative analysis. To meet this need, we are developing the Integrated Genome Browser. IGB is designed to support highly interactive exploration of genome scale data sets, including data from next-generation sequencing experiments, tiling arrays, and EST/genome sequencing projects. Here we describe using IGB to explore the effects of heat, cold, and water deprivation stresses on expression and splicing of SR45a (AT1G07350), a putative regulator of splicing in *Arabidopsis* that is also alternatively spliced. cDNA libraries prepared from *Arabidopsis* plants subjected to heat, water deprivation, and cold stresses were sequenced on the Illumina GAIIX instrument, yielding more than 700 million high-quality 75 base sequencing reads from all experiments combined. Reads were aligned onto the reference genome using TopHat (version 1.2) and visualized using IGB. This analysis revealed increases in SR45a gene expression under heat and water deprivation stress, alternative splice variant switching in heat-treated samples, and heat-related intron retention. Interacting with read alignments using IGB made it possible to dissect the pattern of alternative splicing at the SR45a locus and investigate patterns of splicing that might have been missed otherwise. These results demonstrate the importance of visual inspection and analysis of RNA-Seq alignments when investigating alternatively-spliced genes. IGB is freely available from [www.bioviz.org/](http://www.bioviz.org/)

**121 Multiple Roles of WIN3 in Regulating Disease Resistance, Cell Death, and Flowering Time in *Arabidopsis*****Arabidopsis**Guan-Feng Wang, Savanna Seabolt, Safae Hamdoun, Gina Ng, Jin Park, Hua Lu**University of Maryland Baltimore County**

The salicylic acid (SA) regulatory gene *HOPW1-1-INTERACTING 3 (WIN3)* was previously shown to confer resistance to the biotrophic pathogen *Pseudomonas syringae*. Here we report that *WIN3* controls broad-spectrum disease resistance to the necrotrophic pathogen *Botrytis cinerea* and contributes to basal defense induced by flg22, a 22-amino acid peptide derived from the conserved region of bacterial flagellin proteins. Genetic analysis indicates that *WIN3* acts additively with several known SA regulators, including *PHYTOALEXIN DEFICIENT 4, NONEXPRESSOR OF PR GENES 1 (NPR1)*, and *SA INDUCTION-DEFICIENT 2* in regulating SA accumulation, cell death, and/or disease resistance in the *Arabidopsis* mutant *acd6-1*. Interestingly, expression of *WIN3* is also dependent on these SA regulators and can be activated by cell death, suggesting that *WIN3*-mediated signaling is interconnected with those derived from other SA regulators and cell death. Surprisingly, we found that *WIN3* and *NPR1* synergistically affect flowering time via influencing the expression of flowering regulatory genes *FLOWERING LOCUS C* and *FLOWERING LOCUS T*. Taken together, our data reveal that *WIN3* represents a novel node in the SA signaling networks to regulate plant defense and flowering time. They also highlight that plant innate immunity and development are closely connected processes, precise regulation of which should be important for the fitness of plants.

**122 How Do Environments Regulate Seed Quality?**Hanzi He<sup>1</sup>, Leónie Bentsink<sup>2</sup>, Henk Hilhorst<sup>1</sup>**<sup>1</sup>Wageningen University, Wageningen, The Netherlands, <sup>2</sup>Utrecht University, Utrecht, The Netherlands**

Seed quality is highly dependent on environmental cues during seed formation and filling. It is not clear which environmental factors are the most dominant in this respect. We study the influence of light conditions, temperature, drought/saline and nutritional conditions during seed filling, on a number of seed quality attributes, with a strong emphasis on seed dormancy and seed longevity.

Several genotypes were analysed to investigate the environmental effects, including a set of near isogenic lines (NILs, NILDOG1, NILDOG2, NILDOG3, NILDOG6 and NILDOG22), representing different dormancy and longevity pathways and some abscisic acid related mutants (*aba1-1, dog1-1, dog1-2, cyp707a1, cyp707a2, Atnced6-Atnced9* double mutant).

We grow the plants under standard conditions (5mM nitrate, 20°C, 150W/m<sup>2</sup> and 16 hours day light) and transfer the plants to the different environments when they start flowering. Dormancy levels are assessed by monitoring after-ripening. Fully after-ripened seeds are tested for germination under stress conditions. Results so far clearly indicate that different environments during seed development affect seed quality. New findings will be presented and further studies will be done on more environments.

### **123 ATCSA-1 Is A Critical Factor For UV Tolerance In *Arabidopsis thaliana***

*Sascha Biedermann<sup>1</sup>, Sutton Mooney<sup>2</sup>, Hanjo Hellmann<sup>2</sup>*

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The integrity of the genome is a fundamental prerequisite for the well-being of all living organisms, and effective DNA damage detection mechanisms are decisive to enable the cell to rapidly activate the necessary repair machinery.

We functionally describe the *Arabidopsis thaliana* Cockayne Syndrome Factor 1 (ATCSA-1) protein and its critical role for DNA repair processes. Through Yeast-two-Hybrid assays and protein pulldown analysis we found that ATCSA-1 is part of a CUL4 dependent E3 ligase complex which constitutes a critical factor to initiate repair of UV-B induced DNA lesions. ATCSA-1 is an instable protein, as shown by in vivo protein assays and Western Blot analysis and is degraded in a 26S proteasome dependent manner. GFP fusion constructs unveiled, that ATCSA-1 is localized to the nucleus. Analyses of *atcsa-1, 1* null mutants demonstrates that the protein is necessary for light independent DNA repair processes in Arabidopsis. Furthermore, we provide a detailed analysis of tissue specific expression patterns and UV-B dependent transcriptional profile of Arabidopsis *ATCSA-1* using promoter:GUS constructs and quantitative RT-PCR.

Overall, the results presented here are the first functional description of a plant CSA ortholog and demonstrate the importance of ATCSA-1 for UV-B tolerance.

### **124 Diversifying Selection in Abiotic Stress pathways in *Arabidopsis arenosa***

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Polyplody is an important evolutionary mechanism underlying diversification of flowering plant species. It is also thought to improve adaptive responses to rapidly changing environmental conditions. Our lab is developing *Arabidopsis arenosa*, a close relative of *A. thaliana*, as a model for studying genome evolution, evolutionary divergence, and adaptation. Both diploid and tetraploid populations of *A. arenosa* are extant, with the former occurring exclusively in Southeast Europe, and the latter distributed broadly across Central and Northern Europe. Furthermore, autotetraploid *A. arenosa* is divided into two subspecies. Subspecies *arenosa* occurs in exposed and seasonally dry sites such as roadsides, railways, and agricultural land. Subspecies *borbasi* occurs on and around moist, shaded rock outcrops on mountainsides and river valleys. These subspecies also differ in several morphological, developmental, and life history characters. Here we present whole genome resequencing data of three individuals from subspecies *arenosa* and four individuals from subspecies *borbasi*. Although genetic variation and allele frequency distributions are largely homogenous in the two subspecies, we have identified a small subset of genes that are highly divergent between subspecies. A number of these genes are known to have important roles in diverse abiotic stress responses in *A. thaliana*, including drought, temperature, and UV tolerance. These results have important implications for the relationship between phenotypic plasticity, environmental change, and adaptation, and may shed light on the roles of polyploidy and ecological niche specialization in during evolutionary divergence of plant populations.

### **125 Heat Induces the Splicing of bZIP60 messenger RNA by IRE1 in the Unfolded Protein Response in *Arabidopsis***

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The endoplasmic reticulum (ER) contains a protein quality control (QC) system that monitors protein folding, a process sensitive to adverse environmental conditions. The accumulation of unfolded or misfolded protein sets off the unfolded protein response (UPR) which acts to mitigate stress damage by upregulating chaperones and components of the ER associated protein degradation (ERAD) system. We describe here an arm of the signaling pathway involving the RNA splicing factor IRE1 and its target, bZIP60 mRNA. bZIP60 mRNA was predicted to be a RNA splicing target candidate because it is predicted to fold into a "kissing double hairpin loop" structure characteristic of known IRE1 splicing targets, Hac1 mRNA in yeast and XBPI in mammalian cells. bZIP60 mRNA is spliced in *Arabidopsis* seedlings treated with ER stress agents, tunicamycin (TM) or dithiothreitol (DTT). *Arabidopsis* has two IRE1 genes (IRE1a and -b), and IRE1b was found to be largely responsible for bZIP60 splicing in seedlings. The unspliced form of bZIP60 mRNA encodes a protein with a transmembrane domain located in the ER, while the spliced form encodes a protein with a putative nuclear targeting signal and is located in the nucleus. Binding protein 3 (BIP3) is upregulated by UPR and the upregulation was shown to be dependent on IRE1b and on its splicing action. This was demonstrated by 1) knocking out IRE1b and 2) by altering a single base in the splicing site of bZIP60 mRNA without changing its coding capacity. The splicing of bZIP60 was used to detect ER stress in seedlings in response to adverse environmental conditions, such as heat shock. Heat shock did not immediately upregulate BIP3 expression as it did in response to TM or DTT treatment, however, BIP3 was upregulated by heat shock after a short recovery at room temperature. It was reasoned

that heat shock temporarily debilitated the machinery necessary for the upregulation of BIP3 or that BIP3 upregulation was temporarily outcompeted by the expression of other heat shock response genes. (Supported by NSF IOS091907)

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## 126 Influence of Abiotic Stress on Membrane Proteins Expression Levels in *Arabidopsis* Cell Culture

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Membrane proteins play crucial roles in number of cellular processes. Thus the determination of changes in their expression levels in response to biotic or abiotic stress could contribute to better understanding of its mechanism. However, the extremely hydrophobic nature of proteins possessing transmembrane domains complicates their analysis. Another significant problem in membrane proteomics represents contamination by soluble (nonmembrane) proteins, entrapped in membrane vesicles or bound to the membranes via non-specific interactions. Therefore, we applied recently developed technique towards quantification of membrane proteins in complex mixtures combining reversed-phase chromatography on C<sub>4</sub> resin and mass spectrometric technique using iTRAQ labels. Reversed-phase chromatography on C<sub>4</sub> resin with step wise elution of 2-propanol was used to reduce contamination with hydrophilic proteins and to fractionate of membrane proteins according to their hydrophobicity.

The technique was applied for determination of the influence of abiotic stress evolved by pesticide isoxaben on plasma membrane proteins expression levels. The study was focused mainly on changes of different isoforms of plasma membrane H<sup>+</sup>-ATPase expression levels depending from time of incubation of *Arabidopsis thaliana* cell culture with isoxaben. It was found that isoxaben increases expression levels of plasma membrane H<sup>+</sup>-ATPase 1, H<sup>+</sup>-ATPase 2 and H<sup>+</sup>-ATPase 3, which was supported also by results of western blotting with antibodies against H<sup>+</sup>-ATPase. Used approach seems to be promising for determination of changes of expression levels of membrane proteins in complex mixtures in general.

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## 127 A Dynamic Stress Expression Map of the *Arabidopsis* root

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The development of multicellular organisms is a tightly regulated process involving the precise coordination of thousands of gene products acting at the correct time and place. In plants, development is further complicated by the effect of environmental pressures, which can profoundly alter plant growth. How the environment interacts with the distinct cell types in a plant is thus an important question in developmental biology, yet little is known about this relationship. We have examined the transcriptional response of *Arabidopsis* roots to multiple abiotic stress conditions at high spatial and temporal resolution to understand how developmental processes and stress are linked in the root. Our comprehensive dataset allowed us to examine patterns of gene expression both within a cell type across multiple stresses and across multiple cell types for a particular stress. We show that diverse stresses have distinct effects on root cell-type and developmental-stage transcriptional programs, which in turn lead to specific phenotypic changes. We uncover novel root patterning genes, find new functions of cell types, and reveal new components of transcription networks in the root. Our results demonstrate a complex and multilayered gene regulation, which expands our idea of cell identity and reveals surprising linkages between stress and development at cellular resolution.

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## 128 Functional and Evolutionary Analysis of Plant Adaptation to Salinity

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The build-up of salt in agricultural soils is a widespread problem that limits the growth and yield of important crop species worldwide. *Thellungiella salsuginea* (Thellungiella; formerly *T. halophila*), is a salt tolerant member of the *Brassicaceae* family and is closely related to the salt sensitive species *Arabidopsis thaliana* (Arabidopsis) and the agronomically important Brassica species. *SALT-OVERLY-SENSITIVE1 (SOS1)* encodes a Na<sup>+</sup>/H<sup>+</sup> antiporter that is required for growth in NaCl in both Arabidopsis and Thellungiella. To determine whether evolutionary changes in *SOS1* have contributed to the adaptation of Thellungiella to salinity, the gene is being sequenced in 15 additional *Brassicaceae* species and analyzed to identify specific sites that are under positive selection in lineages leading to Thellungiella. These sites will be functionally evaluated to determine their role in salt tolerance.

To test whether reported differences in the regulation and activity of *SOS1* from Arabidopsis (*AtSOS1*) and Thellungiella (*TsSOS1*) contribute to the increased salt tolerance of Thellungiella, promoters (*SOS1pro*) and coding sequences of the two genes were exchanged. Specifically, *SOS1* sequences from both species were fused to create the following constructs: *AtSOS1pro:AtSOS1*, *TsSOS1pro:TsSOS1*, *AtSOS1pro:TsSOS1*, and *TsSOS1pro:AtSOS1*. Comparison of these constructs in *Atsos1* mutants indicates that the Thellungiella promoter confers greater salt tolerance than the Arabidopsis promoter, regardless of the coding sequence. Furthermore, analysis of *SOS1pro:GFP*

lines of both promoters indicates that this result may be due to an expanded zone of root expression of *TsSOS1pro* compared to *AtSOS1pro*. Finally, analysis of *AtSOS1pro* driving expression of *AtSOS1* from either cDNA or genomic DNA indicates an essential role for introns in regulating proper expression and/or stability of the transcript.

## **129 Molecular And Genetic Characterization Of A Plastid-Specific Programmed Cell Death Pathway**

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In several instances, the execution of programmed cell death (PCD) involves the participation of mitochondria that act as sensors of cellular stress and initiate the onset of the cell death response. Current forms of PCD associated with a mitochondrial dysfunction have been traced back to an ancient hypothetical prokaryotic form acquired by primitive eukaryotic host cells following the endosymbiosis. Core parts of this PCD form are thought to have been conserved during evolution and to be shared among present-day animals, fungi and plants. Here we describe another form of PCD unique to photosynthetic eukaryotes that implicates chloroplasts as being the source and also the target of a cell death. Our findings suggest that this latter cell death program originates from a second endosymbiotic event, in which chloroplasts arose from a cyanobacterial ancestor acquired by a eukaryotic host in which mitochondria were already established. Activation of this pathway leads to a genetically controlled rapid loss of chloroplast integrity and the subsequent collapse of the affected cell. This plastid-derived PCD is initiated by the release of singlet oxygen, one of the reactive oxygen species (ROS).

## **130 Functional Roles of RNA-binding Proteins with RNA Chaperone Activity in Plant Response to Environmental Stresses**

Minkyung Kim, Kyungjin Kwak, Hyunju Jung, Hunseung Kang

**Chonnam National University, Gwangju, Korea**

In recent years, RNA-binding proteins (RBPs) are recognized as key regulatory factors in posttranscriptional regulation of gene expression during stress adaptation in plants. RNA chaperones are RBPs that aid RNA folding process by preventing RNA miss-folding and by resolving miss-folded RNA species. Among the diverse RBPs with potential RNA chaperone activity, the gene families encoding glycine-rich RNA-binding proteins (GRPs) and zinc finger-GRPs in *Arabidopsis thaliana*, *Oryza sativa*, and *Brassica napus* were characterized for their nucleic acid-binding properties, expression patterns, and functional roles in various stress responses. Analyses of the loss-of-function mutants and overexpression transgenic plants revealed that GRPs in different plant species play fundamental roles in cold, salt, or drought stress adaptation process. GRPs contribute to cold and freezing tolerance in *Arabidopsis* and rice, and the ability of GRPs to enhance cold tolerance is closely correlated with their RNA chaperone activities. In addition, analysis of a specific family of RBPs in *A. thaliana* demonstrated that RBPs affect ABA-regulated seed germination and seedling growth of *Arabidopsis*. Taken together, these results demonstrate that the particular family members of GRPs harbor RNA chaperone activity and that the regulation of RNA metabolism by RNA chaperone is important for plant response to diverse environmental stresses.

## **131 G x E GWAS: Detecting Gene-Environment Interaction on a Whole Genome Level**

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Genome-wide association studies (GWAS) have become an obvious general approach for studying the genetics of natural variation. When inbred lines are available, it is possible to study many different traits in different environments for the same genotype. Although a plethora of significant associations of SNPs with different traits have been recently described (some of which have already been verified experimentally), these analyses have been focused on the identification of associations within a single environment. An obvious next step is to use this technique systematically for the identification of gene-environment interactions. Although some attempts to look for gene-environment interactions have already been undertaken, none of them tried to properly model the different effects. To enable this analysis on a genome-wide scale, we implemented an extended version of the broadly used mixed model and validated our statistical model through simulations. We could verify that our model both increase the power to identify gene-environment interaction and control the false discovery rate. Due to the simultaneous testing of both the gene and the gene-environment effect in the model we can identify both, SNPs that are globally important (not influenced by the environment) and SNPs important for gene-environment interaction (having different effects in the respective environment(s)). This method opens the possibility to illustrate the genomic basis for the specific effect of environmental factors (e.g., different abiotic stresses) for a given trait. Thus it is possible to gain insights in the underlying architecture of the respective stress response. Applying the model on real data leads to the identification of novel candidate genes, which were not identified by the marginal analysis, and are clearly important in the G x E setting.

## **132 The Roles of NF-Y Transcription Factors in ABA Responses**

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The heterotrimeric NUCLEAR FACTOR Y (NF-Y) transcription factor has been identified in all sequenced eukaryotes. While metazoans generally have one or two copies of each *NF-Y*, *Arabidopsis*, and other angiosperms, typically have greater than 10 NF-Y genes in the three distinct families (e.g., *Arabidopsis* has 10 *NF-YA*, 13 *NF-YB*, and 13 *NF-YC*). In the plant lineage NF-Y are known to function in drought tolerance, maintenance of nitrogen-fixing nodule meristems, embryo development, and flowering time. Using reverse genetic approaches we have identified NF-YB and NF-YC subunits that affect abscisic acid (ABA) responses in *Arabidopsis*.

Through mutant analysis we show that individual NF-Y transcription factors have both overlapping and antagonistic functions in various ABA responses. In addition, bioinformatic analysis and yeast two-hybrid screens suggest direct interactions between NF-Y and bZIP transcription factors. Because bZIP transcription factors are well-known regulators of ABA responses, these results suggest cooperative NF-Y/bZIP interactions at multiple ABA-responsive promoters. Through the continued study of key NF-Y subunits, we hope to gain a greater understanding of how plants respond and adapt to their ever-changing environment.

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### 133 Anti-insect capability of AtVSP is determined by its stability in the insect digestive canal

JIAJIN LEI

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Arabidopsis vegetative storage protein (AtVSP) is an acid phosphatase that has anti-insect activity [Liu et al., 2005. Plant Physiology 139, 1545–1556]. To investigate AtVSP function as a defensive protein against insects *in planta*, *AtVSP*-overexpressing and-silenced transgenic *Arabidopsis* lines were produced and evaluated for their effect on the polyphagous American grasshopper *Schistocerca americana*. In terms of weight gain and growth rate, there was no significant difference among grasshoppers reared on wild type, overexpressing, or silenced lines, respectively. Furthermore, AtVSP protein was undetectable in either the midgut or frass of grasshoppers reared on transgenic plants, suggesting that AtVSP is unable to withstand proteolytic degradation. To determine the stability of the AtVSP protein in grasshopper midgut, bacterially expressed AtVSP was incubated with gut extracts from various nymphal stages. AtVSP was hydrolyzed rapidly by grasshopper gut extract. We also detected multiple proteases in the midgut of grasshoppers. These protease isoforms may play an important role in disarming AtVSP. Taken together, we conclude that stability of a defensive protein in insect midgut is critical for its anti-insect property.

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### 134 Calcium sensor proteins in the anaerobic response in Arabidopsis

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Upon flooding or water logging, plants become exposed to conditions of low oxygen which trigger a series of adaptive responses at the genetic, biochemical and structural level. One of the most important primary responses in response to low oxygen stress is the induction of a special family of genes called as the "anaerobic response genes" (ANPs). These ANPs include genes encoding enzymes of the glycolytic and fermentation pathway, transcription factors, signal transduction proteins and others involved in adaptation to the anaerobic stress. Microarray and Q-PCR experiments have led to the identification of a calcium sensor ANP - *CML38*, which show acute upregulation of transcript (>600 fold) within four hours of the onset of hypoxia. *CML38* is a member of the calmodulin-like protein (CML) family in *Arabidopsis*. *CML38* is the only calmodulin-like gene that shows transcript upregulation in response to hypoxia, and may represent a unique calcium sensor involved in linking calcium signaling to anaerobic stress signaling. Structural and functional analysis show that *CML38* protein represents a unique subclass of calmodulin-like proteins that typically have basic amphipathic amino terminal extensions and altered properties of EF hand 3 compared to calmodulin. Preliminary investigation of T-DNA insertional mutants indicate that knock out of *CML38* results in an altered adaptive response of *Arabidopsis* seedlings to hypoxic stress. (Supported in part by NSF Grant MCB-0618075).

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### 135 Effects of abiotic stress on splicing patterns in *Arabidopsis thaliana*

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Thanks to the work of several groups, we now know that many putative and known regulators of splicing patterns in plant cells are themselves alternatively spliced, and these patterns change in response to abiotic stresses. We investigated currently-available ESTs and found that for most documented splicing events in *Arabidopsis thaliana*, the vast majority of ESTs that overlap differentially-spliced regions support one major splicing form, raising questions about the role of alternative splicing in regulating gene function: What role, if any, do the less abundant forms play, and do major and minor forms co-occur? What factors or conditions influence production of rarer forms? Do stresses in the environment induce an overall increase in seemingly aberrant splicing events? New high-throughput sequencing technologies are making it increasingly possible to address these and other questions about splicing regulation on a genome-wide scale. We used 75-base Illumina sequencing "reads" to investigate how environmental stresses influence splicing choices. Our experiments confirmed some previously observed results and discovered new candidates for downstream regulation by stress-related splicing patterns in plants.

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### 136 Analysis of Malate Transporters Induced in Roots of Phosphorus Deficient or Aluminum Stressed *Arabidopsis thaliana*

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In acidic soils, phosphorus (P) deficiency and aluminum (Al) toxicity are serious major problems for agriculture. Organic acids secreted from plant roots into the rhizosphere play important roles in insoluble P utilization and Al detoxification. The mechanism of Al detoxification by organic acid secretion has been well investigated and it was known some important genes, such as *Arabidopsis thaliana* aluminum-activated malate transporter (*AtALMT1*). However, the detailed mechanism of organic acid secretion under P deficiency is still unclear. Therefore, in this study, we aimed to investigate the relationships between P-deficiency and Al stresses on the organic acid secretion.

We have recently found that the expression of a homolog of the *AtALMT* family, *AtALMT3*, was up-regulated by P deficiency. Malate exudation was low in a knockdown mutant of *atalmt3* than in WT. *AtALMT3* expression was up-regulated by not only P deficiency but also Al stress. These results indicated that *AtALMT3* is involved in malate transport to the rhizosphere under both P-deficient and Al stressed conditions. Surprisingly, *AtALMT3* expression was down-regulated in also a knockout mutant of *AtALMT1*. Moreover, promoter::GUS analysis showed that the expression of *AtALMT1* was specific in endodermis, on the other hand, mRNA for *AtALMT3* was located in epidermal cells and root hairs. These results indicated that the regulation of malate exudation under P deficiency and Al stress was interacted between each stress. It was also implied that *AtALMT1* and *AtALMT3* play different roles in malate exudation from *Arabidopsis* roots.

### **137 Comparative Functional Analysis of DREB2A and DREB2B in Arabidopsis**

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DREB2A is an ERF/AP2 transcription factor that expresses in response to water- and heat-stresses and contributes to acquisition of tolerance against these stresses via transcriptional activation of stress-specific target genes in *Arabidopsis*. DREB2A is considered to be inactivated under non-stressful conditions by its negative regulatory domain (NRD) and activated in response to stress signals. However, mechanisms that regulate the activity of DREB2A are not clear.

DREB2B is a close homolog of DREB2A that occurred through gene duplication in Brassicaceae. In order to find sequences that are important for the function of DREB2A, we divided each of DREB2A and DREB2B into the N-terminal region containing the DNA-binding domain (NT), NRD, and the C-terminal region containing the activation domain (CT), then compared the function of each part between the two proteins. Partial deletion of NRD, rather than complete deletion, converted DREB2B to a constitutive active form (DREB2B del2), but its activity was lower than that of the constitutive active form of DREB2A (DREB2A CA). Analysis of chimeric proteins between DREB2A and DREB2B suggested that the activity of CT is comparable between DREB2A and DREB2B, whereas the activity of NT from DREB2B is weaker than that from DREB2A. It was also suggested that the negative regulatory activity of NRD from DREB2B is also weaker than that from DREB2A. Microarray analysis of transgenic plants overexpressing *DREB2A CA* or *DREB2B del2* showed that more than 70% of genes upregulated in the *DREB2B del2* plants were also upregulated in the *DREB2A CA* plants. However, promoter analysis of the upregulated genes suggested that the preference for DNA sequences is slightly different between DREB2A and DREB2B, which might be resulted from polymorphisms in NT.

### **138 Strand Specific Transcription in *Arabidopsis thaliana* Suspension Culture Cells Under High Salinity**

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Transcriptional changes due to environmental perturbations have been studied in great detail; however it is unclear how such perturbations affect strand-specific (i.e. sense and antisense) transcription. In addition, the mechanisms responsible for transcriptional regulation of sense and antisense transcripts are largely unexplored. In this study, we used RNA-sequencing to define strand specific transcripts in *Arabidopsis thaliana* T87 suspension culture cells under control and high salinity conditions. We found that, among annotated genes, 15.0% and 14.3% have antisense transcripts under control and high salinity conditions, respectively. At the global scale, we find no significant difference in the expression level distributions of sense and antisense transcripts. Nonetheless, both sense and antisense transcripts associated with annotated genes have significantly higher expression levels than transcripts found in intergenic regions. Antisense transcripts tend to originate from 3' ends of genes compared to a 5' bias for sense transcripts. In addition, we do not see a significant correlation between sense and antisense transcription of genes. Taken together, these observations suggest different modes of regulation for sense and antisense transcripts. Under high salinity conditions, we find 161 genes differentially expressed (>2 fold change, FDR<0.05) in the antisense direction. Given the large number of differentially regulated antisense transcripts, it is likely that some of them play an important role in salt stress response. Our findings indicate substantial antisense transcription in the *A. thaliana* genome with many possibly having regulatory significance under high salinity.

### **139 Post-transcriptional Regulation of the CBL10 Calcium Sensor is Critical for *Arabidopsis* Growth in Saline Conditions**

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Calcium ( $\text{Ca}^{2+}$ ) is a component of pathways that allow plants to respond to numerous endogenous and environmental signals during growth and development.  $\text{Ca}^{2+}$ -binding proteins perceive changes in intracellular  $\text{Ca}^{2+}$  and transduce them into specific cellular responses. CALCINEURIN B-LIKE10 (CBL10) is a  $\text{Ca}^{2+}$ -binding protein in *Arabidopsis* which signals responses needed for plant growth in saline conditions by activating the Salt Overly Sensitive (SOS) pathway. Upon perception of  $\text{Ca}^{2+}$ , CBL10 interacts with and activates SOS2, a protein kinase. SOS2 phosphorylates SOS1, a sodium ( $\text{Na}^+$ ) proton exchanger in the plasma membrane. Once activated, SOS1 transports  $\text{Na}^+$  out of the cell preventing its toxic accumulation.

The *CBL10* primary transcript is alternatively spliced into variants that are differentially regulated during plant growth in salt. In control conditions, two predominant variants are present; one represents the characterized *CBL10* transcript while the second, *CBL10LA*, is a longer transcript due to retention of an intron. During growth in salt, *CBL10* transcript levels remain constant while *CBL10LA* transcript

levels are reduced. *CBL10LA* encodes a truncated protein which is unable to bind Ca<sup>2+</sup> and lacks a phosphorylated residue present in the full length CBL10 protein; however, it can still interact with SOS2.

Based on our data, we have developed a model for how alternative splicing of CBL10 regulates the SOS pathway. In this model, the presence of *CBL10* variants with antagonistic functions would ensure a quick response to saline conditions. In response to increased cellular Na<sup>+</sup>, CBL10LA, a negative regulator, is degraded increasing the effective concentration of CBL10 which signals a salt response through activation of SOS2.

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**140 Three SnRK2 Protein Kinases Involved in ABA Signaling Function for the Control of Seed Dormancy and Abiotic Stress Tolerance in Arabidopsis**

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ABA is an important phytohormone regulating various plant processes, including stress tolerance, seed development and germination. SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3 are redundant ABA-activated SNF1-related protein kinases 2 (SnRK2s) in *Arabidopsis thaliana*. We examined the role of SRK2D, SRK2E and SRK2I in seeds. The triple mutant (*srk2d srk2e srk2i*) was sensitive to desiccation, showed severe growth defects during seed development, exhibited a loss of dormancy and vivipary, and showed highly enhanced insensitivity to ABA (Nakashima et al., 2009, Fujita et al., 2009). It also showed insensitivity to sugars and paclobutrazol (PAC), a gibberellin biosynthesis inhibitor. Disruption of the three protein kinases induced global changes in the expression of ABA/stress-related genes including genes encoding regulatory proteins such as ABA receptors (PYR/PYL/RCARs) and type 2C protein phosphatases (PP2Cs), and functional proteins such as late embryogenesis abundant (LEA) proteins and heat shock proteins (HSPs) in seeds. Stress tolerant assay revealed that the triple mutant seeds were sensitive to cold and heat stresses. These results indicate that these protein kinases are essential for the control of seed dormancy and abiotic stress tolerance through the extensive regulation of global gene expression.

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**141 The Study of the Structure and Function of Arabidopsis Gene ICE2 Using Over Expresses Transgenic Lines**

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Plant resistance to cold stress is an important problem not only for fundamental science but also for practical use in crop production. We studied the effect of ectopic *ICE2* (*INDUCER OF CBF EXPRESSION 2*) over-expression on cold response pathways in *Arabidopsis thaliana*. Previously it was shown that acclimated seedlings over-expressing the *ICE2* gene are resistant to deep freezing [Fursova O.V., Pogorelko G.V., Tarasov V.A. *Gene* 2009 429. pp. 98–103]. Until the end of 2010 year two domains have been incorporated into the structure of this gene: F-box domain incoding by 1st exon and the bHLH family domain incoding by 2 to 5 exones [arabidopsis.org]. In this study we characterized in detail two type of the lines of transgenic plants, one type over-expressing the full *ICE2* gene and one type over-expressing a deleted copy of the *ICE2* gene, without the 1<sup>st</sup> F-box exon. We show that relative to wild-type plants, ectopic *ICE2* over-expression induces expression of not only the CBF regulon gene (*CBF1*) but also of the genes of the abscisic acid (ABA)-dependent cold response pathway (*NCED3*, *RAB18*) in cold condition. Furthermore, the F-box is important for the regulation of *CBF1* and the genes of ABA-dependent cold response *NCED3*, *RAB18*, *RD29B*, but not for the regulation of *RD29A*. Also we made a RACE and computer analysis for characterization of the structure of the gene. Our results show that apparently these are two independent genes.

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**142 Roles of Arabidopsis Chloroplast Localized Molecular Chaperone Hsp90 in the Regulation of Plant Development and Abiotic Stresses**

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Heat shock and other proteotoxic stresses directly cause an accumulation of non-native proteins inside the cell, which induces the expression of heat shock proteins (Hsp). Hsp90, a prominent member in the Hsp family, is a molecular chaperone that has been implicated to play essential roles to help cells resist to and recover from stress conditions and, at the late stage of *de novo* protein folding. In *Arabidopsis thaliana*, Hsp90 isoforms are identified in both cytoplasm/nucleus and major organelles. To understand the role of AtHSP90.5 in chloroplast protein homeostasis, in this study, we generated transgenic plants that express FLAG-tagged chloroplast AtHSP90.5 driven by constitutive cauliflower mosaic virus (CaMV) 35S promoter. Surprisingly, most of the transgenic plants show a chloroplast development defect. The expression levels of both transgene and endogenous AtHSP90.5 gene were analyzed and the abnormal expression level of AtHSP90.5 is found to correlate to the chloroplast development defect. To investigate the role of AtHSP90.5 in chloroplast development and function, Co-immunoprecipitation techniques were employed to determine candidate client proteins of AtHSP90.5 from purified chloroplasts and total cell lysates of plant tissues that show normal growth phenotype, but were grown under both normal and stress conditions. Candidate AtHSP90.5 interacting proteins were analyzed by LC-MS/MS and possible roles of AtHSP90.5 in folding clients proteins are discussed.

**143 Functional Analysis of B-class Heat Shock Transcription Factors in Arabidopsis**

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Heat shock transcription factors (HSFs) are key components of signal transduction in the heat stress response and are highly conserved among eukaryotes. In response to heat stress, HSFs are converted from monomeric to trimeric forms. The trimeric forms of HSFs bind to heat shock elements (HSE) in the promoter region of the target genes with high affinity and regulate the heat stress responsive gene expression. Although animals have only several HSFs, plants have evolved complicated HSF systems involving many HSFs divided into three classes A, B and C based on their sequences of DNA-binding domains and oligomerization domains. A-class HSFs (HSFAs) have transactivation domains, as well as most non-plant HSFs. On the other hand, B-class HSFs (HSFBs) and C-class HSFs (HSFCs) lack transactivation domains. Because of this unique feature, HSFBs and HSFCs are thought to have plant-specific functions, but their roles in the HSR of plants are not known.

We investigated functions of HSFBs in the heat stress responsive gene expression in Arabidopsis, which has 21 HSFs including 5 HSFBs. We found that *HSFB1*, *HSFB2A* and *HSFB2B* were heat-inducible and these HSFBs had repressor activity. Furthermore, these HSFBs interacted with several HSFAs. These results suggest the possibility that HSFBs act as transcriptional repressors and regulate heat stress response in coordination with HSFAs by tuning or shutting down heat-inducible gene expression. In order to test the function of these HSFBs in regulating gene expression, we are now analyzing the expression patterns of heat-inducible genes under heat stress condition in *HSFB* knock-out plants.

**144 SPL Transcription Factor Interacts With Activated Immune Receptors To Regulate Plant Immune Response**

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The NB-LRR family of plant immune receptors can detect pathogens and initiate a series of complex signaling pathways that culminate in programmed cell death (PCD) at the site of pathogen invasion. Extensive transcriptional reprogramming is a prerequisite for successful PDC. Yet, the molecular links connecting pathogen recognition to nuclear transcriptional regulation are not well understood. Recently, several NB-LRRs have been found to localize to the nucleus and their nuclear presence is essential for defense. Here we describe the identification and characterization of Squamosa Promoter binding protein Like- A (SPL-A) transcription factor, that associates with activated immune receptors. SPL-A was identified in a yeast two hybrid screen for proteins that associated with the tobacco TIR-NB-LRR immune receptor N. Fluorescence based complementation and colocalization assays determined that SPL-A associated with only activated N. SPL-A is necessary for N mediated resistance to Tobacco Mosaic Virus. In Arabidopsis, AtSPL-A associated with activated Arabidopsis TIR-NB-LRR, RPS4 to provide resistance against the bacteria *P. syringae* DC3000 (AvrRps4). Knocking down the expression of SPL-A transcripts by either creating transgenic RNAi plants or overexpressing the microRNA mir156 led to increased susceptibility to Pst DC3000 (AvrRps4). Whole genome microarray analysis of infected SPL-A RNAi plants identified a significant subset of defense genes that are positively regulated by SPL-A. Additionally, overexpression of SPL-A induced cell death even in the absence of the pathogen. Together, our data suggests that plant immune receptors can directly regulate defense genes by controlling the activity of the SPL-A transcription factor. This is the first report showing an evolutionarily conserved role for SPL proteins in positively regulating innate immunity and one of the few reports characterizing nuclear interaction partners of NB-LRRs.

**145 Development of transgenic near-isogenic rice lines harboring fungal resistance gene (OgPR1) from wild rice (*Oryza grandiglumis*)**

Jung-Hun Pak

**Busan, Korea**

Previously, we reported the cDNA cloning and expression analysis of pathogenesis-related protein 1(OgPR1) gene from wild rice (*Oryza grandiglumis*). OgPR1 was induced by defense/stress signalling chemicals and showed the resistance against the fungal pathogen, *Botrytis cinerea* and *Magnaporthe grisea* as a result of over-expression in *Arabidopsis* and rice transformation. With rice transgenic lines, progeny test was carried to identify homogenous lines and agronomical traits were investigated. Two high-quality japonica cultivars, Dongjinbyeo and Hwaseongbyeo, were used as recurrent parents. Several isogenic lines of two recurrent parents were developed through the hygromycin selection and histochemical analysis with  $\beta$ -glucuronidase. So far, five isogenic lines were produced from backcross populations to Hwaseongbyeo and waits for yield test this year.

**146 Generation of Transgenic Chinese Cabbage expressing *Arabidopsis* AVP1 H<sup>+</sup>-PPase**

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Chinese cabbage (*Brassica rapa* L) is widely grown in Asia and one of the most important vegetables in Korea as a major material of "Kimchi". In the field, *Brassica rapa* plant is adversely affected by various abiotic stresses such as cold and/or drought during cultivation; for example, seedlings and mature plants of this species show stress-related symptoms including delayed germination, poor growth, frost damage, and poor head. The productivity of *Brassica rapa* is also affected by soil salinization that occurs as a consequence

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of artificial irrigation. *AVP1* encoded a vacuolar H<sup>+</sup>-PPase of *Arabidopsis* which is resistance to salt and water stress. In this study, to develop, Chinese cabbage (*Brassica rapa* L.) with salt and drought tolerant, we introduced *AVP1* H<sup>+</sup>-PPase integrated with GFP in plants under CaMV 35S promoter by *Agrobacterium*-mediated transformation. The transgenic plants overexpressing *AVP1* were selected by GFP and *AVP1*- specific primer. Molecular characterization of transgenic Chinese cabbage overexpressing *AVP1* is under study. Further drought and salt tolerance of transgenic Chinese cabbage plants will be conducted with homozygous plants.

## 147 The Morphometric Landscape Of Root Architectural Plasticity

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Phenotypic plasticity is the ability of a particular genotype to produce diverse phenotypes when exposed to different environmental conditions. Despite advances in our understanding of the theoretical and empirical aspects of phenotypic plasticity, little is known on how multiple environmental signals are integrated in regulating developmental responses. To begin to describe the morphometric space that enables a plant to explore its environment, we performed experiments in which *Arabidopsis* plants were grown under five conditions known to control root architecture: two environmental/ nutritional signals (NO<sub>3</sub>, NH<sub>4</sub>) plus three hormones (IAA, CK, ABA). To quantify the overall root system architecture, we used landmark-based morphometrics in combination with Principal Component Analysis to describe the way in which a root system as a whole occupies the soil space, a novel approach in root architecture studies. This method allowed an intuitive, integrated, and unbiased assessment of the root architecture system compared to traditional measurements of individual traits like primary and lateral root length. Thus, we created a quantitative root architecture plasticity space (RAPS) defined by the Principal Component axes. Four Principal Components captured more than 90% of the variation, some of which were largely driven by particular treatments. Taken as a whole, this morphometric analysis of the root architecture plasticity and its quantification with novel tools will provide a framework to address questions on the evolution of developmental plasticity and adaptation to different environments.

## 148 Light signaling mediates cold acclimation response in *Arabidopsis*

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Plants have evolved a variety of adaptive mechanisms to survive adverse environmental conditions. In the case of freezing temperatures, which negatively affect plant growth and distribution, as well as crop quality and productivity, some species are able to increase their tolerance following low-nonfreezing temperature exposure, an adaptive response named cold acclimation. It has been described that light is required for the increase in freezing tolerance that is produced during cold acclimation in *Arabidopsis*. Consistently, expression analysis in *Arabidopsis* have revealed that light is needed for cold induction of several genes involved in cold acclimation, including the *CBFs*. Previously, we demonstrated that the expression of the *Arabidopsis* light-regulated gene *CAB1* is also induced by low temperature. The cold induction of *CAB1* is regulated at the transcriptional level and *CAB1* does not contain any described low temperature responsive element (LTRE) in its promoter, which suggests that a new LTRE should mediate its cold response. We have performed a deletion analysis of the *CAB1* promoter and have identified such a LTRE. Interestingly, this motif has also been described as a light-responsive element, and constitutes, therefore, a node of interaction between light and low temperature signaling. We have also analyzed the implication of different light signaling intermediates in cold acclimation. Our results indicate that some of them play an important role in this adaptive response by regulating the cold induction of light-responsive genes that protect *Arabidopsis* from the oxidative stress originated by low temperature. These data provide new insights on how cold and light signals integrate to promote the cold acclimation response.

## 149 Cytokinins Increase Flower Fertility and Fruit Set Under Non-Permissive High Temperatures

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High temperatures are known to reduce flower fertility and setting of fruit in many plant species. This effect is considered to involve reductions in pollen production, viability, release from the stigma, or growth of pollen tubes. Ovule development subsequent to fertilization can also exhibit reduced growth rates or abortion due to high temperatures during early stages of growth. The molecular and hormonal mechanisms behind this reduction in reproductive success by heat are not well understood. Existing evidence indicates that cytokinins are essential to pollen production under normal growth temperatures in several plant models. Further, specific to non-permissive high temperatures, sugars have been implicated in reproductive success in tomato. We show here that exogenous application of cytokinins, as well as sucrose, were able to substantially improve fertilization and fruit set under fertility-limiting high temperatures in *Arabidopsis thaliana*. Pod set of bean grown under high temperature conditions during flowering in the field was also significantly increased by cytokinin application. The molecular mechanism of this capacity for cytokinin is proposed to involve sugar movement to and accumulation in the flowers.

## 150 The HyPRP gene *EARLII* has an auxiliary role for germinability and early seedling development under low temperature and salt stress conditions

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The effect of the hybrid proline-rich protein (HyPRP) gene *EARLII* on the rate of germination (germinability) of *Arabidopsis* seeds and seedling growth under low temperature and salt stress conditions was investigated. Comparisons of control, overexpressing (OX), and knockout (KO) lines indicated that higher than wild type levels of *EARLII* improved germinability, root elongation, fresh weight accumulation, and reduction of sodium accumulation in leaves under salt stress, as well as germinability under low-temperature stress. Abscisic acid (ABA) contents remained relatively low under salt stress, suggesting that *EARLII* has an ABA independent effect on germinability under these conditions. Overexpression of *EARLII* during germination enhanced the sensitivity of seeds to exogenously applied ABA, suggesting that *EARLII* has an ABA dependent negative effect on seed germinability under high ABA stress conditions. Well-known stress response marker genes such as *KIN1*, *P5SC1*, *RD22*, or *RAB18* were only slightly affected in OX and KO plants. The pleiotropic effects of *EARLII* during stress and an absence of strong regulatory effects on stress marker genes suggest that this HyPRP gene has an auxiliary role for various stress protection responses in *Arabidopsis*.

## 151 The *Arabidopsis* Homologues of the Yeast *rei1* and *reh1* Genes

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The phylogenetically conserved four zinc finger proteins REIL1 (At4g31420) and REIL2 (At2g24500) are *Arabidopsis* homologues of the yeast 60S ribosomal maturation factors REI1 (YBR267W) and REH1 (YLR387C). The pair of REI1-Like paralogues within the *Arabidopsis* genome and the two yeast paralogues, REI1 (REquired for Isotropic bud growth) and REH1 (REI1 Homologue), resulted from independent gene duplications within each phylum. REI homologues appeared to be delimited to eukaryotic organisms and were single copy genes in most species including monocot plants. Homozygous T-DNA insertion in the *rei2* gene but not in the *rei1* gene caused a conditional biphasic growth deficiency at 10°C cultivation temperature. While growth was unaffected under optimal temperature, the two allelic REIL2.1 and REIL2.2 plant lines generated small, concave, almost spoon-shaped leaves during the first weeks in the cold but changed to wild type leaf morphology and growth rate prior to bolting. Temperature shift experiments demonstrated that this morphological change was determined early in leaf development. Thus a role of REIL2 in the correct timing of the normal *Arabidopsis* leaf development in the cold was indicated. Gene co-expression studies and protein-protein interaction analysis linked REIL2 to other *Arabidopsis* homologues of the 60S ribosomal maturation machinery. Therefore we propose in-depth investigation of the question, if the role of REIL2 in the cold trigger of morphogenetic leaf development is linked to ribosomal maturation or to a different function of this multi-domain protein.

## 152 Determining a possible role of EDR1 in autophagy

*Irene Serrano, Yangnan Gu, Roger Innes*

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Loss-of-function mutations in the *Arabidopsis* gene *EDR1* (*ENHANCED DISEASE RESISTANCE 1*) confer enhanced resistance to the powdery mildew pathogen *Golovinomyces cichoracearum*, and enhanced senescence (Frye and Innes 1998, Wawrzynska *et al.*, 2008). One potential link between these two processes is the regulation of programmed cell death (PCD) via autophagy. Autophagy can be defined as the bulk degradation of cellular components during senescence, starvation and stress responses in plants. In *Arabidopsis* several biological functions of autophagy have been described, including normal plant development, nutrient recycling, environmental stress responses, and more recently, degradation of oxidized proteins and regulation of hypersensitive response-PCD (Hayward and Dinesh-Kumar 2011).

Similar to that observed in an autophagy defective mutant (*atg5*), we found that root growth of *edr1* mutant seedlings is inhibited relative to wild-type when plants are grown on carbon/nitrogen depleted medium, or when they are subjected to stress, consistent with compromised autophagy causing inability to recycle nutrients or break down damaged or toxic materials. Preliminary data indicate that, contrary to *atg5* plants, *edr1* mutants are able to produce autophagosomes, but the number and size of these is reduced relative to wild-type when seedlings are grown on nitrogen depleted medium. A potential role of EDR1 in autophagy is supported by its localization to the trans-Golgi network/early endosomes (Gu and Innes, 2011), which not only plays a critical role in intracellular protein trafficking, but also has been proposed as a source of autophagosomal membranes.

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**153 The Role of *Arabidopsis* NF-YA Transcription Factors in Regulating Abscisic Acid Mediated Drought Responses***Chamindika Siriwardana, Roderick Kumimoto, Ben Holt III***University of Oklahoma, Norman, OK, USA**

Elucidating the molecular mechanisms that regulate plant responses to drought is key to producing drought tolerant crops and biofuels. Some members of the NF-Y transcription factor family have known roles in drought responses. The NF-Y transcription factor family is composed of NF-YA, NF-YB and NF-YC subunits. In the plant kingdom this transcription factor family has undergone a great expansion compared to the animal kingdom (*Arabidopsis* constitutes 10 NF-YA, 13 NF-YB and 13 NF-YC members). In this research we attempt to elucidate the role played by *Arabidopsis* NF-YA transcription factors in regulating abscisic acid (ABA) mediated drought responses. Genetic redundancy, arising from the presence of more than one member of each subunit, has made it challenging to observe phenotypes from single knockdown or knockout mutants in the NF-YA. Therefore our lab has created overexpression lines for all of the NF-YA subunits, where the 35S cauliflower mosaic virus promoter is driving the expression of each *NF-YA*. *NF-YA* overexpression typically leads to severe growth retardation. Although the growth retardation phenotypes are largely consistent across the various 35S:*NF-YA* plant lines, they exhibit variable germination and root growth phenotypes on both normal media and in the presence of exogenous ABA. Additionally, some of these lines show misregulation of well-known ABA-responsive genes. Our current research goal is to identify novel NF-YA protein interacting partners that will help explain their ABA-related phenotypes. We are applying yeast two-hybrid analyses to isolate these interacting proteins. Progress towards this goal will be discussed at the ICAR 2011 meeting.

**154 A vacuole localized β-glucosidase contributes to drought tolerance in *Arabidopsis****Pengtao Wang, Hao Liu, Hongjie Hua, Chun-Peng Song***Laboratory of plant stress biology, Henan University, Kaifeng, China**

Phytohormone abscisic acid (ABA) plays a critical role in plant growth, development and adaptation to various stress conditions. Cellular ABA level is adjusted constantly to respond to changing physiological and environmental conditions. To date, the mechanisms for fine-tuning ABA levels remain elusive. Here we report that *BGLU10*, a member of multigene family of β-glucosidases contributes to drought tolerance in *Arabidopsis*. T-DNA insertion mutant *bglu10* exhibited drought-sensitive phenotype, such as higher rate of water loss, lower leaf temperature, β-glucosidase activity, ABA content, expression of ABA-and drought-responsive genes under drought stress. By contrast, lines engineered to overexpression of *BGLU10* behaved increase in drought resistance than that of wild type, including less transpirational water loss, higher β-glucosidase activity, ABA level, expression of ABA-and stress-responsive genes under drought stress. Transient expression of *BGLU10::GFP* and *γ-TIP1::RFP* in mesophyll cell protoplasts showed *BGLU10* enzyme protein localized in vacuole. *BGLU10* was expressed in various organizations, and was induced by different abiotic stresses, suggesting that *BGLU10* may be involved in variety of stresses and hydrolysis of ABA-GE producing free ABA in plant stress tolerance response.

**155 *Arabidopsis* DRGs: Ribosome Association, Interacting Partners, Association with Heat Stress Granules, and Possible Involvement in Translation Initiation***Joel Stafstrom, Benjamin Nelson, Jennifer Kubic***Northern Illinois University**

DRGs are highly conserved GTP binding proteins. Among plants, animals and fungi, amino acid identity within the DRG1 and DRG2 orthologous groups is 65-70%, and paralogs from one species are about 55% identical. Such conservation suggests that DRGs are likely to carry out essential cell functions. However, little is known about their activities in any organism. *Arabidopsis* contains one DRG1 ortholog (At4g39520) and two DRG2 orthologs (DRG2, At1g17470; DRG3, At1g72660). DRG proteins co-purify with ribosomes. DRG1 and two forms of DRG2 associated with cytosolic monosomes, whereas a 30 kDa proteolytic product of DRG2 associated with polysomes. Transgenic plants containing DRG1 or DRG2 fused with GFP were made. Under nearly all conditions tested, both fusion proteins occurred diffusely in the cytosol and in the nucleus. Following heat stress, both fusion proteins aggregated in granules. Small granules were detected within 20-30 minutes in root tip epidermal cells. After several hours, granules up to 5 μm were seen. Granules dispersed after plants were returned to normal temperatures. Biochemical fractionation of proteins from pea root tips showed co-fractionation of DRGs and small HSPs, suggesting that these granules are heat stress granules (HSGs). A bacterial two-hybrid system and other means were used to identify DFRP1 (At2g20280) as a specific binding partner of DRG1, and DFRP2 (At1g51730) as a specific partner of DRG2. We are analyzing the phenotypes T-DNA knockout mutants in these four genes, including several double mutant combinations. Recent studies in other systems suggest that DRG1, DRG2 and at least one additional gene must be mutated to inhibit the initiation of translation. We are studying the *Arabidopsis* orthologs of these additional genes/proteins.

**156 Engineering the Coenzyme Specificity and Redox Sensitivity of Two Stress-responsive Aldehyde Dehydrogenase Isozymes of *Arabidopsis thaliana****Naim Stiti, Hans-Hubert Kirch, Dorothea Bartels***Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany**

Exposure of plants to environmental stress such as dehydration leads to accumulation of reactive oxygen species, which induce lipid peroxidation and subsequently cause accumulation of highly reactive and toxic aldehydes. One of the evolved mechanisms to counteract the effect of such harmful molecules is to convert aldehydes into less reactive carboxylic acids. The reaction is catalyzed by NAD(P)<sup>+</sup>-dependent aldehyde dehydrogenases (ALDHs).

We investigated kinetic parameters of two stress-inducible *Arabidopsis thaliana* family 3 ALDHs, the cytosolic ALDH3H1 and the chloroplastic isoform ALDH3I1. Coenzyme specificities are correlated with subcellular localization. ALDH3H1 is strictly NAD<sup>+</sup>-dependent, whereas ALDH3I1 is able to use both nicotinamide coenzymes. Site-directed mutagenesis demonstrated that an unusual isoleucine located in position 200 and occupying a central position within the coenzyme-binding cleft is responsible for the ALDH3H1 NAD<sup>+</sup>-specificity. The valine residue is invariant in this location in all other family 3 ALDHs except for ALDH3F1. Enzyme activities of both ALDH isoforms are redox-dependent. Inhibition under oxidizing conditions is correlated with oxidation of thiol groups and homodimer generation. Dimerization and inactivation can be reversed by thiol reducing reagents. Analysis of the redox-sensitivity of generated single cys-mutants of ALDH3H1 and ALDH3I1 showed that cysteines mediating homodimerization are located in the N-terminal region. Homology modeling of protein structures revealed that the redox-sensitive cysteines are located at the surfaces of the subunits of each isoform.

### **157 MicroRNAs Associated with Environmental Stress in *Arabidopsis Thaliana***

*Shawn Thatcher, Dong-Hoon Jeong, Brown Rebecca, Jixian Zhai, Blake Meyers, Pamela Green  
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Environmental stress currently contributes to approximately 50% of crop loss world-wide, with climate change expected to exacerbate this problem. Genomics studies have indicated that differences in stress tolerance arise not only from the presence or absence of stress-associated genes but also from their regulation. In plants, miRNAs are key gene regulators that have been shown to control various developmental processes and also to be involved in response to environmental stresses. In order to further assess the role of miRNAs in environmental stress, a series of stress treatments was carried out in *Arabidopsis thaliana*. Comparisons between small RNA populations of different tissues, mutants and stresses were made via the construction, sequencing and analysis of more than 25 small RNA libraries, resulting in over 150 million sequences. Through this method, several novel miRNAs were discovered. Many new miRNAs coming from previously annotated miRNA precursors were also found. Additionally, some miRNAs and their target genes were shown to be regulated in a novel manner under stress conditions. Despite the prevalence of multiple simultaneous stresses in agriculture, this area remains largely unexplored. To address this, small RNA libraries were also generated from plants subjected to dual stresses. Several miRNAs were found to be regulated in a unique manner under these conditions, highlighting the importance of further study in this area.

### **158 Arabidopsis Damage Associated Molecular Pattern Peptide 1 (AtPep1) and Its Receptors PEPR1 and PEPR2 are Involved in Osmotic Stress Response**

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Surveillance system of plants is able to detect a wide range of endogenous and exogenous signals originating from pathogens, damaged tissues or altered developmental processes. Microbe associated molecular pattern molecules (MAMPs), damage associated molecular pattern molecules (DAMPs), virulence factors, secreted proteins and processed peptides can be recognized directly or indirectly by this surveillance system [1]. *Arabidopsis* DAMP molecule AtPep1 is a 23-aa peptide derived from PROPEP1 and interacts with membrane bound LRR-RLK-type Pattern Recognition Receptors PEPR1 and PEPR2. This interaction triggers signalling [2] that leads to defence response including reactive oxygen species, ethylene and several defence-related transcripts such as PDF1.2 and PR1 [3, 4]. We have investigated this system further and showed that the precursor protein PROPEP1 is localized tonoplast and the abiotic stress inducers including the osmotic environment, oxidative stress and calcium chloride influence the abundance of cleavage product. Furthermore, PROPEP1, AtPep1 and the receptors PEPR1 and PEPR2 are all involved in survival of plants in response to salt and drought stress.

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### **159 Immunity-Related Members Of The DMR6 Family Of Oxidoreductases In *Arabidopsis***

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*Arabidopsis* mutants lacking a functional *DMR6* gene are resistant to infection by the downy mildew *Hyaloperonospora arabidopsis* (*Hpa*). Resistance is associated with enhanced defense gene expression and both resistance and defense was found to require salicylic acid and signalling through the key regulator *NPR1* which signals downstream of salicylic acid. The hypothesis that *DMR6* is a negative regulator of defense was further supported by the finding that overexpression of *DMR6* leads to enhanced susceptibility to a range of pathogens, e.g. *Hpa*, *Phytophthora capsici* and the bacterium *Pseudomonas syringae* pv. *tomato*. *DMR6* is a 2-oxoglutarate iron (II)-dependent oxygenase for which no substrate is known yet. Site-directed mutagenesis confirmed the requirement of conserved catalytic residues for its function as a negative regulator of defense. Structural modeling has allowed the identification of residues important in the predicted substrate binding pocket, the mutation of which strongly reduced the biological activity of the protein. In the *Arabidopsis* genome more than 200 2-oxoglutarate iron (II)-dependent oxygenases are encoded, however, for most no function is known. We have selected a subgroup of *DMR6*-related 2-oxoglutarate iron (II)-dependent oxygenases which are differentially expressed during pathogen infection and in response to the defense-related hormones salicylic acid and/or jasmonic acid. We will report on phenotypic analysis of mutants and overexpression lines of these immunity-related genes, in particular in their altered responses to various pathogens of *Arabidopsis*. The *DMR6*-like oxidoreductases add an additional layer of complexity to the plant immune network.

**160 Unique drought signaling roles of the uncharacterized clade A protein phosphatase 2Cs HAI1, AIP1 and HAI3***Bhaskara Badiger, Paul Verslues***Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan**

The clade A protein phosphatase 2Cs (PP2Cs) have emerged as a hub of abscisic acid (ABA) and stress signaling based on their interaction with the RCAR/PYR/PYL family of ABA receptors as well as other signaling proteins. However, only for six of the nine clade A PP2Cs do the knockout mutants show the defining phenotype of ABA-hypersensitive seed germination that demonstrates their involvement in ABA signaling. The remaining three clade A PP2Cs, *Highly ABA-Induced1 (HAI1)*, *AKT1-Interacting PP2C1 (AIP1)* and *Highly ABA-Induced3 (HAI3)* are of unknown function. We found that mutants of all three of these genes had enhanced proline accumulation in response to low water potential (drought) but not in response to salt stress or ABA. Mutants of *hai1* also had a reduced osmotic potential indicating greater total solute accumulation at low water potential. *aip1* and *hai3* had lesser but significant effect on osmotic potential. For both proline and osmotic potential, T-DNA knockouts of *abi1* and *abi2* had similar, but less intense, phenotypes while mutants of other clade A PP2Cs (*ahg1*, *ahg3*, *hab1*, and *hab2*) did not differ from wild type. This demonstrated that HAI1, AIP1 and HAI3 have unique stress signaling roles. The uniqueness of these three PP2Cs was also shown by observation that *hai1/aip1* and *hai1/hai3* double mutants had ABA-insensitive seed germination rather than the ABA-hypersensitive germination seen in mutants of other clade A PP2Cs. The results show functional specificity within the clade A PP2Cs with AHG1, AHG3, HAB1, and HAB2 being more specific to seed germination; HAI1, AIP2, HAI3 having roles in regulating proline and osmotic adjustment; and ABI1 and ABI2 having broad specificity affecting all of these phenotypes. The molecular basis of the unique signaling role of HAI1 is being investigated using a phosphoproteomic approach.

**161 Cold Days and Warm Nights Induce Flowering by Enhancing FT and SOC1 Expression in *Arabidopsis thaliana****Micael Wendell, Sissel Torre, Jorunn Olsen***Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, ÅS, NORWAY**

Higher ambient temperatures induce flowering in *Arabidopsis thaliana* more rapidly than lower temperatures. Interestingly, thermoperiodic control of flowering has been demonstrated: Cold days in combination with warm nights lead to earlier floral induction than warm days and cold nights or corresponding constant temperatures. We show here that the promotive effect of cold days and warm nights is linked to increased expression of the floral integrator genes *FT* and *SOC1*. Characterization of the diurnal pattern of *FT* expression at different temperature regimes in wild type plants revealed that *FT* expression increased early in the day when exposed to cold days and warm nights in contrast to the other temperature regimes. Consistent with an action of *FT* and *SOC1* through LEAFY (LFY), time to flowering in *ft* and *lfy* mutants was similar under all temperature regimes. We propose that the early expression of *FT* is one of the mechanisms that modulate flowering time in fluctuating temperatures. We also studied if the promotive effect of low day temperature on floral induction was linked to a vernalization effect. Although flowering time in the autonomous-pathway mutants *fve-1* and *fca-1* do not differ between different constant, ambient temperatures, these mutants responded like the wild type to different day and night temperatures. We found no difference in *FLC* expression neither in the *fca-1* mutant or wild type between the different day and night temperature regimes. Additionally, fully vernalized *fca-1* mutants responded like the wild type to the different thermoperiodic treatments. We can thus exclude that cold days in combination with warm nights act through a vernalization effect, and that the autonomous pathway act as a thermosensory pathway with respect to sensing different day and night temperatures.

**162 The Model Plant *Arabidopsis thaliana* for Metabolomic and Proteomic Phenotyping***Christiana Staudinger, Vlora Mehmeti, Wolfram Weckwerth, Stefanie Wienkoop***University of Vienna**

The transfer of data from one plant system to the other is under controversial debate. Parallel analysis of metabolite and protein data, first established for *Arabidopsis thaliana*, is now serving as a model workflow for the investigation of abiotic stress in different plant species/phenotypes. Previous studies have shown, that changing one gene cause different reactions in different plants. In contrast, a systems biology approach that is integrating different molecular levels, allows for more complex response pattern recognition. The advantage of such approach is that it is not picking one single component ("biomarker") that in other plants may play a different role. It will allow for the identification and better understanding of the underlying mechanisms. Here an overview of the technical platform and an integrative metabolite and protein stress response pattern will be presented that seems to be conserved throughout *Arabidopsis* and other plants such as Legumes.

**163 COPPER AMINE OXIDASE 1 (CuAO1) in *Arabidopsis thaliana* is involved in nitric oxide biosynthesis and in abscisic acid mediated stress responses***Rinukshi Wimalasekera<sup>1</sup>, Corina Villar<sup>2</sup>, Tahmina Begum<sup>3</sup>, Günther Scherer<sup>1</sup>***<sup>1</sup>Institute of Floriculture and Wood Science, Leibniz University of Hannover, Hannover, Germany, <sup>2</sup>Institute of Plant Research, ETH, Zurich, Switzerland, <sup>3</sup>ZMBP, University of Tuebingen, Germany**

Polyamines, amine oxidases and nitric oxide (NO) play important roles in diverse stress responses in plants. *COPPER AMINE OXIDASE1 (CuAO1)* in *Arabidopsis* was characterized in abscisic acid (ABA) mediated responses and in NO production. As revealed by qPCR, transcription of *CuAO1* was enhanced in response to ABA. T-DNA insertional knockouts of *CuAO1*, *cuao1-1* and *cuao1-2*

were less sensitive to ABA than wild type (WT) showing higher germination rate and postgermination growth. When grown in ABA supplemented growth medium, knockouts displayed significantly longer primary roots compared to WT indicating less sensitivity to ABA mediated inhibition of root elongation. Compared to ABA treated WT, expression levels of stress responsive genes *RD29A* and *ADH1* were significantly lower in the knockouts. Probable association of *CuAO1* in ABA mediated NO and H<sub>2</sub>O<sub>2</sub> production was assessed. ABA induced NO and H<sub>2</sub>O<sub>2</sub> release as determined by fluorometry was relatively lower in the knockouts in comparison to WT. Fluorescence microscopic observations showed that ABA stimulated NO and H<sub>2</sub>O<sub>2</sub> production in roots of knockouts was lower than in WT. The results suggest that involvement of *CuAO1* in ABA regulated functions and potential association in ABA stimulated NO synthesis.

#### **164 Abstract Withdrawn**

#### **165 Arabidopsis splicing factor variant controls plant growth in response to nutrient conditions**

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Cell proliferation is one of important factors to modulate plant growth. To elucidate cell cycle control, we analyzed *Arabidopsis segregation distortion 5* (*sd5*) that exhibited reduction in cell number resulting in dwarf phenotype. The *sd5* phenotype depends on the environmental nutrient conditions, in particular that of sucrose - a reduction in the level of sucrose and that of the macronutrients recovers the *sd5* phenotype. The corresponding gene of *sd5* encodes a homolog of yeast *DIM1*, a component of the U5 spliceosome. Higher organisms such as mammals and plants possess two *DIM1* homologs, although only a single *DIM1* gene has been found in the yeast genome. *SD5* interacts with *Prp6*, a subunit of the U5 spliceosome. *Arabidopsis* has two homologs, *SD5* and *YLS8*, which show opposite expression patterns and also have a dependency on the nutrients and over-expression of *YLS8* enhances the *sd5* phenotype on sucrose. Thus, *SD5* has antagonistic functions to *YLS8* to adapt to nutrient conditions. RNA immunoprecipitation analysis revealed that specific mRNAs interacted with *SD5* and this expression was reduced in the *sd5* mutant. These observations indicate that *SD5* is involved in a subset of mRNA processing events.

#### **166 AtPHO1 Expression In Guard Cells Influence The Response Of Stomata To Abscisic Acid**

*Celine Zimmerli, Cecile Ribot, Yves Poirier*

**University of Lausanne**

In plants, stomatal opening and closure is driven by ion fluxes that cause fluctuations in cell turgidity, a process that is in turn regulated by the phytohormone abscisic acid (ABA). We report genetic evidence in *Arabidopsis thaliana* that stomatal movements in response to ABA are influenced by *AtPHO1* expression in guard cells. *PHO1* is a mediator of phosphate export that has thus far been associated with phosphate export into xylem tissue. Consequently, the *pho1* mutant has very low phosphate in leaves. Gene expression analysis using microarray and qPCR techniques revealed specific expression and induction of *PHO1* in guard cells following treatment with ABA. The *pho1* mutant was unaffected in its stomatal response to white light, blue light and fusicoccin. However, the stomatal response to ABA treatment, both in terms of induction of closure and inhibition of opening, was severely reduced in this mutant. Normal shoot growth and Pi content was observed following a micrograft of *pho1* shoots onto wild type roots, but the stomatal response to ABA treatment was only partially restored. Specific expression of *AtPHO1* in guard cells of *pho1* mutant plants resulted in partial complementation and reestablishment of ABA sensitivity. In agreement with this result, specific expression knockdown of *AtPHO1* in guard cells of wild type plants using RNAi caused a reduced stomatal response to ABA treatment. Combined, these results imply a role of *AtPHO1* as a transporter and/or signaling component influencing the ABA-mediated stomatal response.

**167 GABA ( $\gamma$ -aminobutyric acid) Promotes Sporulation in the Necrotrophic Fungal Pathogen *Alternaria brassicicola****Christopher Botanga<sup>1</sup>, Angel Gray<sup>1</sup>, Oliver Fiehn<sup>2</sup>, Jane Glazebrook<sup>3</sup>*<sup>1</sup>**Chicago State University, Chicago, IL USA, <sup>2</sup>University of California -Davis, Davis, CA USA, <sup>3</sup>University of Minnesota, St. Paul, MN USA**

In order to ward off invading microbial pathogens, plants must activate a battery of defense responses in a timely manner. Metabolites form a major part of this defense arsenal because they are the ultimate end products resulting from genetic changes and other cellular regulatory processes. Following a metabolite profiling analysis which revealed that the level of GABA increases significantly following the infection of *Arabidopsis* with the necrotrophic fungal pathogen *Alternaria brassicicola*, we assayed the effects of GABA *in vitro* using minimal media in an effort to determine the effects of this metabolite on the growth of the pathogen. The treatment conditions included; no carbon source, 1% glucose, 1% glucose + 5 mM GABA, and 1% glucose + 8 mM GABA. There were no measurable differences in the growth/amount of fungal hyphae at 7 days post-inoculation under these treatment conditions. However, there were significant differences in the amount of spores produced by the pathogen under all the treatment conditions evaluated; ranging from no sporulation,  $4.825 \times 10^5$ ,  $6.25 \times 10^5$ , and  $12.45 \times 10^5$  spores/ml for the no carbon source, 1% glucose, 1% glucose + 5 mM GABA, and 1% glucose + 8 mM GABA, respectively. In a preliminary evaluation, there were no discernable differences in the amount of spores between the 1% glucose treatment and treatments with GABA concentrations of 4mM or less. Our data suggest that while GABA may be involved in stress signaling in *Arabidopsis*, it serves as a nutritional supplement to *A. brassicicola* and therefore promote enhanced disease phenotype.

**168 PHOSPHATIDIC ACID PHOSPHOHYDROLASE1 & 2 regulate phospholipid synthesis at the ER in *Arabidopsis****Christian Craddock, Nicolette Adams, Peter Eastmond***School of Life Sciences, University of Warwick, Wellesbourne, UK**

Phospholipid biosynthesis is essential for the construction of most eukaryotic cell membranes, but how this process is regulated in plants remains poorly understood. In *Arabidopsis thaliana*, two Mg<sup>2+</sup>-dependent phosphatidic acid phosphohydrolases called PAH1 and PAH2 act redundantly to repress phospholipid biosynthesis at the endoplasmic reticulum (ER). Leaves from *pah1 pah2* double mutants contain~1.8-fold more phospholipid than wild type and exhibit changes in ER morphology, which are consistent with membrane over-expansion. The net rate of incorporation of [*methyl-14C*]choline into phosphatidylcholine (PC) is ~1.8-fold greater in the double mutant and the transcript abundance of several key genes that encode enzymes involved in phospholipid synthesis are increased. In particular *PHOSPHORYLETHANOLAMINE N-METHYLTRANSFERASE1* (*PEAMT1*), which catalyses the first committed step of choline synthesis in *Arabidopsis* and defines a variant pathway for PC synthesis not found in yeasts or mammals, is up-regulated at the level of transcription in *pah1 pah2* leaves. The data suggest that PAH1/2 play a regulatory role in phospholipid synthesis that is analogous to that described in *Saccharomyces cerevisiae*. However, the target enzymes differ and key components of the signal transduction pathway do not appear to be conserved.

**169 *In vitro* studies of RNA-Dependent RNA polymerases involved in RNA Silencing***Anthony Devert<sup>1</sup>, Nicolas Fabre<sup>1</sup>, Bruno Canard<sup>2</sup>, Christophe Robaglia<sup>1</sup>, Patrice Crete<sup>1</sup>*<sup>1</sup>**Laboratoire de Génétique et de Biophysique des Plantes (LGBP), CEA, CNRS, Université aix-marseille II, UMR6191, Marseille, France., <sup>2</sup>Architecture et Fonction des Macromolécules Biologiques (AFMB), CNRS, Universités d'Aix-Marseille I et II, UMR 6098, Marseille, France**

RNA Silencing is a ubiquitous mechanism in Eukaryotes that is critical for development and responses to environmental stimuli. RNA-Dependent RNA Polymerases are crucial components of the RNA silencing machinery in plants where they produce double-stranded RNA (dsRNA) from single-stranded RNA (ssRNA) templates. DsRNA then becomes a substrate for a ribonuclease DICER, which convert it into small interfering RNA (siRNA) duplexes that are involved in important endogenous functions including the control of chromatin structure and the regulation of cellular gene expression. In *Arabidopsis* genetic evidences suggest that RDR2 and RDR6 act indifferent biological processes with RDR6 acting in post-transcriptional gene silencing (PTGS) and RDR2 acting in the transcriptional gene silencing pathway (TGS), which blocks transcription via DNA methylation and histone modification. The *in vitro* activity of RDR6 has been well characterized, yet nothing is known about RDR2 activity despite its key role in transcriptional silencing.

We have produced recombinant full-length AtRDR6 and AtRDR2 in *Nicotiana benthamiana* and performed a comparative study of their biochemical activities. We present our results and discuss their compatibility with knowledge obtained from *in vivo* studies and with current available models on AtRDR6 and AtRDR2 endogenous functions.

**170 Molecular Bases of Fe and Mn Transport: Key Transporters and Elemental Imaging***Hannet Roschttardtz, Fanchon Divol, Rémy Cailliatte, Mathilde Séguéla, Daniel Couch, Stéphane Mari, Catherine Curie***BPM, Montpellier, France**

Iron (Fe) and manganese (Mn), which are essential cofactors for numerous metabolic reactions, rely on specific transporters to enter the root and to reach aerial parts and specific organelles. Because an excess of these metals can also be toxic, their movement between cell compartments must be dynamic and tightly controlled. We have characterized key transporters that participate in Fe and/or Mn homeostasis in *Arabidopsis*. NRAMP1 mediates high affinity Mn uptake in root<sup>1</sup> while NRAMP2 plays a key role in providing Mn to the

chloroplast (*unpublished*). We have uncovered new functions for the citrate effluxer FRD3 in the mobilization of Fe during germination and pollen development (*submitted*). In addition, we have characterized two new transporters of the YSL family and demonstrated that they promote Fe efflux from the chloroplast in response to Fe overload and during senescence (*in prep.*). To assist these analyses, we have set up a novel histochemical method that enables to image Fe pools in plant tissues<sup>2</sup>. Thanks to this method, we have established that Fe stores in *Arabidopsis* embryo are concentrated in the vacuoles of endodermal cells<sup>2</sup>. We have now extended this study to the whole plant, and together with X-ray elemental imaging techniques we can now show specific intracellular locations of Fe in several organs.

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### **171 Dynamics of SCFTIR1/AFB Ubiquitin Ligase**

*Kai-Ting Fan<sup>1</sup>, Xiao-Yuan Yang<sup>1</sup>, Adrian Hegeman<sup>2</sup>, Jerry Cohen<sup>2</sup>, William Gray<sup>1</sup>*

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SCFTIR1/AFB, one of the best-studied Cullin-Ring E3 ubiquitin ligases in plants, plays an essential role in auxin-regulated development. SCFTIR1/AFB activity is regulated by both neddylation/deneddylation cycles and cullin binding protein, CAND1. Quantitation of SCFTIR1/AFB subunits and their respective turnover rates will improve our understanding of SCFTIR1/AFB regulation. We used a stable isotope (<sup>2</sup>H<sub>2</sub>O or <sup>15</sup>N) labeling strategy, LC-MS/MS analysis and a novel isotope distribution assignment algorithm to determine the turnover rates of the SCFTIR1/AFB subunits Cullin 1 (CUL1) and the TIR1/AFB F-box proteins in wild-type *Arabidopsis* and various SCF regulatory mutants. While the CUL1 subunit exhibits a shorter half-life in the *csl1-10* mutant – a COP9 signalsome mutant defective in deneddylation – the stabilities of AFB1 and TIR1 appear to be largely unaffected. In contrast, initial studies suggest that CUL1 stability appeared enhanced in *cand1* mutant seedlings.

Measurement of turnover rates for low abundance proteins like F-box proteins often fail with proteomic survey strategies and require a targeted approach. To this end, we have devised a selected reaction monitoring (SRM) method using a triple quadrupole mass spectrometer and stable isotope labeled internal standard peptides generated using the QconCAT strategy (Pratt *et al.*, 2006, *Nat Protoc*, 1: 1029). Our QconCAT gene encodes a concatenation of 3-4 unique tryptic peptides (Q peptides) from each of the four target proteins (TIR1, AFB1, AFB2, and AFB3). Without further enrichment, three AFB1 peptides were observed in crude protein extract from wild-type seedlings using SRM transitions derived from LTQ-Orbitrap HCD MS/MS spectra. Current efforts focus on obtaining both protein turnover and absolute quantification data for *Arabidopsis* TIR1/AFB proteins.

### **172 Abstract Withdrawn**

### **173 The LON2 Protease Contributes to Continued Matrix Protein Import into Peroxisomes**

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Peroxisomes are small, single membrane-bound organelles that compartmentalize hydrogen peroxide production and decomposition as well as critical oxidative reactions, including fatty acid β-oxidation and conversion of the protoauxin indole-3-butyric acid (IBA) into the active auxin indole-3-acetic acid (IAA). Most proteins enter peroxisomes post-translationally with assistance from the receptor *peroxins* PEX5 and PEX7, which recognize peroxisome-targeting signal (PTS) sequences in proteins bound for the peroxisomal matrix. PTS2 proteins are recognized by PEX7 and have their PTS cleaved upon import. After cargo delivery, PEX5 and PEX7 are retrotranslocated to the cytosol and reused in further rounds of import. We are characterizing the molecular functions of *Arabidopsis* LON2, a peroxisomal AAA-ATPase and protease that facilitates the import of matrix proteins. *lon2* mutants are resistant to the promotion of lateral roots by IBA, indicating peroxisomal defects. As *lon2* seedlings age, they accumulate unprocessed PTS2 proteins and display matrix protein import defects. We found that overexpressing PEX7 rescues *lon2* PTS2 processing defects, suggesting a role for LON2 in PEX7 function. Though LON2 is not directly responsible for PTS2 processing, it may assist in matrix protein delivery by degrading cleaved PTS2 peptides to free PEX7 for future rounds of import or by dissociating PEX5-PEX7-cargo complexes after peroxisome entry. We have identified multiple suppressor mutants that rescue *lon2* PTS2 processing and lateral root defects, and we are performing biochemical assays to isolate LON2 interactors. These approaches will expand our understanding of the molecular mechanisms supporting continued peroxisomal matrix protein import and how peroxisome functions contribute to successful seedling development.

### **174 Determining Cross-Species Functionality of Riboswitches**

*Zohaib Ghazi<sup>1</sup>, Barbara Moffatt<sup>2</sup>, Yingfu Li<sup>1</sup>*

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Riboswitches are RNA based regulatory elements that are capable of binding a specific target and regulating the expression of downstream genes. Riboswitches can be very specific and sensitive to their metabolites and are also able to discriminate against analogs to various degrees. We are interested in looking at the effects of riboswitches that are known to be specific and sensitive within their native organism and assessing whether those properties are maintained when expressed in a different species. To investigate this, we have begun cloning a variety of riboswitches from Gram positive bacteria and transforming them into *E.coli*. Their activity in regulating

reporter gene expression in response to concentrations of target metabolite was then analyzed. Results from our experiments provide insights into the potential of engineering riboswitch biosensors that could function in eukaryotic organisms such as *Arabidopsis thaliana*.

**175 Molecular and Biochemical Study Toward Understanding the Cellular Signaling Mechanism of AtRALF1, a Ca<sup>2+</sup> Mobilizing Peptide Hormone**

*Miyoshi Haruta, Michael Sussman*

**University of Wisconsin–Madison**

Rapid changes in the cytoplasmic concentration of calcium are associated with various cellular signaling pathways, including biotic- and abiotic stresses. Using an *in vivo* screening method with aequorin expressing *Arabidopsis* seedlings to identify the major extracellular signal molecules that regulate cytoplasmic calcium, we previously identified and characterized the AtRALF1 polypeptide (*Biochemistry*, 2008, 47, pp 6311–6321). We have now undertaken an investigation of AtRALF1 cellular signaling events using various genomic profiling tools. First, AtRALF1-responsive transcriptome analyses revealed the up-regulation of families of Ca<sup>2+</sup> binding proteins and ethylene transcription factors. The most significantly down-regulated genes are a family of auxin responsive proteins, followed by expansin proteins. Second, yeast two hybrid screening with AtRALF1 as a bait was performed and identified proteases, ribosomal proteins, and cell wall proteins as potential AtRALF1 interactors. Third, we have initiated experiments to identify AtRALF1 binding proteins with *in vitro* studies. AtRALF1 peptide that was either chemically synthesized or heterologously produced in *E. coli* shows comparable biochemical and physiological characteristics to that previously isolated from *Arabidopsis* extracts. Using the tagged version of AtRALF1 peptide with either poly histidines or biotin, we are currently exploring the identification of AtRALF1 binding proteins from plant extracts.

**176 A Role for Glyceraldehyde-3-phosphate Dehydrogenase in Plant Innate Immunity**

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Glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) are important enzymes with diverse cellular regulatory roles in vertebrates including the regulation of reactive oxygen species, but few reports have investigated GAPDH importance outside of their role in glycolysis in plants. We have found that GAPDHs are upregulated during effector triggered immunity at the protein level. A genetic approach was used to investigate the importance of different GAPDH members during plant innate immune responses using the interaction between *Arabidopsis* and the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato* (*Pto*). GAPDH knockout lines were screened for alterations in plant immune responses after inoculation with *Pto*. A subset of knockouts exhibited enhanced disease resistance phenotypes, including more rapid programmed cell death in response to inoculation with avirulent *Pto*. These results indicate that GAPDHs are important proteins involved in the regulation of plant immune responses against microbial pathogens.

**177 Role of Transceptor CHL1 in Nitrate Sensing**

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Sensing and responding to soil nutrient changes is vital for the survival of higher plants. Previous studies showed that CHL1 is a dual-affinity nitrate transporter, involved in both high- and low-affinity nitrate uptakes. When CHL1 is phosphorylated at Threonine 101, it functions as a high-affinity nitrate transporter, whereas T101-dephosphorylated CHL1 is a low-affinity transporter. Our recent study of a decoupled mutant demonstrated that nitrate transporter CHL1 also functions as a nitrate sensor. Studies of transgenic T101A and T101D plants indicated that phosphorylated and dephosphorylated CHL1 lead to a low- and high-level of transcriptional response, respectively. Further studies showed that, in response to low nitrate concentration, protein kinase CIPK23 will phosphorylate T101 of CHL1 to maintain a low-level primary response, whereas exposed to high concentration of nitrate, T101 phosphorylation is prohibited and dephosphorylated CHL1 will lead to high-level primary response. Therefore, using dual-affinity binding and a phosphorylation switch, transceptor CHL1 can sense a wide range of soil nitrate concentrations and then trigger different levels of transcriptional response. Recently, we are interested to find out how a nutrient sensor could exert temporal changes in signaling.

**178 Tryptophan Metabolism in *Arabidopsis*: a Model for Interaction between Primary and Secondary Metabolism**

*Brad Hogan<sup>1</sup>, Angus Wan<sup>1</sup>, Scott Mottarella<sup>1</sup>, La'Kesha Francis<sup>3</sup>, Carolyn Crisp<sup>2</sup>, Judith Bender<sup>2</sup>, John Celenza<sup>1</sup>*

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In plants, tryptophan (Trp) functions not only in protein synthesis, but also as a precursor for indole-3-acetic acid (IAA), and two defense compounds: indole glucosinolates (IGs), and camalexin. We are focusing on Trp metabolism in *Arabidopsis* as a model for studying the interactions between Trp biosynthesis and Trp secondary metabolism. We have examined IG production in the conditional Trp auxotrophic mutants, *trp3-1* and *trp2-1*, which are mutant alleles, respectively, of the *TSA1* and *TSB1* genes. *trp2-1* and *trp3-1* mutants grow poorly in the restrictive high light condition (without a Trp supplement) and have elevated IG levels compared to wild-type Columbia. Thus, *trp2-1* and *trp3-1* mutants apparently synthesize IGs at a cost to growth. These findings suggest a model in which IG synthesis is the major sink for Trp. We have tested this hypothesis by combining *trp2-1* or *trp3-1* with mutants that modulate IG synthesis. Our results show that the elimination of IG production partially suppresses the *trp* mutant phenotype whereas increasing IG synthesis exacerbates the *trp* mutant phenotype. As suggested by the conditional nature of the *trp* mutant auxotrophy and their ability to still make IGs, other Trp synthase genes are likely able to partially compensate for the lack of *TSA1* or *TSB1*. We currently are testing this model by examining *trp* mutant combinations for alterations in IG and camalexin production. In addition, we are using a subfamily

of Myb transcription factor mutants that regulate IG production to ask if there is spatial or temporal control of Trp metabolism. These mutants will also be used to investigate further regulation between primary and secondary Trp metabolism.

### **179 Lumen Thiol Oxidase 1 (LTO1), a novel disulfide bond catalyst at the thylakoid membrane is required for photosynthesis**

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The occurrence of disulfide-bonded proteins in the thylakoid lumen suggests that thiol/disulfide chemistry in this compartment is enzymatically assisted. However the molecular identity of the redox components controlling this process is currently unknown. In the plant *Arabidopsis thaliana*, we have identified AtLTO1 (Lumen Thiol Oxidase 1), a novel redox catalyst at the thylakoid membrane. Using PhoA and LacZ bacterial reporters, we have generated a topological model of plastid AtLTO1 and deduced a luminal location for the protein domains carrying the redox motifs and conserved cysteines. Through heterologous complementation in bacteria, we show that AtLTO1 can partially substitute for the absence of disulfide bond formation in the periplasm, an indication that the plant protein displays sulphydryl oxidizing activity in the thylakoid lumen, the topologically equivalent compartment in the plastid. An insertional mutation within the promoter of the AtLTO1 gene results in a reduced accumulation of the LTO1 transcript associated with a severe photoautotrophic growth defect. Measurements of the photosynthetic activity indicate that the lto1 mutant lines display a limitation in the electron flow from photosystem II. In accord with these measurements, we noted a severe depletion of the structural subunits of photosystem II but no change in the accumulation of the b6f complex and photosystem I. Using yeast 2 hybrid, we show that the soluble thioredoxin-like domain of AtLTO1 interacts with PsbO, a subunit of the oxygen evolving complex in photosystem II that is known to be disulfide bonded. Moreover, using recombinant proteins, we demonstrate that the thioredoxin-like domain of AtLTO1 is able to introduce a disulfide bond in the PsbO target.

### **180 Subunits of the Asymmetric Plastid ClpPR Protease Complex: Mutants, Stoichiometry, Evolution and Functional Implications**

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The Clp protease system expanded in plant plastids compared to its prokaryotic progenitors. The plastid Clp core protease complex in *Arabidopsis thaliana* consists of 5 different catalytic ClpP and 4 different non-catalytic ClpR proteins each in one or more copies and organized in two heptameric rings. To address this complexity, first, we analyzed *CLPP* and *CLPR* mutants extensively. Null alleles for *CLPP3*, *CLPR2* and *CLPR4* showed delayed embryogenesis and albino embryos, with seedling development blocked in the cotyledon stage; this developmental block was overcome under heterotrophic conditions, and seedlings developed into small albino to virescent seedlings. By contrast, null alleles for *CLPP4* and *CLPP5* were embryo lethal. Thus, the ClpPR proteins make different functional contributions. Microscopy and large-scale comparative leaf proteome analyses of *CLPP3* and *CLPR4* null alleles demonstrate a central role of Clp protease in chloroplast biogenesis and protein homeostasis. Second, we determined the exact subunit composition and stoichiometry for the intact core and each ring, using a novel approach. The chloroplast ClpPR protease was affinity-purified from *clpp3* and *clpr4* mutants complemented with C-terminal StrepII-tagged *CLPP3* and *CLPR4*, respectively. The subunit stoichiometry was determined by mass spectrometry-based absolute quantification using stable isotope-labeled proteotypic peptides generated from a synthetic gene. One heptameric ring contained ClpP3,4,5,6 in a 1:2:3:1 ratio. The other ring contained ClpP1 and ClpR1,2,3,4 in a 3:1:1:1 ratio, resulting in only three catalytic sites. These ClpP1/R1-4 proteins are most closely related to the two subunits of the cyanobacterial P3/R complex and the identical P-R ratio suggests conserved adaptation. Furthermore, the plant specific C-terminal extensions of the ClpP/R subunits were not proteolytically removed upon assembly, suggesting a regulatory role in Clp chaperone interaction. These results will now allow testing ClpPR structure-function relationships using rationale design. The quantification workflow is applicable to other protein complexes.

### **181 Transcriptional regulation of the iron deficiency response in *Arabidopsis thaliana***

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Iron is an essential nutrient for all organisms. It is required for fundamental metabolism from respiration to photosynthesis. Although iron is abundant in the earth's crust, plants are continuously challenged to acquire sufficient iron due to the low solubility of the metal in aerobic soil.

All plants, except grasses, use a reduction-based iron uptake. In *Arabidopsis*, *FRO2* encodes the Fe(III) chelate reductase and *IRT1* encodes the Fe(II) transporter functioning in iron uptake at the root-soil interface. FIT is a transcription factor that regulates many but not all iron deficiency induced genes, including the *IRT1* and *FRO2* genes. Although *IRT1* protein is not detectable in iron deficient *fit* plants, *IRT1* mRNA abundance is not completely abolished indicating the presence of additional factors controlling *IRT1* expression. We set up a genetic screen to identify other genes that control *IRT1* expression. Using an *IRT1* promoter-luciferase fusion construct, we screened 15,000 M2 plants and isolated mutant lines that are impaired in luciferase induction under iron deficiency. One recessive mutant *uri* (upstream regulator of *IRT1*) displayed defects in the induction of the reporter as well as the endogenous *IRT1*. As might be expected of a mutant that does not make the iron transporter, the *uri* mutant dies after germination in soil unless fed supplemental iron. The mutant also fails to induce Fe(III) chelate reductase activity. Microarray analysis in the *uri* mutant revealed vast alterations in

expression of iron deficiency regulated genes. *FIT* is not up-regulated, suggesting that *URI*'s role is likely to be upstream of *FIT* in the iron deficiency signaling pathway. We have determined that *URI* encodes a bHLH transcription factor that is not itself iron-regulated at the steady state mRNA level. We propose a network of iron deficiency signaling in *Arabidopsis* where *URI* serves as an upstream regulator of *FIT*, *IRT1* and *FRO2*.

**182 Molecular mechanisms of boric acid transport by NIP7;1, an anther-specific Nodulin Intrinsic Protein**

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Nodulin 26-like intrinsic proteins (NIPs) are a plant specific family of membrane channels which share homology with soybean nodulin 26. Structurally, NIPs share homology to the aquaporin superfamily, and possess the ability to transport water and uncharged metabolites. Based on phylogeny and molecular modeling of the transport pore, NIPs are divided into three subfamilies which possess distinct transporter functions. NIP II proteins in *Arabidopsis* are represented by NIP5;1, NIP6;1 and NIP7;1 which show distinct tissue and organ expression. NIP5;1 (1) and NIP 6;1 (2) have been characterized as boric acid channels expressed in roots and leaf nodes respectively, and which facilitate the uptake and transport of boric acid to critical sink tissues under conditions of limiting boron concentrations in the environment. In the present work we have characterized NIP7;1, the third member of the NIP II subfamily. NIP7;1 is selectively expressed in developing anther tissues, principally in developing pollen microspores of stage 9-11 floral buds. Functional characterization of NIP7;1 shows that unlike NIP5;1 and NIP6;1 which form constitutive boric acid channels, the intrinsic boric acid transport activity of NIP7;1 is extremely low. Molecular modeling suggests that a conserved tyrosine residue (Tyr 81) located in the transport pore stabilizes a closed conformation of the pore. Molecular dynamics simulation suggests that the closed conformation is stabilized by hydrogen bonding between the Tyr81 hydroxyl group and Arg 220 of the canonical "aromatic-arginine" selectivity filter. Substitution of Tyr81 with either a phenylalanine or a cysteine opens the channel to boric acid transport which supports the prediction from the MD simulation. Since boric acid is both essential nutrient as well as a toxic compound at high concentrations, it is proposed that Tyr 81 modulates transport and provides an additional level of regulation of uptake of boric acid in male gametophyte development. (Supported by NSF grant MCB-0618075).

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**183 Evaluation of IBA Transport in *Arabidopsis* Hypocotyls by Stable Isotope Labeling and GC-MS/MS**

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Polar transport of the natural auxins indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) has been described in *Arabidopsis* hypocotyls using [<sup>3</sup>H]IBA and [<sup>3</sup>H]IAA tracers. The reported transport of [<sup>3</sup>H]IBA was twice that of [<sup>3</sup>H]IAA. As the [<sup>3</sup>H] assays employed cannot distinguish IBA from its metabolites, the detected polar transport from applied [<sup>3</sup>H]IBA may be the result of polar transport of the IBA metabolite, IAA. To test this hypothesis, we have established a microscale solid phase extraction and Gas Chromatography-Selected Reaction Monitoring-Mass Spectrometry (GC-SRM-MS) method which allowed us to detect stable-isotope labeled IAA derived from its labeled IBA precursor in a small sample of only a few *Arabidopsis* seedlings. Using this method, we assayed the transport of IBA in *Arabidopsis* hypocotyls by following the movement of [<sup>13</sup>C<sub>1</sub>-indole 2]IBA and the [<sup>13</sup>C<sub>1</sub>]IAA derived from [<sup>13</sup>C<sub>1</sub>]IBA. We also assayed [<sup>13</sup>C<sub>6</sub>]IAA transport in a parallel control experiment. We found that [<sup>13</sup>C<sub>1</sub>]IBA was converted to [<sup>13</sup>C<sub>1</sub>]IAA during the transport period; 26% of the [<sup>13</sup>C<sub>6</sub>]IAA taken up by plants was transported to the basal end, while only 0.7% of the [<sup>13</sup>C<sub>1</sub>]IBA was transported to the basal end, and the transport of [<sup>13</sup>C<sub>1</sub>]IBA was not inhibited by the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA). We further analyzed the transport of [<sup>13</sup>C<sub>1</sub>]IBA in the *ibr1 ibr3 ibr10* mutant, which does not convert IBA to IAA. The transport of [<sup>13</sup>C<sub>1</sub>]IBA in the mutant was not different from the wild type, but the transport of [<sup>13</sup>C<sub>6</sub>]IAA was 40% lower than in the wild type. These results suggest that polar transport of IBA is much lower than previously reported, and IBA uses a transport process distinct from IAA transport. We are now determining if [<sup>13</sup>C<sub>1</sub>]IBA is converted to conjugates during transport.

**184 Gene Identification of Prephenate Aminotransferase Provides Novel Insights into Plant Phenylalanine Biosynthesis**

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L-Phenylalanine (Phe) is an essential aromatic amino acid in human diets and also a precursor of numerous phenolic compounds produced in plants. In vascular plants, ~30% of photosynthetically fixed carbon is allocated to Phe biosynthesis for the production of lignin, a principal plant cell wall component and major obstacle of bioethanol production from lignocellulosic biomass. Despite its importance in both plant and human physiology and metabolism, Phe biosynthesis and its regulation in plants remain poorly understood. Phe is synthesized from prephenate, a product of the shikimate pathway, which can be converted to Phe via phenylpyruvate or arogenate intermediates. Our previous genetic study of arogenate dehydratase (ADT) showed that, unlike in most microorganisms, Phe biosynthesis in plants predominantly operates via the arogenate pathway. In this study, we employed a bioinformatics approach in *Arabidopsis thaliana* and identified the last undiscovered gene in Phe biosynthesis encoding prephenate aminotransferase (PPA-AT), which catalyzes the first step of the arogenate pathway. Biochemical and genetic characterization of PPA-AT enzymes from *Petunia hybrida* further indicated that PPA-AT directs the carbon flux from prephenate toward arogenate making the arogenate pathway predominant in plant Phe biosynthesis.

Comparative analysis of *PPA-AT*- and previously generated *ADT*-RNAi suppression lines of petunia revealed that the regulation of the flux through the shikimate pathway plays a critical role in controlling Phe levels *in planta*. These results provide a foundation for plant metabolic engineering to modulate the production of phenolic compounds synthesized from Phe.

## 185 Characterization of an *Arabidopsis* Aminotransferase Reveals Cross-talk Between Phenylalanine Biosynthesis and Auxin Homeostasis

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Indole-3-acetic acid (IAA), is a central regulator of plant development, plant growth, and plant response to tropic cues. In plants including *Arabidopsis* IAA can be synthesized directly from the amino acid tryptophan [Trp-dependent (TD)] or from an indolic precursor of tryptophan [Trp-independent (TI)]. While there is much support for the TD pathway at the genetic level, the genes involved in the TI pathway remain unknown. The Celenza lab has identified a mutant, called *sall*, that may help distinguish between these two pathways. When *sall* is grown on indole-supplemented (but not Trp-supplemented) medium accumulates three-fold higher IAA compared to wild type and displays growth phenotypes consistent with elevated IAA. These findings suggest that indole is being diverted into IAA in the *sall* mutant, implying that the SAL1 gene may regulate which IAA pathway is used. Positional cloning revealed that SAL1 encodes an uncharacterized aminotransferase (AroAT) conserved in other plant species. However, SAL1 is only weakly similar to TAA1, a characterized *Arabidopsis* AroAT that converts Trp to indole-3-pyruvic acid (IPA) leading to IAA. To demonstrate that SAL1 is an AroAT, we determined that heterologously expressed SAL1 can fully rescue the yeast *aro8 aro9* double mutant that is defective in redundant AroATs that produce (Phe) and tyrosine (Tyr) in yeast. Consistent with a possible role in Phe and Tyr synthesis *in planta*, the mutant shows altered sensitivity to fluorophenylalanine and has reduced levels of phenylpropanoids. Our working model is that SAL1 participates in the Phe/Tyr metabolism and that this perturbation alters indolic metabolism through crosstalk with the Trp branch. Current goals are to characterize SAL1 biochemically and to determine the mechanism of crosstalk by examining genetic interactions between *sall* plants and mutants with altered aromatic amino acid metabolism. In addition, we are performing further metabolite analysis in the *sall* mutant.

## 186 Characterization of Genetic Enhancers of the Auxin-Deficient Mutant *taa1*

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The plant hormone auxin is vital for countless aspects of plant growth and development. To date, only a few auxin biosynthetic genes have been described. *TAA1* has originally been identified genetically in several mutant screens based on the weak ethylene insensitivity, shade avoidance defects, or resistance to auxin transport inhibitors of the knockout mutants. *TAA1* and the two *TAA1*-related genes *TAR1* and *TAR2* encode tryptophan (TRP) aminotransferases that function in the TRP-dependent indole-3-pyruvic acid (IPA) branch of IAA biosynthesis. Unlike *taa1* knockouts, single *tar* mutants do not show any discernable phenotypes, but they enhance the defects of *taa1*. Simultaneous knockouts of multiple *TAA1/TAR* family members results in severe auxin deficiency manifested by the abnormal embryo development, degeneration of root meristems, reduced vasculature, loss of apical dominance, and infertility.

We took advantage of the relatively mild ethylene defects of single *taa1* mutants and performed EMS-mutagenesis of *taa1* DR5:GFP with the goal to identify additional players of auxin biosynthesis in *Arabidopsis*. Screening of the M2 generation in the ethylene triple response assay identified several hundred potential genetic enhancers of *taa1*. Retesting of these lines in the M3 generation in the media supplemented with the ethylene precursor ACC versus with auxin IAA, in combination with the analysis of GFP fluorescence and scoring of a range of auxin-related phenotypes (such as apical hook angle in the dark, root meristem morphology, lateral root number, vasculature patterns in the cotyledons, etc.), has eliminated general auxin response and ethylene signaling mutants and has left us with a manageable number of putative auxin-biosynthesis-related mutants. We are in the process of further characterizing and mapping a subset of these lines. Our ultimate goal is to uncover the missing pieces of the auxin biosynthesis puzzle and shed new light on this essential, but poorly characterized pathway in *Arabidopsis*.

## 187 Adaptation of *Arabidopsis thaliana* to sustained nitrogen availability in the soil

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Nitrogen (N) is one of the most important vital nutrients for crop plant growth and yield. Only about a third of the N content in the soil can be used by the plant. Therefore, improvement of the nitrogen use efficiency (NUE) would be beneficial for agriculture. Understanding the regulatory steps that lead to systemic adaptation to low N conditions might allow improvement of NUE. We have established a simple soil based growth system that closely mimics natural growth conditions but allows plants to be subjected to defined N availability to get an insight into the molecular, biochemical and physiological responses to a sustained N limitation. This leads to a 20% decrease in the relative growth rate resulting in a severe reduction of rosette biomass. Further, leaf initiation rate is slower, floral induction is delayed and seed set is reduced. Plants grown in the low N regime do not show any obvious signs of stress or accumulation stress related secondary metabolites. In addition, the Gln/Glu ratio, a marker for N limitation, indicates no obvious N deficiency. However, both the nitrate concentration and nitrate reductase activity are decreased. This suggests that plants adapt to the low N availability. In this process the root system must have a great influence. Therefore, we have developed a method that allows us to isolate RNA from roots of plants grown in our soil based growth system. With this we were able to examine the whole plant and to identify genes that are

differentially expressed in the root and the shoot under N limitation. This allows us to identify genes that make an important contribution to the adaptation to N limited conditions.

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### 188 A Role for START Lipid/Sterol Binding Domains in Transcription

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Steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domains occur in a variety of proteins. In mammals, START domain-containing proteins exhibit broad sub-cellular distribution including nuclear localization, yet possible functions in transcription are not understood. In a plant-specific configuration, START domains are found in Class III and IV homeodomain leucine zipper (HD-Zip)transcription factors. It is hypothesized that ligand binding of lipids/sterols to the START domain control transcription. To test this idea, START domains were expressed in yeast using a synthetic transcription factor comprised of GAL4 DNA-binding and VP16 activation domains. Mammalian START domains from StAR, MLN64, and PCTP enhanced transcription factor activity. GAL4 DBD fused to the START domain alone resulted in complete loss of activity, indicating that START does not behave as an activation domain. Over-expression of *SUT1*, a positive regulator of sterol biosynthesis, increased reporter activity, consistent with sterols as candidate ligands for START. Conversely, mutation of several predicted ligand-binding residues within StAR's START domain reduced function. Of 25 *Arabidopsis* START domains assayed, five conferred enhanced activity including those from two Class IV HD-Zip transcription factors. Computational modeling of their respective START domains was consistent with a binding pocket that is similar in spatial architecture and volume to that of mammalian counterparts of known crystal structure. In sum, START domains from mammals and plants enhance transcription, likely via ligand binding, pointing towards a regulatory role of the START domain in eukaryotic transcription that is more general than previously thought.

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### 189 Characterizing a Biosynthetic Module for the Formation of a Novel Pathogen-Induced Phytoalexin in *Arabidopsis* Roots

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Plants have evolved a variety of constitutive and induced defense mechanisms against biotic stress. The release of organic defense compounds known as phytoalexins constitutes a powerful induced response to the attack of plant pathogens. However, our knowledge of the function of such chemical defenses in plant roots is still limited. We are characterizing biosynthesis gene modules involved in the production of phytoalexin terpene metabolites in *Arabidopsis* roots. We have identified a single cytochrome P450 monooxygenase of the CYP705A family, which catalyzes the oxidative degradation of the C<sub>30</sub>-triterpene precursor arabidiol thereby causing the release of a volatile acyclic C<sub>11</sub>-homoterpene (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT) and a C<sub>19</sub>-degradation product named arabinol. While DMNT is a common constituent of volatile blends released from insect-damaged foliage, it is emitted from *Arabidopsis* roots after infection by the root-rot pathogen *Pythium irregularare* or the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst) DC3000 suggesting defensive functions of DMNT and arabinol in roots. In contrast to the formation of DMNT from the C<sub>15</sub>-sesquiterpene alcohol (*E*)-nerolidol in leaves, we show that roots produce DMNT via a novel pathway as cleavage product of a C<sub>30</sub>-triterpene. The biosynthetic genes *AtPen1* (arabidiol synthase) and *CYP705A1* are clustered on chromosome 4 with other P450s and glycosyltransferase genes possibly involved in further modification of the C<sub>19</sub>-degradation product. Our findings suggest evolutionary plasticity in the induced formation of homoterpene volatiles in above- and below ground plant tissues.

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### 190 The bZIP Transcription Factor HY5 Modulates the Circadian Expression of the Monoterpene Synthase Gene QH6

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Monoterpenes, including myrcene, ocimene, pinene and limonene, are a group of volatile secondary metabolites which play important role in chemical ecology. Promoter of QH6, a β-pinene synthase gene of *Artemisia annua* which shows a diurnal expression pattern was cloned by genome walking. Some putative light regulated elements including G-box were located in QH6 promoter. Several truncated promoter:GUS transgenic *Arabidopsis* lines were constructed. To reveal the potential regulatory mechanism, the tissue specific expression pattern was compared by GUS stain. GUS activity was found mainly in juvenile leaves. The -1000bp to -800bp fragment was found functional for trichome specific expression. To localize light regulated element, time series expression of GUS gene driven by different truncated promoters under light cycle was examined by quantitative PCR. Yeast One Hybrid Screen suggests that binding of HY5, a bZIP transcription factor, to G-box might account for the diurnal expression. The luciferase reporter system was also introduced to reveal the promoter driven circadian rhythm.

## **191 Analysis of N-End Rule Pathway Components In Arabidopsis: PRT8 is a Novel E3 Ubiquitin Ligase That Targets Proteins With Aliphatic Hydrophobic Amino Terminal Residue**

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The N-end rule is a ubiquitin-dependent proteolytic pathway involved in degradation of various substrates with specific N-terminal amino acid residues. E3 ligases PRT6 and PRT1 are responsible for destabilization of model substrates with basic and aromatic residue at the N-terminus, respectively. Surprisingly, neither of these ligases is involved in degradation of test proteins with an aliphatic hydrophobic amino-terminal residue such as Leu, strongly suggesting the existence of another plant-specific E3 ligase in this pathway. Using a live tissue GUS staining method, we screened F2 pools derived from EMS-mutagenized plants that expressed a GUS transgene with Leu as a first residue. We identified mutants in a locus called PRT8 (PROTEOLYSIS 8), which stabilize the test substrate.

Many components of the plant N-end rule pathway share homology with mammalian proteins. Plant proteins BIG and PRT7 are related to mammalian UBR domain proteins UBR4 and UBR7, respectively. However, in plants the functional importance of these two proteins as E3 ligases is still not known. To deduce the function of these proteins, we isolated a prt7 mutant and analyze big mutants from the SALK collection. These mutants were crossed to plant lines with various ubiquitin fusion reporter constructs to assess their potential roles as ubiquitin ligases, phenotypic and biochemical analysis shall help to understand the biological functions of these two genes.

Deamidation of amino-terminal Asn or Gln is the first step in degradation of substrates with N-terminal Asn or Gln. In mammals, the process employs proteins NTAN1 and NTAQ1, which convert N-terminal Asn and Gln into Asp and Glu, respectively. We suspect similar enzymatic modifications in the plant system and are currently studying plant homologs of NTAN and NTAQ.

The study of new genes from the N-end rule pathway by mutant analysis will certainly add to the molecular understanding of N-end rule pathway functions in plant growth and development.

## **192 Dissecting the Complex Phenotype of MTN-deficient Mutants**

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5'-methylthioadenosine (MTA) is a byproduct of polyamine, nicotianamine and ethylene biosynthesis that is recycled to methionine by 5'-methylthioadenosine nucleosidase (MTN). In Arabidopsis, MTN is encoded by two genes: *AtMTN1* (*At4g38800*) and *AtMTN2* (*At4g34840*). Double mutants have a myriad of developmental defects including altered vasculature, sterility and delayed developmental milestones. We are interested in defining the first abnormal trait associated with reduced MTN activity. Thus, we have developed a series of MTN-deficient lines that differ in their residual MTN activity, using two approaches: crossing different *MTN* knock-down single alleles, and creating transgenic lines expressing an artificial microRNA against *MTN2* in an *mtn1-1* background. The generated lines include (1) *mtn1-4mtn2-1*, (2) *mtn1-1mtn2-1*, (3) *ami 5.3*, and (4) *ami 2.8*. The residual MTN activity in these lines reflects the severity of their phenotypes. The *mtn1-4mtn2-1* mutant has WT MTN activity and a normal phenotype while the *mtn1-1mtn2-1* mutant has 2-3% MTN activity and a complex pleiotropic phenotype. The *ami*MTN lines have intermediate MTN enzyme activities. The anatomy and development of these lines are being assessed and related to their content of key methionine-related metabolites. With this approach we hope to determine the primary effect of MTN deficiency on plant growth and development.

## **193 Studies On The Arabidopsis Fatty Acid Regulators By Coexpression Analysis**

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Fatty acids play crucial roles in signaling transduction and plant development. However, the regulation of the fatty acid biosynthesis pathway is still poorly understood. We have tried to identify the regulators of fatty acid biosynthesis by omics based co-expression analysis in Arabidopsis. Fifty-two fatty acid biosynthesis genes including de novo synthases, desaturases and elongases were selected as guide genes. Calculation of the correlation between all Arabidopsis genes with each guide gene by Arabidopsis co-expression dating mining tools was performed with a Pearson correlation coefficient (PCC) cut-off of 0.55 and a gene correlated with more than seven guide genes was supposed to be involved in regulation of fatty acid biosynthesis. As a result, seven transcription factors that correlated with at least seven guide genes were selected, and analysis of corresponding T-DNA insertion lines indeed showed the altered total fatty acid composition of mature seeds. qRT-PCR analysis revealed the suppressed expression of the guide genes in the mutants. Details and how these genes regulate the fatty acid biosynthesis will be presented.

**194 Mutations in RIG2, a Membrane E3 Ubiquitin Ligase Which Interacts With GLUTAMINE DUMPER1, Suppress *gdu1-1D* Phenotype**

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The over-expression of the plant-specific membrane protein GLUTAMINE DUMPER1 (GDU1) induces the secretion of glutamine at the leaf margins and increased amino acid content in apoplasm and phloem sap (*gdu1-1D* mutant). However, the molecular mechanism for GDU1 function in plant cells is still unclear. To understand the role of GDU1 protein and its physiological function, we searched for genes involved in the same process as GDU1.

A yeast two-hybrid screening identified an *Arabidopsis* RING finger E3 ubiquitin ligase (RIG2) as a GDU1-interacting protein. RIG2 was shown to be membrane-associated. RIG2 and GDU1 transiently expressed in *Nicotiana benthamiana* leaves could be co-immunoprecipitated, showing that the two proteins interact with each other *in planta*. A screening for ethyl methanesulfonate-mutagenized mutants suppressing *gdu1-1D* phenotype identified several of such mutants. Interestingly, two of the mutations (*Loss Of GDU1: log2, log5*) were recessive, and shown by genetic mapping to be localized in *RIG2*. Both mutants exhibited wild type phenotype, loss of glutamine secretion, amino acid over-accumulation and the reduced size typical of *gdu1-1D*. Over-expressing artificial miRNAs that target *RIG2* in *GDU1* over-expression lines led to partial suppression of the *gdu1-1D* phenotype. Decrease in RIG2 activity by the *log2* mutation or the expression of the artificial miRNAs did not affect GDU1 protein over-accumulation. These results suggest that RIG2 acts downstream from GDU1, possibly as a subunit necessary for its function.

**195 The *Arabidopsis CYP724A1* Gene Encodes a Functional Brassinosteroid C-22 Hydroxylase**

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Brassinosteroids (BRs) are an important class of signaling molecules in plants. A necessary step in the biosynthesis of bioactive BRs is the hydroxylation at C-22. This key committed step, a focal point for regulation in the BR biosynthesis pathway, is catalyzed by P450 enzymes of the CYPB90 and CYP724B families in rice and tomato. Crucifers lack genes encoding CYP724B enzymes and *Arabidopsis* null mutants of *DWARF4* (encoding CYP90B1) lack bioactive BRs and display a severe BR-deficiency phenotype. *CYP724A1* (At5g14400) is a poorly expressed gene of *Arabidopsis* that encodes a P450 with less than 55% sequence identity to CYP724B enzymes. Here we show that ectopic overexpression of *CYP724A1* in null *dwarf4* mutants functionally complements their BR-deficiency largely restoring normal growth and development. This demonstrates that *CYP724A1* is a functional enzyme and indicates that, like CYP724Bs, enzymes of the CYP724A sub-family have BR C-22 hydroxylase activity. Progress in defining the role of *CYP724A1* in *Arabidopsis* is also reported.

**196 AthaMap-assisted Prediction of microRNA Targets**Lorenz Biilow, Julio Bolívar, Reinhard Hehl**Technical University of Braunschweig, Germany**

AthaMap is a database for gene expression regulation in *Arabidopsis thaliana*. The database was now extended to include post-transcriptional regulation by microRNAs. Among smallRNAs, microRNAs play an important role in the control of plant gene expression. The *Arabidopsis* genome encodes at least 176 microRNA genes. Potential target sites of microRNAs were bioinformatically determined within the genome of *Arabidopsis thaliana* and have been annotated to AthaMap. As expected, genes have been identified as putative targets of microRNAs but intergenic regions seem to be targeted as well. The effect on expression of microRNA target genes have been analyzed and expression levels of these genes are relatively low. The effect on expression of genes adjacent to intergenic regions targeted by microRNAs was also studied. On the AthaMap website, a new function has been implemented to identify all annotated microRNA targets.

**197 Exploring the molecular network of glucosinolate biosynthesis using bioinformatic tools**Yazhou Chen<sup>1,2</sup>, Xiufeng Yan<sup>2</sup>, Sixue Chen<sup>1</sup>**<sup>1</sup>University of Florida, Gainesville, USA, <sup>2</sup>Northeast Forestry University, Harbin, China**

Glucosinolates constitute a major group of secondary metabolites in *Arabidopsis*, which play an important role in plant interaction with pathogens and insects. Advances in glucosinolate research have defined the biosynthetic pathways. However, cross-talk and interaction between glucosinolate pathway and other molecular pathways are largely unknown. Here three bioinformatics tools were used to explore novel components and pathway connections in glucosinolate network. Although none of the software tools were perfect to predict glucosinolate genes, combination of results generated by all the tools led to successful prediction of all known glucosinolate genes. This approach was used to predict new genes in glucosinolate network. A total of 330 genes were found with high potential to relate to glucosinolate biosynthesis. Among them 94 genes were considered as top candidates because their individual connection to a known glucosinolate gene was predicated by all the software tools. Microarray data of candidate gene mutants were used for validation of the results. The mutants of nine genes predicted by glucosinolate seed genes all exhibited changes in the expression of glucosinolate genes. Four of the genes have been well-known to functionally interact with glucosinolate biosynthesis. These results indicate that the approach we took provides a powerful way to reveal new players in glucosinolate networks. Creation of an *in silico* network of glucosinolate biosynthesis will allow the generation of many testable hypotheses and ultimately enable predictive biology.

**198 Investigation of Gravitropism Using Auxin Mutants and Multidimensional Data Modeling**Misuk Cho, Nathan Miller, Edgar Spalding**University of Wisconsin, Madison, Wisconsin, USA**

Relationship between growth rate and curvature in response to gravity in *Arabidopsis* root of auxin mutants. We have used a custom high throughput, high resolution machine-vision platform to acquire a large data set describing the range of gravitropism response patterns displayed by the wild type seedling root. Mathematical modeling demonstrated that the wild-type behavior could be largely explained by two parameters, one possibly relating to the size of the root elongation zone and the other to the magnitude of the gravity-induced growth differential. Auxin has been considered central to the gravitropism mechanism since Chodlony and Went first proposed that a gradient of this hormone across a root or stem was responsible for the growth differential that created curvature. To test the relationship between auxin transport and the model parameters 1 and 2, and to associate genetic functions with the model's parameter space, the behavior of 11 auxin and tropism mutants were quantified and subjected to the modeling exercise. The auxin transport related mutants studied were aux1, axr4-2, pin2, pin3, and abc4-1. The auxin perception and signaling mutants studied were tir1, axr1-3, axr2-1, axr3-1, and shy2-1. The automated image acquisition and analysis methods quantified the gravitropic response in these mutants with 2-min temporal and micron-level spatial resolution in control conditions, with NAA, or with NPA. Modeling of the results is in progress. We hope to be able to attribute the two key parameters of the gravitropism model to specific combinations of auxin-related gene functions.

**199 Enhanced Y1H assays to elucidate *Arabidopsis* gene regulatory networks**Allison Gaudinier<sup>1</sup>, Lifang Zhang<sup>3</sup>, John Reece-Hoyes<sup>2</sup>, Mallorie Taylor-Teeples<sup>1</sup>, Albertha Walhout<sup>2</sup>, Doreen Ware<sup>3</sup>, Siobhan Brady<sup>1</sup>**<sup>1</sup>UC Davis, Davis, CA, USA, <sup>2</sup>University of Massachusetts Medical School, Worcester, MA, USA, <sup>3</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA**

An essential factor in studying developmental processes and responses to the environment is understanding the gene regulatory networks (GRNs) which control such mechanisms. To elucidate this regulation, assays are needed to determine the transcription factor (TF) interactions with gene promoters. We have adapted a Gateway-compatible yeast one-hybrid (Y1H) assay to screen TF interactions with genes expressed in the stele tissue of the *Arabidopsis* root. Our collection of nearly all stele expressed TFs consists of full length and sequence confirmed genes. This high-throughput robotic mating approach uses a sensitive yeast high-copy TF vector and two reporter assays as an internal confirmation for interactions. The enhanced Y1H (eY1H) screen allows us to use a gene-centered approach to rapidly and systematically map GRNs.

**200 PIN Mediated Auxin Redistribution During Phototropism In *Arabidopsis* Hypocotyls***Tim Hohm<sup>1,3</sup>, Christian Fankhauser<sup>2</sup>, Sven Bergmann<sup>1,3</sup>*<sup>1</sup>**Institute of Medical Genetics, University of Lausanne, Lausanne, Switzerland, <sup>2</sup>Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland, <sup>3</sup>Swiss Institute of Bioinformatics, Lausanne, Switzerland**

Being sessile organisms, plants possess various mechanisms to react to different and changing environmental stimuli. One of these mechanisms allows plants to adjust their growth direction to the direction of incoming blue light. This phototropic response involves sensing of light by photoreceptors, here mainly the membrane-associated proteins phot1 and phot2, redirection of the flux of the hormone auxin, as well as other downstream signaling events. Although these key players in phototropism in *Arabidopsis thaliana* are known, detailed means of interaction remain hidden.

The redirection of auxin fluxes is of primary interest. Following the Chododny Went hypothesis, it is commonly accepted that an accumulation of auxin on the shaded side is a necessary prerequisite for the bending reaction observed in phototropism. Still, little is known about the process leading to this auxin redistribution.

To elucidate possible mechanisms, we investigate changes in localization of auxin efflux facilitators of the PIN family that are supposed to play a predominant role in polar auxin transport. The changes in localization of PIN3 are to be monitored using state of the art microscopy, complemented by quantitative modeling for PIN redistribution. This study is focused on capturing early events of redistribution, immediately after onset of blue light irradiation.

**201 VirtualPlant: A Software Platform to Support Next-Generation Systems Biology Research***Manpreet Katari<sup>1</sup>, Rodrigo Gutierrez<sup>2</sup>, Gabriel Krouk<sup>1</sup>, Rebecca Davidson<sup>1</sup>, Tamara Tershakovec<sup>1</sup>, Dennis Sasha<sup>1</sup>, Gloria Coruzzi<sup>1</sup>*<sup>1</sup>**New York University, New York, NY, USA, <sup>2</sup>Pontificia Universidad Católica de Chile, Santiago, Chile**

VirtualPlant enables biologists to mine lists of genes, microarray experiments, next-generation sequencing data and gene networks to address biology's grand challenge questions. VirtualPlant achieves this by enabling researchers to integrate, analyze, and visualize genomic data in a systems biology context. VirtualPlant simplifies data analysis by integrating the tools into a single platform. The unique "gene cart" functionality enables iterative data analysis and suggest additional rounds of experimentation.

During ICAR 2006, VirtualPlant was made publicly available to the *Arabidopsis* community ([www.virtualplant.org](http://www.virtualplant.org)). Five years later VirtualPlant has 750 registered users from 39 different countries around the world including users from 15 different companies. Noteworthy updates to VirtualPlant in regards to features, data, and tools are:

**Biomaps:** The gene set enrichment tool, now has an interactive graph written in Flash with the ability to save the GO-heirarchy image as a high quality figure.

**GeneSect:** A new tool that uses non-parametric randomization test to determine whether overlap between two gene sets are significant.

**RNA-seq:** A new tool is being developed where users can submit the read counts for each gene in the genome and then use tools such as edgeR to determine differentially expressed genes.

**Tair 10:** The *Arabidopsis* annotation has been updated to Tair 10. A feature in the gene cart allows users to convert their gene lists from an older version of Tair to the newer version.

**Multi-Species:** VirtualPlant can support any number of species or ecotypes. Rice is already available and we are in the process of supporting more sequenced plant genomes.

**Comparative Analysis:** Currently users can align their sequencing using Blast to identify putative orthologs based on top match. Other methods and databases will be supported in the near future.

Please see the following manuscript for details and example case studies: Katari et al. "VirtualPlant: a software platform to support systems biology research." *Plant Physiol.* 2010 Feb;152(2):500-15.

**202 Identification of Promoter Motifs and Constitutive and Tissue-Specific Promoters in *Glycine Max*****using a Data Mining Approach***Kathleen Keating, Matthew Hudson***University of Illinois at Urbana-Champaign**

Transcription is regulated by the sequence upstream of a gene, the promoter. Proteins bind to specific conserved sequences, or motifs, to control the expression of the gene. It is thought that genes with similar expression patterns will have the same motifs statistically overrepresented in their promoters. Using this postulation, we identified highly expressed probable constitutive and tissue-specific promoters (e.g. root-specific, leaf-specific) based on an analysis of transcript profiling data and the soybean (*Glycine max*) genome sequence. These probable tissue-specific and constitutive promoters were then used for motif analysis.

With the availability of sequencing data for soybean, data mining techniques are feasible to identify promoters of a specific interest. Using tissue-specific soybean EST libraries from NCBI's Unigene database, we were able to elucidate probable constitutive and tissue-specific genes. The promoter sequence (defined as 2,000 bp upstream of transcription start site) for each candidate gene was used for motif analysis. Each group (constitutive, tissue-specific) appeared to have over-representation of some known motifs. However, the probable root-specific promoter group was the only group with a statistically significant novel motif.

Currently, promoter:GFP constructs are being made for candidate constitutive and tissue-specific promoters. After these constructs are transformed into *Arabidopsis*, we can confirm their expression patterns. Thus, our objectives are to (i) characterize promoters identified by data mining and to (ii) identify motifs that confer specific patterns of expression (e.g. constitutive, root-specific, leaf-specific).

The results of this work will contribute to the understanding of transcriptional control and co-regulation of genes.

## **203 Elucidating the *Arabidopsis* Auxin Response Using Smooth Curve Regression Analysis of a Mutant Time Course Transcriptomics Data Set**

Kim Kenobi

**University of Nottingham, Nottingham, UK**

The Centre for Plant Integrative Biology (CPIB) at the University of Nottingham have collected a time-course transcriptomics data set for *Arabidopsis* roots following application of auxin. The data were collected on wildtype and *arf7arf19* plants. We consider a regression approach using a basis of cubic splines (smooth curves) to shed light on the genes that are involved in the *Arabidopsis* response to auxin.

We fitted a linear model using the regression splines for each gene across all of the experimental conditions (wildtype control, wildtype with auxin, *arf7arf19* control, *arf7arf19* with auxin). In the experiment, ARF7 does not show any significant change on application of auxin, which is to be expected since it is known to be constitutively expressed. However ARF19, which is known to play a key role in the *Arabidopsis* auxin response, shows a distinctive pattern, with a sharp rise followed by a tailing off in the wildtype on application of auxin.

We used the fitted coefficients (together with the associated covariance structure) from the regression analysis to define a variety of metrics for comparing the profiles of different genes. We focussed on ARF19 as a central gene in the auxin response, and looked for other genes whose profiles are similar to that of ARF19 in terms of the different metrics we used.

When we used different metrics, some genes appeared repeatedly as being similar to ARF19 in their response to auxin. These included genes that are annotated in the TAIR10 classification as being involved in the auxin response, such as auxin-inducible 2-11 and indole-3-acetic acid inducible 11, which supports the idea that our method is identifying genes that are genuinely involved in the *Arabidopsis* response to auxin treatment.

This work is generating new hypotheses about the genetic basis of the *Arabidopsis* auxin response in roots, which we will go on to test biologically.

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## **204 Abstract Withdrawn**

## **205 Leaf Senescence Database: a comprehensive resource for plant leaf senescence research**

*Zhonghai Li<sup>1</sup>, Xiaochuan Liu<sup>1,2</sup>, Jinying Peng<sup>1</sup>, Wenyang Li<sup>1</sup>, Wenrong He<sup>1</sup>, Jingchu Luo<sup>1,2</sup>, Hongwei Guo<sup>1</sup>*

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Leaf senescence has been recognized as the last phase of plant development, a highly ordered process regulated by genes known as senescence associated genes (SAGs). Leaf senescence is induced as part of plant development but can also be prematurely induced as a result of environmental changes or harvesting. Premature senescence leads to reduced yield and quality of crops and this is likely to be of increasing concern in times of climate change and parallel population growth. Therefore, it might be expected that more research should be focused on this important topic in the future as increased understanding of the senescence traits will lead to the development of crops with improved yield, stress tolerance and shelf life.

By broad literature survey, we have developed a leaf senescence database (LSD, <http://www.eplantsenescence.org/>) that contains a total of 1145 senescence associated genes (SAGs) from 21 species. These SAGs were retrieved based on genetic, genomic, proteomic, physiological or other experimental evidence, and were classified into different categories according to their functions in leaf senescence or morphological phenotypes when mutated. We made extensive annotations for these SAGs by both manual and computational approaches, and users can either browse or search the database to obtain information including literatures, mutants, phenotypes, expression profiles, miRNA interactions, orthologs in other plants and cross links to other databases. We have also integrated a bioinformatics analysis platform WebLab into LSD, which allows users to perform extensive sequence analysis of their interested SAGs. The SAG sequences in LSD can also be downloaded readily for bulk analysis.

We believe that the LSD contains the largest number of SAGs to date and represents the most comprehensive and informative plant senescence-related database, which would facilitate the systems biology research and comparative studies on plant aging. We hope that LSD may become a useful resource for the research community, especially in the study of regulating mechanism of plant leaf senescence, but can also be useful for crop science and breeding in agriculture.

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## **206 An Incoherent Feed Forward Loop Defines Discrete Expression Patterns During Early *Arabidopsis thaliana* Trichome Development**

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**<sup>1</sup>Dept. Molecular Biology, The Ohio State University, Columbus, OH, USA, <sup>2</sup>Plant Biotechnology Center, The Ohio State University, Columbus, OH, USA**

The development of trichomes from pluripotent epidermal cells in *Arabidopsis thaliana* provides a powerful model for the study of gene regulatory networks involved in plant cell differentiation. We have previously shown that the R2R3-MYB protein GL1 and bHLH transcription factor GL3 together directly regulate approximately 20 genes involved in trichome initiation, including the WRKY transcription factor gene *TTG2* [1, 2]. To better establish the trichome initiation network, we combined literature-based ChIP analyses with ChIP-chip experiments using *TTG2*-GFP transgenic plants and GFP antibodies. Among approximately 370 genes identified as

TTG2 direct targets, many were shared by GL3 (with or without GL1). In addition to the TTG2 genome-wide location, gene expression experiments indicated that several GL3/TTG2 common targets showed opposite regulation by GL3 and TTG2. Subsequent expression analyses conducted on mutant strains suggest that GL3, TTG2, and target genes participate in an Incoherent Feed Forward Loop (I-FFL) in which the target gene is up-regulated by GL3 and down-regulated by TTG2. To explore the I-FFL on GL3 and TTG2 gene regulation, we took advantage of dexamethasone (Dex) induced system by making transgenic plants that express GL3 fused to glucocorticoid receptor (GR) in *gl3 egl3* double mutants or *gl3 egl3 ttg2* triple mutant. Trichome phenotypes of GL3-GR in *gl3 egl3 ttg2* triple mutant after Dex induction mimicked *ttg2* mutant phenotype, confirming that TTG2 is involved in trichome maturation but not initiation. We propose that it is this network architecture that is responsible for the narrow window of gene expression for several GL3 targets that occurs at early stages of trichome development.

[1] Morohashi, et al., (2009). PLoS Genet 5(2): e1000396.

[2] Morohashi, et al., (2007). Plant Phys 145: 736.

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**207 Share And Visualize Genome-Scale Data Sets Using Integrated Genome Browser**

*David Norris<sup>3</sup>, Hiral Vora<sup>2</sup>, Alyssa Gulledge<sup>1</sup>, Michael Lawrence<sup>4</sup>, Lance Froehman<sup>4</sup>, Ann Loraine<sup>2</sup>*

<sup>1</sup>**University of North Carolina at Charlotte, <sup>2</sup>Dept. of Bioinformatics and Genomics, UNC Charlotte, <sup>3</sup>Dept. of Computer Science, UNC Charlotte, <sup>4</sup>Genentech Research and Early Development**

Integrated Genome Browser is a fast, flexible, and free Java-based genome visualization tool used by more than 8,000 scientists worldwide to visualize genome-scale data sets, including data from next-generation sequencing experiments, tiling arrays, and EST/genome sequencing projects. IGB is 100% open source and is available from [www.bioviz.org/igb](http://www.bioviz.org/igb) or from Sourceforge.net as part of the larger Genoviz project. IGB is easy to download and install and runs on any platform. Major features of IGB include: ability to view RNA-Seq, ChIP-Seq, and tiling array data aligned onto a reference genome; real-time animated zooming; incremental or whole-chromosome data loading; convenient access to public data sets from TAIR and Ensembl; linkouts to external databases; and native support for widely used genomics file formats without the need for conversion. IGB is convenient for data providers as well as users. Groups doing genome or transcriptome sequencing projects can easily display and share their data using the simple file-based IGB QuickLoad system. The Genoviz project also includes a library of graphical visualization components called the Genoviz SDK that developers can use to create and deploy their own visualization software for users. The IGB project provides on-line instructional videos, example data sets, a comprehensive User's Guide for IGB, and tutorials focusing on visualization and analysis of high throughput sequencing data. These resources provide opportunities for plant scientists to evaluate how well new sequencing technologies will address their scientific questions of interest.

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**208 Robustness of Crosstalk from Transcriptomics Data in *Arabidopsis thaliana***

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The levels of cellular organization, from gene transcription to translation to protein-protein interaction and metabolism, operate via tightly regulated mutual interactions facilitating organismal adaptability and various stress responses. Characterizing the mutual interactions between genes, transcription factors, and proteins involved in signaling, termed crosstalk, is therefore crucial for understanding and controlling cell's functionality. Based on the type of data used in the analysis, the existing methods for identifying crosstalk can be divided into two groups: (1) proteomics-based, relying on integration of protein-protein interaction data with existing pathway information and (2) transcriptomics-based, employing high-throughput transcriptomics data sets from different conditions. Here we design, analyze, and test crosstalk detection algorithms that employ only transcriptomics data and, thus, cope with the lack of detailed protein-interaction networks for *Arabidopsis thaliana* as model plant species. Our network-based approach for detection of signaling crosstalk from transcriptomics data is independent of any hidden parameters and exhibits robustness of the obtained results. In addition, we analyze the state-of-the art methods which employ a bioclustering-based approach and assess their robustness with respect to the obtained genes putatively involved in crosstalk. We used transcriptomics data sets from *Arabidopsis thaliana* under 31 different experimental conditions: 5 nitrate, 4 sulfur, 2 iron and 20 hormone experiments to demonstrate that the bioclustering approach lacks robustness as: (1) the order of input signals is neglected and (2) the bioclustering-based method depends on several "hidden" parameters whose effect on the final outcome of the analysis seems difficult to estimate.

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**209 Expressolog Identification in Plant Species**

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In order to identify "expressologs" (orthologs exhibiting the highest expression profile ranking) among a variety of plant species, bioinformatic methods were used in order to first identify sequence orthologs and subsequently to rank these orthologs based on expression profile similarity.

Analyses conducted on these data suggested that expressologs exhibited greater functional equivalency. A comparison of drought response in *A. thaliana* and *Populus* showed that expressologs exhibited a higher correlation when computed using stress data as opposed to developmental data. This suggested that the use of condition-specific data sets is more appropriate when examining specific conditions.

Analysis was conducted in order to investigate the hypothesis that neutral evolution was a predominant factor in gene expression divergence. Some evidence was found for selection acting on expression pattern maintenance. Further analysis will be required in order to confirm the type of selection acting to maintain expression patterns across species.

We have created a tool, called the Expressolog Tree Viewer for the Bio-Array Resource at <http://bar.utoronto.ca>, for

exploring expressologs in 6 plant species, along with an intuitive interface for visualizing both sequence and expression pattern similarity. In addition to our Arabidopsis and poplar eFP Browsers, new eFP Browsers were also created to aid in the exploration of gene expression data from the 4 other species in this study.

## **210 High-Throughput Systematic Genetic Interaction Analysis of MAPK Signaling in Arabidopsis**

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**<sup>1</sup>Genome Center of Wisconsin and Department of Horticulture, University of Wisconsin-Madison, Madison, WI, USA, <sup>2</sup>Department of Botany, University of Wisconsin-Madison, Madison, WI, USA**

Well-developed genetic resources have made reverse genetic analysis a standard method for studying gene function in Arabidopsis. In order to take reverse-genetics to the next level, we have pioneered the systematic generation and analysis of large numbers of double mutant plants using a novel high-throughput pipeline developed in our laboratory. Our genetic interaction analysis includes two major parts: (1) a systematic double mutant producing pipeline, and (2) high-throughput quantitative phenotypic analysis.

We are focusing on exploring genetic interactions within MAP kinase (MAPK) pathways. Therefore, our target library includes 76 single-mutant lines for all of the Arabidopsis genes expected to be involved in MAPK signaling for which suitable T-DNA alleles are available. To query this target library for interactions, we chose mutants the three most well-studied MAPKs: *MPK3*, *MPK4*, and *MPK6*. We systematically created double-mutants between each query mutant and all the members of the target library. Furthermore, we applied this high-throughput pipeline to produce all possible double mutants within the MAPK gene family. There are 13 suitable T-DNA insertion single mutant lines in this gene family which can generate 78 different double-mutants. The resulting double-mutant lines will be screened for quantitative and qualitative phenotypic differences to search for evidence of genetic interaction. For a total of 306 possible combinations, we have to date successfully generated 286 double-mutants and observing 2 genetic interactions based on preliminary observations.

In order to increase the possibility of observing genetic interactions, we developed a high-throughput quantitative phenotypic analysis system. A computer program has been designed to automatically measure Arabidopsis seed size, root length, and hypocytol length from seedling images collected with a high resolution flat-bed scanner. Using this strategy to gather and analyze phenotypic data in a high-throughput fashion, we will screen all 306 double mutants for phenotypes differing from either single mutant parent, revealing possible genetic interactions between the genes.

## **211 A Gene Regulatory Network for Vascular Development and Secondary Cell Wall Biosynthesis in the Arabidopsis Root**

Mallorie Taylor-Teeple<sup>1</sup>, Sebastian Ahnert<sup>2</sup>, Allison Gaudinier<sup>1</sup>, Siobhan Brady<sup>1</sup>

**<sup>1</sup>University of California, Davis, <sup>2</sup>Kings College, University of Cambridge, UK**

The maturation of xylem tissue in the plant *Arabidopsis thaliana* relies on the timely and successive execution of several developmental programs including protoxylem and metaxylem cell specification as well as metabolic pathways involved in secondary cell wall synthesis. Using a high-throughput yeast-one-hybrid system, we present a gene regulatory network that coordinates xylem differentiation and secondary cell wall biosynthesis. This network reveals a novel relationship between the cell cycle, two developmental programs involved in metaxylem specification and the phenylpropanoid pathway. This network also reveals that a number of genes encoding enzymes involved in the phenylpropanoid pathway are regulated directly by a large number of transcription factors known to control secondary cell wall biosynthesis as well as transcription factors involved in response to biotic and abiotic stresses. This highlights the redundancy of the network surround secondary cell wall biosynthesis as well as the dual role of the phenylpropanoid pathway.

Along with data from the *Arabidopsis* root spatiotemporal map, this set of novel interactions provides novel insights into how vascular development is regulated both spatially and temporally.

**212 Suppression of Defense Responses in Distantly Related Plants by Homologous RXLR Effectors**

*Ryan Anderson<sup>1</sup>, Devdutta Deb<sup>1</sup>, Megan Casady<sup>1</sup>, Rachel Fee<sup>1</sup>, Brett Tyler<sup>2</sup>, John McDowell<sup>1</sup>*

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Diverse pathogens secrete effector proteins that enter plant cells to manipulate host cellular processes. Bioinformatic analysis suggests that the genome of *Hyaloperonospora arabidopsis* (*Hpa*), the downy mildew of *Arabidopsis thaliana*, encodes at least 130 RXLR effector proteins. The majority of predicted *Hpa* effector genes share no homologs in other oomycete species. However, a small subset of predicted *Hpa* effectors are conserved between *Hpa* and related species in the *Phytophthora* genus. Here, we describe functional characterization of the *Hpa* effector *Ha96* and a homologous gene, *Ps163*, from the soybean pathogen *Phytophthora sojae*. Both effectors are induced during the host-pathogen interaction. Furthermore, *Ha96* and *Ps163* carry a functional cell entry motif that is sufficient for translocation into the host cell. Transient assays in soybean indicate that both genes suppress diverse elicitors of programmed cell death, including the *P. sojae* elicitor Avr4/6. Transient expression of *Ps163* in *Nicotiana benthamiana* triggers a cell death response that requires RAR1 and Hsp90-1. Stably transformed *Arabidopsis* plants expressing either *Ha96* or *Ps163* exhibit suppression of PAMP triggered immunity (PTI) during bacterial infection. The *Ha96* and *Ps163* transgenes suppress RPP4-mediated resistance to avirulent *Hpa* EMOY2 and basal resistance to virulent *Hpa* EMC05. Quantitative real-time PCR indicates that several defense genes are suppressed in the effector expressing plants during the *Hpa* EMOY2/ Col-0 incompatible interaction. These experiments demonstrate that homologous effectors from related oomycetes suppress defense mechanisms in distantly related plant species.

**213 Two-Component Elements Mediate Interactions Between Cytokinin and Salicylic Acid in Plant Immunity**

*Cris Argueso<sup>1,2</sup>, Fernando Ferreira<sup>2</sup>, Petra Epple<sup>2</sup>, Jennifer To<sup>2</sup>, Claire Hutchison<sup>2</sup>, G. Eric Schaller<sup>3</sup>, Jeff Dangl<sup>2</sup>, Joseph Kieber<sup>2</sup>*

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Plants defend themselves against pathogens through a two-tiered immune system. In the first layer of defense, plants perceive microbial-associated molecular patterns and activate a suite of responses known as pattern-triggered immunity (PTI) that is sufficient to halt the growth of non-pathogenic microbes. PTI also contributes to limiting the growth of virulent microbes. Cytokinins are plant hormones involved in the regulation of many aspects of plant development and responses to the environment. In *Arabidopsis*, cytokinin signaling involves a phosphorelay pathway similar to two-component response systems used by bacteria and yeast to perceive and react to various environmental stimuli. In this study, we asked whether cytokinin and components of cytokinin signaling contributes to limiting the growth of a pathogenic isolate of the oomycete *Hyaloperonospora arabidopsis* (*Hpa*). We demonstrate that treatment of *Arabidopsis* plants with cytokinin regulates PTI responses to *Hpa*. We show that high concentrations of cytokinin lead to decreased susceptibility through a process that requires salicylic acid (SA) accumulation and activation of PTI defense gene expression; surprisingly treatment with lower concentrations of cytokinin results in increased pathogen growth, mediated by negative regulation of SA on cytokinin signaling. These functions for cytokinin in plant immunity require an intact host cytokinin phosphorelay system, and are mediated in part by type-A ARR, which act as negative regulators of basal and PTI-induced gene expression. Our results support a model in which cytokinin up-regulates defense responses via an elevation of SA function, which in turn feedback-inhibits cytokinin signaling.

**214 *Arabidopsis thaliana* and involved in the defense response against *Pseudomonas syringae***

*Grace Armijo, Consuelo García, Aldo Seguel, Luis Leon, Paula Salinas, David Leiva, Loreto Holguíne*

**P. Universidad Católica de Chile, Santiago, Chile**

Plants have evolved complex systems to respond and adapt to stressful conditions. The control of these mechanisms is mainly mediated by plant hormones, such as salicylic acid (SA). The role of SA has been mainly characterized in the defense response induced by biotrophic pathogens that are specifically recognized by the plant.

Previously in our laboratory, using *Arabidopsis thaliana* as a model, we identified a group of genes activated by SA, in which the *LLP* gene (coding for a lectin-like-protein) has the highest level of activation. *LLP* protein shows similarity to proteins of the legume lectin family and has not an associated biological function. *LLP* is activated by inoculation with the avirulent bacteria *Pseudomonas syringae* pv. tomato AvrRpm1 (Pst AvrRpm1) by a SA-dependent pathway.

To evaluate the role of *LLP* in the defense response, we isolated and characterized a homozygous mutant line null for *LLP*. In parallel, we developed transgenic lines overexpressing *LLP* fused to c-Myc epitope or to GFP protein. Our results of subcellular localization, by using confocal microscopy, indicate that *LLP*-GFP is located in the plasma membrane of the plant cell. Then we made a loss or gain of function analysis, by evaluating the proliferation of *Pseudomonas* in the null and overexpressor lines. We determined that *LLP* is involved in the defense response to PstAvrRpm1, reducing bacterial proliferation and increasing cell death in infected tissues. Currently we are investigating the specific role of this gene in the defense response.

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**215 Comparative Analysis of AvrB and AvrRpm1 Recognition in Arabidopsis and Soybean***Tom Ashfield, Thomas Redditt, Andrew Russell, Ryan Kessens, Natalie Rodibaugh, Roger Innes***Indiana University, Bloomington, IN, USA**

Resistance (R) genes that mediate recognition of the *Pseudomonas syringae* effector proteins AvrB and AvrRpm1 are found in both Arabidopsis and soybean. In Arabidopsis, recognition of both pathogen proteins is accomplished by a single R protein, RPM1. RPM1-mediated resistance relies on a second plant protein, RIN4, which is targeted for phosphorylation in an effector-dependent manner. In soybean, detection of AvrB and AvrRpm1 is mediated by distinct R genes, *Rpg1b* and *Rpg1r*, respectively. We wish to understand the evolutionary relationship between the soybean R genes and how they manage to distinguish between AvrB and AvrRpm1. *Rpg1b* and *Rpg1r* map to the same complex NB-LRR cluster. We have previously cloned *Rpg1b* and have now identified a strong candidate for *Rpg1r* (*cRpg1r*), both of which are CC-NB-LRR type genes. Several gene conversion events have been detected in *Rpg1b*, one of which has introduced the NB-ARC region from *cRpg1r*, or a closely related parologue. Analysis of NB-LRR paralogues from the *Rpg1* cluster has identified residues under positive selection. While a bias for location within the predicted solvent exposed face of the LRRs was observed, sites under selection were also identified elsewhere, including within the NB-ARC. Comparative modeling of the tertiary structure of the *Rpg1b* NB-ARC is being used to gain insights into the possible significance of these sites. Four RIN4 homologues (gmRIN4s) have been identified in soybean and these are co-orthologous with the Arabidopsis gene. All four gmRIN4s are cleavable by AvrRpt2, which also strongly suppresses AvrB and AvrRpm1 recognition in soybean. To varying degrees, the gmRIN4s interact with AvrB in Y2H assays. The putative role of the gmRIN4s in the ability of soybean to distinguish between AvrB and AvrRpm1 is being investigated further by targeting family members using VIGS and by transient co-expression with *Rpg1b* and *cRpg1r*.

**216 Characterization of Candidate Programmed Cell Death Inducers in Arabidopsis***Shawn Bachan<sup>1</sup>, Shisong Ma<sup>1</sup>, Matthew Porto<sup>1</sup>, Michael Snyder<sup>2</sup>, S.P Dinesh-Kumar<sup>1</sup>***<sup>1</sup>University of California at Davis, Davis, CA, USA, <sup>2</sup>Stanford University, Stanford, CA, USA**

Plants have developed sophisticated mechanisms of pathogen recognition, and in turn will specifically impede systemic infection by producing visible, restricted programmed cell death (PCD) termed the hypersensitive response (HR). Although HR-PCD is morphologically well characterized, the regulators that control the initiation, execution, and termination of PCD are not well understood. Under the Arabidopsis 2010 grant, we have to-date transiently overexpressed and purified over 14,000 *Arabidopsis* proteins in *Nicotiana benthamiana* and have generated high-density protein microarrays for the high-throughput investigation of molecular interactions. Interestingly, during this process, we have uncovered a subset of Arabidopsis open reading frames (ORFs) that induce HR-like PCD when overexpressed in *N. benthamiana*. We will present our data on the characterization of these candidate PCD inducers. An in-depth characterization of these potential positive regulators of PCD will greatly advance our knowledge of PCD pathway(s) and their integral role during the plant defense response.

**217 Investigating the Effector Complement of the Arabidopsis Obligate Biotrophic Pathogen, *Albugo laibachii* Nc14***Kate Bailey, Torsten Schultz-Larsen, Eric Kemen, Ariane Kemen, Alexandre Robert-Seilaniantz, Anastasia Gardiner, Jonathan Jones***The Sainsbury Laboratory, Norwich**

White blister rust of brassica sp. caused by the obligate biotrophic oomycete pathogens of the *Albuganacea* is a commercially important disease causing serious crop losses worldwide. *Albugo laibachii* (*Al*) Nc14, isolated in Norwich, is the first fully sequenced member of the *Albuginaceae* (Kemen et. al. in press) and, as a pathogen of Arabidopsis provides an interesting tool for the study of host-pathogen interactions within the *Al* clade. Arabidopsis accessions show variation for resistance to *Albugo* sp and several R genes have been cloned and characterised, however the effector complement of these pathogens remains unstudied. Analysis of the *Al* Nc14 secretome showed 16 genes encoding putative RXLR-type effector proteins, which have been demonstrated to be effectors in other oomycete pathogens. Further mining of the secretome revealed a second class of putative secreted effector proteins containing a conserved N-terminal CHXC motif. In addition, homologues of the *P. infestans* Crinkler effectors have also been identified. Here, we describe the selection and analysis of candidate effectors utilizing the Effector Detector Vector (EDV) delivery system. We show that both RXLR and CHXC candidate effectors are capable of enhancing virulence of plant pathogenic bacteria when expressed in this heterologous system, and subsequent downstream analysis of these candidates is described.

**218 Abstract Withdrawn**

**219 UPR Signaling Pathway is Important in the Establishment of Defense Response in *Arabidopsis thaliana****Francisca Blanco, Adrián Moreno, Ariel Orellana***Núcleo Milenio en Biotecnología Celular Vegetal. FONDAP Center for Genome Regulation. Centro de Biotecnología Vegetal. Universidad Andrés Bello, Santiago, Chile.**

Salicylic acid (SA) is a key hormone in the defense response in plants and such response requires the synthesis of glycosylated proteins (PRs). The transcriptional activation of *PR* genes depends of the interaction between the coactivator NPR1 and TGAs bZIP transcription factors. Moreover it has been described that the exogenous application of SA activates genes related to endoplasmic reticulum stress (UPR, unfolded protein response) and this requires the NPR1 coactivator. Interestingly the transcription factors involved are not known. There are two transcription factors related to UPR in plants, bZIP28 and bZIP60.

Based on this we proposed that the transcriptional activation of UPR genes required during the defense response is regulated by the interaction between bZIP28 and bZIP60 with NPR1.

We evaluate the involvement of bZIP28 and bZIP60 and the importance of NPR1 in the expression of UPR genes this in response to SA and tunicamycin treatments. Using qRT-PCR we analyzed the expression of UPR marker genes in WT and mutants plants (*bzip60*, *bzip28* and *npr1-1*). Also we analyzed the phenotype of *bZIP60* and *bZIP28* mutant plants during the infection with pathogens related to the basal and induced defense responses.

We achieve to identify different group of genes considering the dependence of the transcription factors analyzed. Interestingly the absence of the bZIP60 and bZIP28 genes renders the plants more susceptible to biotic stress comparing with wild type plants.

These results suggest a complex regulatory crosstalk between the classic components described for defense response and the UPR signaling pathways. Interestingly this represents the discovery of new components of the defense response in *Arabidopsis thaliana*.

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**220 Proteic Signals Involved in Systemic Acquired Resistance (SAR) in Plants***Heiko Breitenbach<sup>1</sup>, Hakan Sarioglu<sup>2</sup>, Thomas Colby<sup>3</sup>, Lucia Jorda<sup>3</sup>, Jane Parker<sup>3</sup>, A. Corina Vlot<sup>1,3</sup>*

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Systemic acquired resistance (SAR) plays a fundamental role in protecting plants against various diseases. SAR is a state of heightened defense that provides long-lasting, broad spectrum resistance to microbial pathogens and is activated systemically following a primary infection. Prior to SAR establishment, mobile signals are transported from the infected site through the phloem to the systemic leaves. SAR-like disease resistance can be induced by over expression of the bacterial effector AvrRpm1 from a dexamethasone-inducible transgene. Thus induced resistance reduces growth of virulent bacteria in systemic, non-avrRpm1-expressing tissues. So far, salicylic acid (SA), methyl salicylate (MeSA), proteins/peptides and lipid(-derived) compounds are proposed to have a critical role during SAR long-distance signaling. Furthermore, ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) encodes one of the main regulators of SA signaling and is essential for SAR signal generation and/or transmission (Jorda, Vlot, and Parker, personal communication). By comparing the molecular profile of AvrRpm1-expressing wild type plants against similar extracts from eds1 mutant plants we aim to identify new proteic SAR signals or signaling regulators. To this end, proteins were isolated from the apoplast of *Arabidopsis* plants expressing AvrRpm1. 2D-gel-analysis and subsequent protein identification by MALDI-TOF-MS led to the identification of seven proteins that reproducibly accumulate in the apoplast of AvrRpm1-expressing plants in an EDS1-dependent manner. Complementary direct LC-MS/MS (Orbitrap) analysis of 1D-gel resolved and trypsin-digested apoplast extracts led to the identification of two additional potential SAR involved proteins. These proteins will be characterized further for a role in SAR in plants over and under expressing the corresponding genes.

**221 Insect eggs suppress plant defense against herbivores in *Arabidopsis****Friederike Bruesow, Caroline Gouhier-Darimont, Philippe Reymond***University of Lausanne, Lausanne, Switzerland**

Insect egg deposition represents a threat for a plant, as larvae hatching from the egg will ultimately feed on their host. We found that oviposition by the butterfly *Pieris brassicae* triggers cellular and molecular changes that are similar to the changes caused by biotrophic pathogens in *Arabidopsis thaliana* and that the plant defense signal salicylic acid (SA) accumulates at the site of oviposition. This is unexpected since the SA pathway controls the defense against fungal and bacterial pathogens whereas it negatively interacts with the jasmonic acid (JA) pathway, which is crucial for the defense against herbivores. Application of *P. brassicae* or *Spodoptera littoralis* egg extract onto leaves reduced the induction of insect-responsive genes after challenge with caterpillars, showing for the first time that egg-derived elicitors suppress plant defense via the SA pathway. Consequently, larval growth of the generalist herbivore *S. littoralis*, but not of the specialist *P. brassicae*, was significantly higher on plants treated with egg extract than on control plants. These data revealed an intriguing facet of the crosstalk between SA- and JA-signaling pathways and suggest that insects have evolved a way to suppress the induction of defense genes by laying eggs that release elicitors. We are currently studying the nature of these elicitor(s).

## 222 The *Xanthomonas* Type III Effector XopD Targets the Arabidopsis Transcription Factor AtMYB30 to Suppress Plant Defence

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Plant and animal pathogens inject type III effectors (T3Es) into host cells to suppress host immunity and promote successful infection. XopD, a T3E from *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), has been proposed to promote bacterial growth by targeting plant transcription factors and/or regulators. Here, we show that XopD from the B100 strain of *Xanthomonas campestris* pv. *campestris* (*Xcc*) is able to target AtMYB30, a transcription factor that positively regulates Arabidopsis defence and associated cell death responses to bacteria. XopD specifically interacts with AtMYB30, resulting in repression of its transcriptional activity and suppression of the Arabidopsis defence response. We therefore propose that sequestration of AtMYB30 by XopD illustrates a strategy developed by *Xanthomonas* to subvert plant defence and promote bacterial growth. Finally, our results support the notion that XopD-dependent bacterial strategies deployed to suppress plant disease resistance may differ depending on the *Xanthomonas*/host plant interaction.

## 223 Investigating the Association Between Age-Related Resistance and the Transition to Flowering in *Arabidopsis thaliana*

*Philip Carella, Marisa Melas, Daniel Wilson, Robin Cameron*

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The Age-Related Resistance (ARR) response in *Arabidopsis thaliana* is a developmentally regulated disease resistance pathway. As the plant ages, it becomes increasingly resistant to normally virulent pathogens including the bacterium *Pseudomonas syringae* and the oomycete *Hyaloperonospora arabidopsidis*. A 10- to 100-fold reduction in pathogen growth is observed in mature ARR-competent compared to young ARR-incompetent plants (3 weeks post germination [wpg]). The onset of ARR-competence has been associated with the transition to flowering, such that plants grown in short day conditions flower and display ARR later at 6 wpg, while long day grown plants flower and display ARR earlier at 4 wpg. In *Arabidopsis*, several converging and overlapping pathways control the transition from the vegetative to reproductive phase, including the photoperiod pathway (changes in day length), autonomous pathway (independent of environment), gibberellic acid pathway (hormonal), vernalization (exposure to cold), and more recently, the aging pathway (age-dependent). We assayed ARR in short day grown early and late flowering mutants (*svp*, *ld*, *co*) affecting various flowering pathways to determine if ARR competence is associated with the transition to flowering or with developmental age as measured by rosette leaf number. Our preliminary data suggests that in short day grown plants, the transition to flowering is not an essential component for ARR competence. Instead it appears that plants reach a certain developmental age, in terms of rosette leaf number, before they become competent for the ARR response.

## 224 Impact of Increased Host Ploidy on the Sustained Growth and Reproduction of an Obligate Biotroph, the Powdery Mildew *Golovinomyces orontii*

*Divya Chandran, Joshua Rickert, Mary Wildermuth*

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*Golovinomyces orontii* infection site-specific profiling identified *Arabidopsis* genes and associated cellular processes with altered expression during the growth and reproductive phase of a compatible powdery mildew (PM) and suggested host cell cycle modulation at the infection site. Host endoreduplication, not cell division, was impacted at the PM infection site at 5 days post infection (dpi). Host ploidy increased in mesophyll cells underlying the haustorium-containing epidermal cell but not in cells distal to the infection (Chandran *et al.* 2009). Induced host endoreduplication was first observed at 5 dpi, the external fungal growth and reproduction phase, and not at earlier time points associated with fungal establishment (e.g. penetration and/or haustorial development). Mutants in *MYB3R4*, a known transcriptional activator of mitosis, were compromised in induced endoreduplication. While *MYB3R4* expression was up-regulated at the infection site, known targets of *MYB3R4* were down-regulated, suggesting that it may have dual functionality, in this case acting as a regulator of induced endoreduplication. Expression profiling on mesophyll cells at the infection site in *myb3r4* and wild type plants allows for identification of *MYB3R4* targets and host processes/components whose expression is impacted by increased ploidy. In *myb3r4* mutants, PM growth and reproduction is reduced, with no associated cell death. An assessment of similar published mutants with enhanced PM resistance found that *pmr5* (Vogel *et al.* 2004) is deficient in PM-induced endoreduplication. Localized, induced, host endoreduplication at/adjacent to nutrient exchange sites has been observed in other plant-biotrophic interactions (endosymbiotic rhizobia and parasitic root nematodes), with reduced host endoreduplication resulting in decreased biotroph growth and/or development in these systems. This suggests induced endoreduplication may be a common strategy employed by obligate biotrophs to support the enhanced metabolic demands associated with these interactions (Wildermuth 2010). Thus, our findings may fuel translational research of agronomic and ecological import.

## 225 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* and *sfd1* 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 a 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 accumulation and 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.

## 226 A Receptor-like Cytoplasmic Kinase Is Involved In The Activation of A Plant Innate Immune Receptor

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Plants have evolved sophisticated surveillance systems to recognize pathogen effectors delivered into host cells. It remains largely unknown how resistant proteins perceive virulent effectors. RPM1 is an NB-LRR immune receptor that recognizes the *Pseudomonas syringae* effectors AvrB and AvrRpm1. Both effectors associate with RIN4, an immune regulator, and it has been hypothesized that RPM1 recognizes the posttranslational modification of RIN4. Using a co-immunoprecipitation approach, we identified a set of RIN4 interacting proteins. RIPK, one of the interacting proteins, can directly interact with AvrB and RIN4. RIPK can directly phosphorylate RIN4 at amino acids T21, S160, and T166. RIN4 phosphorylation at T166 is specifically induced by AvrB and AvrRpm1, leading to the activation of the RPM1 immune receptor. Furthermore, *ripk* knockout lines display reduced RPM1-mediated defense responses. These data indicate that AvrB may specifically target RLCK complexes or mimic the activity of host kinases to induce RIN4 phosphorylation, leading to the activation of immune responses. Additional results analyzing the importance of this kinase and other closely related kinases during immune signaling will be reported.

## 227 RESISTANCE TO FUSARIUM OXYSPORUM 3 (RFO3) is an S domain kinase

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The filamentous mold *Fusarium oxysporum* (FO) is ubiquitously found in soil and within plant roots without causing disease. However, rare host-specific lineages of FO instigate wilt disease in over a hundred cultivated plants (including cotton and date palms here in Southern California).

Our lab is using an experimental pathosystem to explore the molecular nature of plant host resistance and fungal virulence. Despite the economic impact, little is known about plant wilt diseases, and soil-borne infectious diseases in general, beyond histological and physiological descriptions.

At least six *RFO* loci quantitatively contribute to a remarkable diversity in FO resistance that is observed among wild accessions of *Arabidopsis thaliana*. Thus far, our lab has identified two other *RFO* genes, namely *RFO1* and *RFO2*. *RFO1* is synonymous with *WALL-ASSOCIATED KINASE-LIKE KINASE 22 (WAKL22)* and encodes a receptor like kinase (RLK). *RFO1* is an unusual resistance gene because (i) most RLKs associated with resistance have an extracellular leucine-rich repeat domain (eLRR); and (ii) *RFO1* confers broad resistance to three phylogenetically-distinct FO lineages, while most resistance genes provide lineage-specific resistance.

*RFO2* is synonymous with *A. thaliana RECEPTOR-LIKE PROTEIN 3 (AtRLP3)*. Uncharacteristic for a receptor-like protein that confers resistance, *RFO2*'s eLRR is highly conserved among plants species and related to peptide hormone receptors.

Here, we present our efforts to clone and characterize *RESISTANCE TO FUSARIUM OXYSPORUM 3 (RFO3)*. The candidate gene for *RFO3* is an S domain RLK. In *Arabidopsis*, the S domain kinases form a family of approximately 40 genes of which most have unknown function. However, in Brassica, S domain kinase *S GENE FAMILY RECEPTOR 2 (SFR2)* has been found in concurrence with defense to bacterial pathogen *Xanthomonas campestris* and wound response. Also in Brassica, S domain kinase, *S RECEPTOR KINASE 1 (SRK1)*, is involved in self incompatibility.

**228 An mRNA Export Component Plays a Role in Plant Immunity**Oliver Dong<sup>1</sup>, Hugo Germain<sup>2</sup>, Xin Li<sup>1</sup><sup>1</sup>**Michael Smith Laboratories, the University of British Columbia Vancouver, BC, Canada, <sup>2</sup>Laurentian Forestry Center, 1055, rue du P.E.P.S., C.P. 10380, Stn. Sainte-Foy, Quebec, Canada**

Effector-triggered immunity (ETI) in plants is largely mediated by Resistance (R) proteins that specifically recognize effectors released by pathogens (Avr proteins). Upon this R - Avr recognition, the immunity in the host is turned on through a series of downstream events leading to the induction of the expression of the defense response genes. A dominant mutation in an R gene *SNC1* results in constitutive high level of defense response gene expression without the presence of the pathogen effectors. This renders the mutant with enhanced disease resistance and when homozygous, dwarfism and curly leaves due to fitness cost. The unique autoimmune phenotypes of *snc1* provides us with a great tool to genetically study ETI. From the Modifier of *snc1* (MOS) screen, we identified MOS11, a nuclear protein required for efficient mRNA export from the nucleus. *mos11* is able to mostly suppress the *snc1* phenotypes. Its human homolog is CIP29, an RNA co-chaperone that facilitate the RNA unwinding activity of the RNA helicase DDX39. The human CIP29 complex involves protein components whose homologs can be identified in Arabidopsis. Through reverse genetics analysis of these Arabidopsis homologs of the human CIP29 complex components, we found that one mutant is able to suppress the *snc1* phenotype in a similar way as *mos11* does. Current work is investigating the detailed phenotypic analysis of this mutant including susceptibility to various pathogens, the subcellular localization of the gene product and its physical interaction with MOS11.

**229 Function of PBS1 Phosphorylation in PAMP Triggered Immunity**Ullrich Dubiella, Roger Innes**Indiana University, Bloomington (IN), USA**

The *Arabidopsis thaliana* protein PBS1 (AvrPphB Susceptible 1) belongs to a subfamily of receptor-like cytoplasmic kinases (RLCK) with 45 members. Cleavage of PBS1 by the bacterial effector protein AvrPphB leads to activation of the CC-NBS-LRR type R-Protein RPS5 and therefore to the induction of the hypersensitive response (HR) (Shao et al, 2003). In addition to PBS1, eight other members of this RLCK subfamily are cleaved by AvrPphB in vivo, among them BIK1(Botrytis-induced kinase 1) and PBL1 (PBS1 like 1) to suppress plant innate immunity (Zhang et al, 2010). PBS1, BIK1 and PBL1 have all been shown to interact with the flagellin-receptor FLS2 and this interaction is diminished after flg22 induction. These findings indicate that this subfamily of RLCKs plays a general role in PAMP induced signaling (Zhang et al, 2010).

Here we show that PBS1 is phosphorylated *in vivo* in a flg22 dependent manner. We will discuss the influence of these phosphorylations on protein stability and their role in innate immunity responses and the HR activation by RPS5.

Shao F, Golstein C, Ade J, Stoutemyer M, Dixon JE & Innes RW (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector. *Science* **301**:1230-1233

Zhang J, Li W, Xiang T, Liu Z, Laluk K, Ding X, Zou Y, Gao M, Zhang X, Chen S, Mengiste T, Zhang Y & Zhou J (2010) Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host Microbe* **7**:290-301

**230 Quantitative Proteomics Reveals Dynamic Changes at the Plant Plasma Membrane during Immune Responses**James Elmore<sup>1,2</sup>, Jun Liu<sup>1,2</sup>, Brett Phinney<sup>1,3</sup>, Gitta Coaker<sup>1,2</sup><sup>1</sup>**University of California at Davis, Davis, CA, USA, <sup>2</sup>Department of Plant Pathology, <sup>3</sup>UC Davis Genome Center Proteomics Core Facility**

Most classes of plant pathogens remain extracellular during their lifecycle. As a result, the plant plasma membrane mediates critical aspects of plant immunity including pathogen recognition, signal transduction, and downstream defense responses. Investigating how the plasma membrane proteome changes during these events will identify novel components of plant defense responses and lead to a better understanding of plant immune signaling. We have used label-free shotgun proteomics to investigate plasma membrane dynamics during effector-triggered immunity (ETI).

Transgenic Arabidopsis plants expressing the bacterial effector AvrRpt2 under the control of a dexamethasone(Dex)-inducible promoter were used to initiate ETI. Expression of the AvrRpt2 protease results in RIN4 cleavage and activation of the disease resistance (R) protein RPS2. Plasma membrane vesicles were isolated 6 hours post-Dex treatment and subjected to gel-enhanced liquid chromatography tandem mass spectrometry (Gel LC-MS/MS) for protein identifications. We employed spectral counting to quantify relative protein abundance between treatments. The QSpec statistical framework was used to assign significance to differentially regulated proteins. Over 2300 proteins were identified across 3 biological replicates and 24% are significantly changing during ETI. Proteins that are up-regulated at the plasma membrane during ETI include proteins involved in membrane scaffolding and transport, primary and secondary metabolism, and known regulators of plant immune responses. Functional validation of significantly differentially regulated proteins is currently underway. These experiments highlight the dynamic nature of the plasma membrane proteome during plant defense responses.

**231 A nuclear kinase is required for plant immunity against *Pseudomonas syringae* pv. *maculicola***

Zhengqing Fu, Rajinikanth Mohan, Shan Zhu, Xinnian Dong

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Plant pathogen *Pseudomonas syringae* pv. *maculicola* relies on the type III secretion system and effectors it injects into host plant to suppress plant innate immunity and to cause diseases. Some of these effectors became recognized by the host plants carrying corresponding resistance genes and are then called avirulent (Avr) proteins. Local infection by pathogen carrying *Avr* genes not only turns on defense by showing hypersensitive response in the local tissue, but also triggers broad spectrum disease resistance throughout the whole plant, a phenomenon called systemic acquired resistance (SAR). Through genetic screens, our lab identified a locus called *NPR1* that is required for SAR. It has been shown that phosphorylation of *NPR1* protein plays an essential role in activating SAR, but the kinase responsible for this phosphorylation has not been found. Here we show that we identified a nuclear kinase that is required for *NPR1* function and SAR. T-DNA insertion knockout plants of this kinase are defective in SAR and *PR* (pathogenesis-related) gene expression, a marker for plant defense. Before treatment by plant hormone salicylic acid, *NPR1* is sequestered in the cytosol as oligomer. After induction by salicylic acid, *NPR1* is reduced into monomer and translocated into nucleus to interact with TGA transcription factors to turn on plant defense. In the mutant plants of this kinase, *NPR1* can not form *NPR1* monomer even after salicylic acid treatment. More importantly, *NPR1* protein is phosphorylated at the predicted phosphorylation site of this kinase after salicylic acid treatment. Currently we are investigating if this kinase phosphorylates *NPR1* directly.

**232 Genetic Approaches to Identify the *Arabidopsis* Virulence Targets of the Bacterial Pathogenicity****Factor HopAM1**Theresa Law<sup>1</sup>, Meredith Horton<sup>2</sup>, Derek Lundberg<sup>1</sup>, Ajay Goel<sup>3</sup>, Chiharu Akimoto-Tomiya<sup>4</sup>, Michael Iakovidis<sup>1</sup>, Jeffery Dangl<sup>1</sup>, Sarah Grant<sup>1</sup><sup>1</sup>University of North Carolina, Chapel Hill, NC, USA, <sup>2</sup>Department of Education Wake Forest University, Winston-Salem, NC, USA, <sup>3</sup>Dupont India, Hyderabad, India, <sup>4</sup>NIAS, Tsukuba, Japan

Type III effectors of Gram-negative bacteria are injected directly into host cells, where they inactivate host pathogen defenses by interacting with host proteins. HopAM1 is a typical type III effector from *Pseudomonas syringae*: it can suppress plant defense responses and render a weakly virulent *P. syringae* strain more virulent. What makes HopAM1 unique among the many type III effectors being studied is a clearly visible HopAM1-dependent phenotype that we have directly related to its virulence function using host genetics. Following EMS mutagenesis, we identified a collection of confirmed *Arabidopsis* mutants that lose the virulence phenotype. We are now mapping the mutations to identify loci responsible for the virulence phenotype. HopAM1 also triggers the hypersensitive response typical of activation of NB-LRR proteins on some *Arabidopsis* ecotypes when delivered from *Pseudomonas* strains. We are mapping loci that segregate between HopAM1-dependent-HR-responsive and non-responsive ecotypes. The proteins that function as intracellular 'receptors' for HR induced by pathogen effectors (NB-LRR proteins) can physically associate with virulence targets of those effectors. Hence, our genetic screens provide two independent approaches to characterize the function of the HopAM1 type III effector in suppressing plant defenses. Identification of the host targets of individual type III effectors like HopAM1 will define essential components of the complex plant defense response.

**233 Nuclear localization of the bacterial effector AvrRps4 is required to induce resistance**Katharina Heidrich<sup>1</sup>, Lennart Wirthmueller<sup>2</sup>, Jane Parker<sup>1</sup><sup>1</sup>Max Planck Institute for Plant Breeding Research, Cologne, Germany, <sup>2</sup>The Sainsbury Laboratory, Norwich, UK

Microbial pathogens rely on effectors for pathogenicity. The phytopathogenic bacterium *Pseudomonas syringae* secretes effector proteins to the host cytoplasm via its type III secretion system. Recognition of effector proteins by plant NB-LRR receptors usually results in localized programmed cell death. *P. syringae* type III effector AvrRps4 is recognized by two *Arabidopsis* TIR-NB-LRR receptors, RPS4 and RRS1. We previously showed that nuclear localization of RPS4 is required for its resistance function but it remained unclear how and where in the host cell AvrRps4 is perceived by its cognate R proteins. To explore in which subcellular compartment AvrRps4 recognition takes place, transgenic *Arabidopsis* lines were generated expressing AvrRps4 fused to a functional or mutated nuclear localization (NLS) or nuclear export signal (NES) and tested for AvrRps4 resistance. Also, *P. syringae* strains expressing AvrRps4-NLS or -NES were tested for induction of resistance by means of restriction of bacterial growth. Our results show that increased export of AvrRps4 from the nucleus fails to induce a full defense response whereas AvrRps4 targeted to the nucleus triggers resistance. Collectively, the data suggest that at least one essential step of RPS4/RRS1-mediated AvrRps4 recognition occurs in the nucleus. Interestingly, AvrRps4 targeted to the nucleus induces resistance but fails to elicit a host cell death response. The restriction of bacterial multiplication can thus be uncoupled from induction of cell death.

**234 Complex Regulation of the *R* Gene *SNC1* Revealed by *bon1* Enhancers and Suppressors**

Mingyue Gou, Zhilong Bao, Zhenying Shi, Donglei Yang, Jian Hua

Cornell University

The NB-LRR gene *SNC1* is negatively regulated by the evolutionarily conserved *BON1* gene, and the activation of *SNC1* in *bon1* leads to constitutive defense responses. To understand how *R* genes are normally repressed, we characterized enhancers and suppressors of the *bon1* mutants. Here we report the identification of *cpr30* as an enhancer and *mos1* as a suppressor of *bon1*, revealing complex regulation on the *SNC1* activity. *CPR30* encodes an F-box protein implicated in protein degradation, and the *cpr30* mutant has constitutive

defense similarly to the *bon1* mutant. We found that *cpr30* enhances the *bon1* phenotype and the loss of *SNC1* function suppressed *cpr30* totally and *cpr30 bon1* largely. The accumulation of *SNC1* protein appears to be affected by the expression level of CPR30, suggesting a regulation of the *SNC1* protein stability by CPR30. We also identified *sbo3*, a suppressor of *bon1* as a mutant of *MOS1* (Modifier of *snc1-1*). A few putative suppressors of *bon1* were subsequently identified through analyzing *MOS1* related genes. The identities of these genes indicate that a transcriptional regulation of *SNC1* at the chromatin level is critical for its activation and repression. Thus the activity of *SNC1* is under tight control at multiple levels.

### **235 Characterization of hybrid necrosis in *Arabidopsis thaliana***

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When plants are exposed to temperatures higher than those they are adapted to, a variety of responses are observed. One of these is the inability to mount a proper response to pathogen attack. Hybrid necrosis is a type of postzygotic-incompatibility that has been reported in many plant species, including *Arabidopsis thaliana*. Several studies of hybrid necrosis have demonstrated that it is the result of epistatic interactions between genes associated with pathogen responses that spontaneously trigger an autoimmune response. Like many pathogen responses, hybrid necrosis is suppressed at elevated temperatures. It is not known what mechanisms underlie this response. Here we sought to understand the suppression of necrosis at elevated temperatures by identifying genetic modifiers in three different cases in *A. thaliana*. We also characterised general suppressors of hybrid necrosis, as they rarely were common between the three studied cases. Certain identified suppressors suggest that the temperature response is somewhat controlled by temperature-dependent changes in chromatin. Disruption of the perception or production of certain hormones had different effects on hybrid necrosis. A lack of Salicylic Acid appears to partially rescue some cases and a lack of Abscisic Acid enhances other cases. It has been shown elsewhere that a specific point mutation in Resistance genes can suppress their temperature sensitivity. This mutation was tested in one case of hybrid necrosis but did not appear to have similar effects. Overall, the suppression of hybrid necrosis does not appear to be controlled by one simple pathway, instead it is more likely that many endogenous factors control the suppression as well as the threshold temperature at which it occurs.

### **236 Proteomic Analysis of the Plant-Pathogen Interface**

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Plants are constantly infiltrated by pathogens through natural openings and wounds. However, pathogenesis and virulence leading to systemic disease is rare as plants possess an active immune system to detect microbes and trigger immune responses. A virulence strategy employed by *Pseudomonas syringae* is the injection of type III secreted effectors (TTSE) directly into host cells via a molecular syringe known as the type III secretion system (TTSS). While it is clear that TTSE interact with host proteins to disable plant immunity, the precise targets of many TTSE remain unclear. One avenue of insight into host targets of TTSE is the proteomic identification of TTSE/host protein complexes. Here we describe the purification of high molecular weight HopF2<sup>Pto</sup> complexes from transgenic *Arabidopsis* via gel-filtration and immuno-affinity chromatography. Liquid chromatography tandem mass spectrometry was subsequently used to identify components of HopF2<sup>Pto</sup> complexes revealing novel targets of HopF2<sup>Pto</sup> in *Arabidopsis*.

### **237 The *chs3-2D* Mutation in the CHS3 LIM Domain Activates Constitutive Disease Resistance in *Arabidopsis***

*Kaeli Johnson<sup>2</sup>, Dongling Bi<sup>1</sup>, Yan Huang<sup>2</sup>, Zhaohai Zhu<sup>1</sup>, Xin Li<sup>2</sup>, Yuelin Zhang<sup>1</sup>*

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Plants possess a suite of Resistance (R) proteins which are essential for the activation of defense responses upon pathogen infection. CHILLING SENSITIVE 3 (CHS3) is an R protein belonging to the TIR-NB-LRR class with a C-terminal zinc-binding LIM (Lin-11, Isl-1, Mec-3) domain. Previous studies have implicated CHS3 in cold stress and defense response, although the role of the LIM domain remains unclear. In this study, a dominant *chs3* mutant (*chs3-2D*) isolated in a screen for NPR1-independent negative defense regulators was characterized. The *chs3-2D npr1-1* mutant displayed dwarfed, curled leaf morphology as well as elevated SA levels. The mutation resulted in constitutive resistance to the virulent oomycete *Hyaloperonospora arabidopsis* Noco2. Complementation experiments showed that *chs3-2D* is a gain-of-function mutation. The nuclear localization of the protein was not affected by the mutation. Another *chs3* mutant (*chs3-3D*) containing a mutation which appears to be in the LRR-LIM linker region was recently isolated in a *mos4* suppressor screen undertaken in our lab. Revertants of *chs3-2D* identified in a genetic screen will be presented. Analysis of these loss-of-function and gain-of-function mutation sites will provide structural and functional details for the different domains of this R protein.

**238 Functional Sites Of Leucine-Rich Repeats Of Pattern Recognition Receptors In The Plant Immune System***Teresa Koller, Laura Helft, Andrew Bent***University of Wisconsin-Madison, Madison, WI, USA**

Many pattern recognition receptors and R gene products in the plant immune system, and other plant receptor proteins, contain large leucine-rich repeat domains (LRRs). In several cases a direct interaction of immune system LRRs with a danger signal or a pathogen-derived ligand has been demonstrated. We are studying the specificity and plasticity of the LRRs of EFR and FLS2, which detect bacterial EF-Tu and flagellin, respectively. FLS2 orthologs are present in a wide variety of plant species. EFR seems to exist only in members of the Brassicaceae. Based on the assumption that amino acids involved in ligand interaction are evolutionarily conserved, we used our recently released Repeat Conservation Mapping computational tool to discover functional sites within the LRR of EFR (see also Helft *et al.* presentation, and <http://www.plantpath.wisc.edu/RCM>). EFR LRRs from multiple Brassicaceae species were cloned and sequenced. Capacity for detection of elf18 (the recognized epitope of EF-Tu) was confirmed by transient expression in *Nicotiana benthamiana* of domain-swap Arabidopsis EFR derivates carrying these Brassicaceae-derived EFR LRRs. The EFR LRR sequences were then used in conservation mapping to discover conserved amino acid clusters on the predicted surface of the folded protein. Several conserved clusters were identified. Thus additionally to ligand specificity, we hypothesize that conservation mapping has identified LRR sites involved in receptor multimerization, interactions with co-receptors, and/or receptor glycosylation and processing. The functions of these evolutionarily conserved amino acid clusters will be studied in more detail. We are also initiating use of yeast cell surface display as a platform for *in vitro* evolution of the LRRs of EFR and FLS2 toward novel ligand specificities. These latter studies explore the feasibility of engineering novel receptors with specificity for pathogen ligands of interest.

**239 Rowing up and down the MAMP-triggered calcium stream***Mark Kwaaitaal<sup>1,3</sup>, Rik Huisman<sup>1</sup>, Jens Maintz<sup>1</sup>, Anja Reinstädler<sup>1</sup>, Ralph Panstruga<sup>1,2</sup>*

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Binding of microbial epitopes to cognate pattern recognition receptors (PRRs) and receptor kinase activation triggers a plethora of cellular responses. In recent years, the PRRs detecting the active epitopes of the microbe-associated molecular patterns (MAMPs) of bacterial flagellin, the bacterial elongation factor Ef-Tu and chitin (flg22, elf18 and chitin polymers, respectively) have been characterized. One of the earliest detectable events after MAMP application is a rapid rise in cytosolic calcium levels. Knowledge about the signaling events leading to the opening of calcium channels and about the channels involved is scarce. Using a combination of approaches, including both forward and reverse genetics and unbiased and targeted chemical screens we attempt to isolate the molecular components controlling MAMP-triggered calcium fluxes. As read-out we make use of transgenic plants stably expressing the yellow cameleon 3.6 and aequorin biosensors. Our studies suggest a prominent role for amino acid-activated or -controlled calcium fluxes, likely through ionotropic glutamate receptor-like channels (iGluRs). The interference with amino acid-mediated calcium fluxes modulates MAPK activity and the transcriptional activation of defense genes. Therefore, in contrast to lipopolysaccharide (LPS), flg22, elf18 and chitin seem to employ iGluR-like channels to direct calcium influx.

**240 AtCML9, a calmodulin-like protein, contributes to plant defence responses***Louis-Jerome Leba, Cecilia Cheval, Christian Mazars, Benoit Ranty, Jean-Philippe Galaud, Didier Aldon  
Université Toulouse -CNRS, France*

Calcium signals are recognized as primary mediators in immune responses against potential invaders and recent studies on Ca<sup>2+</sup> signaling components have demonstrated a critical role of calmodulin and calcium-dependent protein kinases in plant immunity. To further investigate Ca<sup>2+</sup> signal transduction during pathogen attacks, we have analyzed *Arabidopsis* mutants in calmodulin like (CML) sensors that were up-regulated during infection with *Pseudomonas syringae* strains or following the perception of pathogen associated molecular patterns (PAMPs), such as flagellin. We showed that *cml9* knockout mutants were more susceptible to virulent strains of *P.syringae* whereas transgenic plants over-expressing *CML9* displayed less pronounced disease symptoms that correlated to a delayed bacterial growth in infected leaves. Loss- and gain of functions of *CML9* also altered callose deposition following bacterial PAMPs treatment indicating a putative role of CML9 in basal defence responses.

To better understand the involvement of CML9 in plant immunity, expression of plant defence associated marker genes were analysed using RNA from different genotypes by a high throughput qPCR approach (BioMark System). Our work constitutes a first step in deciphering the molecular mechanism of CML9 function in plant innate immunity.

**241 Regulation of RNA Silencing and Hormone Responses by RAV/EDF Transcription Factors***Mathew Lewsey<sup>1</sup>, Anna Stepanova<sup>2</sup>, Joseph Ecker<sup>1</sup>*

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RAV/EDF transcription factors (TFs) are involved in diverse plant defensive and hormonal pathways. There are six RAV/EDF TF family members in *Arabidopsis thaliana*, all of which possess AP2 and B3 DNA binding domains. EDF2 regulates expression of biotic and abiotic stress responsive genes, including those induced by methyl jasmonate and wounding. It is also required for suppression of RNA silencing by the silencing suppressors of two unrelated viruses, HcPro (of tobacco etch virus) and P38 (of turnip crinkle virus).

HcPro requires *EDF2* for induction of the endogenous suppressors of RNA silencing, *FIERY1* and *CML38*. The RAV/EDFs are therefore potential novel integrators of hormone signaling and RNA silencing. To further understand the functions of these TFs, we investigated their importance in the response to ethylene (ET). We found that expression of *EDFs 1-4* is ET-inducible and that EIN3, the key transcriptional activator of ET signaling, binds the *EDF1* promoter. This EIN3 binding site was sufficient to direct ET-responsive expression of a *GUS* transgene *in planta*. Furthermore, knockout mutations indicate that all four *EDFs* are required for normal ET sensitivity, and overexpression of a truncated *EDF1* caused constitutive activation and repression of different branches of the ET response pathway. Knowledge of the *in planta* binding targets of the RAV/EDF TFs would provide great insight into their mode(s) of action, but these are currently unknown. We are employing chromatin immunoprecipitation coupled with deep sequencing to identify these targets. A recombineering approach is being used to generate *A. thaliana* lines expressing RAV/EDFs tagged with a Ypet epitope. The advantages of this approach are that it will maintain genomic contexts and native gene expression patterns. We will investigate how the binding patterns of RAV/EDFs change in response to hormone stimuli using these tools, and will characterize the associated transcriptional responses by RNAseq. This will result in much greater understanding of the roles of RAV/EDFs in specific signaling pathways and as integrators of multiple pathways.

## 242 Growth regulation in response to cross-kingdom communication

*Louisa Liberman, Philip Benfey*

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Organisms sense and adapt to their environment to efficiently use resources and avoid predators and toxins. As sessile organisms plants depend on rapid adaptation to external cues for survival. Plant roots provide stability while facilitating nutrient and water uptake from soil teeming with microbes, fungi and invertebrates, all competing for resources. Cooperative relationships exist between plants and microbes, but molecular mechanisms underlying mutualism remain unclear. Complementary approaches have been used to determine the effect of beneficial microbes on root architecture and transcriptional regulation in *Arabidopsis thaliana*. Phenotypic changes in root growth and architecture upon exposure to multiple beneficial microbes were observed. Candidate mutant lines as well as *Arabidopsis* ecotypes were screened to determine their role in sensing and responding to bacterial signals. Transcriptional profiling will be used to identify genes that are differentially regulated in response to growth-promoting bacteria. The ultimate goal is to elucidate the molecular mechanisms underlying root-microbe interactions that result in increased growth.

## 243 Receptor-like Cytoplasmic Kinases in Plant Innate Immunity

*Zuh-Jyh Lin, Jun Liu, Gitta Coaker*

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*Arabidopsis thaliana* receptor-like cytoplasmic kinases (RLCKs), are a subset of the receptor like kinases (RLKs) but lack both extracellular and transmembrane domains. Several members of the *Arabidopsis* RLCK subfamily VII have been conclusively linked to plant innate immunity. Members PBS1 and RIPK are crucial components in RPS5 and RPM1 mediated effector triggered immunity, respectively, while BIK1 has been shown to participate in the signaling cascade triggered by flagellin perception. Furthermore, numerous RLCK-VII's have been shown to be cleaved by the *Pseudomonas syringae* effector AvrPphB and can be correlated with the dampening of PAMP triggered immunity in plants expressing AvrPphB. The RLCK subfamily VII is rather large, consisting of 46 members, and given the existing lines of evidence it is probable that additional RLCK-VII's may be involved in plant immunity. To identify these, public *Arabidopsis* microarray experiments were examined for RLCK-VII's exhibiting differential regulation in response to biotic stress. TDNA insertion lines for these particular RLCK-VII's were then obtained and subjected to disease phenotyping; two were found to exhibit enhanced disease resistance and additional efforts will be undertaken to elucidate their role in plant innate immunity. These efforts will entail an examination of well-established plant immune responses in the mutants for aberrant phenotypes and a search for protein interactors via yeast two hybrid and immunoprecipitation experiments.

## 244 Molecular Characterization of *mlo*-based Powdery Mildew Resistance and the Role of Heterotrimeric G-Protein Signaling in *Arabidopsis* Defense

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Powdery mildew is a common fungal disease of monocotyledonous and dicotyledonous plant species. Successful pathogenesis by the biotrophic fungus depends on the presence of plant-specific MLO (MILDEW RESISTANCE LOCUS O) proteins, as mutations in particular *MLO* genes confer durable powdery mildew resistance in barley, tomato and *Arabidopsis*. In the absence of MLO, fungal spores fail to invade the host epidermal cell, resulting in an early termination of fungal pathogenesis.

MLO proteins define a family of heptahelical plasma membrane-localized proteins, reminiscent of G-protein coupled receptors (GPCRs) in metazoans that activate heterotrimeric G-protein signaling. A genetic approach was chosen in this study to assess the role of MLO proteins as putative plant GPCRs and results from these experiments demonstrate that powdery mildew susceptibility conferred by MLO is independent of the heterotrimeric G-protein complex. However, data from this analysis suggest a function of the heterotrimeric G-protein in basal defense mechanisms against powdery mildew fungi as well as in the integration of MAMP (microbe-associated molecular patterns) perception into downstream immune responses.

Metabolomic analysis performed in this study indicate that the adapted powdery mildew fungus, *Golovinomyces orontii*, is able to suppress the accumulation of the defense-relevant indolic glucosinolate, 4MI3G (4-methoxyindol-3-ylmethylglucosinolate) in *Arabidopsis*, thereby inhibiting the PEN2-dependent glucosinolate defense pathway. This inhibition requires functional MLO, suggesting that successful

defense suppression either operates through the MLO protein or that it requires the formation of post-invasive fungal infection structures. Other data obtained here demonstrate that MLO proteins negatively regulate transcriptional activation of defense-related genes in response to powdery mildew challenge as well as upon MAMP treatment, implicating MLO functions in MAMP-triggered defense signaling.

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## 245 Arabidopsis *MPL1* (*MYZUS PERSICAE INDUCED LIPASE1*) Mediated Resistance Against Green Peach Aphid

Aphid

*Joe Louis<sup>1</sup>, Katarzyna-Lorenc Kukula<sup>1</sup>, Vijay Singh<sup>1</sup>, John Reese<sup>2</sup>, Jyoti Shah<sup>1</sup>*

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The phloem-specific feeding Green peach aphid (GPA; *Myzus persicae*) utilizes its slender stylet to feed continuously from the sieve elements thereby causing extensive damage to plants. We have utilized the model plant *Arabidopsis thaliana* to characterize host interaction with GPA and previously demonstrated that the *PHYTOALEXIN DEFICIENT4* (*PAD4*) gene as an important modulator of antixenosis (feeding deterrence) and antibiosis (affect aphid fecundity) against GPA. Here, we demonstrated the involvement of another, previously uncharacterized gene *MPL1* (*MYZUS PERSICAE-INDUCED LIPASE1*), in Arabidopsis defense against GPA. Like *PAD4*, *MPL1* expression is induced in response to GPA infestation and *MPL1* is required for limiting insect infestation. However, unlike *PAD4*, *MPL1* is not required for antixenosis. Electrical Penetration Graph technique revealed that the *mpl1* mutant allele does not impact GPA feeding behavior. *MPL1* is required for the accumulation of an antibiosis activity. Petiole exudates collected from leaves of the *mpl1* mutant lacked an antibiosis factor that is present in similar exudates collected from wild type leaves. Furthermore, overexpression of *MPL1* resulted in enhanced antibiosis against the GPA, without any effect on antixenosis. Constitutively elevated expression of *MPL1* is also required for the heightened antibiosis that is observed in the *ssi2* (*suppressor of salicylic acid-insensitivity2*) mutant. Taken together, these results support the hypothesis that the *MPL1* gene is required for antibiotic defense against the GPA. The *MPL1* protein contains a conserved triad of Ser, Asp and His residues that are found in most α/β fold acyl hydrolases/lipases. Since, recombinant *MPL1* protein exhibits lipase activity, we propose that in Arabidopsis a lipid(s), or a product thereof, is involved in antibiosis to GPA.

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## 246 Dissection of Membrane Trafficking in Plant Immunity

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To successfully infect plants, filamentous pathogens such as oomycetes penetrate host tissues and project haustorial structures inside the host cell for the uptake of nutrients. It has been described since years that plant cells respond with the substantial subcellular rearrangements, which are needed for accommodating the haustoria and are thought to provide means for the pathogen to deliver molecules to reprogram plant processes. To address the role of membrane trafficking in plant-pathogen interactions, we examined subcellular localization of fluorescent protein-tagged membrane compartments in *Arabidopsis* upon infection with *Hyaloperonospora arabidopsidis* (*Hpa*). Using life cell imaging we found that all tested markers for early and late endosomes including multivesicular bodies (MVBs) localized around the haustorial projections. Similarly, markers for Golgi compartments were associated with haustoria. By contrast, only few plasma membrane markers labelled haustoria, and often these occurred at encased haustoria. We will also present results obtained from FM4-64 endosomal tracing and chemical interference with endocytosis. Our data point at a role for endosomal trafficking in plants cells accommodating and interacting with *Hpa* haustoria.

To genetically dissect endosomal trafficking in *Hpa* infections, we applied quantitative high throughput confocal imaging and identified mutants with altered levels of MVBs, as detected with the fluorescent biosensor GFP-2xFYVE. Two mutants, *fel2* and *fel9* display a high increase in MVB numbers compared to wild-type reference plants. These mutants are likely affected in endosome trafficking as revealed by chemical interference. We will discuss phenotypic characterization at the subcellular level and upon *Hpa* infection, and present gene loci revealed by classical mapping and whole genome sequence analysis. Altogether, *fel2* and *fel9* are novel membrane trafficking mutants, allowing us to better understand the molecular mechanisms underlying the subcellular rearrangements in plant-pathogen infections.

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## 247 Immunity-Related Members of the DMR6 Family of Oxidoreductases in Arabidopsis

*Nora Ludwig, Joyce Elberse, Tieme Zeilmaker, Guido Van den Ackerveken*

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*Arabidopsis* mutants lacking a functional *DMR6* gene are resistant to infection by the downy mildew *Hyaloperonospora arabidopsidis* (*Hpa*). Resistance is associated with enhanced defense gene expression and both resistance and defense was found to require salicylic acid and signalling through the key regulator *NPR1* which signals downstream of salicylic acid. The hypothesis that *DMR6* is a negative regulator of defense was further supported by the finding that overexpression of *DMR6* leads to enhanced susceptibility to a range of pathogens, e.g. *Hpa*, *Phytophthora capsici* and the bacterium *Pseudomonas syringae* pv. *tomato*. *DMR6* is a 2-oxoglutarate iron (II)-dependent oxygenase for which no substrate is known yet. Site-directed mutagenesis confirmed the requirement of conserved catalytic residues for its function as a negative regulator of defense. Structural modeling has allowed the identification of residues important in the predicted substrate binding pocket, the mutation of which strongly reduced the biological activity of the protein. In the *Arabidopsis* genome more than 200 2-oxoglutarate iron (II)-dependent oxygenases are encoded, however, for most no function is known. We have selected a subgroup of *DMR6*-related 2-oxoglutarate iron (II)-dependent oxygenases which are differentially expressed during pathogen infection and in response to the defense-related hormones salicylic acid and/or jasmonic acid. We will report on phenotypic analysis

of mutants and overexpression lines of these immunity-related genes, in particular in their altered responses to various pathogens of *Arabidopsis*. The DMR6-like oxidoreductases add an additional layer of complexity to the plant immune network.

## 248 Suppression of immunity in diverse plants by the conserved RXLR effectors Ha23 from

### *Hyaloperonospora arabidopsis* and Ps73 from *Phytophthora sojae*

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Numerous plant pathogens secrete effector proteins that are exported to the interior of host cells. Effectors have been well characterized in bacteria but the roles of effectors in oomycete and fungal pathogenicity are poorly understood. Bioinformatic analysis of recently obtained genome sequences from oomycete pathogens *Phytophthora sojae*, *P. ramorum*, *P. infestans*, and *Hyaloperonospora arabidopsis* have led to the identification of a large number of candidate effector genes. These effector genes are defined by characteristic motifs (signal peptide, RxLR and dEER) that target the effectors into plant cells. These effector genes are very diverse and appear to turn over rapidly. However, some candidate RXLR effector genes are conserved between the *Arabidopsis* pathogen *H. arabidopsis* and *Phytophthora* spp., suggesting that they fulfill key roles in pathogenicity. The goal of this project is to characterize a selected set of conserved effector candidates by comparing the function of homologous effector genes in *H. arabidopsis* and the soybean pathogen *Phytophthora sojae*. Our primary objectives center on identifying effector functions and *in planta* targets using both transient assays and stably transformed plants. The Ha23 effector is induced at early time points during infection of *Arabidopsis*. Ha23 triggers an ecotype-specific defense response in *Arabidopsis*, suggesting that it is recognized by the host. Ha23 can suppress PAMP triggered immunity and enhance bacterial virulence when delivered through the *Pseudomonas syringae* type III secretion system or when expressed as a stable transgene in *Arabidopsis*. *Arabidopsis* Ha23 transgenic plants also display enhanced susceptibility to *H. arabidopsis* and attenuated induction of defense marker genes. In soybean, Ha23 can suppress cell death triggered by the *P. sojae* gene Avr4/6, but does not suppress cell death triggered by the oomycete elicitors INF1 or NEP1. Many of these attributes are shared by Ps73.

## 249 Dissecting DIR1 and DIR1-like Involvement During Systemic Acquired Resistance in *Arabidopsis* and Cucumber

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Systemic Acquired Resistance (SAR) is a defense response induced by an initial infection that leads to the production of a long distance signal(s). This signal moves to and is perceived by distant leaves resulting in resistance to normally virulent pathogens. DIR1 is involved in SAR long distance signaling as demonstrated by its presence in phloem sap of SAR-induced wild type, but not mock-induced or *dir1-1* plants. Occasionally a DIR1-sized band appeared in protein gel blots of *dir1-1* exudates suggesting that a DIR1-like protein may exist. Recombinant protein expression in yeast indicates the DIR1 antibody recognizes DIR1 and DIR1-like. Although, DIR1 and DIR1-like are 88% similar at the amino acid level, only *dir1-1* was identified in a forward genetic screen and *dir1-1* is rarely SAR-competent. Furthermore, homology modeling of DIR1-like, revealed clues as to why DIR1-like rarely compensates for the SAR defect in *dir1-1*. However, DIR1-like can compensate for the SAR defect in *dir1-1* when expressed in one leaf via transient transformation by *Agrobacterium*. A cucumber-*Arabidopsis* SAR model was established to take advantage of the ability to collect undiluted phloem samples from cucumber petioles. Phloem exudates collected from cucumber leaves induced for SAR complement the SAR defect in *Arabidopsis dir1-1*. To determine if putative cucumber DIR1 orthologues are responsible for this complementation, our Agro-SAR assay will be used to express individual orthologues during SAR in *dir1-1* *Arabidopsis* plants. These studies may provide further evidence that DIR1 or DIR1-like SAR long distance signaling mechanisms are conserved in both cucumber and *Arabidopsis*.

## 250 Auxin plays multiple roles in promoting susceptibility to *Pseudomonas syringae*

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The bacterial pathogen *Pseudomonas syringae* manipulates plant hormone signaling to cause disease. Multiple lines of evidence indicate that auxin, a key hormone controlling plant growth and development, promotes host susceptibility to this pathogen. Treatment with exogenous auxin leads to more severe disease symptoms (Chen et al. PNAS, 2007; Navarro et al. Science, 2006). Transgenic plants that over-express the *YUCCA1* auxin biosynthesis gene (*35S:YUCCA*), and accumulate elevated auxin levels, have increased susceptibility to *P. syringae*. Auxin may act to increase susceptibility by suppressing host defenses. To investigate the mechanism for increased susceptibility in *35S:YUCCA* plants, we examined whether gene-for-gene resistance is compromised in these plants. Infection with strains expressing avirulence genes indicates *35S:YUCCA* plants have a partial loss of resistance compared to wild type plants and exhibit a weaker hypersensitive response. Wang et al. (Curr Biol, 2007) report that auxin acts antagonistically with salicylic acid (SA)-mediated defenses. To determine whether auxin promotes disease susceptibility by suppressing SA signaling, we crossed the *sid2-2* mutation, which blocks pathogen-induced SA synthesis, into the *35S:YUCCA* line. The new line exhibits significantly enhanced susceptibility to *P. syringae*, with a higher level of susceptibility than either *sid2-2* or *35S:YUCCA* alone, suggesting auxin is affecting pathogen virulence and/or host susceptibility via an additional mechanism that is independent of SA. Additionally, when multiple TIR1/AFB auxin receptors are knocked out, pathogen growth in planta is similar to that in wild type plants, suggesting that the virulence effect of auxin does not require TIR1/AFB-mediated signaling. Thus, we are investigating potential direct effects of auxin on the pathogen itself, which may promote its virulence.

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251 Abstract Withdrawn

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**252 Involvement of ER body in the Strategy for Environmental Adaptation of *Arabidopsis thaliana*.**

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Unlike animals, plants are not able to escape from adverse circumstances. To avoid external stress, plants develop new function in their organelles, especially in the endoplasmic reticulum (ER), which is the most flexible and adaptable organelle. Furthermore, plants can generate a specialized compartment from the ER to accumulate an enormous amount of proteins that are actively synthesized on ER. In transgenic *Arabidopsis thaliana* expressing green fluorescent protein (GFP) with an ER-retention signal (GFP- HDEL, His-Asp-Glu-Leu), ER bodies appear as spindle- shaped GFP-fluorescent structures (~10 µm long and ~1µm wide). ER body is one of ER-related organelles, and highly accumulates a specific protein, PYK10, a β-glucosidase with an ER-retention signal. Functions of ER bodies are still unclear. ER bodies are present in the epidermal cells of cotyledons and hypocotyls (constitutive ER bodies) of young seedlings, and disappear in those of mature tissues of *Arabidopsis*. When mature leaves are wounded, ER bodies are induced around the wounded site of the leaves (inducible ER bodies). Therefore, the induction of ER bodies might be involved in self-defense of the plants. The aims to clarify the functional differences of these two ER bodies, we compared constitutive ER bodies with inducible ER bodies in wounded cotyledons of *Arabidopsis* seedlings.

First, we focus on the number of ER bodies, and established the method for counting ER bodies in the cell from Z- stacking images of confocal microscopy. Using this method, we found more ER bodies are induced in the cotyledons of wounded plants than unwounded plants. Numbers of ER bodies are increased not only in injured local cotyledons but also in the uninjured systemic cotyledons after wounding. We also show the Quantitative polymerase chain reaction analysis for components of constitutive and inducible ER bodies and then discussed their possible it functions.

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**253 Functional Characterization of RNA-binding Proteins and MicroRNAs as Posttranscriptional****Regulators in Plant Defense Responses**

Youngju Park, HwaJung Lee, Hunseung Kang

**Chonnam National University, Korea**

RNA-binding proteins (RBPs) and microRNAs (miRNAs) are recognized as central regulators in posttranscriptional response of plants to diverse pathogens. Here, we investigated the pathogen-responsive expression patterns and the roles of RBPs and miRNAs in defense response in *Arabidopsis thaliana*. Glycine-rich RNA-binding protein7 (GRP7), the RBP of which is ADP-ribosylated by HopU1 and quells the host immunity, conferred defense against a fungus and virus as well as bacteria. Analysis of loss-of-function mutants and overexpression transgenic plants of pathogen-regulated RBPs demonstrated that a specific type of RBP confers defense against diverse pathogens. The expression patterns of individual miRNA in *A. thaliana* treated with pathogen or elicitor were analyzed to understand the potential roles of each miRNA in response to pathogens. Comprehensive analysis of miRNAs upon pathogen or elicitor treatment showed that specific miRNA family members are modulated by pathogen infection or elicitor treatment. The transgenic *Arabidopsis* plants overexpressing either miR400 or miR844 displayed altered response to pathogens. These results indicate the importance and potential roles of RBPs and miRNAs as posttranscriptional regulators in plant defense response.

**254 Metabolic Incompatibility between *Arabidopsis* and the biotrophic pathogen *Hyaloperonospora arabidopsisidis****Johannes Stuttmann<sup>1</sup>, Hans-Michael Hubberten<sup>2</sup>, Rainer Hoefgen<sup>2</sup>, Jane Parker<sup>1</sup>*<sup>1</sup>**Max-Planck Institute for Plant Breeding Research, Dept. Plant-Microbe Interactions, Cologne, Germany, <sup>2</sup>Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany**

The reliance of biotrophic pathogens on living plant cells to propagate implies strong interdependence between host metabolism and nutrient uptake by the pathogen. Thus, additional factors besides the plant immune system might determine host suitability. A loss of inhibition allele of *ASPARTATE KINASE2* and a loss of function allele of *DIHYDRODIPICOLINATE SYNTHASE2* were isolated in a screen for *Arabidopsis* mutants with increased resistance to the obligate biotrophic oomycete *Hyaloperonospora arabidopsisidis* (Hpa). These mutations perturb, by different mechanisms, plant amino acid homeostasis leading to accumulation of the aspartate-derived amino acids methionine, threonine and isoleucine. We present data showing that the plant host metabolic state can, in specific ways, influence the ability of adapted biotrophic strains to colonize tissues and therefore may be a factor determining host suitability. Funding by a Deutsche Forschungsgemeinschaft 'SFB 635' grant.

**255 The Roles of the CC and LRR Domains of the RPS5 NB-LRR Protein in Pathogen Recognition***Dong Qi, Roger Innes***Dept Biology, Indiana University, Bloomington, Indiana, USA**

The *Arabidopsis* RPS5 protein is a coiled coil-nucleotide binding-leucine-rich repeat (CC-NB-LRR) disease resistance protein. It mediates recognition of the Type III effector protein AvrPphB, a cysteine protease from *Pseudomonas syringae*. RPS5 is activated by the cleavage of PBS1, an *Arabidopsis* kinase, by AvrPphB. Transient co-expression of RPS5, PBS1 and AvrPphB in *Nicotiana benthamiana* leaves induces a hypersensitive response (HR). Mutations in the RPS5 CC domain abolished the HR triggered by RPS5 and a previously reported auto-active mutant D266E, but did not affect any known inter/intra-molecular interactions of RPS5. Interestingly, the RPS5 CC domain was functionally substituted with the corresponding region of RPS2, another *Arabidopsis* CC-NB-LRR R-protein, which mediates recognition of the Type III effector AvrRpt2. This result suggests that RPS5 and RPS2 may share a similar signaling mechanism upon activation. The first twenty amino acids of RPS5 contains both myristylation and palmitoylation motifs and we determined that this region was necessary and sufficient to direct RPS5 to the plasma membrane (PM). Modification of RPS5 acylation residues, G2 and C4, affected RPS5 PM localization, protein stability, and function in an additive manner. Single acylation mutants were still capable of recognizing PBS1 cleavage. Substitution of the RPS5 LRR domain with the RPS2 LRR domain led to auto-activation, suggesting that the NB and LRR domains of RPS5 have co-evolved and the LRR may function to inhibit nucleotide exchange. Mutation of a single acylation signal (G2/3A) blocked HR induction by this RPS5-RPS2 chimera, and this protein was not activated by PBS1 cleavage. Partial deletions in the RPS5 LRR region also disabled RPS5 activation by PBS1 cleavage but not HR induced by the auto-active mutant D266E. In addition, the RPS5 LRR region was co-immunoprecipitated with recombinant PBS1 cleavage products but not the full-length PBS1 protein. These observations indicate that the RPS5 LRR domain plays a direct role in recognizing PBS1 cleavage by AvrPphB.

**256 JMJ27, an *Arabidopsis* JmjC Domain-Containing H3K9 Histone Demethylase Is Required for Defense****Against *Pseudomonas Syringae****Aditya Dutta, Julie Caruana, Ramesh Raina***Syracuse University, Syracuse, NY, USA**

Histone modification is known to regulate a wide variety of biological processes, and studies in recent years have revealed a role for chromatin remodeling in plant defense. Here we report JMJ27, an *Arabidopsis* JHDM2 family jumonji-domain containing protein, is involved in regulating defense against pathogens. *jmj27* mutants are compromised in resistance against both virulent and avirulent strains of the bacterial pathogen *Pseudomonas syringae*. JMJ27 is a nuclear protein containing a zinc finger motif, and a catalytic JmjC domain with conserved Fe(II) and  $\alpha$ -ketoglutarate binding sites. JMJ27 has H3K9me1/2/3 demethylase activity both *in vitro* and *in vivo*. Demethylation by JMJ27 requires Fe(II) and  $\alpha$ -ketoglutarate and the rate of removal of H3K9me1/2 marks is more efficient than removal of the H3K9me3 mark. Together these results demonstrate that JMJ27 is an H3K9me1/2/3 demethylase and is required for resistance against bacterial pathogens.

**257 Conservation of RIN4 function in *Arabidopsis* and Soybean***Thomas Redditt, Tom Ashfield, Andrew Russell, Natalie Rodibaugh, Roger Innes***Indiana University Bloomington, Bloomington, IN, USA**

The major goal of the Innes lab is to better understand the molecular mechanisms that lead to disease resistance in plants. My project focuses on the recognition of the bacterial virulence factors AvrB and AvrRpm1, and the resulting disease resistance response mediated by the soybean disease resistance proteins, Rpg1-b and Rpg1-r. Previous work has shown that both AvrB and AvrRpm1 are recognized by RPM1 in *Arabidopsis* and that this recognition is dependent upon the presence of another host protein, RIN4. My goal is to determine if soybean and *Arabidopsis* use similar molecular mechanisms to recognize and respond to AvrB and AvrRpm1. One method used was to complement *rpm1* and *rin4* mutant *Arabidopsis* with the orthologous soybean genes. *Arabidopsis* plants mutant for *rps2* and *rin4* were transformed with each of the four co-orthologous soybean *RIN4* genes (*GmRin4A,B,C*, and *D*). Also, *Arabidopsis* plants mutant for *rpm1 rps2* and *rin4* were transformed with *Rpg1-b*, one *GmRin4*, or both. Preliminary results show that *GmRin4C*, but not *GmRin4A* or *GmRin4B*, is able to complement the *Arabidopsis rin4* mutation in RPM1-mediated defense against AvrB and AvrRpm1, suggesting a

functional difference between the GmRin4s. However, preliminary data suggest that *Rpg1-b* is unable to complement for AvrB recognition in *rpm1* mutant plants, even in the presence of any *GmRin4*. Future directions will focus on identifying the biochemical activities of AvrB and AvrRpm1 that lead to detection in both Arabidopsis and Soybean. Preliminary data indicate that AvrB and AvrRpm1 modify RIN4s differentially. Specifically, AvrRpm1, but not AvrB, is able to modify GmRin4C and GmRin4D, but not GmRin4A or GmRin4B *in planta*

## 258 *Arabidopsis ABP30.6*, a Novel Actin-Bundling Protein, Contribute to Resistance to *Botrytis cinerea*

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The actin cytoskeleton is a highly organized and dynamic structure present in all eukaryotic cells where it plays important role in many processes, including intracellular transport, cell growth, division and morphogenesis, or responses to environmental stimuli. In cells, the formation of higher-order structures, such as bundles and cables, is regulated by specific families of actin-binding proteins (ABPs). ABP30.6 is a novel actin-bundling protein in *Arabidopsis*. The function of ABP30.6 is not clear. We reported here that ABP30.6 play an important role in *Arabidopsis* defense reaction. Plants overaccumulating ABP30.6 display enhanced resistance to *Botrytis cinerea*. Conversely, plants lacking ABP30.6 exhibit enhanced disease susceptibility. Microarray analysis showed that many pathogenesis-related protein genes (PRs) genes are regulated by ABP30.6. Further analyses indicated that expression of genes involved in jasmonate (JA) response are strongly up-regulated, including PDF1.2a, MYC2, VSP2 et al. These data suggest that ABP30.6 contribute to resistance against fungal pathogens through a regulatory network that may involve JA signaling pathway.

## 259 A Tale of Two Effectors From *Albugo laibachii* Nc14

*Torsten Schultz-Larsen, Eric Kemen, Kate Bailey, Ariane Kemen, Anastasia Gardiner, Jonathan Jones*

**The Sainsbury Laboratory, Norwich, UK**

A striking attribute of *Albugo laibachii* (*Al*) is its capacity to suppress host plant defences, conferring susceptibility not only to *Al*, but also to parasites normally resisted by the host (Cooper *et al.*, 2008, MPMI 21:745-56). The recent completion of the *Al* Nc14 genome enabled identification of candidate effectors, which were screened for contribution to virulence of *Pseudomonas syringae* (*Pst*) DC3000 infection of *Arabidopsis* (Kemen *et al.*, in press).

Here, we present the characterization of two candidate effectors from different classes of *Al* effectors: a short secreted protein SSP6, and the CHXC type effector CHXC1. CHXC1 is a secreted HECT type E3 ubiquitin ligase, which localizes to the plant nucleus after transient expression in *Nicotiana benthamiana*. We find that CHXC1 confers enhanced virulence of *Pst* DC3000 lux on Nd-0, but not Col-0, when delivered by T3SS. Interestingly, CHXC1 is present in the related species *Albugo candida* 20DD5 (*Ac* 20DD5), suggesting it to be a core effector. In contrast, SSP6 is absent from *Ac* 20DD5 but exists in multiple polymorphic copies in *Al* haplotypes. Transient heterologous expression of SSP6-A shows that it localizes to the plasma membrane and causes a weak necrosis.

Based on these findings we propose two evolutionary classes of effectors: Fast evolving effectors without conserved functions, and core effectors with conserved functional domains.

## 260 The bHLH transcription factors MYC2, MYC3 and MYC4 regulate glucosinolates biosynthesis in *A.thaliana*

*Fabian Schweizer, Philippe Reymond*

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Plant defense against herbivore insects is mainly regulated by the phytohormone jasmonate-isoleucine through its receptor complex COI1/JAZ. Insect herbivory induces around 1500 genes in Arabidopsis, most of which depend on a functional jasmonate pathway (1/3). In order to identify genes that play a direct role against herbivores, we screened for insect-induced transcription factors (TF). One of the most insect induced TFs is *MYC2*, which together with *MYC3* and *MYC4* has been shown to partially regulate some JA-Ile-dependent genes and responses and also physically interact with the receptor complex. Although the triple mutant *myc2/3/4* does not completely phenocopy the jasmonate-insensitive mutant *coi1-1*, it remains as sensitive to insect herbivores as <>*coi1-1*. In this study, we show that insect sensitivity can be explained by the lack of expression of some crucial genes of the biosynthesis of glucosinolates (GS), which are the major pathogen and insect deterrents in crucifer plants. Furthermore, this down-regulation results in a nearly undetectable level of GS in *myc2/3/4* when compared to wild-type plants. Interestingly, the absence of GS does not only impact insect performance but also feeding behaviour.

## 261 DNA Repair Proteins Are Directly Involved in Regulation of Gene Expression during Plant Immune Response

*Junqi Song<sup>1,2</sup>, Wendy Durrant<sup>1</sup>, Shui Wang<sup>1</sup>, Shunping Yan<sup>1</sup>, EK Han Tan<sup>1</sup>, Xinnian Dong<sup>1</sup>*

**<sup>1</sup>Duke University, Durham, NC 27708, <sup>2</sup>UW-Madison, Madison, WI 53706**

Systemic acquired resistance (SAR), an inducible plant defense response to local infection, requires the signaling molecule salicylic acid (SA) and the transcriptional coactivator NPR1, with concerted activation of *pathogenesis-related* (*PR*) genes. *Arabidopsis sni1* is an *npr1* suppressor and derepression of defense genes in *sni1* causes reduced growth and fertility and increased homologous recombination. Characterizing suppressors of *sni1*, we identify the DNA damage repair proteins SSN2 and RAD51D as genetic and physical interactors of SNI1. During plant defense, SSN2 and possibly RAD51D replace the transcription repressor SNI1 at pathogenesis-related gene promoters. In the presence of SNI1, NPR1 is also required for SSN2 binding. Thus, coordinated action of SNI1, SSN2-RAD51D and NPR1 ensures the tight control of plant immune gene expression. Given that the SSN2-RAD51D complex is conserved in eukaryotes,

their dual function in homologous recombination and transcription regulation of plant defense genes suggests a general link between these two stress responses.

## 262 AtVDAC1 Regulates Defense Response Against Bacterial Pathogen.

*Chika Tateda<sup>1,2</sup>, Kanako Watanabe<sup>1</sup>, Tomonobu Kusano<sup>1</sup>, Yoshihiro Takahashi<sup>1</sup>*

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The voltage-dependent anion channel (VDAC), a major outer mitochondrial membrane protein, is thought to play an important role in energy production and apoptotic cell death in mammalian systems. However, the function of plant VDACs is largely unknown. We reported that VDACs are involved in non-host pathogen resistance and also in Bax-mediated cell death using *Nicotiana benthamiana* as host plant. These data suggest that plant mitochondria are important for plant defense and cell death regulation. In this study, we performed the molecular and genetic characterization of all *VDAC* genes present in *A. thaliana*. Moreover, we examined the defense potential of mitochondrial *vdac1* knock out plants against bacterial pathogens. Based on these results, we discuss the possible functions of individual *Arabidopsis* VDAC members in vegetative and reproductive growth, and in pathogen defense.

## 263 Investigating the Transcriptional Regulation of *RRS1* Genes in Response to Biotic and Abiotic Stresses.

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*Ralstonia solanacearum* is a Gram-negative soil-borne β-proteobacterium that causes bacterial wilt disease in diverse and important food crops such as tomato, potato, banana and ginger. *RRS1-R*, a resistance gene of *Arabidopsis thaliana*, ecotype Nidersenz, confers resistance to many strains of the pathogen. The *RRS1-R* gene product is a TIR-NBS-LRR (TNL) protein which C-terminal domain corresponds to a WRKY transcription factor domain. An allelic form of *RRS1-R*, *RRS1-S* is present in a susceptible plant from the ecotype Columbia. The RRS1 proteins recognize PopP2, the corresponding avirulence protein of *Ralstonia solanacearum* (Deslandes *et al.* 2002). The work presented here, aimed at the localization of *RRS1-R* and *RRS1-S* gene expression in plants. Gene expression pattern was studied in response to biotic, abiotic stresses and in response to the expression of PopP2.

## 264 Three members of the CBP60 family of proteins are differentially involved in salicylic acid mediated defences in *Arabidopsis*

*William Truman<sup>1</sup>, Kenichi Tsuda<sup>1</sup>, Suma Sreekanta<sup>1</sup>, You Lu<sup>1</sup>, Lin Wang<sup>2</sup>, Jane Glazebrook<sup>1</sup>*

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Salicylic acid (SA) is an important signalling compound for defense responses in *Arabidopsis* and plays a key role in resistance to biotrophic pathogens such as *Pseudomonas syringae* pv *maculicola* (*Pma*). Isochorismate synthase (*ICS1*) is an essential enzyme component in the biosynthesis of SA; two homologous genes have been identified as regulators of *ICS1* expression: Calmodulin Binding Protein 60g (*CBP60g*) and Systemic Acquired Resistance Deficient 1 (*SARD1*). Both *cbp60g* and *sard1* mutants are compromised in *ICS1* expression, SA accumulation and resistance to *Pma* infection with the double mutant exhibiting stronger symptoms indicating partial redundancy in function.

Both *CBP60g* and *SARD1* have been shown to bind to the promoter region of *ICS1*. The growth of *Pma* ES4326 in the *cbp60g sard1* double mutant is significantly greater than in the *ICS1* null mutant *sid2-1* implicating these DNA binding proteins in the regulation of additional defense responses. Gene expression profiling of *cbp60g sard1* mutants has revealed several additional potential targets for *CBP60g/SARD1* regulation. A DNA motif bound by *CBP60g* and *SARD1*, GAAATT, was significantly over-represented in promoters of *CBP60g/SARD1* dependent genes, suggesting that expression of these genes is modulated by *CBP60g/SARD1* binding.

Of the eight members of the CBP60 gene family *CBP60a* is most closely related to *CBP60g* and *SARD1*. A TDNA insertion knock out mutation in this gene shows enhanced resistance to *Pma* ES4326 infection compared with wildtype. Singly, and in combination with *cbp60g* and *sard1* mutants, *cbp60a* plants have significantly elevated levels of SA compared with wildtype plants. Upon infection, the triple mutant *cbp60a cbp60g sard1* accumulates significantly more SA than the *cbp60g sard1* double mutant. *CBP60a* shares a high degree of sequence homology with *CBP60g* and *SARD1* across the central protein domain identified as being sufficient for DNA binding. The potential for *CBP60a* to bind to various DNA motifs and promoter regions is currently being investigated.

Wang et al. PLOS Pathog 5(2), 2009

Zhang et al. PNAS 107(42), 2010

## 265 SGT1 and chloroplast-generated ROS: The new players in coronatine-induced chlorosis and *Pseudomonas syringae* pv. *tomato* disease associated cell death

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Coronatine producing pathovars of *Pseudomonas syringae* including pvs. *tomato*, *maculicola*, and *glycinea* causes important diseases on tomato, crucifers and soybeans, respectively, and produces symptoms with necrotic lesions surrounded by chlorosis. The chlorosis is mainly attributed to a jasmonic acid (JA)-isoleucine analogue, coronatine (COR). However, the significance of COR-induced

chlorosis in localized lesion development is poorly understood. Taking advantage of a chlorotic phenotype elicited by COR on *Nicotiana benthamiana* leaves and virus-induced gene silencing as a rapid forward/reverse genetic screening tool, we identified new player that link disease associated chlorosis and necrosis development. Silencing of *SGT1* (suppressor of G2 allele of *skp1*) abolished COR-induced chlorosis. Furthermore, in Arabidopsis, *AtSGT1b* but not *AtSGT1a*, was required for COR responses and *P. syringae* pv. tomato DC3000 (*Pst* DC3000) symptom (water soaked lesion) development, and silencing of *SGT1* in tomato resulted in reduction of disease-associated cell death and chlorosis suggesting a connection between COR-induced chlorosis and cell death. In addition, a forward genetic screen identified a gene encoding 2-Cys peroxiredoxin (*Prxs*) when silenced produced a spreading hypersensitive/necrosis-like phenotype instead of chlorosis after COR application in a *COII*-dependent manner. Loss-of-function analysis of 2-Cys Prxs and NADPH dependent thioredoxin reductase C (NTRC), the central players of chloroplast redox detoxification system resulted in spreading accelerated *Pst* DC3000 disease associated cell death with enhanced ROS accumulation in a COR-dependent manner in tomato and Arabidopsis. These results suggested that COR-induced ROS generated in chloroplast were required for localized cell death (necrosis), and NTRC/Prxs function as negative regulator of pathogen-induced cell death and thereby limits the runaway cell death. Taken, together our results demonstrated a new role for coronatine in *P. syringae* pv. *tomato* disease development.

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**266 Plant SUMO Paralogs Are Negative Regulators Of The Innate Immune Response**

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A critical aspect of a successful defense response is the ability of a plant to rapidly mount a defense response upon pathogen attack. This defense response heavily relies on transcriptional reprogramming in the first hours after invasion. In non-infected plants the defense response is suppressed by SUMO (small ubiquitin-like modifier). SUMO (small ubiquitin-like modifier) is a post-translational modification that modulates the activity and the recruitment of chromatin-modifying enzymes to transcription complexes. Currently, little is known concerning the role of chromatin remodeling on defense gene regulation. We have a set of candidate SUMO targets implicated in defense signaling and we are using complementation studies to identify the role of SUMOylation on gene regulation by these targets. In addition, we are performing transcriptomics to identify gene regulation by SUMO. Our research on the model system *Arabidopsis thaliana* indicates that SUMO acts as a 'hand-break' of the plant defense system.

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**267 Quantitative PhosphoProteomic Analysis of R-gene Mediated Immune Signaling.**

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Understanding the non-adaptive plant immune system is of great scientific and agronomical importance. Pathogen derived surface molecules (e.g. flagellin) are recognized by receptors and trigger a basal defense, restricting pathogen growth (PTI). Pathogens can secrete effector proteins that target components of basal defense to increase virulence. Resistance (R-) proteins recognize effectors or their activity on targets ("modified self"), activating a strong and rapid defense response leading to resistance (ETI). Signaling events downstream of R-proteins are largely unknown and it is not yet understood if and how PTI and ETI pathways are integrated. Genetics and Transcriptomics have contributed most of what we know today. To complement these approaches, we use proteomics methods to quantify changes in protein expression and phosphorylation levels in response to immune signaling in Arabidopsis. We used conditional expression of a single bacterial effector protein (avrRpm1), activating the R-protein RPM1. Quantitative (phospho-)proteomic analyses were done using LC-MS/MS. Of the ~5000 proteins and ~1000 phosphoproteins measured, 7 to 20% were perturbed, respectively. The majority of these changes (70%) were not predicted by gene expression data, suggesting an important role for post-transcriptional regulation. A striking overlap between phosphorylation-events during PTI and ETI signaling was observed. We are currently conducting functional analysis of proteins and phosphorylation events using knockout mutants, amiRNA knockdowns and overexpression of phospho-mimetic or phospho-dead proteins. In addition, we are developing simple and rapid MS-based assays to quantitatively analyze selected proteins/phosphorylations, called MRM (Multiple Reaction Monitoring).

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**268 Regulation of Salicylic Acid Signaling and Response by the GH3 acyl adenylase PBS3**

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The Arabidopsis GH3 acyl adenylase PBS3 (GH3.12) mediates induced total salicylic acid (SA) accumulation and response, with mutants compromised in resistance to *Pseudomonas syringae* pathovars. Though PBS3 impacts SA metabolism and response, it is active on 4-substituted benzoates not SA (2-hydroxybenzoate), with elevated SA inhibiting PBS3 activity. Contrary to previous reports that restrict PBS3 to Arabidopsis and its close relatives, our integrated syntetic, phylogenetic, and functional analysis identified PBS3 syntelogs in poplar, grape, columbine, maize, and rice suggesting descent from a common ancestral chromosome dating to before the eudicot/monocot split and the possibility of conserved function. To examine the mechanism by which PBS3 impacts SA metabolism and response free from biotic influence (e.g. effectors targeting this pathway), an abiotic response assay was developed. Following treatment, two phases of free SA accumulation are observed, with the second phase accompanied by formation of glucose-conjugated SA (SAG) and the expression of SA-associated genes such as PR1. Expression of the SA biosynthetic gene isochorismate synthase 1 (ICS1) and the ICS1 protein parallels SA formation with two peaks of response. It had been previously proposed that PBS3 acts upstream of SA. However, our analysis of a pbs3 null mutant indicates that though induced total SA and PR1 expression are dramatically reduced, ICS1 transcript and protein expression are not compromised. Instead, HPLC analyses suggest SA catabolism is enhanced in the pbs3 mutant. Taken together our findings suggest PBS3 acts downstream of SA synthesis to facilitate the accumulation of SA (free SA and SAG) to

levels required for the robust activation of SA-dependent responses by limiting SA catabolism. PBS3 activity is then inhibited by elevated SA consistent with a role for PBS3 in fine-tuning stress-induced SA metabolism.

## 269 Identification of mobile defense-inducing signals in plants

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Bacterial elicitors are recognized by receptors in plants. Subsequently, defence mechanisms are activated and long distance signals move from the infected to the systemic site to initiate systemic acquired resistance (SAR). Local infection of *Arabidopsis thaliana* plants by *Pseudomonas syringae* pv tomato encoding the effector AvrRpm1 induces the activation of SAR in systemic tissues. SAR can also be induced by activating a dexamethasone-inducible transgene driving *AvrRpm1* expression. Initiation of SAR in Col-0 *DEX::AvrRpm1-HA* plants was confirmed by expression of the SAR marker gene *PR1* in systemic, non-*AvrRpm1*-expressing tissues of DEX-treated plants. In addition, pathogen growth was reduced in untreated, distal leaves of DEX-treated as compared to untreated plants. Similar to the *eds1* mutant in response to bacterial infection, *eds1 DEX::AvrRpm1-HA* plants failed to initiate SAR upon local induction of *AvrRpm1* expression. The aim of the current work is to get a better understanding of SAR and SAR-related metabolites that contribute to systemic defence. The *eds1* plants are incapable of generating and/or transmitting SAR signals (Jorda, Vlot, and Parker, personal communication). Seven *EDS1*-dependent metabolites were detected by LC-MS and the SAR-inducing compound(s) seem(s) to be present in a highly lipophilic fraction of MeOH extracts. Further LC-MS and GC-MS analyses are in progress to narrow down active components inducing SAR.

## 270 DNA Damage Response Potentiates Immune Response In Plants

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DNA damage response (DDR) and immune response are two major mechanisms for the survival of all living organisms. While DDR is essential to maintain genome stability, the immune response is required to defend "non-self" invaders. Although they have been well-studied independently and considerable progresses have been made, the relationship between them remains elusive. In *Arabidopsis*, an unknown protein SNI1 negatively regulates defense gene expression. In a genetic study, we found that the mutation of RAD17 and ATR, two key regulators of DDR, suppressed the phenotypes of the *sni1* mutant, suggesting that SNI1 functions in DDR. Consistently, we found that DDR was constitutively activated in *sni1*. Intriguingly, DNA damage treatment mimicked the *sni1* mutant and synergistically enhanced defense gene expression induced by immune inducer. More importantly, the DDR mutant *rad17* and *atr* were hypersensitive to pathogen attack. Our results suggest that SNI1 represses defense gene expression by inhibiting DDR and provide a functional link between DDR and immunity.

## 271 A Signaling Cascade Activated by *Pseudomonas syringae* Through Abscisic Acid And Jasmonic Acid Signaling Suppresses Salicylic Acid Accumulation

*Xiao-yu Zheng<sup>1</sup>, Natalie Spivey<sup>1</sup>, Weiqing Zeng<sup>2</sup>, Sheng Yang He<sup>2</sup>, Xinnian Dong<sup>2</sup>*

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Successful phytopathogen can target plant hormone signaling network to attenuate plant defense responses, employing the antagonistic crosstalk between different hormone signaling. The phytopathogen *Pseudomonas syringae* can activate abscisic acid (ABA) and jasmonic acid (JA) signaling by effectors. Several *P. syringae* strains can also produce a phytotoxin, coronatine, which is believed to function as a JA-Ile mimic. The antagonism between ABA/JA and salicylic acid (SA) signaling attenuates SA-mediated defense, the major defense mechanism against *P. syringae*. However, the molecular mechanism mediating this antagonism remains unclear. We identified three AtMYC2-regulated homologous transcription factors (TFs) mediating this antagonism. They are induced by *P. syringae* pv. *maculicola* (*Psm*) and this induction is dependent on *Arabidopsis* ABA signaling, JA signaling, and *Psm*-produced coronatine. Chromatin-IP experiment showed that AtMYC2 bound to the promoters of these three genes, suggesting that *Psm* induces these three TFs through ABA/JA-activated AtMYC2. Stomata assay showed that the three TFs were necessary for coronatine-induced stomata reopening. Besides their functions in stomata, we found these three TFs also affect plant defense after *Psm* enters apoplast. We identified two direct downstream targets of the three TFs, which are associated with SA accumulation. Intriguingly, the triple mutant of the three TFs accumulated higher level of SA than wild type upon *Psm* infection, and exhibited enhanced resistance to *Psm*, suggesting their roles in suppressing SA-triggered defense.

**272 The Role of the Conserved Protein, CACTIN, in Arabidopsis. An Exciting, Essential, and Unique Eukaryotic Gene.**Katherine Baldwin, Patrick Masson**University of Wisconsin, Madison, WI, USA**

*CACTIN* is a conserved gene found across eukaryotes and encodes a protein without recognizable functional domains. Mutations in the *CACTIN* gene result in lethality in *Drosophila*, *C. elegans*, *Toxoplasma*, and *Arabidopsis*. In flies, CACTIN plays a role in the NF- $\kappa$ B pathway. There is no recognized NF- $\kappa$ B pathway in plants or *C. elegans*. Our studies using *Arabidopsis* suggest that CACTIN plays a critical role in embryogenesis and possibly a function in root gravitropism. Like in animal systems, *Arabidopsis* fluorescently tagged CACTIN localizes to nuclear speckles. By yeast-two-hybrid, we found that CACTIN binds to a putative component of the spliceosome. We propose that CACTIN maybe a peripheral part of the eukaryotic spliceosome. We present further experiments in *Arabidopsis* to test this hypothesis as well as examining its functional conservation between plants and animals.

**273 AGD1, a Class 1 ARF-GAP that Localizes to Punctate Bodies of the Endomembrane System, Regulates Multiple Components of Root Hair Growth in Arabidopsis**Cheolmin Yoo<sup>1</sup>, Satoshi Naramoto<sup>2</sup>, J. Alan Sparks<sup>1</sup>, Hiroo Fukuda<sup>2</sup>, Elison Blancaflor<sup>1</sup><sup>1</sup>**Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA, <sup>2</sup>University of Tokyo, Tokyo, Japan**

Mutations to the *Arabidopsis thaliana* *AGD1* gene, which encodes a class 1 Adenosine Diphosphate (ADP)-Ribosylation Factor (ARF)-Guanosine TriNucleotide (GTPase) Activating Protein (GAP), triggers the formation of wavy root hairs. To better understand the position of AGD1 within the intricate signaling pathways that govern root hair polarity, we studied the localization of a functional AGD1-green fluorescent protein (GFP) fusion in living root hairs, evaluated various components of root hair tip growth in *agd1* mutants, and analyzed double mutants of *agd1* and other loci involved in root hair development. AGD1-GFP decorated punctate bodies in the root hair that were reminiscent of fluorescently labeled endomembrane compartments. However, AGD1-GFP foci were distinct from several endomembrane markers and localization of AGD1 punctate structures persisted despite exposure to Brefeldin A or Wortmannin. Targeting of RabA4b-GTPase, ROP2-GTPase and phosphatidylinositol-4-phosphate, which are essential components of tip growth maintenance, were altered in *agd1* root hairs. Furthermore, dampened tip cytosolic calcium oscillations and disrupted tonoplast dynamics were observed in root hairs of *agd1*. Double mutant analysis indicated that *RHD4*, which encodes a phosphatidylinositol-4-phosphate phosphatase, is epistatic to *AGD1*. On the other hand, double mutants to *AGD1* and *ACT2*, a root hair-expressed vegetative actin isoform, displayed additive root hair defects. Taken together, our results support a model that positions *AGD1* downstream of phosphoinositide metabolism in controlling cytoskeletal, cytosolic calcium , ROP2 and RabA4b-mediated root hair polarity.

**274 Dissecting The Requirement For Plant RanGAP1 Subcellular Targeting And GAP Activity For Its Cellular And Developmental Functions**Joanna Boruc, Thushani Rodrigo-Peiris, Iris Meier**The Ohio State University, Columbus (OH), USA**

RanGAP is an accessory protein of Ran signaling, which is involved in nucleocytoplasmic transport, spindle organization and post-mitotic nuclear assembly. These functions of RanGAP are conserved across higher eukaryotic organisms. However, we have recently discovered a novel function for higher plant RanGAP in cytokinesis, possibly reflecting the phragmoplast-dependent division of plant cells. We have shown that *Arabidopsis* RanGAP1 is a continuous marker of the cell division plane and it is required for proper cell division and plant development, likely independent of its role in nucleocytoplasmic trafficking. We have created *Arabidopsis* mutant lines with decreasing RanGAP levels and increasing phenotypic severity. These mutants are a valuable tool to dissect the requirement for the GAP activity and/or mitotic subcellular addresses of RanGAP for its role in plant cell division and development. We have identified a point mutation in the targeting domain of RanGAP that blocks all subcellular positioning during mitosis, but which still complements a temperature-sensitive yeast RanGAP mutant strain. Moreover, we have constructed point mutations that block the GAP activity, but do not interfere with the subcellular positioning. Through the complementation of the mutant plants with these mutant versions of the protein, we are currently testing which features of *Arabidopsis* RanGAP are required for its roles in cell division and plant development. These data will further evaluate our hypothesis of a separate evolution of RanGAP targeting mechanisms and its subfunctionalization in different kingdoms.

**275 GT-2 Family Transcription Factors Regulate Cell Growth in Arabidopsis**Christian Breuer, Ayako Kawamura, Keiko Sugimoto**RIKEN Plant Science Center**

Plant organ growth is regulated by two distinct processes, first, the cell number which is controlled by cell proliferation, and second, the size of individual cells, which is regulated by cell expansion and cell growth. In *Arabidopsis*, various studies have shown a positive correlation between cell size and ploidy, but the molecular mechanisms underlying this observation are still unclear.

We have previously identified GT-2-LIKE1 (GTL1) as a repressor of ploidy-dependent cell growth in trichomes. GTL1 is a nuclear protein, and further expression analysis revealed that GTL1 is expressed in various cell types, except meristematic cells, throughout plant development, suggesting that GTL1 might function as a general transcriptional regulator of plant cell growth. Ubiquitous expression of *GTL1* arrests early seedling growth, and thus, we have used strong cell- and tissue-specific promoters to allow an assessment of the potential role of *GTL1* in regulating growth, ploidy and size of plant cells. Detailed examination of these ectopic lines confirms that *GTL1*

can negatively regulate cell growth and ploidy of any cell type tested in *Arabidopsis*. In addition, we are able to show the essential role of endoreduplication for early differentiation processes in plant cells.

*GTL1* is a member of the GT2 trihelix transcription factor family has two closely related homologs in *Arabidopsis*, *DF1* and *GT2*. Loss-of-function alleles for *DF1* and *GT2* do not exhibit any obvious phenotypes. Furthermore *GTL1*, *DF1* and *GT2* show similar expression patterns and ectopic expression studies of *DF1* and *GT2* also cause a dramatic decrease in cellular growth. These results suggest a conserved function among the three homologs, and confirming our initial assumption of functional redundancy within the gene family. Investigations to reveal direct down-stream targets of these transcription factors are currently ongoing and are considered as a subject for discussion.

## 276 Identification of Nuclear Genes Encoding Chloroplast-Localized Proteins Required for Embryo Development in *Arabidopsis*

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We describe here the diversity of chloroplast proteins required for embryo development in *Arabidopsis*. Interfering with certain chloroplast functions has long been known to result in embryo lethality. What has not been reported before is a comprehensive screen for embryo-defective (*emb*) mutants altered in chloroplast proteins. From a collection of transposon and T-DNA insertion lines at the RIKEN chloroplast function database (<http://rarge.psc.riken.jp/chloroplast/>) that initially appeared to lack homozygotes and segregate for defective seeds, we identified 23 additional examples of *EMB* genes that likely encode chloroplast-localized proteins. Fourteen gene identities were confirmed with allelism tests involving duplicate mutant alleles. We then queried journal publications and the SeedGenes database ([www.seedgenes.org](http://www.seedgenes.org)) to establish a comprehensive dataset of 381 nuclear genes encoding chloroplast proteins of *Arabidopsis* associated with an embryo-defective (119 genes), plant-pigment (121 genes), gametophyte (3 genes) or alternate (138 genes) phenotype. Loci were ranked based on the level of certainty that the gene responsible for the phenotype had been identified and the protein product localized to chloroplasts. Embryo development is frequently arrested when amino acid, vitamin, or nucleotide biosynthesis is disrupted but proceeds when photosynthesis is compromised and when levels of chlorophyll, carotenoids, or terpenoids are reduced. Chloroplast translation is also required for embryo development, with genes encoding chloroplast ribosomal and pentatricopeptide repeat (PPR) proteins well-represented among *EMB* datasets. The essential chloroplast *accD* locus, which is necessary for fatty acid biosynthesis, appears to explain why chloroplast translation is required for embryo development in *Arabidopsis*. Plant species that can tolerate the loss of chloroplast translation, including *Brassica* and maize, have evolved a compensation mechanism that allows an alternate form of the enzyme produced in the cytosol to carry out fatty acid biosynthesis in the chloroplast. *Research supported by the NSF Arabidopsis 2010 Program.*

## 277 Abstract Withdrawn

## 278 SWEET Sugar Transporters For Cellular Efflux Highjacked For Nutrition Of Pathogens

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Sugar efflux is essential for processes requiring cellular exchange of carbon and energy in multicellular organisms. Examples in plants include nectar secretion, pollen nutrition, release of sugars from mesophyll cells as a first step for phloem loading, seed nutrition, and carbon sequestration in storage tissues. Despite the widespread importance, sugar effluxers have remained elusive. A novel class of sugar transporters, the SWEETs, was identified using FRET glucose nanosensors, which dynamically monitor subcellular glucose levels in live cells (Chen et al., 2010). The SWEETs form a broadly conserved gene family with 17 members in *Arabidopsis*, 7 in *Chaenorhabditis elegans* and 1 in human. AtSWEET1 and AtSWEET8/RPG1 function as uniporters. AtSWEET8/RPG1 is expressed in the tapetum and is essential for pollen viability (Guan et al., 2008). The rice homologs OsSWEET11/Xa13/Os8N3 and OsSWEET14/Os11N3 are highjacked by bacterial pathogens by means of direct binding of a bacterial effector to the promoter (Chen et al., 2010 and Antony et al., 2010). OsSWEET11 promoter mutations that abrogate this binding are responsible for resistance to *Xanthomonas oryzae* in a wide spectrum of rice lines. Both OsSWEET11 and OsSWEET14 mediate glucose transport when expressed in HEK293T cells or *Xenopus* oocytes, suggestive of a role in local sugar efflux induced by the pathogens. This mechanism of pathogen nutrition may be general because several pathogens induce *Arabidopsis* SWEETs during infection, though each pathogen tested induces a different set of SWEETs.

## 279 The Localization of APYRASE1/2 and Their Roles in Regulating of Growth and Development

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Apyrases (EC 3.6.1.5) are known as enzymes that hydrolyze both di- and triphosphate nucleotides and are widely identified in eukaryotic cells. They are categorized as E-type ATPases, which have the characteristics of broad divalent cation ( $Mg^{2+}$ ,  $Ca^{2+}$ ) requirement for activation, and insensitivity to inhibitors of F-type, P-type, and V-type ATPases. Apyrases are well characterized in animals and yeast. In animals it plays a crucial role in terminating signal transduction initiated by extracellular nucleotides extracellular ATP (eATP). In yeast, GDA1 is involved in regulating protein glycosylation, sugar level control and membrane integrity.

In *Arabidopsis*, seven apyrases were identified. Currently, only Apyrase 1 and Apyrase 2 have been well characterized in the literature. Previous research showed that the single knock-out mutants did not show any discernible phenotype due to the functional redundancy of APY1 and APY2. However, the double knockout (DKO) pollen failed to germinate, which implicated this step as the cause of the lethality of apyrase double knockout mutants. In addition, suppression of apyrases in the RNAi lines display a dwarf phenotype in overall vegetative growth and have dramatically reduced growth in primary root and etiolated hypocotyls. In this work, APY1/2 tagged with C-terminal green fluorescence protein (GFP) can rescue the double knockout successfully, which indicates that the fusion proteins have the proper localization and function. Confocal microscopy of fluorescently tagged APY1 and APY 2 shows they mainly reside in Golgi vesicles. The correlation of the localization of APY1 and APY2 and their regulation of growth and development will be examined in the future.

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## **280 Membrane Bound Regulators of Actin Depolymerizing Factor (ADF)**

*Katrina Cuddy, Paris Grey, David Oppenheimer*

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Actin filament turnover is required for many actin-dependent cellular processes including cell motility and membrane trafficking. Members of the actin depolymerizing factor/cofilin (ADF) family of actin binding proteins are essential for severing/depolymerizing actin filaments. Because ADF plays a central role in severing actin filaments, understanding the regulation of ADF activity is essential for understanding actin dynamics. We recently identified a new regulator of ADF in plants named RPA for REGULATOR OF PLANT ADF. The *RPA1* gene was isolated by map-based cloning of a gene responsible for normal trichome shape in *Arabidopsis*. The actin cytoskeleton in *rpa1* mutants showed an increase in the number of thick actin filament bundles in developing trichome cells, culminating in an "actin knot" and numerous actin rings surrounding the cell nucleus. Our *in vitro* analysis of RPA1 function showed that it interacts with ADF and inhibits actin binding to ADF. RPA1 is a member of a moderately sized gene family. Interestingly, about half of the members possess a putative transmembrane domain (TM) at their N-terminus. To test the hypothesis that the putative TM domain targets the RPA proteins to particular membrane compartments, we are constructing GFP fusions to one of the TM-containing RPA10 protein. In addition we are conducting *in vitro* transcription/translation of the RPA10 protein in the presence of eukaryotic microsomes to test for co-translational membrane insertion. The results of these experiments will be discussed. Understanding the function of membrane association of RPA proteins will provide key insight into the role of actin dynamics in membrane trafficking.

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## **281 Dissecting the pathway of delivering tail-anchored proteins to the plant outer nuclear envelope**

*Mintu Desai, Iris Meier*

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Tail-anchored (TA) proteins are characterized by a C-terminal transmembrane domain (TMD) that mediates post-translational insertion into the membrane of the endoplasmic reticulum (ER) as well as the outer membranes of mitochondria and chloroplasts. They participate in important cellular activities, acting as ER-associated enzymes, vesicle-fusing SNARES, protein translocation complexes, and plasma membrane receptors. However, the specificity of their sorting to different cellular membranes is not well understood. We have identified a plant-unique TA protein (WIP1) that is specifically associated with the outer nuclear envelope (ONE) and have mapped a 36-amino-acid domain (WIP1-Transmembrane Domain Fragment, WIP1-TDF) sufficient for ONE targeting. Further dissection of the targeting signal by TMD swap, mutation and deletion constructs indicates that the most C-terminal 9 amino acids are sufficient for ONE targeting when combined with a generic (oligo-L) TMD. GET (Guided Entry of Tail Anchored Proteins) is a major targeting pathway for TA proteins in yeast. We have identified three putative *GET3p/Asna-1* orthologs from *Arabidopsis*, AtGET3a, b and c. The yeast ARR4::HIS ( $\Delta$ get3) temperature sensitive mutant was rescued by AtGET3b and AtGET3c, and AtGET3b binds to WIP1 in a yeast two-hybrid assay. Transient *in-vivo* localization of GET3p orthologs show ER and cytoplasmic localization, consistent with their proposed function. The domain requirement for the WIP1-AtGET3b interaction are being mapped and compared with the requirement for WIP1 ONE targeting. T-DNA insertion lines for AtGET3b and AtGET3c have been identified and will be investigated for WIP1 ONE-targeting defects. Together, our data will present the first precisely defined targeting signal for ONE-localized plant proteins and will begin dissecting the cytosolic pathway involved in TA protein delivery to the ONE.

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## **282 AtCenp-E2, a Kinesin-7, Plays a Conserved Role in Mitosis and is Important for Meiosis Progression**

*Kristophe Diaz, Denise Butler , Akielia Mayers , Adán Colón-Carmona*

**University of Massachusetts, Boston**

Kinesin motor proteins are crucial for all stages of mitosis progression. In *Arabidopsis thaliana*, there are 61 kinesins organized in 14 functional subfamilies. Among them, two *Arabidopsis* kinesin-7, called *Arabidopsis thaliana* centromere associated protein E (AtCenp-E) 1 and AtCenp-E2, are hypothesized to be involved in chromosomes segregation during mitosis. In mammalian system, dividing cells with a kinesin-7 knockdown mutation show aberrant metaphases and display lagging chromosomes located at spindle poles in addition to a disruption of the spindle assembly checkpoint. Using a plant reverse genetic approach, we isolated and characterized a T-DNA mutation that clearly demonstrates that AtCenp-E2 plays an important and conserved role in mitosis. AtCenpe-E2 is involved in monitoring and maintaining proper chromosomes organization from pro-metaphase to anaphase. It also participates in the plant spindle assembly checkpoint. Strikingly, changes in AtCenp-E2 gene expression resulted in aneuploidy in meristematic cells. In addition, our study demonstrates that this plant kinesin-7 is involved in meiosis progression, indicating the discovery of a mitotic regulatory gene that is important for gamete production.

**283 secRFP, as a Powerful Marker for Plant Secretory Pathway Mutant Screening***Wenyan Du, Federica Brandizzi***Michigan State University, MI, US**

Soluble proteins that are secreted by default start their journey at the endoplasmic reticulum (ER), pass through the Golgi and finally reach the apoplast by mechanisms that are largely unknown. Using fluorescent marker in mutant screening with confocal microscopy is a powerful approach to study this complicated pathway. These markers might be Golgi markers, such as ST GFP and ER markers, such as ssGFPHEDEL. secRFP is a protein based on a sporamin signal sequence-mRFP fusion (Faso et al., 2009). This protein is usually secreted outside of the plasma membrane and detectable in the apoplast and ER bodies. Compared to other markers, secRFP provides more easily ways to distinguish a mutant phenotype from wild type. More importantly, it not only enable us to explore how proteins been transport from ER to the Golgi, but also what happens in post Golgi trafficking, which is less studied. Several secRFP mutants have been identified so far. The related proteins would be important in secretory pathway. The most recent findings of this screen are presented in the poster.

**284 Early Signaling in Plant Immunity***Tenai Eguen, Zhouxin Shen, Steve Briggs, Earl Kang, Michelle Lee***University of California, San Diego**

PAMP triggered immunity (PTI) and Effector triggered Immunity (ETI) are two forms of disease resistance mounted by plants to fight pathogen infection. Either ETI or PTI can trigger systemic acquired resistance (SAR), which requires elevated Salicylic Acid (SA) levels and consequent redox activation of NPR1. Exogenous application of BTH can serve as a potent substitute for elevated SA levels. We used liquid chromatography electro-spray ionization tandem mass spectrometry (LC ESI MSMS) to survey the arabidopsis leaf proteome for changes induced by BTH treatment for 5 minutes. Changes in protein levels were determined by extracting total protein, converting proteins to tryptic peptides, labeling the peptides with iTRAQ mass tags, and comparing the iTRAQ tag intensities from the different samples to each other. The levels of 7153 proteins were compared and 21 were found to increase in abundance while 28 decreased. A total of 2,384 phosphopeptides were identified derived from 1,105 proteins. The levels of 19 phosphopeptides increased in response to BTH while 24 decreased. Changes in redox state were determined by using LC ESI MSMS to observe differential binding to a mutant thioredoxin column which selectively binds proteins containing disulfide bonds. Of the 2,260 identified proteins, 196 were observed to bind only after BTH treatment while 53 proteins lost binding. Several of the proteins that changed in response to BTH are known to play important roles in jasmonic acid signaling, stress tolerance, hypersensitive response, reactive oxygen species (ROS) signaling, or cell death. The observed changes in abundance, phosphorylation and redox state in response to infection provide us with important clues on how early defense signaling works.

**285 Four amino acids guide the assembly or disassembly of *Arabidopsis* histone H3.3-containing nucleosomes***Leilei Shi<sup>1</sup>, Jing Wang<sup>1</sup>, Fang Hong<sup>1</sup>, David Spector<sup>2</sup>, Yuda Fang<sup>1</sup>*<sup>1</sup>**Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China, <sup>2</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA**

The histone variant H3.3 and the canonical histone H3.1, which differ in only 4-5 amino acid positions, are co-expressed in complex multicellular eukaryotes from fly to human and plant. H3.3 is mainly associated with active chromatin by replacing H3.1 through chaperones such as HIRA, DAXX, ATRX or DEK and plays important roles in the germline, epigenetic memory and reprogramming. However, the signals within H3.3 that serve as a guide for its dynamic deposition or depletion in plant chromatin are not clear. *Arabidopsis* histone H3.3 differs from H3.1 by four amino acid sites 31, 41, 87 and 90. While histone H3.1 is highly enriched in chromocenters, H3.3 is present in nucleolar foci in addition to being diffusely distributed in the nucleoplasm. We have evaluated the function of the four amino acids that differ between H3.1 and H3.3. We will show how these four amino acids guide the assembly and disassembly of *Arabidopsis* histone H3.3-containing nucleosomes in the nucleolar chromatin.

**286 Characterisation Of The ER Accessory Protein AXR4***Alison Ferguson, Ranjan Swarup***University of Nottingham, Nottingham, United Kingdom**

ER accessory proteins are a novel class of endoplasmic reticulum (ER) proteins that facilitate the exit of specific polytopic membrane proteins from the ER. They are important for the correct targeting of their cognate polytopic membrane protein to the plasma membrane and their absence leads to abnormal accumulation of their targets in the ER (Kota and Ljungdahl, 2005).

AXR4 has previously been shown to be involved in the correct targeting of AUX1 (a trans-membrane protein involved in transport of plant hormone auxin) to the plasma membrane (Dharmasiri et al., 2006). AUX1 belongs to a small multigene family in *Arabidopsis* and recent genetic and molecular evidences suggest that AXR4 also appears to regulate trafficking of AUX1 homologs LAX2 and LAX3. Efforts are underway to investigate the molecular basis of AXR4 role in regulating trafficking of these auxin transporters. Recent results using heterologous expression systems indicate that AXR4 directly interacts with AUX1. Currently efforts are being made to investigate *in planta* interactions. In addition, molecular, bioinformatic and protein modelling approaches are being used to pin point functionally important residues in AXR4.

Dharmasiri S, Swarup R, Mockaitis K, Dharmasiri N, Singh SK, Kowalchyk M, Marchant A, Mills S, Sandberg G, Bennett MJ, Estelle M (2006). AXR4 is required for localisation of the auxin influx facilitator AUX1. *Science* 312: 1218-1220.

Kota J, Ljungdahl PO (2005). Specialised membrane-localized chaperones prevent aggregation of polytopic proteins in the ER. *J. Cell Biol* 168: 79-88.

**287 Identification of a Novel Endosome Associated Protein that Promotes Movement of the SHORT-ROOT Transcription Factor**

*Koji Koizumi, Shuang Wu, Kimberly Gallagher*  
**University of Pennsylvania**

For an organism to develop and function properly, its cells must be able to share information. One way in which plant cells do this is through the directed transport of transcription factors. Our research focuses on the movement of a root expressed transcription factor, SHORT-ROOT (SHR) that is responsible for proper patterning of the vasculature and ground tissue, as well as maintaining indeterminate growth. We have been able to show that SHR movement occurs via plasmodesmata (PD). We have also identified a novel protein, SIEL (SHORT-ROOT INTERACTING EMBRYONIC LETHAL) that interacts with SHR and promotes its movement. SIEL also interacts with other non-cell-autonomous proteins including CAPRICE (CPC), TARGET OF MONOPTEROUS 7 (TMO7) and AGAMOUS-LIKE (AGL21). We find SIEL associated with nuclei and endosomes. Live imaging of YFP-SIEL reveals that the protein moves rapidly within the cell. We suggest that SIEL is an endosome associated intracellular shuttle that assists in the trafficking of cargo proteins to sub-cellular domains including the plasmodesmata (PD), where the cargo can then exit the cell.

**288 Arabidopsis ARCP Protein DDH1/CSI1 Is A Novel Microtubule-associated Protein Which Is Required For Microtubule Stability**

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**Institute of Plant Physiology & Ecology, Chinese Academy of Sciences, Shanghai, China**

Studies on the Arabidopsis Armadillo repeat containing protein Defective Dehiscence1 (DDH1), which is also known as cellulose synthase-interactive protein 1 (CSI1), showed that knockout mutant of DDH1 exhibits defective anther dehiscence as well as root cell radial expansion, and both can be rescued by introducing the mammalian microtubule stabilizing protein MAP4. Taking advantage of anti-DDH1 antibody, we demonstrate that DDH1 coimmunoprecipitates with  $\alpha$ -tubulin in vivo and colocalizes with cortical microtubules in root cells, which indicates DDH1 a novel microtubule-associated protein. Further studies showed that cortical microtubules are hypersensitive to microtubule-disrupting drug oryzalin and dehydration treatment. Although cortical microtubules have been shown to guide the CESA complex in varies reports, our results suggest a reciprocal interaction between microtubule and CESA which is mediated by DDH1 as a scaffold protein.

**289 Calcium signaling during the Arabidopsis gravitropic response**

*Won-Gyu Choi<sup>1</sup>, Gabriele Monshausen<sup>2</sup>, Simon Gilroy<sup>1</sup>*

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Plants adapt to a changing environment by entraining their growth to prevailing conditions. Such 'plastic' development requires a highly dynamic integration of growth phenomena/growth regulation with signal perception and transduction systems. Such a signaling system is thought to operate during the gravitropic growth response that allows the root system to navigate through the complex environment of the soil. Experimental evidence suggests that  $\text{Ca}^{2+}$  changes are associated with gravitropism in roots. However, it has been difficult to functionally link such  $\text{Ca}^{2+}$  changes to either the signal perception or response machinery. Additionally, any relationship to signal transmission and the control of growth, for example through modulation of the auxin transport/response system, remains unknown. We have used a combination of confocal imaging of plants expressing the YC3.6 GFP-based  $\text{Ca}^{2+}$  sensor and high-resolution morphometric growth analysis with a range of gravitropic mutants to help define how tropic growth and  $\text{Ca}^{2+}$  signaling are related. We have found that asymmetric  $\text{Ca}^{2+}$  signals occur in the gravi-responding regions of the root after several minutes of stimulation. One such  $\text{Ca}^{2+}$  signal appears to relate to growth-associated dynamics of root pH. These changes are linked to auxin and are dependent on auxin transport mediated by AUX1 specifically expressed in cells of the lateral root cap and root epidermis. They operate independently of the TIR1 auxin perception system. In addition to such growth-related dynamics, highly cell-type-specific, asymmetrical  $\text{Ca}^{2+}$  signals also occur in cells of the root cap that do not exhibit a growth response to gravi-stimulation. The root cap is thought to represent the site of gravi-perception and these  $\text{Ca}^{2+}$  changes appear most likely connected to relaying directional gravitropic information from the cap. This work is funded by NASA and NSF.

**290 Developmental Traits Contributing to Heterosis in *Arabidopsis* Hybrids Between C24, Landsberg *erecta*, and Columbia Accessions.**

Michael Groszmann<sup>1,2</sup>, Ian Greaves<sup>1,3</sup>, Amanda Huen<sup>1</sup>, Yingjie Yu<sup>1,4</sup>, Mark Talbot<sup>1</sup>, Maria Alonso-Peral<sup>1</sup>, Jean Finnegan<sup>1</sup>, William Peacock<sup>1</sup>, Elizabeth Dennis<sup>1</sup>

<sup>1</sup>CSIRO Plant Industry, Canberra, ACT, Australia, <sup>2</sup>NSW Agricultural Genomics Centre, Wagga Wagga, NSW, Australia., <sup>3</sup>Department of Genome Biology - John Curtin School of Medical Research, Australian National University, ACT, Australia., <sup>4</sup>School of Life Sciences - Northeast Normal University, Changchun - Jilin Province, China.

Hybrid vigor, or heterosis, is characterised by the superior performance of a hybrid over its parents in traits such as growth rate, biomass, and stress tolerance, leading to yield increases. Hybrids are extensively utilised for many agricultural and horticultural crops including maize, rice, sunflower and canola, and account for a large proportion of global crop production. A large effort is being made to unravel the molecular causes generating hybrid heterosis which will lead to improvements in crop production required to secure future food production. Our lab is currently using hybrids between *Arabidopsis* accessions as a model for dissecting the genetic and epigenetic contributions of hybrid heterosis. As part of this work we are characterising the heterotic developmental traits of hybrids derived from C24, Landsberg *erecta*, and Columbia accessions. The degree of heterosis differs between each hybrid combination. The enhanced growth of the hybrids is not linear throughout their life cycle, with 'bursts' of heterosis occurring at certain stages of development, with the first onset observed during embryogenesis. The patterns of growth differ to some degree between the hybrid combinations, as does the developmental cause of the increased growth (i.e. cell number, cell size, etc). Here we show some of our characterisation of the hybrids, highlighting the developmental time points and tissues that will be our focus for subsequent molecular analysis in our attempts to dissect the mechanisms of heterosis.

**291 Defining The Role of Endomembrane Trafficking in EDR1-KEG Controlled Programmed Cell Death**

Yanngan Gu, Roger Innes

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Loss-of-function mutations in the *Arabidopsis thaliana* ENHANCED DISEASE RESISTANCE 1 (*EDR1*) gene confer enhanced programmed cell death (PCD) under both abiotic and biotic stress conditions. All *edr1*-mediated phenotypes can be suppressed by a specific missense mutation (*keg-4*) in the *KEEP ON GOING (KEG)* gene, which encodes a multi-domain protein that includes a RING E3 ligase domain, a kinase domain, Ankyrin repeats, and HERC2-like repeats. Using confocal laser scanning microscopy and fluorescent organelle markers, we determined that KEG localizes to *trans*-Golgi network/early endosome vesicles (TGN/EE). Localization of KEG to the TGN/EE is blocked by the vesicle trafficking inhibitor Brefeldin A, but not by Wortmannin or Concanamycin A, suggesting that KEG dissociates from TGN/EE vesicles when forward trafficking is blocked. Loss-of-function mutations in KEG cause severe defects in hypocotyl cell expansion that are independent of ABI5-mediated signaling pathways. Electron microscopic ultra-structure analysis revealed severe defects in both cellulose microfibril orientation and central vacuole morphology in *keg* hypocotyl cells. Abnormal membrane structures were found to accumulate inside of the central vacuoles of *keg* root cells. These data suggest that KEG plays a central role in maintaining endomembrane trafficking systems. In addition, we found that KEG physically associates with EDR1 inside plant cells and can recruit EDR1 to TGN/EE vesicles from the endoplasmic reticulum and cytosol. We hypothesize that TGN/EE vesicles function as signaling platforms during EDR1-mediated stress responses. In support of this hypothesis we found that EDR1 accumulates on TGN/EE vesicles in *Arabidopsis* protoplasts upon heat shock, and that *edr1* mutant plants undergo enhanced PCD following transfer to high temperature growth conditions. These observations suggest that TGN/EE localization of EDR1 is crucial for cell death suppression. Collectively, these data suggest that EDR1 and KEG function together to regulate endocytic trafficking and/or formation of signaling complexes on TGN/EE vesicles during stress responses.

**292 Coordination of Phosphatidylinositol-4-OH kinase PI4Kβ1 and Phosphatidylinositol-4-phosphate Phosphatase RHD4 Activities During *Arabidopsis* Root Hair Growth**

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Root hairs are model system to study tip growth. In *Arabidopsis*, these epidermal cells form hair-like structures that emerge from the root shaft. Upon hair initiation, these cells transition from diffuse to tip-restricted cell growth. This is accompanied by reorientation of cell secretory pathways, and cell wall components are selectively targeted to the apex of the hair cell. We previously showed the regulatory Rab GTPase, RabA4b, selectively marks cell wall-containing compartments that accumulate in growing root hair apices during tip-growth. RabA4b recruits the lipid kinase, PI4Kβ1, to plant secretory vesicles, and this enzyme, which converts PI to PI(4)P plays important roles during tip-growth as loss of PI4Kβ1 and the highly conserved PI4Kβ2 results in root hair defects. Further underscoring the importance of PI(4)P during polarized membrane trafficking, we found that the root hair development mutant (root-hair-defective4) encodes a Sac1p-like PI(4)P phosphatase, and PI(4)P was shown to be predominantly localized to apical plasma membranes in the tips of growing root hairs. Examination of the subcellular localization of PI4Kβ1 and RHD4, indicated both these proteins were co-recruited to RabA4b-labeled membranes. To better understand how PI-4Kβ1 kinase and RHD4 phosphatase activities might be coordinated on RabA4b-compartments during tip-growth we examined the phenotypes of rhd4, pi4kβ1/β2 double- and rhd4/pi4kβ1/β2 triple-mutants. Further we have examined the effects of these mutants on subcellular dynamics of RabA4b-labeled compartments and the subcellular distribution of PI(4)P using the EYFP-hFAPP1 biosensor. Finally, we present evidence that PI4Kβ1 and RHD4 are regulated by cytoplasmic Ca<sup>2+</sup>

through interactions with EF-hand containing  $\text{Ca}^{2+}$ -binding proteins. We discuss implications of  $\text{Ca}^{2+}$  regulation on PI(4)P dynamics and present a working model for coordinate action of PI4K $\beta$ 1 and RHD4 during polarized membrane trafficking during root hair tip-growth.

**293 Tonoplast Membrane Protein Mislocalizes to the ER In *impaired traffic to tonoplast* Mutants**

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Tonoplast membrane proteins transport molecules in and out of the vacuole including ions, lipids, sugars, hormones and defense molecules. The transport of molecules through the tonoplast membrane proteins is essential for vacuolar function. Two trafficking pathways for tonoplast membrane proteins through the endomembrane system have been proposed; the one comprises passage through the Golgi and delivery to the pre-vacuolar compartment (PVC) of the lytic vacuole and in the other, the tonoplast intrinsic proteins (TIPs) move directly from the ER to the PVC of the protein storage vacuole (PSV). However, the trafficking mechanisms of tonoplast membrane proteins are largely unknown. To identify proteins that regulate the targeting of TIPs, we screened an EMS mutagenized line that carries the GFP- $\delta$ -TIP, and mCherry-HDEL markers for mutants where the tonoplast marker was mis-localized. Six *impaired traffic to tonoplast* (*itt*) mutants with mis-localized GFP- $\delta$ -TIP and abnormal vacuole morphology were identified by confocal microscopy. In *itt2*, *itt4*, and *itt6* mutants, GFP- $\delta$ -TIP co-localizes with mCherry-HDEL, which indicates that the tonoplast marker accumulated at the ER. We have initiated mapping experiments to determine the identity of the *ITT* loci. Initial characterization of mutants and mapping experiments will be presented.

**294 Transgenic polyglutamine proteins show length-dependent aggregation in *Arabidopsis***

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Proteins containing long tracts of consecutive glutamines (polyQ) are known to aggregate in cells of a wide variety of organisms including bacteria, fungi, invertebrates and mammals. Expression of polyQ proteins, encoded by dominant CAG-expanded alleles, is thought to be the root cause of several human neurological disorders. PolyQ proteins show a threshold for aggregation that depends both on the length of the polyQ tract and the state of the proteostasis systems. The Columbia genome encodes a number of polyQ-containing proteins however none have a predicted polyQ tract length of over 24 glutamines, which is below the threshold for aggregation detected in other organisms. Using transgenic GFP-polyQ reporters, we show that polyQ proteins containing tracts of 88 or 103 glutamines mislocalize to punctate or reticulate structures, compared to the diffuse localization of reporters with 46, or 15 glutamines, or GFP alone. By western blotting, we also detect high molecular weight forms of the polyQ-GFP proteins, indicative of intracellular aggregation, specifically in the lines expressing longer polyQ tracts (88 and 103 glutamines). Together our results indicate that polyQ proteins show length-dependent aggregation similar to animal and fungal models.

**295 MSL2 and MSL3 Provide a Functional Link Between Membrane Stretch and Chloroplast Division**

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*Arabidopsis* chloroplasts must divide repeatedly in order to maintain a population of approximately 100 chloroplasts per mesophyll cell (Lopez-Juez and Pyke, 2005). Numerous proteins required for chloroplast division have recently been identified, and the structural and functional relationships between components of the division apparatus are beginning to be elucidated (Yang et al., 2008). However, the mechanisms by which division is regulated in response to chloroplast size, environmental stimuli, or developmental factors are largely unstudied. We previously reported that that two homologs of the bacterial mechanosensitive (MS) channel MscS, MscsS-Like (MSL) 2 and MSL3, are localized to the chloroplast envelope where they are required for normal plastid shape and size (Haswell and Meyerowitz, 2006). To further investigate the enlarged chloroplasts found in the *msl2msl3* mutant, we characterized FtsZ ring assembly, an early step in chloroplast division. FtsZ ring assembly at the middle of the chloroplast is controlled both by components evolved from the bacterial fission apparatus (AtMinE and AtMinD), and by plant-specific components (MCD1 and ARC3) (Nakanishi et al., 2009). We found that, similar to *arc11* (a lesion in *AtMinD*), *arc3*, and *mcd1* chloroplasts, *msl2msl3* chloroplasts exhibited multiple FtsZ rings. Triple mutant analyses indicated that *MSL2* and *MSL3* affect FtsZ ring assembly through the same pathway as *AtMinD*, *AtMinE*, and *ARC3*. Finally, we found that an *E. coli* strain lacking MscS also showed aberrant FtsZ ring assembly. These results establish MSL proteins as novel regulators of chloroplast division, and demonstrate that their role is evolutionarily conserved. We propose that mechanical stress—perhaps resulting from chloroplast expansion or formation of the contractile ring—regulates FtsZ ring placement in both plants and bacteria.

**296 Assessing the Function of Matrix Attachment Region-Binding Filament-Like Protein (MFP1) in**

***Arabidopsis thaliana***

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Matrix attachment region-binding filament-like protein 1 (MFP1) is a long coiled-coiled DNA-binding protein which is found in the thylakoid membrane in the chloroplasts of plants. It consists of an N-terminal transmembrane domain anchoring it in the thylakoid membrane with a long coiled-coil domain exposed to the stroma. This structure is similar to that of golgins, which are proteins found anchored in the Golgi membranes that are involved in giving the Golgi body its characteristic stacked structure. We hypothesize that due to its structural similarity MFP1 may serve a similar purpose in the thylakoid membranes, which also exhibit a stacked structure. A knockout mutant lacking MFP1 displays no obvious phenotype visible to the naked eye. However, preliminary comparison of cells from

wild-type and mutant seedlings using transmission electron microscopy indicates a slightly decreased chloroplast size with decreased density of thylakoid membranes within the chloroplasts of plants lacking MFP1. Since a change in thylakoid structure could compromise the plant's ability to photosynthesize, further experiments will be done to compare photosynthetic efficiency in the knockout mutant versus the wild-type plants.

## **297 Activity of the MCM Complex in the Endosperm of *Arabidopsis***

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The MINICHROMOSOME MAINTENANCE complex is conserved across all eukaryotes; it consists of 6 subunits (MCM2-7) which form a helical structure. The complex is essential for DNA replication as it separates the two strands of DNA at the replication fork, enabling access by DNA polymerase and other proteins. During *Arabidopsis* endosperm development, several rounds of nuclear division occur without cytokinesis forming a syncytium. This early division of the endosperm has an effect on final seed size. In *Arabidopsis*, mutants of MCM subunits cause seed abortion at various stages of development, characterized by a lack of nuclear division in the embryo and endosperm. The maternal plant contributes significantly to this phenotype, suggesting a lack of MCM protein accumulation in the central cell can cause seed abortion, or alternatively, that parental bias occurs in the expression of MCM genes. Late abortion is characterized by aberrant embryo growth, or large endosperm nuclei, or both. We are focussing on the role of the MCM5 and MCM7 subunits in the endosperm. Selectively rescuing *MCM5/MCM7* mutations in the endosperm, and specific disruption of *MCM5/MCM7* expression (using amiRNA constructs), will enable us to distinguish between phenotypes caused by a lack of endosperm expression, and those caused by a lack of maternal protein. In addition, we can analyse the contribution of the paternal genome to *MCM5/MCM7* expression using GFP fusion constructs and qPCR. These complementary experiments will allow us to determine the functions of MCM subunits in the endosperm, irrespective of their activity in other tissues.

## **298 Abstract Withdrawn**

## **299 An insight into the function of the HUA2 gene family**

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Coordinate control of processes that occur at the transition from vegetative to reproductive phase in plants is not well understood. *HUA2* has been shown to positively regulate two MADS box genes, *FLOWERING LOCUS C (FLC)* affecting flowering time, and *AGAMOUS (AG)* affecting floral patterning, thus having implication for the coordinate control of induction and maintenance of floral state. We have previously demonstrated that the natural variant of *HUA2-Sy-0* is uncoupling the effects on *FLC* and *AG*.

*HUA2* is a member of a small gene family that includes *HUA2-like* genes *HULK1*, *HULK2* and *HULK3*. Except for *HUA2*, single mutation in the gene family does not produce a visible phenotype, suggesting a functional redundancy within the gene family. Double and triple mutants within the gene family members result in various pleiotropic phenotypes. The quadruple mutant is lethal, suggesting that the *HUA2* gene family is essential for *Arabidopsis* development.

To gain more insight into the function of the *HUA2* protein family, we have identified *HUA2/HULK* interacting proteins. We have shown that the members of the *HUA2* protein family interact with proteins involved in pre-mRNA splicing, AtPRP40, FCA, RBP45 and UBP1, in both yeast two-hybrid and *in vitro* pull-down assay. *HUA2/HULK* proteins localize in the nucleus and affect the 3'-end processing of *FCA*, *AG* and *UI-70K*, suggesting that the members of the *HUA2* protein family function in pre-mRNA processing.

**300 Proteomics dissection of the *Arabidopsis thaliana* vacuolar proteome. New insights into the composition and molecular mass of protein complexes of plant vacuole**

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The vacuole is a multifunctional organelle characteristic of plant cells, playing a central role in cellular physiologies. The vacuole allows the storage of many metabolites, ensures care of turgor pressure, pH regulation and ionic homeostasis via the storage or release of solutes and ions through transporters present at the vacuolar membrane, the tonoplast. Among functions, the vacuole has a key role in cellular protection by neutralizing the compounds that could interfere with the normal metabolic pathways processes. It allows sequestration or degradation of xenobiotics and toxic compounds. Cellular detoxification mainly depends on the vacuole, but the mechanisms of transport and storage of toxics in the vacuole is still unclear. To identify proteins involved in these mechanisms we have developed a procedure to prepare intact highly purified vacuoles. We used protoplasts isolated from *Arabidopsis thaliana* cell cultures. Based on the specific activity of the vacuolar marker?  $\alpha$ -mannosidase, preparations showed the necessary degree of purity for proteomic study.

We were interesting in the characterization of the soluble and membrane vacuolar fractions. Analysis of the vacuolar sap has identified over 500 proteins, 70% of them are enzymes. Behind the 950 tonoplastic proteins described around 130 transporters were characterized.

To go into insight the vacuolar proteome fine location of proteins were carried out by the use of shave-and-conquer concept and quantification based on spectral counting. We reassessed acute vacuolar protein location (ie full membrane or associated to the external or internal membrane) and vacuole/extravacuole distribution of some soluble protease. Using biochemical and proteomics approaches, we present the first evidence of active proteasome / subtilase degradative pathway associated to the plant tonoplaste. Then to look into the supra molecular proteins organization blue-native polyacrylamide gel electrophoresis (BN/SDS-PAGE) was used. We applied this dividing method in native condition to reveal the presence of putative complexes in the soluble and tonoplastic vacuolar proteome such as complexes of ubiquitin specific peptidase and proteasome system.

Altogether, the present proteomic work constitutes the basis to study the dynamics of the vacuolar proteome in response to several stresses.

**301 Capping Protein: a Membrane-Associated Actin-Binding Protein in Arabidopsis**

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 USA**

The actin cytoskeleton is a dynamic structure, and a major regulator of cell morphogenesis, sexual reproduction and cellular responses to extracellular stimuli. Cytoskeletal function is coordinated through a plethora of actin-binding proteins (ABPs). Many of these proteins are regulated directly by binding to phospholipids. Heterodimeric capping protein (CP) is a major actin cytoskeleton regulator; it binds to filament plus-ends with high affinity and regulates filament assembly and disassembly reactions. Filament ends can be uncapped by direct interaction of CP with phosphatidic acid, a major component of plant membranes. Whether CP associates with membranes in plant cells remains an open question.

Complementary biochemical approaches were used to estimate CP cellular abundance and to elucidate possible CP-membrane association. CP was demonstrated to be moderately abundant in the cell, but likely sufficient to cap all available filament ends. Differential centrifugation and sucrose density gradients provide initial evidence that CP associates with membrane-bound organelles. This association may have profound implications for many regulated membrane functions. For example, it may facilitate mediating crosstalk between the actin cytoskeleton and a wide spectrum of essential cellular functions and processes. In particular, it may promote regulated actin assembly on cellular compartments, thereby enhancing processes like intracellular vesicle trafficking, endocytosis and post-endocytic traffic through the endosomal membrane system. Identification of the particular compartment(s) containing CP is the next step in this investigation.

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**302 Cell Biology of the trans-acting siRNA pathway in plants**

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RNA silencing is a regulatory mechanism essential for many processes during development. This mechanism is characterized by the sequence-specific inhibition of gene expression by small RNA molecules. Among the several pathways of RNA silencing, the TAS3 trans-acting siRNA pathway, which combines features of both miRNA and siRNA pathways, is unique to plant and controls several key aspects of plants development. In this pathway AGO7, a member of the ARGONAUTE family, interacts specifically with miR390 to target and cut the *TAS3* transcript priming it for production of siRNAs called tasiARFs by SGS3, RDR6 and DCL4 action. This pathway is conserved across all land plants. By their repressing activity on Auxin Response Factor members, ARF2, ARF3 and ARF4, the tasiARFs control phase change and leaf patterning. We have shown that, in addition, the tasiARFs play an essential role in control of lateral root growth (Marin, Jouannet et al., 2010, *The Plant Cell*). However our knowledge of the subcellular organization of this pathway remains essentially unknown. For this reason we have chosen to study the subcellular localization of the different members of the TAS3 pathway, by focusing on AGO7 which represents an important and specific trigger of this pathway. We show that AGO7 accumulates in

cytoplasmic foci and provide evidences for the functional importance of this cytoplasmic localization. In addition we show that several other components of the pathway are also located in cytoplasmic foci, like AGO7. Our results identify cytoplasmic hotspots for the processing of the TAS3 precursor.

### **303 An Arabidopsis ABCA Family Gene Important for Seed Storage Lipid**

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ABC transporters are highly represented in plants than in animals and they are known to transport diverse substances such as phytohormones, alkaloids, pigments, heavy metals, drugs, and lipids. ABCA family members of ABC transporters are well-known lipid transporters in animal systems. However, the function of plant ABCA family members has not been characterized yet. To identify ABCA genes important for seed storage lipid, we examined seeds from T-DNA insertion mutants of many ABCA family ABC transporters for altered seed morphology and germination. A loss-of-function mutation of an ABCA member resulted in small, shrunken seeds and a delay in seed germination. This mutation also decreased the content of triacylglycerol (TAG), a general storage form of lipid in dry seed, by approximately 35% compared to the wild type. De-esterification of the TAG and analysis of the resultant fatty acids revealed that the contents of all fatty acids analyzed (16:0, 16:1, 16:2, 16:3, 18:0, 18:1, 18:2, 18:3, 20:0, 22:0, 22:1) were reduced in the mutant dry seeds to a more or less similar extent. However, protein content of the mutant was indistinguishable from that of the wild type. The 35Spro::ABCAGDNA Arabidopsis plants produced significantly larger dry seeds than the wild type, which weighed as much as 123% more than those of wild type. The triacylglycerol content of the ABCA overexpressing seeds were increased up to 132% of the wild-type per dry seed basis. The growth and overall morphology of the ABCA overexpressing Arabidopsis plants appeared similar to that of the wild-type plants. This result implies that the ABCA has a crucial role in triacylglycerol storage in seed.

### **304 Testing for Interaction between the Exocyst Complex and Myosin XI Family Members in Cell Expansion**

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Plant cell growth requires the regulated trafficking of membrane-bound vesicles to the plasma membrane at sites of expansion. The myosin XI family of motor proteins is proposed to facilitate vesicle transport, whereas the exocyst complex is thought to be important for vesicle tethering prior to fusion with the plasma membrane. We hypothesize that the exocyst complex and certain myosin XI family members functionally interact (directly or indirectly) in trafficking pathways leading to cell expansion.

Root hairs are an excellent model for investigating plant cell growth. These cells undergo an unusual amount of elongation, allowing for wide range of phenotypic change. Expansion occurs only at the extreme apex of the cell (i.e., tip growth), and is dependent on polarized secretion, making these cells a good model for investigating vesicle trafficking. Additionally, both myosin and exocyst family members are known to be individually important for normal root hair growth, although single mutations have only quantitative effects on final root hair length.

We obtained plants with disrupted expression of either myosin or exocyst genes, and generated a series of mutant combinations interfering with both exocyst and myosin XI function. We found that certain myosin/exocyst mutant combinations produce shorter root hairs than either single mutant parent. Therefore, our current results indicate that some myosin and exocyst family members show a synthetic genetic interaction, consistent with functional interaction between these molecules. Ongoing experiments aim to test the contributions of myosin and exocyst proteins to non-polarized (i.e., diffuse) cell growth, and to identify the nature of the compartments influenced by these two sets of proteins.

### **305 DAYSLEEPER, An Essential Domesticated Transposase in *Arabidopsis***

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*DAYSLEEPER* is an evolutionary conserved domesticated transposase in *Arabidopsis*, derived from the hAT-family of transposons. *DAYSLEEPER* was found to be essential for plant development, since *DAYSLEEPER* knock-out mutants do not progress past the early seedling stage (Bundock *et al.* 2005; *Nature*, 436:282-284). *DAYSLEEPER* can bind DNA, but its exact function has not been determined to date. We found that *DAYSLEEPER*-like genes are present in all examined angiosperm species, including basal angiosperms. We named these genes *SLEEPER*-genes and found that these genes are functionally conserved in *Oryza sativa*, *Vitis vinifera* and *Arabidopsis thaliana*. *SLEEPER*-genes are not found in gymnosperms, mosses or algae. Furthermore, we have identified several *DAYSLEEPER* interacting proteins, using a yeast two hybrid approach and verified these interactions using Bi-molecular Fluorescence Complementation (BiFC) in *Arabidopsis* protoplasts. These interactors include, among others, CSN5A, sub unit of the COP9-signalosome and RRP6A, part of the exosome system. An interactor of particular interest is VPS2.3, which is one of 3 VPS2 genes found in *Arabidopsis*. VPS2 is an integral part of the ESCRT-III machinery, which is highly conserved in eukaryotes and is involved in vesicle formation. We found that *DAYSLEEPER* can also bind VPS2.1 and 2.2 in protoplasts. Moreover, we propose a model of domestication in which a transcribed transposase sheds its transposon-context and acquires new regulatory sequences through a retrocopy process.

**306 Reactive Oxygen Species Facilitate Lateral Root Emergence in Arabidopsis**Daniel Lewis, Gloria Muday**Wake Forest University**

The initiation and outgrowth of lateral roots is an important aspect of root system architecture which is regulated by intrinsic and extrinsic factors. Reactive oxygen species (ROS), usually derived from either nitric oxide or hydrogen peroxide, regulate many physiological functions in plants, including cell proliferation, cell expansion, and the response to pathogens. ROS regulates these processes by acting as signaling molecules or by regulating the activity of proteins, the availability of redox substrates, or the destruction of biological molecules. We are combining a physiological analysis of ROS function in lateral root development with analysis of genes involved in ROS production which were induced by auxin with kinetics consistent with a role in root branching in a recent microarray experiment. Time lapse imaging with the fluorescent dye oxyburst, which monitors extracellular ROS, revealed a maximum of ROS that surrounded emerging lateral root primordia (LRP). Cells in the vicinity of the emerging LRP exhibited higher levels of intracellular ROS as visualized by H<sub>2</sub>DCF-BSA. We tested whether ROS was necessary for lateral root emergence by using the synthetic thiol DTT, which acts like the endogenous reactive oxygen scavenger GSH, and found that increasing ROS scavenging had a negative impact on lateral root emergence. The nitric oxide-specific dye DAF did not exhibit increased fluorescence in the region of emerging LRP, suggesting that hydrogen peroxide or its derivatives may be the active ROS in this process, a hypothesis that we are currently testing. Our working model is that ROS is produced as a result of auxin-induced expression of ROS synthesizing enzymes and secretion of these ROS by cells surrounding the primordia may facilitate cell separation by changing cell wall chemistry. (Supported by National Science Foundation Grant # IOB-0820717)

**307 The ATG1/ATG13 Protein Kinase Complex is Both a Regulator and A Target of Autophagic Recycling in Arabidopsis**Faqiang Li, Anongpat Suttangkakul, Taijoon Chung, Richard Vierstra**Department of Genetics, University of Wisconsin-Madison, , Madison, WI, USA**

Autophagy is an intracellular recycling route in eukaryotes whereby organelles and cytoplasm are sequestered in vesicles, which are subsequently delivered to the vacuole for breakdown. The process is induced by various nutrient-responsive signaling cascades converging on the Autophagy-Related (ATG)-1/ATG13 kinase complex. Here, we describe the ATG1/13 complex in *Arabidopsis thaliana* and show that it is both a regulator and a target of autophagy. Plants missing ATG13 are hypersensitive to nutrient limitations and senesce prematurely similar to mutants negating other components of the ATG system. Synthesis of the ATG12-ATG5 and ATG8-phosphatidylethanolamine adducts, which are essential for autophagy, still occurs in ATG13-deficient plants, but not the biogenesis of ATG8-decorated autophagic bodies, indicating that the complex regulates downstream events required for autophagosome enclosure and/or delivery. Surprisingly, both ATG1a and ATG13a protein levels drop dramatically during nutrient starvation and rise again upon nutrient addition. This turnover is abrogated by inhibition of the ATG system, indicating that the ATG1/13 complex becomes a target of autophagy. Consistent with this mechanism, ATG1a is delivered to the vacuole with ATG8-decorated autophagic bodies. Given its responsiveness to nutrient demands, the turnover of the ATG1/13 kinase likely provides a dynamic mechanism to tightly connect autophagy to a plant's nutritional status.

**308 An N-Glycan-Dependent Endoplasmic Reticulum-Associated Degradation System in Arabidopsis**Wei Su<sup>1</sup>, Zhi Hong<sup>1,2</sup>, Yidan Liu<sup>1</sup>, Yang Xia<sup>1</sup>, Jianming Li<sup>1</sup>**<sup>1</sup>Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109-1048, <sup>2</sup>School of Life Science, Nanjing University, Nanjing, Jiangsu Province, China**

Asparagine-linked glycans (N-glycans) have recently emerged as crucial signals for protein folding, quality control, and endoplasmic reticulum-associated degradation (ERAD) in yeast and mammalian cells. While similar quality control systems were reported in plants, little is known about their biochemical mechanisms, especially their relationships with N-glycans. Our recent genetic studies revealed that the dwarf phenotype of two brassinosteroid receptor mutants, *bri1-5* and *bri1-9* each carrying a single amino acid change, are caused by ER retention of the mutated bri1 proteins and their subsequent degradation by an ERAD process. Mutations in *EMS-mutagenised Bri1 Suppressors 1 and 2* (*EBS1* and *EBS2*) encoding two components of an overzealous ER quality control system result in escape of mutated bri1 proteins from the ER and their correct localization to the plasma membrane where the mutated bri1 proteins initiate the brassinosteroid signaling, leading to phenotypic rescue of the brassinosteroid-insensitive dwarf phenotype of *bri1-9*. Genetic screens looking for additional *bri1-9* suppressors resulted in identification of four additional *ebs* mutants (*ebs3 - ebs6*) with increased *bri1-9* abundance and regained brassinosteroid sensitivity. Positional cloning of these four suppressor genes and biochemical studies of their gene products not only revealed a conserved N-glycan signal that tags a misfolded/mutated glycoprotein for ERAD but also identified two key components of the Arabidopsis ERAD machinery. Our research on the *ebs* project is supported by a National Institutes of Health Research Grant GM060519.

**309 The NEV and AGD6 ARF-GAPs Redundantly Control Plant Development.**Christian Burr<sup>1</sup>, Iris Chen<sup>1</sup>, Sara Orlowski<sup>1</sup>, Mark Daniels<sup>2</sup>, Sarah Liljegren<sup>1</sup>**<sup>1</sup>University of North Carolina, Chapel Hill, NC, USA, <sup>2</sup>University of Virginia, Charlottesville, VA, USA**

Members of the ADP-ribosylation factor-GTPase-activating protein (ARF-GAP) family regulate membrane trafficking by inactivating ARF G-proteins and promoting efficient cargo loading into vesicles. In *Arabidopsis*, mutations disrupting the function of the Age2-like ARF-GAP NEVERSHED (NEV) block organ abscission (Liljegren et al., 2009). *nev* mutant flowers have a unique set of trafficking

defects, including changes in the structure of the Golgi, the location of the *trans*-Golgi network, and the hyperaccumulation of extracellular vesicles. In yeast, Age2 shares redundant roles with the Gcs1 ARF GAP in regulating traffic from the *trans*-Golgi network to the plasma membrane, endosome, and vacuole. Genetic analysis of *NEV* and *ARF-GAP DOMAIN6 (AGD6)*, one of two Gcs1-like ARF-GAP genes found in *Arabidopsis*, has revealed that *nev agd6* double mutants are small, sterile, and show defects in cell expansion and meristem size. Our co-localization studies with endomembrane markers have shown that NEV localizes to the *trans*-Golgi network and recycling endosomes. We have developed markers to pinpoint the subcellular location of AGD6 and are using transmission electron microscopy to examine the trafficking defects in *nev agd6* roots and leaves. By dissecting how a set of ARF-GAPs regulate membrane trafficking in a multicellular organism, we aim to explore the integral links between the movement of specific signaling molecules and cell polarity, cell division, cell expansion, and cell wall remodeling during multiple phases of plant development.

### **310 AtMAP65-1 and AtMAP65-2 Positively Regulate Axial Cell Growth in Etiolated *Arabidopsis* Hypocotyls**

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Plant morphogenesis depends upon the organization of interphase microtubules into functional arrays. Microtubule-associated proteins (MAPs) are key regulators of array organization. Members of the MAP65/ASE1/PRC gene family are proposed to be important organizers of interphase arrays as they bundle microtubules *in vitro*, localize to interphase microtubules in plant cells, and may stabilize microtubules. We investigated the function of two highly similar proteins, *AtMAP65-1* and *AtMAP65-2*, in interphase microtubule array organization and cell growth by live-cell imaging and genetic analysis. We created fluorescent-protein fusions driven by native promoters that co-localized *AtMAP65-1* and *AtMAP65-2* to anti-parallel interphase microtubule bundles in all epidermal hypocotyl cells. *AtMAP65-1* and -2 labeling showed episodes of linear extension and retraction coincident with microtubule growth and shortening. Dynamic co-localization of *AtMAP65-1* and -2 with microtubules during bundle formation provided *in planta* evidence that plant cortical microtubule bundles are not stabilized by MAP65-1 or -2, and that bundling is accomplished through a polymerization-dependent microtubule-microtubule templating mechanism. Analysis of etiolated hypocotyl length in *map65-1* and *map65-2* mutants revealed an important role in axial cell growth, complemented by the transgenic fluorescent protein reporters. Surprisingly, *map65-1/map65-2* double mutant hypocotyl cells formed interphase microtubule arrays that were indistinguishable from control arrays. Double *map65-1/map65-2* mutants did not show morphological defects commonly associated with defects in array organization such as cell swelling and twisting. We conclude that *AtMAP65-1* and *AtMAP65-2* play a critical role in the microtubule-dependent mechanism for axial cell growth in the hypocotyl, independent of any mechanical role in microtubule array organization.

### **311 RNS2, a Conserved Member of the RNase T2 Family, Is Necessary For rRNA Decay in Plants**

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Ribosomes are essential components of all cells. Ribosome synthesis and assembly are well-studied; however, the pathways of normal ribosome decay, especially rRNA decay, are not known. Some information on ribosome recycling derives from studies on starved yeast cells that use a specialized type of autophagy, called ribophagy, to differentially target ribosomes for degradation. The RNase T2 family is composed of endonucleases that are either extracellular or targeted to the secretory pathway. Members of this family are found in all eukaryotes, suggesting that they perform an important role. Phylogenetic analyses have shown that plant RNase T2 enzymes can be classified in three classes, and that Class II enzymes may carry out a housekeeping function. Patterns of gene expression suggest that plant RNase T2 enzymes may degrade RNA when phosphate is limiting, and we hypothesized that they could also participate in recycling of RNA, particularly rRNA, to maintain normal cellular homeostasis. We found that RNS2, the only Class II RNase T2 in *Arabidopsis*, is necessary for normal decay of rRNA. Mutants lacking RNS2 activity accumulate RNA intracellularly, mainly in the vacuole. *In vivo*-labeling experiments showed that both 18S and 28S rRNA subunits have a longer half-life in the mutants than in WT plants. In addition, mutants with reduced or absent RNS2 display constitutive autophagy. These phenotypes and the subcellular localization of RNS2 in the endoplasmic reticulum and the vacuole suggest that RNS2 participates in a ribophagy-like mechanism that targets ribosomes for recycling under normal growth conditions. We suspect that lack of proper rRNA recycling alters cellular homeostasis, which results in induction of macroautophagy as a compensatory mechanism.

### **312 Sensitivity in Flowering Time Regulation by Coupling Noncoding Transcript Splicing and Chromatin**

**Silencing in *FLC* repression**

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Non-coding RNA is now recognized as having a central role in chromatin regulation. In *Arabidopsis*, targeted 3' processing of an antisense transcripts of the floral repressor *FLC* at a proximal site is promoted by plant specific RRM-domain RNA binding proteins and conserved 3' end processing factors. Proximal polyadenylation of antisense transcripts triggers removal of histone 3 Lysine 4 dimethylation (H3K4me2) by the histone demethylase FLD and mediates chromatin silencing of the locus to promote flowering. We now identify a requirement for the conserved pre-mRNA splicing factor, PRP8, in this mechanism. Inefficient splicing of antisense introns leads to reduced antisense proximal polyadenylation, disruption of the chromatin silencing mechanism and delayed flowering. Our findings highlight the regulatory potential of introns within long non-coding transcripts. We propose that coupling between chromatin structure, antisense transcript splicing and 3' processing that are interconnected with transcription by RNA Pol II to form opposing feedback

loops. The interconnectivity of co-transcriptional processes at the antisense transcript allows for sensitive fine-tuning of expression of the corresponding sense gene, rationalizing how a specific phenotype can be generated by hypomorphic mutations in core pre-mRNA maturation machinery components.

### 313 Dissecting the pathway of delivering tail-anchored proteins to the plant outer nuclear envelope

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Tail-anchored (TA) proteins are characterized by a C-terminal transmembrane domain (TMD) that mediates post-translational insertion into the membrane of the endoplasmic reticulum (ER) as well as the outer membranes of mitochondria and chloroplasts. They participate in important cellular activities, acting as ER-associated enzymes, vesicle-fusing SNARES, protein translocation complexes, and plasma membrane receptors. However, the specificity of their sorting to different cellular membranes is not well understood. We have identified a plant-unique TA protein (WIP1) that is specifically associated with the outer nuclear envelope (ONE) and have mapped a 36-amino-acid domain (WIP1-Transmembrane Domain Fragment, WIP1-TDF) sufficient for ONE targeting. Further dissection of the targeting signal by TMD swap, mutation and deletion constructs indicates that the most C-terminal 9 amino acids are sufficient for ONE targeting when combined with a generic (oligo-L) TMD. GET (Guided Entry of Tail Anchored Proteins) is a major targeting pathway for TA proteins in yeast. We have identified three putative *GET3p/Asna-1* orthologs from Arabidopsis, AtGET3a, b and c. The yeast ARR4::HIS (*get3*) temperature sensitive mutant was rescued by AtGET3b and AtGET3c, and AtGET3b binds to WIP1 in a yeast two-hybrid assay. Transient *in-vivo* localization of GET3p orthologs show ER and cytoplasmic localization, consistent with their proposed function. The domain requirement for the WIP1-AtGET3b interaction are being mapped and compared with the requirement for WIP1 ONE targeting. T-DNA insertion lines for AtGET3b and AtGET3c have been identified and will be investigated for WIP1 ONE-targeting defects. Together, our data will present the first precisely defined targeting signal for ONE-localized plant proteins and will begin dissecting the cytosolic pathway involved in TA protein delivery to the ONE.

### 314 The Heterodimeric Enzyme that Modifies the Wobble Position of Cytosolic tRNAs is Required for Seed Development in Arabidopsis and is a Member of a Diverse Family of Zinc-Dependent Deaminases

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Most eukaryotic tRNAs are produced from a primary transcript that undergoes extensive processing before translation. One modification known to be essential in yeast involves the conversion of adenine to inosine at the wobble ( $A_{34}$ ) position of the anticodon, a reaction catalyzed by heterodimeric (TAD2/TAD3) tRNA adenosine deaminases (ADATs). Here we identify the Arabidopsis homologs of *TAD2* (At1g48175) and *TAD3* (At5g24670), which modify selected tRNAs for cytosolic translation, confirm their function in *S. cerevisiae*, and show that both genes are required for seed development. Because the Arabidopsis TAD2 catalytic subunit rescues the yeast *tad2* mutant, the AtTAD2/ScTAD3 heterodimer must be functional. In contrast, the yeast *tad3* mutant was rescued with *AtTAD2* and *AtTAD3* combined but not with *AtTAD2* or *AtTAD3* alone, reflecting likely structural limitations to the formation of ScTAD2/AtTAD3 heterodimers. The Arabidopsis gene (At1g01760) that putatively encodes the TAD1 deaminase responsible for  $A_{37}$  modification adjacent to the anticodon is not essential, consistent with published results with yeast. These results extend work elsewhere on the adenosine deaminase (TADA; At1g68720) that modifies the wobble position of chloroplast tRNA<sup>Arg</sup> in Arabidopsis. We discuss here the probable functions of the remaining zinc-dependent deaminase-domain proteins in Arabidopsis, which include a putative guanine deaminase, two proteins likely to be associated with riboflavin biosynthesis, and a tandem array of eight genes encoding cytidine deaminase-like proteins clustered in the same orientation on chromosome 4. This unusual array provides an interesting example of a gene duplication event unique to plants that led to widespread functional degeneration. *Research supported by the NSF Arabidopsis 2010 Program.*

### 315 Abstract Withdrawn

### 316 Biotic Stress Induces the Unfolded Protein Response (UPR) Through the Unconventional Splicing of bZIP60 mRNA Mediated by IRE1 in *Arabidopsis thaliana*

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The unfolded protein response (UPR) is a signaling pathway that is activated by the accumulation of misfolded proteins in the endoplasmic reticulum. It is a widespread process that has been described in organisms ranging from yeasts to mammals. The IRE1 branch of UPR in *Arabidopsis thaliana* is composed of IRE1-1 and IRE1-2. However, little is still known about the importance of this signaling pathway in plants. Here, we show that processing of the bZIP60 mRNA, which is stimulated under endoplasmic reticulum stress conditions, is absent in plants that have mutated IRE1 genes. Given that pathogen infection activates UPR, we analyzed whether the IRE1/bZIP60 branch of UPR is involved in this process. Our results show that IRE1 double mutants and bZIP60 mutants (bzip60-1) are more sensitive to pathogens than wild-type plants. Furthermore, bZIP60 mRNA processing occurs when plants are treated with salicylic acid, which suggests that the IRE1/bZIP60 signaling pathway plays an important role in biotic stress. Finally, bZIP60 mRNA processing was observed in anthers in the absence of ER stressors, biotic and abiotic stress. This indicates that this branch of UPR is active to basal conditions in some organs.

### 317 The Reticulon-like Proteins BTI1 and BTI2 Regulate the Intracellular Trafficking and Activity of the FLS2 Membrane Receptor

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Receptors localized in the plasma membrane are critical for the recognition of pathogens. In plants, membrane-associated immune receptors bind pathogen molecules and subsequently initiate signal transduction cascades that stimulate the immune system. We have employed *Arabidopsis* protein microarrays to identify proteins that physically interact with the cytosolic domain of the immune receptor FLS2. Our aim is to contribute to a better understanding of the molecular determinants that are necessary for FLS2 receptor functions and regulate its activity. In the screen, we identified a membrane-localized protein called BTI1 (*VirB2*-interacting) as one of the putative FLS2-binding proteins. The interaction between BTI1 and FLS2 was further confirmed *in planta* by using split-luciferase complementation and co-immunoprecipitation. The association between BTI1 and FLS2 was greatly impaired when LCR2, a unique structural element from the N-terminal region of BTI1, was removed. FLS2 was also found to co-immunoprecipitate with the closest BTI1 homolog, BTI2. Stable *bti1/bti2* and BTI1 overexpressing (*BTI1ox*) lines showed a significantly reduced activation of the FLS2-dependent signal transduction pathways and exhibited increased susceptibility to bacterial pathogens. *N. benthamiana* plants transiently expressing BTI1-HA or BTI2-HA showed accumulation of FLS2-GFP in the ER and impaired FLS2-GFP transport to the plasma membrane. We found that LCR2 and two Tyr-dependent sorting motifs from BTI1 are critical for its effects on FLS2-GFP transport. Moreover, phase partitioning and confocal microscopy revealed that in both *bti1bti2* and *BTI1ox*, a lower amount of endogenous FLS2 was detected at the membrane compared to the wild type. Our results are consistent with a model in which BTI1 and BTI2 function in a concentration-dependent manner in the transport of newly synthesized FLS2 by regulating FLS2 export from the ER.

### 318 Ethylene Signaling from the Endoplasmic Reticulum to the Nucleus Mediated by EIN2

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The gaseous plant hormone ethylene can trigger myriad physiological and morphological responses in plants. Through genetic screens many components of the ethylene signaling pathway have been identified. These components mainly belong to two categories, the positive regulators and the negative regulators. Genetics and biochemistry studies showed that ethylene signaling occurs in a largely linear pathway, in which ETR1 and its family members are inactivated upon perception of ethylene at the ER membrane. CTR1 acts downstream and when it interacts with ETR1 is inactivated. This interaction is predicted to lead to derepression of EIN2 and subsequently to activation of EIN3 and other members of the EIN3/EIL family of transcriptional regulators. While many of the proteins in the ethylene signaling pathway have been studied extensively during the past decade, very little is known about the function(s) of EIN2. Here we demonstrate that the subcellular localization of EIN2 is the endoplasmic reticulum (ER) membrane in *Arabidopsis*. Through a variety of genetic and cell biological studies, we found that in the absence of ethylene, EIN2 is exclusively localized in the ER in *Arabidopsis*. Upon ethylene treatment, EIN2 is cleaved, and a portion of the protein (CEND) is translocated from the ER to the nucleus. We propose a model in which plants exposed to ethylene gas transmit a signal to EIN2 from ETR1-CTR1, causing the EIN2 CEND to be cleaved through an unknown mechanism, and translocated to the nucleus resulting in EIN3/EIL1 dependent transcriptional activation. Further studies are in progress to test this model, to understand the mechanisms controlling cleavage/ nuclear translocation and to identify nuclear proteins that interact with EIN2 CEND.

### 319 Division Plane Orientation in Plant Cells

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Proper orientation of cell division planes is crucial for development in many organisms. While mechanisms of asymmetric divisions are relatively well studied in *Arabidopsis thaliana*, less is known about factors controlling symmetric division orientation. The preprophase band (PPB), a plant specific microtubule and microfilament structure, is thought to predict the future division plane before the cell enters mitosis [1]. But, as the cell enters metaphase, the PPB is disassembled. How then does the PPB predict the division plane? Recent work has identified several potential landmark proteins, one of which is TANGLED, that localize to the division site when the PPB is formed and stay throughout mitosis and cytokinesis [2, 3]. We have identified two separate domains of TANGLED: one is necessary and sufficient for co-localization with the PPB, and the other is necessary and sufficient for localization at the division site later in mitosis after the PPB has disassembled. This second domain also directly interacts with another protein required for correct division plane orientation [4], the kinesin POK1. In addition, we have identified a suite of TANGLED interacting proteins that illuminate our understanding of the plant division site.

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### 320 Investigating the Secretory Pathway: From Imaging to Gene

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Plant cells, like all other eukaryotic cells, have a complex endomembrane system highly interconnected by vesicle trafficking for transport of lipids, sugars, and proteins along the secretory pathway. This system is highly mobile and dynamic, yet every organelle is able to preserve its identity and structure. Using stable lines of *Arabidopsis* expressing specific markers for the Golgi apparatus or the endoplasmic reticulum and treating these plants with ethyl methanesulfonate (EMS), we created random mutations in the *Arabidopsis* genome. From this point on, we were "hunting" for the phenotype! The screening of M2 populations using confocal microscopy and forward genetics has given us a valuable tool with which to identify the key factors regulating the secretory pathway and maintenance of the organelle structure.

### 321 Expression and localization divergence in the evolution of the Filament-like protein 4 (FLIP4) family in *Arabidopsis thaliana*

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The *Arabidopsis* genome contains multiple chromosomal regions with a high level of homology, suggesting recent genetic duplication. Homologous genes in these regions may have escaped elimination as pseudogenes through functional divergence, thus selectively preserving families of similar genes. One candidate for this process is the *FLIP4* gene family in *Arabidopsis*. This family consists of two genes in duplicated regions of chromosomes 3 and 5, respectively, which are conserved as single-copy genes in other plant species such as tomato and rice. They code for acidic proteins of about 50 kDa in size with a C-terminal coiled-coil domain. Tomato *FLIP4* and *Arabidopsis* *FLIP4-2* both interact with the WPP domain of RanGAP and activate reporter gene expression in yeast, suggesting a common molecular mechanism of function. However, in comparison to other *FLIP4* proteins, *Arabidopsis* *FLIP4-2* contains a functional N-terminal chloroplast targeting peptide which facilitates its localization to the chloroplasts of leaves. In contrast, *Arabidopsis* *FLIP4-1* mRNA is expressed in the reproductive pathway and specifically in the sperm cells of pollen grains. We suggest that divergence of both regulatory DNA sequences resulting in cell-type specific gene expression patterns as well as protein targeting signals resulting in differential subcellular targeting of gene products has contributed to the preservation of both members of the *FLIP4* gene family after gene duplication in *Arabidopsis*.

### 322 Chemical Genetics Uncovers Inhibitors of a Golgi-independent Pathway for Tonoplast Membrane Proteins

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The vacuole is an essential and dynamic organelle in plant cells that depends on constant deposition of membrane proteins. The transport of membrane proteins from the endoplasmic reticulum (ER) to the vacuolar membrane, or tonoplast, occurs by vesicle trafficking via two proposed pathways. Our goal is to characterize the molecular mechanism for trafficking of tonoplast intrinsic proteins (TIPs) in *Arabidopsis* using a chemical genetic approach. Using a small chemical library and a double-marker transgenic line expressing GFP- $\delta$ -TIP and mCherry-HDEL, we identified 32 chemicals that induce the accumulation of the tonoplast marker in the ER. One chemical, referred to as C834, specifically affects the subcellular localization of the  $\delta$ - and  $\alpha$ -TIP markers, but not that of the  $\gamma$ -TIP marker in roots and mature embryos. Moreover, Brefeldin-A (BFA), a chemical inhibitor that affects the formation of the Golgi-associated coat proteins, induces the accumulation of the  $\gamma$ TIP marker in BFA-compartments, but does not affect  $\delta$ - and  $\alpha$ -TIP. These results indicate the presence of at least two distinct trafficking pathways for tonoplast proteins in *Arabidopsis*, one that is Golgi-dependent and one that is Golgi-independent. C834 is a specific inhibitor of the Golgi-independent pathway and is being used to identify regulatory components of this trafficking pathway.

### 323 Tracing the endocytic route and signalling of BR receptor-ligand complex

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Endocytosis is an integral part of signal transduction as besides signal attenuation, it provides spatial and temporal dimensions to signalling events. The BRASSINOSTEROID INSENSITIVE1 (BRI1) receptor perceives its ligand, the BR hormone, at the cell surface and undergoes constitutive endocytosis. Until now the endocytic trafficking of the receptor-ligand complexes and its relevance to

signalling in plants remains unknown. Here we developed a bioactive, fluorescently labelled BR, Alexa Fluor 647 castasterone (AFCS) and visualized in living plant cells the endocytic route of BRI1-AFCS complexes. Genetic or pharmacological interference with function of TGN/EEs inhibited disassociation of BRI1 receptor-ligand complex and impaired BR signalling. Our data provide insights into how trafficking of receptor-ligand complexes mediates subcellular processing of the complex, receptor downregulation and regeneration ultimately determining throughput of hormonal signalling.

### **324 Investigating the Role of Polarized Vesicle Secretion in Early Pollen Pistil Interactions in the Brassicaceae**

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Compatible pollen-pistil interactions in the Brassicaceae are believed to trigger a series of cellular events in the stigma for pollen acceptance. Our main interest is to study the role of vesicle secretion and the exocyst complex in these responses. Polarized secretion has been shown in yeast and animal systems to be promoted by the eight-subunit exocyst complex which tethers secretory vesicles to the plasma membrane for fusion and exocytosis. Previously, we have found that Exo70A1, a predicted subunit of the exocyst complex, is essential in the stigma for compatible pollen-pistil interactions in *Arabidopsis thaliana* and *Brassica napus*. Based on this discovery, we hypothesize that Exo70A1 functions as part of the exocyst complex to tether secretory vesicles to the plasma membrane at the pollen attachment site to deliver essential stigmatic resources for the compatible pollen. This is thought to result in water transfer to the pollen grain for hydration as well as the expansion of the papillar cell wall to promote pollen tube penetration for the subsequent fertilization. We are interested in observing the proposed secretory activity in the stigmatic papillae following compatible pollinations at the ultrastructural level using the transmission electron microscope. To date, we have examined the presence of secretory vesicles at the plasma membrane for self-compatible pollinations in *A. thaliana* and *B. napus*, and cross-compatible pollinations in *A. lyrata*. In contrast, this polarized secretion is expected to be absent in self-incompatible pollinations correlating with self-pollen rejection, and this was examined using self-incompatible pollinations in *A. lyrata* and *B. napus*. Finally, the fate of secretory vesicles following compatible pollinations was examined in exo70A1 mutants where the exocyst complex would be predicted to be disrupted.

### **325 Monitoring dynamic changes in ER Ca<sup>2+</sup> levels using the FRET-based Ca<sup>2+</sup> sensor D1ER**

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As a second messenger, cytosolic calcium plays an important role in regulating plant growth and developmental processes. While it has been suggested that the signature of Ca<sup>2+</sup> changes encodes information determining the specificity of downstream cellular responses, much remains to be elucidated about how these Ca<sup>2+</sup> changes are generated at the molecular and subcellular level. In animals, the endoplasmic reticulum has been identified as a main site of intracellular Ca<sup>2+</sup> sequestration and release and a similar function has been proposed for the ER in plant cells. To investigate the role of the ER in shaping plant Ca<sup>2+</sup> signals in response to external stimuli, we have generated stably transformed *Arabidopsis* expressing the FRET-based ratiometric Ca<sup>2+</sup> biosensor D1 targeted to the lumen of the ER (D1ER)<sup>1</sup>. Using live-cell confocal imaging, we show that mechanical stimulation of *Arabidopsis* roots not only triggers an elevation of cytosolic Ca<sup>2+</sup> levels but also leads to a rapid increase in the Ca<sup>2+</sup>-dependent D1ER ratio, the kinetics of which closely mimic those of the cytosolic Ca<sup>2+</sup> signal. This D1ER ratio increase likely reflects an increase in ER Ca<sup>2+</sup> levels. Inhibition of mechanically-induced cytosolic Ca<sup>2+</sup> transients by pre-treatment of *Arabidopsis* with the Ca<sup>2+</sup> channel blocker La<sup>3+</sup> also abolished the D1ER ratio change, indicating that the Ca<sup>2+</sup> elevation in the ER is linked to the elevation of Ca<sup>2+</sup> concentration in the cytosol. We propose that in response to mechanical stimulation, the ER shapes the cellular Ca<sup>2+</sup> signal by rapidly sequestering free Ca<sup>2+</sup> entering the cytoplasm and may thus modulate the amplitude and duration of cytosolic Ca<sup>2+</sup> transients. This work was supported by NSF (MCB- 0641288).

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### **326 ESCRT proteins are required for starch turnover**

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Endosomes are organelles involved in the sorting of membrane proteins into the vacuole for degradation. For this purpose, the endosomal sorting complex required for transport (ESCRT) drives membrane proteins from the endosomal membrane into intraluminal vesicles. These vesicles are released into the vacuolar lumen after endosome-vacuole fusion. The first ESCRT cargos identified in plants were auxin transport facilitators such as PIN1 and AUX1 which mislocalize to the tonoplast in a mutant of the ESCRT-related *CHMP1A* and *B* genes. Missorting of plasma membrane proteins leads to severe defects in *chmp1a;b* embryos but some of them reach maturity and are able to germinate.

We analyzed *chmp1a;b* mutant seedlings to determine post-embryonic processes requiring CHMP1 function. Mutant plants establish most cell types and all plant organs but show morphogenesis and patterning defects like altered cell elongation and clustered stomata. Surprisingly and unlike embryos, mutant seedlings display enlarged plastids and form an extensive network of stromules. Plastid enlargement is at least to some extent caused by accumulation of starch due to impaired starch mobilization. This defect is more pronounced when plants are grown on high sucrose medium indicating that sucrose uptake is not abolished. While endosomal sorting is a prime function of the ESCRT complex, some of its components are also implicated in non-endosomal processes such as chromatin modification and messenger RNA localization. Although we cannot exclude the possibility that plastid defects are caused by non-endosomal functions

of CHMP1 we have evidence indicating that conventional cargo missorting causes some of the observed defects. Stunted growth and enlarged starch filled plastids have been described in several alleles of the plasma membrane localized protein SUCROSE-PROTON SYMPORTER2 (SUC2). We are currently investigating whether SUC2 expression and localization is altered in *chmp1a;b* mutant plants.

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### 327 ADF4 is Important for Actin Turnover in the Cortical Array of Hypocotyl Epidermal Cells

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Advances in single actin filament imaging have provided unique opportunities to study the dynamic behavior of the plant actin cytoskeleton. Recently, we proposed a model describing the growth and turnover of individual actin filaments in plant cells, based on a novel property called stochastic dynamics. Actin stochastic dynamics is characterized by rapid growth at filament barbed ends (~1.7  $\mu\text{m/s}$ ) that is offset by prolific severing activity and disassembly of filaments. Combining these imaging advances with the power of reverse-genetics in *Arabidopsis*, allows us an unparalleled opportunity to study how specific actin-binding proteins modulate the stochastic dynamics *in vivo*. We hypothesize that ADF/cofilin is one of two classes of actin-binding protein that contribute to filament disassembly via its ability to sever ADP-actin filaments. We used total internal reflection fluorescence microscopy (TIRFM) to visualize and quantify the severing activity of recombinant ADF4 *in vitro*, thereby confirming the biochemical activity of this protein. We also used time-lapse variable angle epifluorescence microscopy (VAEM) and reverse genetics to explore the organization and dynamics of the actin cytoskeleton in living epidermal cells. Homozygous *adf4* mutant plants have several phenotypes, including: longer dark-grown hypocotyls, longer epidermal cells, and longer roots compared to wild-type plants. A reduced severing frequency is the only *in vivo* actin-based stochastic dynamics parameter measured that was significantly different from wild-type controls. Altered turnover of single actin filaments in *adf4* mutant cells also results in changes in actin cytoskeletal architecture. Specifically, *adf4* cells have quantitative increases in actin filament bundling and a reduction in filament density. Our observations provide strong evidence that ADF4 plays a role in filament severing and turnover in the cortical array of living epidermal cells.

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### 328 Integrity of the early secretory organelles in plant cells

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Plant secretory proteins are synthesized in the endoplasmic reticulum (ER) and then transported to the Golgi apparatus to be distributed to the plasma membrane or to the vacuoles/lysosomes. The Golgi is also involved in receiving materials from distal compartments for further recycling to other destinations within cells. The ER and Golgi apparatus are highly structured organelles made of domains that are morphologically and functionally distinct. The factors that control how the ER and Golgi achieve and maintain their structure are largely unknown, especially in plants. Similarly, how these organelles maintain their identity despite the intense communication with other organelles is a fundamental question with only a limited number of answers. To address these questions we have developed genetics screens based on force confocal microscopy and forward genetics to isolate novel mutants bearing altered organization of the ER and Golgi. We have identified a Golgi mutant, thus expanding the knowledge on factors that control the integrity of key organelles of the secretory pathway. Our most recent findings for these screens will be presented in this talk.

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### 329 The Exocyst Complex in Cytokinesis of the Plant Cell

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The exocyst complex is crucial for polarized secretion. It participates in targeting and tethering of secretory vesicles to secretory domains of the plasma membrane. The exocyst is highly conserved across eukaryotes and consists of SEC3, SEC5, SEC6, SEC8, SEC10, SEC15, EXO70, and EXO84 subunits.

Several *Arabidopsis* mutants in exocyst subunits are defective in polar growth of root hairs, pollen tubes, and hypocotyls. Especially, dwarfish *exo70A1* and heavily dwarfed *exo84b* mutants are severely affected. In *exo84b* mutants, cell division of leaf epidermal cells and guard cells is distinctively compromised, including incomplete cell walls, cell wall stubs or aberrant cell plates. Detailed time-lapse microscopy revealed that *exo70A1* mutant cells exhibit a specific defect in cell plate assembly. We found that GFP-tagged exocyst subunits SEC6, SEC8, SEC15b, EXO70A1, and EXO84b are localized predominantly along the plasma membrane. In cytokinesis, all of them show their localization maxima at cell plate initiation and cell plate maturation – stages with a high demand for vesicle fusion.

We conclude that the exocyst complex is involved in secretory processes during cytokinesis in *Arabidopsis* cells that underlie cell plate initiation, cell plate maturation and formation of new primary cell wall.

### **330 The Dynamics of Actin Filament Arrays is required for Vacuolar Fusion of Guard Cells during Stomatal Opening in *Arabidopsis***

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The dynamics of actin filaments (AFs) and vacuoles in guard cells are involved in the regulation of stomatal movement. It remains unclear whether there is any interaction between AF dynamics and vacuolar changes during stomatal movement. Here, we report the relationship between AF dynamics and vacuolar fusion revealed by the pharmacological experiment and the characterization of *actin-related protein 2/3* (*arp2* and *arp3*) mutants. Our results show that cytochalasin D-induced depolymerization of AFs also leads to an increase of small unfused vacuoles during stomatal opening in wild-type plants. Light-induced stomatal opening is retarded and vacuolar fusion in the guard cells is impaired in *arp2/3* mutants that AF arrays are aberrant in comparison to the wild-type plant. In addition, in wild-type plants, AFs surround the separate small vacuoles tightly and form a ring that encircles the boundary membrane of fusing vacuoles in guard cells during stomatal opening. In contrast, in the *arp3* mutant, most AFs and actin patches are accumulated around the nucleus of guard cells, and few AFs or actin patches are colocalized with large vacuoles. AFs do not tightly surround the unfused small vacuoles, which may result in the impairment of vacuolar fusion in guard cells of the mutants and stomatal opening retarded further. Our results suggest that AF dynamics regulates possibly the vacuolar fusion of guard cells during stomatal opening through the colocalization and interplay between AFs and vacuoles in guard cells.

### **331 Functional Analysis of B1-type Cyclins during Plant Growth and Stress Response**

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A tight control of cell proliferation is the key for development and growth of all organisms. In contrast to animals, flowering plants as for instance *Arabidopsis*, contain very large families of cyclin genes but the function of most – often all - of the individual members is not understood. Here we present the complete characterization of the B1 type class of cyclins that build a family with 4 members and one pseudo gene. We present the expression of all members during plant growth as well as the localization during the cell cycle. This together with single and multiple mutant analyses demonstrate that B1-type cyclins are key regulators of mitosis throughout plant development. In particular, B1-type cyclin activity appears to be required for organizing microtubules during mitosis and the free nuclear divisions during endosperm growth are one of the most affected processes when B1-type cyclin activity drops. In addition to their developmental role B1-type cyclins have been proposed to be decisive under stress conditions, especially during DNA damage, since it was previously observed that CYCB1;1 is upregulated after treatment with bleomycin that is known to cause DNA double strand breaks. This led to the hypothesis that high activity of CYCB1s is used as a sensor in the cell to direct the type of DNA repair pathway used, i.e. homologous recombination (HR) versus the more error-prone non-homologous end-joining pathway (NHEJ). Using cyclin mutants as well as gene and promoter swap lines and promoter reporter lines we test here the importance for B1-type cyclins during DNA stress. In addition, we present here the co-depletion of B1-type cyclin with key factors in DNA repair pathways allowing the precise dissection of B1-type cyclin activity during plant growth and stress response.

### **332 The ER-Localized TWD1 Immunophilin Is Necessary for Localization of Multidrug Resistance-Like Proteins Required for Polar Auxin Transport in *Arabidopsis* Roots**

*Guosheng Wu, Marisa Otegui, Edgar Spalding*

**University of Wisconsin**

Immunophilins are studied in both plants and animals for their roles in folding and trafficking of proteins. Previous studies in *Arabidopsis thaliana* established a physical and functional interaction between the TWISTED DWARF1 (TWD1) immunophilin and some Multidrug Resistance ABC transporters (ABCB) that are required for auxin transport. In this work, confocal microscopy of fluorescently tagged TWD1 shows it to reside at the endoplasmic reticulum. Mutations in TWD1 caused mislocalization of ABCB1, ABCB4, and ABCB19 to the ER instead of the plasma membrane as shown by confocal microscopy of fluorescently tagged fusion proteins and transmission electron microscopy of immunogold-labeled samples in the case of ABCB19. Localization of the unrelated PIN-FORMED2 auxin transporter or plasma membrane marker proteins was not affected by loss of TWD1. Abnormal spread of auxin signaling into the elongation zone of *twd1* roots, attributable to mislocalized ABCBs and suppressed by an auxin transport inhibitor, appeared to cause the twisted cell files characteristic of *twd1* roots. The cytoskeleton is an important regulator of plant growth and morphology. Disruption of microtubule arrangement by mutations or some specific drugs causes some aberrant cell arrangement, resulting in some distorted organs such as twisted roots or hypocotyls. The high similarity of root phenotypes between *twd1* and some microtubule mutants indicates that phytohormone auxin may be an important player in regulating the organization of microtubules. To study the molecular mechanism of auxin role in cytoskeleton, mRFP- $\alpha$ -Tubulin has been crossed into the *twd1* and *abcb1 abcb19* backgrounds. The effects of the mutations on the microtubules, which are potentially related to the twisted phenotype, will be analyzed.

**333 Metabolic Sugar Signal Promotes *Arabidopsis* Meristem G2 to M Transition***Anna Skylar, Frances Sung, Xuelin Wu***University of Southern California**

Most organs in higher plants are generated postembryonically from the meristems, which harbor continuously dividing stem cells throughout a plant's life cycle. In addition to developmental regulations, mitotic activities in the meristematic tissues are modulated by nutritional cues, including carbon source availability. Here we further analyze the relationship between the sugar signal and seedling meristem establishment, taking advantage of our previous observation that exogenously supplied metabolic sugars can rescue the meristem growth arrest phenotype of the *Arabidopsis stip* mutant seedlings. Our results show that metabolic sugars reactivate the *stip* meristems by activating the expression of key cell cycle regulators, and therefore, promoting G2 to M transition in *Arabidopsis* meristematic tissues. One of the early events in this process is the transcriptional repression of *TSS*, a genetic suppressor of the *stip* mutations, by sugar signals, suggesting that *TSS* may act as an integrator of developmental and nutritional signals in regulating meristematic proliferation. We also present evidence that metabolic sugar signals are required for the activation of mitotic entry during *de novo* meristem formation from G2 arrested cells. Our observations, together with the recent findings that nutrient deprivation leads to G2 arrest of animal germline stem cells, suggest that carbohydrate availability-regulated G2 to M transition may represent a common mechanism in stem cell division regulation in multicellular organisms.

**334 An Intragenic Mutation Restores the Function of a Defective Brassinosteroid Receptor on the Membrane in *Arabidopsis****Yang Xia, Jianming Li***University of Michigan, Ann Arbor, MI, USA**

Endoplasmic reticular-mediated quality control (ERQC) is a highly conserved protein quality control system in eukaryotic organisms, which retains misfolded/incompletely folded proteins in the ER for additional rounds of chaperone-assisted folding but eliminates terminally misfolded proteins by ER-associated degradation (ERAD) that involves cytosolic proteasome-mediated proteolysis. Most of our knowledge on ERQC/ERAD came from genetic/biochemical studies in yeast and mammalian systems. By contrast, our understanding of similar processes in plants is rather limited, largely due to lack of convenient model proteins for genetic screens and gene discovery. We recently discovered that a mutated brassinosteroid (BR) receptor, *bri1-5*, which carries a cysteine69-to-tyrosine (Cys69Tyr) mutation in its extracellular domain, is retained in the ER by at least three independent ER retention mechanisms and is subsequently degraded by a proteasome-independent ERAD mechanism, thus revealing an excellent model substrate for genetic and mechanistic studies of ERQC/ERAD processes in plants. Here, we report that an intragenic second-site mutation near the position of Cys69, isolated from an EMS-mutagenized suppressor screening of the *bri1-5* dwarf mutant, prevents the recognition of the doubly-mutated receptor by the *Arabidopsis* ERQC system, restoring the cell surface expressing of the mutated BR receptor and its ability to active the BR signaling pathway. Currently, we are conducting genetic/biochemical studies to understand the underlying mechanism by which this second-site mutation compensates the structural defects of the Cys69Tyr mutation, which could shed new light on the structure-function relationship of the BR receptor and the mechanisms of the plant ERQC system.

**335 The importance of PHOSPHATIDYL SERINE SYNTHASE1 in microspore development of *Arabidopsis thaliana****Yasuyo Yamaoka<sup>1</sup>, Junya Mizoi<sup>1,2</sup>, Yuki Fujiki<sup>1</sup>, Ikuo Nishida<sup>1</sup>*<sup>1</sup>Saitama University, Saitama, Japan, <sup>2</sup>Current: The University of Tokyo, Tokyo, Japan

Phosphatidylserine (PS) has many important biological roles, but little is known about its role in plants. We show here that genetic disruption of PS biosynthesis decreased heterozygote fertility due to inhibition of pollen maturation. *At1g15110*, designated *PSSI*, encodes a base-exchange-type PS synthase. Promoter-GUS assays showed *PSSI* expression in developing anther pollen and tapetum. A few seeds with *pss1-1* and *pss1-2* knockout alleles escaped embryonic lethality but developed into sterile dwarf mutant plants. *PSSI* is essential for PS biosynthesis, because *pss1* knockout plants contained no PS. Reciprocal crossing revealed reduced *pss1* transmission via male gametophytes, predicting a rate of 61.6% *pss1-1* pollen defects in *PSSI/pss1-1* plants. Alexander's staining of inseparable *qrt1-1 PSSI/pss1-1* quartets revealed a rate of 42% three and four dead pollen grains, suggesting sporophytic *pss1-1* cell death effects. Analysis with the nuclear stain DAPI showed that all tetrads from *PSSI/pss1-1* anthers retain their nuclei, whereas unicellular microspores were sometimes anucleate. Transgenic *Arabidopsis* expressing a GFP-LactC2 construct that binds PS revealed vesicular staining in tetrads and bicellular microspores and nuclear membrane staining in unicellular microspores. Hence, distribution and/or transport of PS across membranes were dynamically regulated in pollen microspores. However, among unicellular microspores from *PSSI/pss1-2 GFP-LactC2* plants, all anucleate microspores showed little GFP-LactC2 fluorescence, suggesting that *pss1-2* microspores are more sensitive to sporophytic defects or show partial gametophytic defects.

**336 SYN3 Is Required For Chromosome Synapsis and Condensation during Meiosis in *Arabidopsis****Li Yuan, Xiaohui Yang, Christopher Makaroff***Department of Chemistry and Biochemistry, Miami University, Oxford, Ohio, USA**

Cohesin complexes are critical for holding sister chromatids together during nuclear division and also play important roles in the compaction of chromosomes and their bipolar attachment to the spindle, DNA double strand break repair, and the regulation of gene expression. Cohesin complexes consist of a heterodimer of Structural Maintenance of Chromosome (SMC) proteins, SMC1 and SMC3,

and two non-SMC proteins, SCC3 and an  $\alpha$ -kleisin protein, either SCC1 or REC8, which participate in mitosis and meiosis, respectively. Arabidopsis contains four  $\alpha$ -kleisin proteins, SYN1-4. SYN1 is the REC8 paralog and is essential for meiosis. SYN2 and SYN4 appear to have redundant functions and participate in mitosis. SYN3 is an essential protein that is found throughout the plant and enriched in the nucleolus. In order to further investigate the role(s) of SYN3 we generated and analyzed plants that express *SYN3* RNAi from either DMC1 meiotic promoter or a DEX-inducible construct. *AtSYN3*-RNAi lines show reduced fertility and defects in both male and female meiosis. Meiocytes showed a reduction in synapsis and defects in homologous chromosome pairing. Chromosome condensation was also affected. Immunolocalization analyses showed that meiotic cohesin complexes and ASY distribution patterns were normal. Increased numbers of AtRAD51 recombination foci, and a dramatic reduction in the synaptonemal complex protein AtZYP1 were however observed in the lines. qPCR found that mRNA levels for *AtMLH1*, and *AtRCK* were reduced in the RNAi lines while *AtSPO11-2* and *AtZYP1a* levels were increased. It was previously shown that over expression of *ZIP1* in yeast blocks SC formation and causes defects in synapsis. We postulate that the meiotic defects in *SYN3*-RNAi plants may result from a similar mechanism. More importantly, our alterations in *SYN3* levels affect meiotic gene expression, raising the possibility that *SYN3* may play an active role in controlling gene expression.

### **337 Functions for tethering complex exocyst in *Arabidopsis* exocytosis and PM recycling**

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Exocytosis in plants is the cellular process involved in all aspects of plant cell polarity, morphogenesis and differentiation starting from the cytokinesis. Yet we know so little about the mechanisms regulating this crucial process in the biogenesis of plant cell surfaces. Central for initiating exocytotically active domains in plant cell cortex are along with Rho/Rop and Rab GTPases regulatory modules, also their effectors including exocytotic vesicles tethering complex exocyst. Over last years we have shown that also in *Arabidopsis* complex exocyst exists as a biochemical entity and is involved in these processes including polarized cell expansion (pollen tubes, root hairs), polarized cell wall deposition (seed coats), cell division and innate plant immunity involved in the basal resistance against *Arabidopsis* microbial pathogens. *Arabidopsis* exocyst encompass eight subunits as in other eukaryotes and possibly some other adaptor proteins (e.g. involved in the seed coat deposition) which along with the multiplicity of EXO70 isoforms create potential for many putative functional versions of exocyst – depending on the tissue differentiation but most probably also within a single cell. We will present our progress in the molecular analysis of exocyst functions in *Arabidopsis* and its involvement in the dynamics of exocytosis using advanced microscopy techniques.

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### **338 The Exocyst: A Vital Role In The Pollen-Stigma Interactions In *Arabidopsis thaliana***

*Yara Zayed, Laura Chapman, Daphne Goring*

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The exocyst complex is proposed to be a crucial component in the stigmas of *Arabidopsis thaliana* for accepting compatible pollen during the early stages of pollen-pistil interactions. The proposed role of the exocyst is to tether secretory vesicles to the inner leaflet of the stigmatic plasma membrane for vesicle fusion. Following this, resources are then proposed to be released for pollen adhesion, hydration, germination and pollen tube growth. The exocyst consists of eight putative subunits: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84. We are interested in investigating the roles of each subunit individually in the compatible pollen response in *A. thaliana*. For example, previously published work from the Goring group showed that the Exo70A1 was involved in compatible pollen-pistil interactions where stigmas of *Exo70A1* T-DNA knockout *A. thaliana* plants failed to permit wild-type pollen grains to either hydrate or form pollen tubes. We are currently examining the roles of the remaining subunits using stigma-expressed RNAi or antisense strategies, in combination with T-DNA knockout lines to address genetic redundancy for duplicated genes. For example, the Sec15 subunit is represented by the gene pair, *Sec15a* and *Sec15b*. When the more highly expressed *Sec15b* gene was suppressed with a stigma-expressed RNAi construct in Col-0, very little change was observed in the ability of the transgenic stigma to accept compatible pollen. *A. thaliana sec15a* homozygous T-DNA knockout plants were then transformed with this RNAi construct, and preliminary results show that this strategy is more successful in blocking the compatible pollen response in the transgenic stigmas.

### **339 Formation and function of a ROP signaling scaffold at specialized domains of the Endoplasmic Reticulum**

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The leaf epidermis is a biomechanical shell, the growth of which can affect the behavior of underlying cell layers and the overall architectural properties of the leaf. Understanding the genetic and molecular basis of its morphogenesis is an important problem in basic research and applied plant science. *Arabidopsis* pavement cell and trichome morphology mutants continue to identify new genes and biochemical pathways that affect the growth process; however, it is unclear how these complicated networks of proteins interact with the endomembrane and cytoskeletal systems to initiate and maintain polarized growth. The ROP small GTPase exchange factor *SPIKE1*

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and "distorted" actin based growth control system is a perfect example. Genetic and biochemical analyses provide a logic model for information flow from the formation of a ROP activation scaffold through a series of heteromeric protein complexes (WAVE/SCAR and ARP2/3) that generate an actin filament nucleation response. The cellular deployment of the pathway is unknown. It is commonly believed that during polarized growth ROP activation must be restricted to specific cortical domains. Contrary to this notion, our most recent results suggest that abundant subdomains of the ER termed ER exit sites serve as a distributed network of SPK1-ROP activation sites. We will provide an update of our live cell imaging, genetic, and biochemical analyses that indicate that SPK1 signals diverge from ERES to control distinct trafficking and cytoskeletal activities. We will discuss how signal generation and cytoskeletal responses are compartmentalized in the cell and how this unexpected cellular deployment might regulate the cytoskeleton and morphogenesis at the spatial scale of a cell.

**340 Induction and Development of Tracheary Elements in In Vitro Cultures of Arabidopsis***Anika Benske, Jenny Bolivar, Tanya Falbel, Sara Patterson***Department of Horticulture, University Wisconsin-Madison, Madison, WI, US**

Three different tissue culture systems of wildtype Columbia *Arabidopsis* have been established in order to further investigate cell wall lignin content and tracheid development. The three *in vitro* cultures established are callus, suspensions, and nodules. The cultures are started from seedlings plated on MS growth medium, that are then placed onto a hormone medium under dark conditions for approximately a month to induce callus growth. From healthy callus tissue, cell suspensions are made by breaking up callus tissue and moving the culture to a liquid medium, which is then placed on an orbital shaker to free single cells. Cell suspensions eventually cluster into larger clumps, forming nodules. These nodules are moved into roller bottles and are kept turning at 1 RPM under lights. Within all of these culture systems, select hormones are added to induce tracheid development. Tracheary elements, composed of lignified cell walls, allow water to move throughout the plant and provide the plant with its structural support. Forming a thick, protective barrier, lignin impedes the plant biomass digestibility potential by impeding access of digestive enzymes to hemicellulose and cellulose. Modified cells with reduced lignin content will be analyzed to give insights to cell wall modifications. In addition to the wildtype *in vitro* cultures, cultures of a reduced-lignin mutant, *ref3*, will be established in order to investigate differences in lignin content from a biochemical approach, as well as a microscopic analysis.

**341 Abstract Withdrawn****342 Root Hair-Specific EXPANSIN A7 Is Required for Root Hair Elongation in Arabidopsis***Changfa Lin, Hee-Seung Choi, Hyung-Taeg Cho***Seoul National University, Seoul, Korea**

Expansins are non-hydrolytic cell wall-loosening proteins that are involved in the cell wall modifications that underlie many plant developmental processes. Root hair growth requires the accumulation of cell wall materials and dynamic cell wall modification at the tip region. Although several lines of indirect evidence support the idea that expansin-mediated wall modification occurs during root hair growth, the involvement of these proteins remains to be demonstrated *in vivo*. In this study, we used RNA interference (RNAi) to examine the biological function of *Arabidopsis thaliana* EXPANSIN A7 (AtEXPA7), which is expressed specifically in the root hair cell. The root hairspecific AtEXPA7 promoter was used to drive RNAi expression, which targeted two independent regions in the AtEXPA7 transcript. Quantitative reverse transcriptase-PCR analyses were used to examine AtEXPA7 transcript levels. In four independent RNAi transformant lines, RNAi expression reduced AtEXPA7 transcript levels by 25-58% compared to controls. Accordingly, the root hairs of RNAi transformant lines were 25-48% shorter than control plants and exhibited a broader range of lengths than the controls. Our results provide *in vivo* evidence that expansins are required for root hair tip growth.

**343 Two ABC Transporters Which Deposit Steryl Glucoside on Pollen Coat Are Important for Pollen Fitness***Hyunjoo Choi<sup>1</sup>, Yu-Young Kim<sup>1</sup>, Kiyoshi Ohyama<sup>2,3</sup>, Toshiya Muranaka<sup>2,4</sup>, Youngsook Lee<sup>1</sup>***<sup>1</sup>POSTECH, Pohang, Korea, <sup>2</sup>RIKEN Plant Science Center, Yokohama, Japan, <sup>3</sup>Tokyo Institute of Technology, Tokyo, Japan, <sup>4</sup>Osaka University, Osaka, Japan**

Pollen grains are coated with many lipophilic materials which collectively protect pollen against various stresses. To find candidate transporters that are involved in transport of such lipid materials, we searched ABC transporter genes that are highly expressed in anther. From *in silico* microarray database, together with quantitative reverse transcriptase-PCR and promoter-GUS assay, we found that *ABCG9* and *ABCG31* are highly expressed in anther and the two genes are highly co-expressed. Knockingout of both genes did not affect overall

plant development, but vital staining of pollen revealed that the mutant pollens were less viable than wild-type pollen, and many of them were shriveled and collapsed when exposed to air. When exposed to cold shock during flowering period, *abcg9abcg31* plants could not produce as many seeds as the wild type or the single knockout mutants. Electron microscopic observation of the *abcg9abcg31* pollen coat revealed many irregular structures such as vesicles and electron-translucent stick-shaped structures, whereas wild-type pollen coat was smooth and orderly. Our extensive analyses of lipid composition of the pollen found that steryl glucoside was reduced to about half in the *abcg9abcg31* pollen, and no changes in steryl ester and free sterol. A mutant deficient in steryl glucoside synthesis, *ugt80A2B1*, was also similarly reduced in pollen viability under normal condition, and its pollens often collapsed when exposed to air. Together, these results indicate that steryl glucoside is one of the important materials for pollen fitness and the two ABC transporters contribute to accumulation of steryl glucoside on pollen coat.

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### 344 Characterization of MUM ENHANCER 4, a gene required for mucilage production in *Arabidopsis thaliana*

*Uday Divi, Andrej Arsovski, Tamara Western  
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Pectin is a major component of the plant cell wall that contributes to wall strength, porosity and cell-cell adhesion. Mechanisms underlying biosynthesis, secretion and modification of pectin are poorly understood. The mucilage secretory cells (MSCs) of the *Arabidopsis* seed coat are specialized to synthesize large amounts of pectinaceous mucilage that is extruded upon seed hydration. Thus the MSCs serve as excellent tool to study pectin related genes. Many genes affecting MSC differentiation and mucilage release have been identified, including *MUCILAGE-MODIFIED4 (MUM4)*. *MUM4* encodes a UDP-L-rhamnose synthase required for synthesis of rhamnogalacturonan I (RGI), a main pectic component of mucilage. Recently, a screen for genetic enhancers of *mum4* identified six *mum enhancers (men1–6)*. Out of these, only *men4* single mutants exhibited reduced mucilage and rhamnose levels compared to wild type seeds, suggesting that *MEN4* is involved in pectin production. *MEN4* has been further characterized and a possible connection between mucilage and the phytohormone abscisic acid is being studied.

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### 345 The *Arabidopsis* Deficient in Cutin Ferulate (DCF) Encodes a Transferase Required for Ferulylation of ω-Hydroxy Fatty Acids in Cutin Polymers

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The cuticle is a complex aliphatic polymeric layer connected to the cell wall and covers surfaces of all aerial plant organs. The cuticle prevents non-stomatal water loss, regulates gas exchange and acts as a barrier against pathogen infection. The plant cuticle is synthesized by epidermal cells and predominantly consists of a polymer matrix (cutin) and intracuticular, and epicuticular waxes. Cutin monomers are primarily aliphatic C<sub>16</sub> and C<sub>18</sub> unsubstituted, ω-hydroxy, and α,ω-dicarboxylic fatty acids. Phenolics such as ferulate and *p*-coumarate esters also contribute to a minor extent to the cutin polymer. Here we present the characterization of a novel acyl-CoA dependent acyltransferase (BAHD family), which is encoded by a gene designated *Defective in Cutin Ferulylation (DCF)*. The DCF protein is responsible for ferulylation of ω-hydroxy fatty acids incorporated in cutin polymers of aerial *Arabidopsis* organs. The enzyme specifically transfers hydroxycinnamic acids using ω-hydroxy fatty acids as acyl acceptor and hydroxycinnamoyl-CoAs, preferentially feruloyl-CoA and sinapoyl-CoA, as acyl donors *in vitro*. *Arabidopsis* mutant lines carrying *DCF* loss-of-function alleles are devoid of rosette leaf cutin ferulate and exhibit a 50% reduction in ferulic acid contents in stem cutin extracts. *DCF* is specifically expressed in the epidermis throughout all green *Arabidopsis* organs. The DCF protein localizes to the cytosol suggesting that ferulylation of cutin monomers takes place in the cytoplasm.

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### 346 Identification and Analysis of Seed Coat Epidermal-Specific Promoter in *Arabidopsis thaliana* and *Brassica napus*

*Elahe Esfandiari<sup>1</sup>, Zhaoqing Jin<sup>1</sup>, Ashraf Abdeen<sup>2</sup>, Jonathan Griffiths<sup>1</sup>, Tamara Western<sup>2</sup>, George Haughn<sup>1</sup>  
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During differentiation of *Arabidopsis thaliana* seed coat, dramatic changes occur including cytoplasmic rearrangement, proanthocyanidin biosynthesis, and production of secondary cell walls. The epidermal cells undergo an especially pronounced transformation highlighted by the synthesis and secretion of copious amounts of dispensable, pectinaceous mucilage. Thus, this cell type represents an excellent platform to study the biosynthesis and modification of cell wall components, particularly pectin. One tool required for molecular genetic analysis is a promoter that drives expression specific to this cell layer. To identify such a promoter, we analyzed *Arabidopsis* seed coat microarray data for genes specifically expressed in the seed coat. This led to the identification of 14 candidate genes. Based on RT-PCR results, 9 of these genes showed a seed-specific expression pattern. The transcriptional regulatory region of each of these candidate genes was fused to the GUS reporter gene. A histochemical GUS assay demonstrated that only one of the promoters, *SEED COAT-SPECIFIC PROMOTER (SCSP)* is able to express *GUS* specifically in the seed coat where expression was detected in the epidermal and palisade cell layers. qRT-PCR data using wild type seed coat RNA suggests that the promoter is particularly active at 7 days post anthesis. The *SCSP* was able to direct transcription of *GUS* in a similar pattern in the *Brassica napus* (Canola) seed coat. Thus, in addition to its application in studying the plant cell wall, this promoter will provide an experimental tool for expressing high-valued recombinant proteins as well as modifying seed coat traits in economically important crops.

**347 Re-examining the Role of Apoplastic Calcium in Cell Wall Modification**

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Calcium is a unique plant nutrient with specialized signalling and structural roles. The apoplast provides the transport pathway for calcium ions ( $\text{Ca}^{2+}$ ) through the plant but also binds  $\text{Ca}^{2+}$ , to demethylesterified pectin, to afford strength to the extracellular matrix<sup>1</sup>. We have identified an *Arabidopsis thaliana* mutant with aberrant regulation of apoplastic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]$ )<sup>2</sup>. Associated with a high apoplastic  $[\text{Ca}^{2+}]$  there are multiple leaf phenotypes including increased cell wall thickness, decreased extensibility and reduced stomatal aperture<sup>2</sup> demonstrating that apoplastic  $[\text{Ca}^{2+}]$  needs to be tightly regulated to ensure plant productivity is optimal<sup>1,2,3</sup>. All these phenotypes can be conditionally suppressed by growing the plant in a sufficient but reduced  $\text{Ca}^{2+}$  supply and as such this provides a tool for the study of processes that are regulated by apoplastic  $[\text{Ca}^{2+}]$ . The genetic basis for these cell-wall associated phenotypes has been examined and Comprehensive Microarray Polymer Profiling (CoMPP) performed, which shows that changes in the expression of certain genes (particular members of the pectin methylesterase, cellulose synthase and polygalacturonase families) correlate with particular modifications in cell wall composition and physical properties. A new working model will be presented that examines the complexities in the role and regulation of apoplastic  $\text{Ca}^{2+}$  as a structural and signalling element that modulates cell wall properties.

<sup>1</sup>Gillham et al. (2011) *Journal of Experimental Botany* **62**:2231-2259

<sup>2</sup>Conn et al. (2011) *The Plant Cell* **23**:240-257

<sup>3</sup>Dayod et al. (2010) *Protoplasma* **247**:215-231

**348 The FEI2 RLK/SOS5 Pathway Regulate the Synthesis of Cellulose in *Arabidopsis* Seed Coat Mucilage Via CESA5**

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The seeds of *Arabidopsis thaliana* and many other plants are surrounded by a pectinaceous mucilage that aids in seed hydration and germination. Mucilage is synthesized during seed development within maternally derived seed coat mucilage secretory cells (MSCs), and is released to surround the seed upon imbibition. The FEI1/FEI2 receptor-like kinases and the SOS5 extracellular GPI-anchored protein were previously shown to act on a pathway regulating the synthesis of cellulose in *Arabidopsis* roots. Recently, we demonstrated that both FEI2 and SOS5 also play a role in the synthesis of seed mucilage. Disruption of FEI2 or SOS5 leads to a reduction in the rays of cellulose observed across the seed mucilage inner layer, which alters the structure of the mucilage in response to hydration. Mutations in CESA5, which disrupts an isoform of cellulose synthase involved in primary cell wall synthesis, and in KORRIGAN1 (KOR1), which disrupts a gene encoding an endo-1,4- $\beta$ -glucanase involved in cellulose biosynthesis, result in a similar seed mucilage phenotype. These results indicate that CESA5/KOR1-derived cellulose plays an important role in the synthesis and structure of seed coat mucilage and that the FEI2/SOS5 pathway plays a role in the regulation of cellulose synthesis in MSCs. These results establish a novel structural role for cellulose in anchoring the pectic component of seed coat mucilage to the seed surface.

**349 Identification and Characterization of Candidate Genes involved in Secondary Cell Wall Formation**

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The plant secondary cell walls are composed of cross-linked networks of cellulose, hemicellulose, and lignin. Lignin is the primary component of secondary cell wall that provides structural rigidity to the cell wall and also makes it difficult for cell wall deconstruction/ or decomposition. We use *Arabidopsis* *in vitro* tracheary element (TE) differentiation as a model system to study the secondary cell wall (SCW) formation. Pre-existing microarray data sets (Brown et al., 2005) have been used to identify candidate genes involved SCW formation, whose expression correlated with the differentiation of TEs. We are currently testing the role of these candidate genes by loss of function mutations in *Arabidopsis*, and overexpression of these candidate genes *in planta* and in T87W cultured cells. SCW components analysis in differentiating transgenic TE cells and *in planta* loss-of-function allele analysis will be used to assess the functional roles of the candidate genes during SCW formation. A better understanding of SCW formation, especially of lignin synthesis, transport and polymerization, will provide tools for the production of crops with enhanced properties for bioenergy production.

**350 REDUCED WALL ACETYLATION Leads To Impairment In Cuticle, Trichome Cell Death And Enhanced Resistance Against *Botrytis cinerea***

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The epidermal cuticle and cell wall serve as the first barriers against invading pathogens. Previous work on a subset of mutants compromised in cuticle and cell wall integrity has indicated that the cuticular lipids and cell wall polysaccharides contribute to defence not just as mechanical barriers but also as sensors for incoming infections. We have recently identified a mutant of *Arabidopsis thaliana*,

*reduced wall acetylation 2 (rwa2)*, defective in cell wall acetylation. Detailed cell wall analyses identified that *rwa2* has 20% reduction in acetylation of cell wall polymers. The reduction in cell wall acetylation has a profound effect on the plant surface as *rwa2* has collapsed trichomes and a more permeable cuticle, despite having a thicker cell wall and cuticle as evidenced by TEM analysis. In addition, *rwa2* showed enhanced resistance to the necrotic fungal pathogen *Botrytis cinerea*. Preliminary results suggest that the enhanced resistance against *B. cinerea* is due to faster activation of plant defense responses, such as indole glucosinolates, possibly due to altered structure and composition of the cuticle and cell wall. These results suggest an intricate link between cell wall structure, cuticle deposition and pathogen responses.

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**351 The Interconversion of UDP-Arabinopyranose and UDP-Arabinofuranose is Indispensable for Plant Development in *Arabidopsis thaliana***

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L-arabinose, an important constituent of plant cell walls, is found predominantly in the furanose rather than in the thermodynamically more stable pyranose form. Nucleotide sugar mutases have been demonstrated to interconvert UDP-L-arabinopyranose (UDP-Arap) and UDP-L-arabinofuranose (UDP-Araf) in rice. These enzymes belong to a small gene family encoding the previously named Reversibly Glycosylated Proteins (RGPs). RGPs are plant-specific cytosolic proteins, which tend to associate with the endomembrane system. In *Arabidopsis* the RGP protein family consists of five closely related members. We characterized all five RGPs regarding their expression pattern and subcellular localizations in transgenic *Arabidopsis* plants. Enzymatic activity assays of recombinant proteins expressed in *E.coli* identified three of the *Arabidopsis* RGP protein family members as UDP-L-arabinose mutases that catalyze the formation of UDP-Araf from UDP-Arap. Co-immunoprecipitation and subsequent LC-ESI-MS/MS analysis revealed a distinct interaction network between RGPs in different *Arabidopsis* organs. Examination of cell wall polysaccharide preparations from *RGP1* and *RGP2* knockout mutants showed a significant reduction in total L-arabinose content (12-31%) compared to wild-type plants. Concomitant down regulation of *RGP1* and *RGP2* expression results in plants almost completely deficient in cell wall-derived L-arabinose and exhibiting severe developmental defects.

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**352 Three-Dimensional Architecture of *Arabidopsis* Cell Walls at Molecular Resolution as Revealed by Electron Tomography**

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Plant cell walls are complex structures composed of polysaccharides, lignin and some glycoprotein. Although biochemical analyses have provided extensive chemical compositional information about plant cell walls, the exact spatial organization of the cell wall components is still not known with certainty. In-depth knowledge of plant cell wall architecture will be useful for cell wall researchers working towards efficient production of industrial products such as biofuel, paper, textile, and timber as well for agricultural research, including fruit ripening, and pathogen and stress resistance. Our primary research objective is to develop realistic three-dimensional (3D) plant cell wall model(s) at molecular resolution by *in situ* 3D imaging with electron tomography. As a first step towards this objective, we tested different sample preparation methods for electron tomography on *Arabidopsis* stems, including 1) microwave-assisted chemical fixation, dehydration and resin embedding 2) high-pressure freezing, freeze-substitution and resin embedding 3) plunge freezing and vitreous sectioning for cryo-electron tomography. We find that there is a trade-off between high-throughput processing and highest quality of structural preservation among the different sample preparation methods, and a correlation of data obtained from different sample preparation methods is required to obtain comprehensive and accurate information. Cell walls of *Arabidopsis* are being studied with the goal of developing a realistic cell wall model for dicotyledon cell wall. Primary cell walls from different cell types of hypocotyl and adult stems are being studied to understand the underlying similarities and variations in their 3D architecture. For each cell wall sample, tilt series of TEM images are collected and reconstructed into 3D tomograms, which are then segmented and analyzed to develop 3D models of the cell walls. Preliminary 3D models of the *Arabidopsis* cell wall samples studied will be presented.

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**353 The Role of the FEI Receptor Like-Kinases in Regulating Plant Cell Wall Function**

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**University of North Carolina at Chapel Hill**

The cell wall is a highly dynamic structure that changes in response to both environmental and developmental cues. It is required to ensure proper growth and development, protect against pathogenic attack, and is the foundation of cellulosic biofuel. Despite the importance of understanding the cell wall, signaling pathways regulating cell wall function remain poorly understood. FEI is a member of the leucine rich receptor-like kinase family and is involved in regulating cell wall function in *Arabidopsis thaliana*. Mutations in FEI1 and FEI2 disrupt anisotropic cell expansion and cellulose synthesis in the root. Here we show that the disruption of cellulose biosynthesis in *fei1 fei2* may not be a consequence of the degradation of the cellulose synthase protein complex because the catalytic subunit of cellulose synthase, CesA6, is abundant in both *fei1 fei2* and wild-type seedlings. In addition, using bimolecular complementation we have found that the FEI receptors interact with the enzyme that synthesizes 1-aminocyclopropane-1-carboxylic acid, or ACC, the precursor to ethylene in

the ethylene biosynthetic pathway. Finally, because FEI1 and FEI2 define a novel signaling pathway, we performed a suppressor screen to identify other components that may play a role in this pathway and are currently cloning the genes corresponding to suppressors of the *fei1 fei2* phenotype. Characterization of these suppressors will likely shed light on how the cell wall perceives and responds to signals that initiate change in both wall architecture and function

### **354 A Screen of Arabidopsis Insertion Lines Identifies Candidate Cell Wall Digestibility Genes**

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Through collaboration between UW-Madison and MSU in a project funded through the Great Lakes Bioenergy Research Center (GLBRC), a collection of 1,200 Arabidopsis T-DNA lines with insertions in cell wall biosynthesis-related genes have been screened for differences in cell wall digestibility. Digestibility is defined as the percent yield based on total dry weight of free Glucose (Glu) and Xylose (Xyl) after chemical pretreatment and incubation with a commercial cellulase mixture. Stem and leaf tissue was collected from the 1,200 lines at two growth stages and screened using the automated high-throughput digestibility platform (HTDP). Outlying lines that displayed either a significant increase or decrease in Glu or Xyl yield were re-tested, validated, and further characterized chemically, genetically, and phenotypically. We will present a summary of our screening results and the initial characterization of verified outlier lines. On-going work primarily focuses on phenotypic and genetic interactions of an elite list of genes selected from digestibility and cell wall composition analyses. This work will assist in the identification of novel gene interactions and signaling pathways regulating cell wall assembly and disassembly. Future work will include transfer of the knowledge gained in this study of Arabidopsis to grasses and woody plants.

### **355 CFL1, A WW Domain Protein, Regulates Cuticle Development by affecting the Activity of a Class IV Homeodomain Transcription Factor**

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Plants have a chemically heterogeneous lipophilic layer, the cuticle, which protects them from biotic and abiotic stresses and takes part in some vital developmental processes. The mechanisms that regulate cuticle development are far from well understood. We identified a rice (*Oryza sativa*) dominant mutant, *curly flag leaf 1* (*cfl1*), and showed that over-expression of *CFL1*, which encodes a WW domain protein, was responsible for the phenotype. To characterize the function of *CFL1*, we over-expressed both rice and Arabidopsis *CFL1* in *Arabidopsis thaliana*; these transgenic plants showed severely impaired cuticle development. Furthermore, reduced expression of Arabidopsis *CFL1* resulted in reinforcement of cuticle structure. Arabidopsis *CFL1* was expressed predominantly in specialized epidermal cells and in regions where dehiscence and abscission occur. *In vitro* and *in vivo* assays showed that Arabidopsis *CFL1* interacts with a class IV homeodomain-leucine zipper transcription factor which also takes part in regulation of cuticle development. Expression of two important cuticle development-associated genes, *BDG* and *FDH*, were down-regulated in At *CFL1*-over-expressor plants. Our results suggest that rice and Arabidopsis *CFL1* negatively regulate cuticle development together with the transcription factor, which regulates the downstream genes such as *BDG* and *FDH*.

**356 ABRC: A Central Hub for *Arabidopsis* Teaching Resources**

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The Arabidopsis Biological Resource Center (ABRC) was established twenty years ago with a goal to represent a central repository for Arabidopsis research, a mission that ABRC continues to serve. Following sequencing of the Arabidopsis genome in 2000, a number of different resources have been generated, accompanied by immense knowledge of the function of many Arabidopsis genes. The core part of the ABRC collection is represented by mutant and transgenic stocks, characterized by community efforts. Taking advantage of their variety, as well as their characterized genotype and phenotype, these stocks have been used to compose several teaching resources, which can be found and ordered through the TAIR database ([http://arabidopsis.org/abrc/catalog/education\\_kits\\_1.html](http://arabidopsis.org/abrc/catalog/education_kits_1.html)). Some of these teaching resources have been designed by ABRC staff. ABRC teaching modules are named "Greening the classroom" and are available at <http://www.abrcoutreach.osu.edu/>. "Greening the classroom" modules developed to date cover biological concepts such as heredity, natural diversity, adaptation, survival and developmental changes. The modules are aligned with K-12 national and state standards and have been accepted as a part of Columbus City Schools official curriculum. A new donation form, designed specifically for new teaching resources, will be presented. A donation drive, based on the increasing need for hands-on teaching materials using plants and the potential that Arabidopsis resources have to offer to fulfill this need, will be announced.

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**357 The iPlant Collaborative**

*Victoria Bryan*

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iPlant is currently building cyberinfrastructure in support of two Grand Challenges; iPlant's Tree of Life (iTOL) and iPlant Genotype to Phenotype (iPG2P). The iTOL project will facilitate creation of phylogenetic trees for up to 500,000 species of green plants, enable the dissemination of data associated with large trees, visualize large trees, and implement scalable "post-tree" analysis tools to foster integration with other sciences. The iPG2P project will provide scalable analysis, integration and storage tools to facilitate the prediction of a plant's phenotype given the plant's genetic makeup and sufficient environmental information about where it is grown.

In addition to Grand Challenge related tools, iPlant is supporting the development of many smaller, related projects. These include a high-throughput image analysis platform to provide support for automated phenotyping, cloud computing development to provide use of virtual machine images, and development of semantic web technologies that will facilitate future web-based data and tool discovery. Several smaller "Seed Projects" are also underway to provide initial development of CI for plant nutrition, plant adaptation, tree biology, and botanical geospatial diversity.

iPlant's cyberinfrastructure will also serve as the foundation for development of educational software. For the first time students will be able to use the same tools and data as research scientists.

**358 A Mutant in Every Gene? Genome Coverage by ABRC Resources**

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In the 20 years since its establishment, the stock collection at the Arabidopsis Biological Resource Center has become an essential resource for Arabidopsis research and for plant research in general. T-DNA insertion lines represent the most frequently ordered ABRC stocks and ORF entry clones are the most popular non-seed resource. The core of ABRC's seed collection is represented by characterized mutant lines. These are high quality stocks that are both genotypically and phenotypically characterized. The starting material for these resources has been the large populations of mutant lines generated for forward and reverse genetic screens. Insertions in the majority of genes in the Arabidopsis genome have been achieved through generation of flank sequenced insertion populations, particularly the SALK T-DNA-insertion mutant collection. Next-generation sequencing promises to identify insertions in many of the remaining genes. T-DNA-insertion mutant populations will continue to be a source of characterized mutant lines and in this way contribute to the core mutant collection. Large scale projects generating ORF entry clones have contributed to significant coverage of the genome by this type of resource, which also represents starting material for studies of gene function. An analysis of genome coverage by ABRC seed and clone resources, focusing on both individual protein-coding genes and on gene families, will be presented. Target resources for donation and development will be identified.

This material is based upon work supported by the National Science Foundation, Award Number DBI-1049341. Any opinions, findings, and conclusions or recommendations are those of the author(s) and do not necessarily reflect the views of the NSF.

**359 Report on Plant Resource Project in RIKEN BRC**

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RIKEN BioResource Center (BRC) Experimental Plant Division promotes collection, preservation and distribution of Arabidopsis and other model plant resources. The major resources are RIKEN Arabidopsis Full-Length cDNA (RAFL) clone, RIKEN Arabidopsis Transposon-tagged Mutant (RATM) line and Arabidopsis Full-length cDNA over-eXpressor (FOX) mutant line. Additionally, we have

started distribution of Arabidopsis T87 cultured cell line to overseas country. The resources are maintained and distributed under the appropriate quality control. Since 2002, we have distributed 36,910 materials to the world research community.

In 2007, we released a new database, SABRE (Systematic consolidation of Arabidopsis and other Botanical REsource) that provides information of plant cDNA resources in connection with Arabidopsis genes annotated by TAIR. Currently, cDNA and EST resources of Arabidopsis, *Thellungiella halophila*, model moss (*Physcomitrella patens*), model tree (poplar), cassava and tobacco are loaded on the SABRE. This year, we are going to add cDNA resources provided through National BioResource Project (NBRP) to the SABRE. The additional data includes full-length cDNA from wheat, barley, tomato, legume and morning glory. The expanded SABRE database will provide all plant researchers easy way to access to the Arabidopsis genome information.

To view RIKEN resources and SABRE, please visit following addresses:

<http://www.brc.riken.jp/lab/epd/Eng/>

<http://saber.epd.brc.riken.jp/sabre/SABRE0101.cgi>

### **360 An Equation For A Vibrant Database: Curators + Journals + Community = Success**

*Donghui Li, Tanya Berardini, Raymond Chetty, Bob Muller, Eva Huala*

**TAIR, Carnegie Institution for Science, Stanford, CA, USA**

In 2008, The Arabidopsis Information Resource (TAIR) and the journal Plant Physiology began a collaboration to create an efficient mechanism for rapid and reliable transfer of the genetic and molecular data on Arabidopsis published in the journal into TAIR's public database. Since then, over 10 more plant journals have joined TAIR in the effort to involve the research community in direct data submission. TAIR now hosts a universal online data submission tool that allows authors with publications from any journal to submit their data directly to our curators. Once registered in the TAIR database, submitters can begin annotating as soon as they enter a DOI or PMID into the on-line form. The journals incorporate the URL for this form at critical points in the manuscript submission process. Hosting the submission tool ourselves allows us to streamline the data integration process and spend more time reviewing the annotations themselves instead of dealing with differences in journal-specific data formats. The TAIR-hosted form also makes the cost of collaborating with TAIR negligible for each journal which should allow for the rapid expansion of the set of publishers who promote direct data submission. Contributions from the community not only enrich the database and help to keep it current, but they also allow the database curators to focus on other publications that might otherwise not be read. All submissions are reviewed before integration into the database. This layer of review guarantees that the basic standards of annotation practiced at TAIR are applied to these data as well. The combination of all three communities - biocurators, journal publishers and researchers - working together ensure that the data available through TAIR are current and dynamic. We will present the features of the online tool and statistics on user submissions over the past two years.

### **361 A Comprehensive Dataset of Genes with a Loss-of-Function Mutant Phenotype in *Arabidopsis thaliana***

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The identification of mutant phenotypes in *Arabidopsis thaliana* has proven to be a powerful tool in plant biology. Despite remarkable advances enabled by mutant analysis, a detailed list of genes with a loss-of-function mutant phenotype in Arabidopsis has not been compiled. The initial dataset that we published 8 years ago (Meinke et al.; Plant Physiol 131: 409-418) is outdated and curated information on mutant phenotypes remains difficult to obtain. We present here a comprehensive dataset of more than 2,100 genes with a loss-of-function mutant phenotype in Arabidopsis. Phenotype descriptions were gathered from the SeedGenes database and from manual curation of the scientific literature. Genes were placed into one of four groups based on phenotype: essential, morphological, cellular or biochemical, and conditional. More specialized phenotype classes and subsets were also established. Gene identities were classified as either confirmed (through molecular complementation or the analysis of multiple sequenced alleles) or not confirmed. Relationships between mutant phenotype and protein function, genetic redundancy, and subcellular localization were explored. A complementary dataset of more than 350 genes that give a mutant phenotype only when disrupted in combination with a putative paralog was also compiled. The importance of these genes in confirming functional redundancy and enhancing the value of single gene datasets is discussed. With additional input and curation from members of the Arabidopsis community, we believe these datasets will provide a valuable foundation for exploring the relationship between genotype and phenotype in a model plant. Such datasets will also be valuable for comparative studies with other organisms. Toward this end, we have begun to explore whether knockouts of orthologous genes in different plant species often exhibit similar mutant phenotypes, with initial emphasis on tomato and rice. *Research supported by the NSF Arabidopsis 2010 Program.*

### **362 An international bioinformatics infrastructure to serve the Arabidopsis community**

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The Arabidopsis community has diverse, complex and growing bioinformatics needs, the result of increasingly sophisticated methods of data collection, visualization, analysis, and comparison. There are extensive tools and resources for information storage, curation and retrieval of Arabidopsis data that have been developed over recent years primarily through the activities of The Arabidopsis Information Resource (TAIR), the Nottingham Arabidopsis Stock Centre (NASC) and the Arabidopsis Biological Resource Center (ABRC), among others. However, the rapid expansion in many data types, the international basis of the Arabidopsis community, and changing priorities of the funding agencies all suggest the need for changes in the way informatics infrastructure is developed and maintained. There is a need for a single, core resource that is integrated into a larger international consortium of investigators. This could consist of a distributed

system of data, tools and resources, accessed via a single information portal and funded by a variety of sources, under shared international management of an International Arabidopsis Informatics Consortium (IAIC). We are developing a network that will support the development of ideas, collaborations and projects leading to novel informatics tools and resources for plant biology, and in particular, for Arabidopsis.

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**363 Effects of Sterilization Methods on Germination of Arabidopsis Seeds**

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The method used for surface-sterilization of Arabidopsis seeds can be critical for seed quality and viability. To investigate the effects of different sterilization techniques on seed germination, two experiments were performed. In the first experiment, increasing concentrations of bleach were applied to Columbia wild type seeds for different periods of time. It was shown that the bleach concentration had no effect on seed germination for up to 20 minutes soaking time. While lower bleach concentrations had modest influence on germination rate, higher concentrations had significant detrimental effect for soaking times of more than 20 minutes. In the second experiment, two commonly used sterilization protocols (bleach and chlorine gas) were used to test the dependence of the germination rate on the sterilization method in seed stocks shown to have different germination rates in the absence of sterilization agent. Neither bleach nor chlorine gas caused a considerable decrease in germination of seeds which had a high germination rate in the absence of sterilization. However, both sterilization techniques significantly affected germination of seeds with lower germination rates in the absence of sterilization. A correlation between initial seed quality and the germination rate after sterilization was observed. Based on these results, a standardized protocol for bleach sterilization was developed, as well as a recommended sterilization technique for high-throughput and other studies potentially using seeds of different quality.

This material is based upon work supported by the National Science Foundation, Award Number DBI-1049341. Any opinions, findings, and conclusions or recommendations are those of the author(s) and do not necessarily reflect the views of the NSF.

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**364 A Gateway to Elucidating Protein Function in Arabidopsis**

*The Arabidopsis Interactome Mapping Consortium<sup>1,2</sup>*

**<sup>1</sup>The Salk Institute for Biological Studies, La Jolla, CA USA, <sup>2</sup>Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Boston, MA, USA**

Nearly all cellular processes rely on biophysical interactions occurring between specific proteins. To facilitate the identification of such interactions on a proteome-wide scale in plants, we created an Arabidopsis Gateway ORFeome collection containing an unbiased set of ~12,000 pENTR ORF clones ready for recombination into various protein expression systems. In collaboration with the Center for Cancer Systems Biology at the Dana-Farber Cancer Institute, ~8500 unique loci from the At-ORFeome were individually interrogated using a stringent matrix-based yeast-two-hybrid screening pipeline. Over 6000 interactions were recovered for approximately one third of the interrogated proteins and assembled into a highly connected network named Arabidopsis Interactome-1 (AI-1). The high quality of AI-1 interactions was demonstrated using a plant-based, *in vitro* protein-protein interaction assay termed wNAPPA in which AI-1 interactors were detected at a similar rate to a set of literature-derived positive reference pairs. The high quality of AI-1 interactions was also apparent from the investigation of various modules involving multiple members from plant-specific gene families, several of which are uncharacterized. Importantly, interactions in AI-1 linked many of these poorly described proteins to well-characterized processes. These novel interactions should greatly advance our understanding of the role that these proteins play in plant cellular processes.

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**365 Navigating NCBI resources for Plant Genomics**

*Anjana Vatsan, Vyacheslav Chetvernin, William Klimke, Sergey Resenchuk, Brian Smith-White, Igor Tolstoy, Deanna Church, Donna Maglott, Tatiana Tatusova*

**National Center for Biotechnology Information, Bethesda, Maryland, USA**

The National Center for Biotechnology Information (NCBI) provides storage and analysis of plant map and genome data from a variety of sources and makes it available to the scientific community as interactive web resource. This is supported by several resources including Entrez Gene, reference sequence (RefSeq) data sets and the MapViewer which provides display of sequence and genetic maps from 46 plants. These resources are extensively cross-linked and facilitate navigation across a broad spectrum of biological information. By placing all organisms into a single system, NCBI can offer comparative studies of maps and sequences via MapViewer, UniGene, Homologene, BLAST and the protein cluster database (ProtClustDB).

MapViewer resource for Arabidopsis genome displays cDNA and ESTs from 18 plants aligned to the Arabidopsis sequence map. ProtClustdb currently has 71,000 clusters composed of 373,000 proteins from seven higher plant genomes (*Arabidopsis thaliana*, *Arabidopsis lyrata*, *Vitis vinifera*, *Populus trichocarpa*, *Ricinus communis*, *Oryza sativa japonica*, *Sorghum bicolor*), two lower vascular plant genomes (*Physcomitrella patens*, *Selaginella bicolor*), six algae genomes (*Chlamydomonas reinhardtii*, *Volvox carteri*, *Thalassiosira pseudonana*, *Ostreococcus lucimarinus*, *Phaeodactylum tricornutum*, *Micromonas pusilla*) and three nucleomorph genomes (*Guillardia theta*, *Hemiselmis andersenii* and *Bigelowiella natans*). These resources will be described at length.

**366 Content Advances and New Developments in the T-DNA Insertion Allele Collection GABI-Kat**

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The GABI-Kat collection is the 2nd-largest publicly available resource for T-DNA insertion lines of *Arabidopsis thaliana* worldwide. The collection has been intensively used in reverse genetics and functional genomics since ten years. There is still a high demand for GABI-Kat insertion alleles that is documented by continuing requests from the international plant research community. Also, the mutant alleles are successfully used as a resource in the DUPLO project that systematically generates homozygous double mutants for paralogous gene pairs. The collection can be accessed through the web-based user interface "SimpleSearch" (see [www.gabi-kat.de](http://www.gabi-kat.de)), which is connected to an FST (flanking sequence tag) database describing which genes have been disrupted.

During the last two years, several measures resulted in a massive improvement of the quality of the GABI-Kat collection:

- (i) the annotation of all T-DNA insertion predictions has been updated to the TAIRv10 genome sequence and annotation dataset;
- (ii) all existing FSTs have been re-mapped to the genome sequence with optimised parameters, resulting in the prediction of new insertion alleles for ~5,500 additional genes;
- (iii) the collection of in-house confirmation results has reached a critical information content that allowed to correct a large part of the problematic FST-to-line links;
- (iv) a fraction of the collection with weak FST yield was re-analyzed by generating new FSTs.

As a result of these parallel actions, the percentage of Ath nuclear protein coding genes covered with (predicted) GABI-Kat insertion alleles was increased from 63.7% to 84.8%. In addition, the reliability of the collection has been significantly improved by increasing the confirmation success rate from 78% to 83%. Finally, about 20,000 new FSTs were submitted to EMBL/GenBank in February 2011. The new data are available in an update of the SimpleSearch database (v24 of 2011-03-07). Further updates of the SimpleSearch tool are under way to make new features like paralogous insertion predictions from the same FST of which only one can be confirmed available to users.

Contact us at [info@gabi-kat.de](mailto:info@gabi-kat.de)

**367 A Deep Plant Alignment Database Integrated with Proteomic Data Constructed Using New Sequence Clustering and Alignment Algorithms***Andrew Carroll***UC Berkeley, California, CA**

The number of completed genomes and amount of sequence data has exploded in recent years, with tens of plant genomes having been completed and many more on the horizon. The amount of data is increasing beyond the ability of algorithms to comprehensively analyze them. A key step in the analysis of sequence data is the process of sequence alignment, which determines which residues in a protein are homologous, allowing for comparisons between proteins and protein regions.

Here I present a new process of sequence clustering and sequence alignment which is far faster than currently available methods. Using this process, alignments of all gene families in all completed genomes can be constructed on a personal computer in a reasonable amount of time. I have used this to generate an alignment database of all plant gene families.

I have combined this with available proteomic data to determine the conservation around post-translationally modified sites in order to build new prediction models based on the conservation data, and to identify new consensus sequences for post-translational modification. In addition, I have developed algorithms to identify regions which may indicate class-specific functions in sub-families.

This resource is in a state of construction and rapid evolution. It is presently located at <http://www.universalphylogeny.com>. The goal of this work is to provide comprehensive information on proteins and protein families to inform investigation of these families through experiments such as mutagenesis, cross-family complementation, domain exchanges, and to allow them to better understand the evolution and properties of their gene family.

**368 Integration of Systems Biology and Genetic Approaches indicates that the COP9 Signalosome is an ancient regulator of the DNA damage response***Osnat Atias<sup>1</sup>, Yair Halimi<sup>1</sup>, Shaul Pollack<sup>1</sup>, Claus Schwechheimer<sup>2</sup>, Benny Chor<sup>3</sup>, Daniel Chamovitz<sup>1</sup>*

**<sup>1</sup>Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Israel, <sup>2</sup>Department of Plant Systems Biology, Technische Universität München - Weihenstephan, Germany, <sup>3</sup>School of Computer Science, Tel Aviv University, Israel**

The COP9 signalosome (CSN) is a highly-conserved regulatory protein complex that in higher eukaryotes consists of eight subunits named CSN1 to CSN8. Loss-of-function of any of these subunits in *Arabidopsis* leads to a classic *cop/det/fus* phenotype. The most studied CSN function is regulation of protein degradation where its role as a deneddylase regulating culling-based E3-ubiquitin ligases is well established. However, aside from this biochemical activity, what is common to the biological function of the CSN in such diverse organisms as *Arabidopsis* and *Drosophila*? Does the high level of sequence and structural conservation of the complex among diverse organisms imply a conserved functional role for the CSN complex? These are the naïve questions that we are attempting to answer by using computational methods for comparing microarray experiments performed in these two organisms. We have developed a novel method for integrating and comparing data from diverse organisms. Among other results, our data point to a conserved function for the CSN in regulating the DNA damage response, particularly a gene module that includes the small subunit of ribonucleotide reductase (RNR2). Interestingly, we independently identified RNR2 as interacting with CSN7, with the subcellular localization of RNR2 being dependent on CSN7 and on DNA damage. While mutants in the CSN display a constitutive induction of the DNA-damage response, the ability of the mutants to further respond to DNA damage is compromised.

**369 Comprehensive Phylogenetic Analysis of the F-Box Gene Superfamily in Plants Reveals Divergent Evolutionary Histories Indicative of Genomic Drift***Zhihua Hua<sup>1</sup>, Cheng Zou<sup>2</sup>, Shin-Han Shiu<sup>2</sup>, Richard Vierstra<sup>1</sup>*

**<sup>1</sup>University of Wisconsin-Madison, Madison, (Wisconsin), USA, <sup>2</sup>Michigan State University, East Lansing, (Michigan), USA**

The emergence of multigene families is a major contributor to the evolution of complex traits and speciation. Here, we examined the phylogenetic relationships of *F-Box* (*FBX*) genes, composing one of the largest superfamilies in the plant kingdom. *FBX* proteins are the target recognition subunit of SCF ubiquitin ligases, where they individually recruit specific substrates for ubiquitylation. Through the extensive analysis of 10,811 *FBX* loci from 18 plant species, ranging from an alga to numerous monocots and eudicots, we discovered strikingly diverse evolutionary histories. The number of *FBX* loci varies widely and appears independent of the growth habit and life cycle of land plants, with a little as 198 predicted for *Carica papaya* to as many as 1350 predicted for *Arabidopsis lyrata*. This number differs substantially even among related species, with evidence for extensive gains/losses. Despite this extraordinary inter-species variation, one subset of *FBX* genes was conserved among most species examined. Together with evidence of strong purifying selection and expression, the corresponding ligases likely direct essential ubiquitylation events. From a reverse genetic analysis of this conserved subset in *Arabidopsis thaliana*, we identified SCF ligases that control embryo development and the response to reactive oxygen species. Another *FBX* subset was lineage specific, showed relaxed purifying selection, and was enriched in loci with little evidence of expression, suggesting that they control more limited, species-specific processes or arose from genomic drift and thus may provide reservoirs for innovation. Numerous *FBX* loci were also predicted to be pseudogenes with their numbers in each species tightly correlated with total *FBX* gene numbers. Taken together, it appears that the *FBX* superfamily has independently undergone substantial birth/death in many plant lineages, with its size and rapid evolution potentially reflecting a central role for ubiquitylation in driving plant fitness.

### **370 Comprehensive Phylogenetic Analysis of the F-Box Gene Superfamily in Plants Reveals Divergent Evolutionary Histories Indicative of Genomic Drift**

*Zhihua Hua<sup>1</sup>, Cheng Zou<sup>2</sup>, Shin-Han Shiu<sup>2</sup>, Richard Vierstra<sup>1</sup>*

<sup>1</sup>**University of Wisconsin-Madison, Madison, (Wisconsin), USA, <sup>2</sup>Michigan State University, East Lansing, (Michigan), USA**

The emergence of multigene families is a major contributor to the evolution of complex traits and speciation. Here, we examined the phylogenetic relationships of *F-Box (FBX)* genes that compose the largest and most polymorphic gene superfamily in the plant kingdom. FBX proteins are the target recognition subunit of SCF ubiquitin ligases, where they individually recruit specific substrates for ubiquitylation. Through the extensive analysis of 10,811 *FBX* loci from 18 plant species, ranging from an alga to numerous monocots and eudicots, we discovered strikingly diverse evolutionary histories. The number of *FBX* loci varies widely and appears independent of the growth habit and life cycle of land plants, with a little as 198 predicted for *Carica papaya* to as many as 1350 predicted for *Arabidopsis lyrata*. This number differs substantially even among related species, with evidence for extensive gains/losses. Despite this extraordinary inter-species variation, one subset of *FBX* genes was conserved among most species examined. Together with evidence of strong purifying selection and expression, the corresponding ligases likely direct essential ubiquitylation events. From a reverse genetic analysis of this conserved subset in *Arabidopsis thaliana*, we identified SCF ligases that control embryo development and the response to reactive oxygen species. Another *FBX* subset was lineage specific, showed relaxed purifying selection, and was enriched in loci with little evidence of expression, suggesting that they control more limited, species-specific processes or arose from genomic drift and thus may provide reservoirs for innovation. Numerous *FBX* loci were also predicted to be pseudogenes with their numbers in each species tightly correlated with total *FBX* gene numbers. Taken together, it appears that the *FBX* superfamily has independently undergone substantial birth/death in many plant lineages, with its size and rapid evolution potentially reflecting a central role for ubiquitylation in driving plant fitness.

### **371 From EST to RNAseq: A New Strategy to Annotate the Arabidopsis Genome for TAIR10**

*Philippe Lamesch<sup>1</sup>, David Swarbreck<sup>2</sup>, Eva Huala<sup>1</sup>*

<sup>1</sup>**Carnegie Institution for Science, Stanford, CA, USA, <sup>2</sup>TGAC, Norwich, Norfolk, UK**

In 2005, TAIR took over responsibility from TIGR to improve the gene structure annotation of the *Arabidopsis* genome. Since then, TAIR has published five genome releases, TAIR6-TAIR10, each improving the gene structure and type of thousands of genes. The curation process at TAIR evolved over the years as it was guided by the types of expression data available. Earlier releases mostly used the vast amount of cDNA/EST expression data from Genbank available at that time. In later releases TAIR curators integrated additional data types such as mass spec peptide and short read sequence data, the latter representing the main data source used for TAIR10.

In this talk we will present our latest release, TAIR10, which was published in December 2010. We will discuss how TAIR curators completely reinvented their annotation pipeline in order to make use of the vast amount of RNAseq data that became available in 2010 and how this effort resulted in the largest number of new gene models ever added to a TAIR release. We will walk the audience through our 4-step annotation process using RNAseq data and the tools that were used to carry out each of these steps, such as Tophat, Supersplat, Augustus, and Cufflinks. While customized to the annotation of the *Arabidopsis* genome, this method can be considered a model for genome annotation of many other eukaryotic genomes for which short read data is available. Statistics and numbers of the TAIR10 release will also be presented.

### **372 A Reduction in 24nt Small RNA in Arabidopsis Hybrids May Contribute to Hybrid Vigor**

*Ying Li<sup>1</sup>, Kranthi Varala<sup>1</sup>, Matthew Hudson<sup>1,2</sup>*

<sup>1</sup>**University of Illinois, Urbana, IL, US, <sup>2</sup>Energy Biotechnology Institute, University of Illinois, Urbana, IL, US**

Heterosis, also known as hybrid vigor, refers to the phenomenon wherein a F1 hybrid produced from crossing two cultivars of the same species or two different species displays superior phenotypes compared to the inbred parents. Regardless of its practical application and scientific importance, the molecular mechanism underlying heterosis is not completely understood. In recent decades, knowledge on regulatory roles of small RNAs has greatly helped improving our understanding of many basic biological questions. We therefore applied a global small RNA profiling approach using next-gen sequencing technique to characterize the inheritance of small RNA expression patterns in *Arabidopsis* reciprocal hybrids. Two *Arabidopsis thaliana* accessions, *Columbia* and *Landsberg erecta*, were crossed reciprocally to produce hybrids. The small RNA expression patterns of both parents and two hybrids were compared. We report that the most common expression patterns of small RNA in the hybrids are negative dominance and additive expression. Analysis of the genomic origin of those differentially expressed small RNAs suggested that they are mostly 24nt siRNA associated with genes and transposable elements. Interestingly, the transposon-associated siRNA are mostly additively inherited while the gene-associated siRNA are mainly down-regulated. Overall, down-regulation of siRNA is the most distinguished feature of small RNA expression pattern in *Arabidopsis* hybrids.

### **373 A Data Model of Root Gravitropism**

*Nathan Miller<sup>1</sup>, Tessa Durham Brooks<sup>2</sup>, Misuk Cho<sup>1</sup>, Edgar Spalding<sup>1</sup>*

<sup>1</sup>**University of WI-Madison, Madison, WI, USA, <sup>2</sup>Doane College, Crete, NE, USA**

Root gravitropism is a rapid manifestation of processes fundamental to plant development such as hormone transport and tight regulation of cell expansion. It can be quantified with high spatiotemporal resolution by algorithmically extracting and analyzing the root midline in each frame of a time series of digital images. A set of 1100 trials (separate movies) of wild-type *Arabidopsis* roots was collected in a systematically controlled set of conditions to produce a large and varied data set. A combination of principal and independent

component analysis and a non-linear embedding technique was used to distill the midlines down to three statistically independent parameters that captured ~95% of the variance. From these three parameters the midline response surface ( $x,y,t$ ) of any given bending root could be reconstructed. The first parameter was found to control a relationship between tip angle and growth rate. Sensitivity analysis of this parameter showed a 10  $\mu\text{m}/\text{hr}$  shift in growth rate caused a 5.8 degree shift in tip angle. A second parameter controlled the magnitude of curvature all along the midline, and therefore the total tip angle, without a major effect on growth rate (range of 0.17  $\mu\text{m}/\text{hr}$  over the entire dataset). The third parameter controlled the initial shape of the root at the onset of gravitropic stimulation but had little effect on the ensuing response (affecting tip angle by less than 3 degrees and growth rate by less than 0.6  $\mu\text{m}/\text{hr}$ ). Its role in the model is to specify the initial condition. Modeling the wild type response in this way provides a simple metric against which mutant responses can be compared and spatiotemporal phenotypes quantified.

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### 374 Quantitation of Cellular Dynamics in Growing *Arabidopsis* Roots with Light Sheet Microscopy

*Giovanni Sena<sup>1</sup>, Zak Frentz<sup>2</sup>, Kenneth Birnbaum<sup>1</sup>, Stanislas Leibler<sup>2,3</sup>*

<sup>1</sup>New York University, New York, U.S.A., <sup>2</sup>The Rockefeller University, New York, U.S.A., <sup>3</sup>The Institute for Advanced Study, Princeton, U.S.A.

To understand dynamic developmental processes, living tissues must be imaged frequently and for extended periods of time. Root development is extensively studied at cellular resolution to understand basic mechanisms underlying pattern formation and maintenance in plants. Unfortunately, ensuring continuous specimen access, while preserving physiological conditions and preventing photo-damage, poses major barriers to measurements of cellular dynamics in indeterminately growing organs such as plant roots.

We present a simple and relatively inexpensive system that integrates optical sectioning through light sheet fluorescence microscopy with hydroponic culture, that enables us to image at cellular resolution a vertically growing *Arabidopsis* root every few minutes and for several consecutive days.

We present novel automated routines to track the root tip as it grows, track cellular nuclei and identify cell divisions. We demonstrate the system's capabilities by collecting data on cell divisions and nuclear dynamics.

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### 375 Extensive Genomic and Transcriptomic Variation in the 19 Founders of the *Arabidopsis* MAGIC Lines

*Joshua Steffen<sup>1</sup>, Jonas Behr<sup>5</sup>, Philipp Drewe<sup>5</sup>, Katie Hillebrand<sup>2</sup>, Paula Kover<sup>3</sup>, Rune Lyngsoe<sup>6</sup>, Richard Mott<sup>4</sup>, Edward Osborne<sup>1</sup>, Gunnar Rätsch<sup>5</sup>, Sebastian Schultheiss<sup>5</sup>, Vipin Sreedharan<sup>5</sup>, Oliver Stegle<sup>5</sup>, Chris Toomajian<sup>2</sup>, Gan Xiangchao<sup>4</sup>, Richard Clark<sup>1</sup>*

<sup>1</sup>University of Utah, Biology, Salt Lake City, UT, USA, <sup>2</sup>Kansas State University, Plant Pathology, Manhattan, KS, USA, <sup>3</sup>University of Bath, Life Sciences, Manchester, M13 9PT, United Kingdom, <sup>4</sup>University of Oxford, WTCHG, Oxford, OX3 7BN, United Kingdom, <sup>5</sup>Max Planck Society, FML, Tübingen, 72076, Germany, <sup>6</sup>University of Oxford, Statistics, Oxford, OX3 7BN, United Kingdom

Much of our knowledge about genome structure and function in *Arabidopsis* comes from studies with the single reference accession, Col-0. To broadly understand both sequence and functional variation in *Arabidopsis*, we have Illumina sequenced the genomes of 18 diverse accessions that are the founders of the Multiparent Advanced Generation Inter-Cross (MAGIC) nested association mapping population. Using a combination of read alignment and *de novo* methods, we assembled the unique to moderately repetitive fraction (~80%) of each accession's genome with an error rate of about 1 nucleotide error per 10kb. From the assemblies, we identified more than 3 million SNPs, and more than 1 million indels that (non-redundantly) alter more than 10% of the reference genome sequence. To inform the genome assemblies, we also produced the seedling transcriptomes of each accession (Illumina RNA-seq), and we are now extending this work to also produce floral bud and root transcriptomes. By combining computational methods with the RNA-seq data, we annotated each of the MAGIC founder genomes, in the process identifying thousands of alternative gene models or new genes not predicted in or absent from the Col-0 genome. In an initial analysis, we have used the DNA sequence and transcriptome data to understand the genetic basis of gene expression variation in unprecedented detail. We identify more than 9,000 differentially expressed genes at the seedling stage alone. Much of this expression variation is obviously explained by structural changes, or is associated with other *cis* polymorphisms, often nearby transcriptional start sites. Moreover, with strand-specific RNA-seq, we find extensive antisense transcription, including at genes for which antisense or non-coding transcripts have not previously been reported. The comprehensive DNA sequence, gene annotation and expression data will be fundamental for studies to understand phenotypic variation of ecological and agronomic relevance in these lines, and in other *Arabidopsis* populations.

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### 376 Novel and Known Post-transcriptional Regulatory Sequences are Conserved across Plant Families

*Justin Vaughn, Bijoyita Roy, Albrecht von Arnim*

**University of Tennessee, Knoxville, TN, USA**

Gene regulatory regimes at the post-transcriptional level operate via mRNA sequence motifs. For example, ~30% of mRNAs contain at least one small open reading frame (uORF) upstream of the major ORF. Here we present results from a comparative transcriptome study between *Arabidopsis* and five other families of dicot plants aimed at examining uORF conservation specifically and UTR conservation in general. We identified several hundred conserved RNA motifs of 5-30 nucleotides in length. Within the 5' UTR, purine-rich motifs were overrepresented. In contrast, in the 3' UTR more complex motifs were common, some of which are probable target sites for RNA binding proteins or miRNAs, and some of which may serve as sites for subcellular localization of mRNAs. These data have implications for the RNA regulon concept. Surprisingly, AUG was the most conserved triplet in the 5' UTR in all plant lineages evaluated. Given that conserved-peptide uORFs are rare, a large proportion of the associated uORFs must function in a peptide-independent fashion,

whereas many others may evolve neutrally. Previous research has established that components of the basal translation machinery, such as subunits of eukaryotic initiation factor 3 and of the 60S ribosomal subunit, compensate for the inhibitory effect of certain uORFs. A computational model of the translation initiation process was implemented to show how eIF3 may contribute to the competence of the translation machinery for re-initiation. Supported by DOE DE-FG02-96ER20223 and NSF DBI-0820047.

### **377 Construction of A Novel Conceptual Coexpression Network for Biological Knowledge Discovery**

*Hairong Wei*

**Michigan Technological University**

Existing coexpression construction methods suffer some flaws mainly because transcription noise contained in existing gene expression data is not taken into account. For this reason, selection of more robust gene association methods for network construction can lead to significant improvement of biological knowledge discovery via network construction and decomposition. In this presentation, I will show several robust methods and their efficiency in identifying functionally associated genes followed by an introduction of a new conceptual coexpression construction method, shared coexpression connectivity matrix (SCCM), in which each entry represents the number of shared coexpressed genes between two regulatory genes or two genes of interest. This sparse and symmetric matrix embodies a new concept of coexpression networks where genes are associated in the context of other shared coexpressed genes. To discover the novel biological knowledge from this network, one can use graph decomposition methods to decompose SCCM into subnetworks, each has a dedicated function under experimental condition. We will show a novel heuristic algorithm termed "Triple-Link" to decompose SCCM. We applied this framework to gene expression data from human stem cells and *Arabidopsis* roots and discover a large amount of novel knowledge.

### **378 Construction and Validation of a Brassinosteroid Gene Regulatory Network (GRN) in the Control of Plant Growth and Development in *Arabidopsis thaliana***

*Huaxun Ye, Lei Li, Jaroslaw Zola, Maneesha Aluru, Hongqing Guo, Sarah Anderson, Peng Liu, Steve Rodermel, Srinivas Aluru, Yanhai Yin*

**Iowa State University, Ames, Iowa, USA**

Plant steroid hormone brassinosteroids (BRs) regulate growth, development and plant responses to environmental changes. Recent microarray studies revealed that BRs regulate thousands of target genes; but the transcriptional network for such regulation remains to be fully defined. BRs function through membrane receptor BRI1 and other signaling components to regulate the activities of BES1 and BZR1 family transcription factors. To understand how BES1 regulates gene expression and BR responses, we have identified BES1 direct target genes by Chromatin Immunoprecipitation and genomic tiling arrays (ChIP-chip). Transcription factors are highly enriched in BES1 target genes. To understand how these BES1 targeted transcription factors (BTFs) control gene expression, we used ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks) to build a GRN based on public gene expression data. Interestingly, many of the BTFs form extensive connections, suggesting functional interactions among them. The GRN confirms many known interactions in the BR signaling pathway and also predicts new hypotheses about BR-regulated gene expression and BR signaling. For example, we have recently confirmed that BES1 represses two related transcription factors, GLK1 and GLK2, to repress chloroplast development, which reveals the mechanisms for the long-standing observation that BRs function to regulate photomorphogenesis. We have been carrying out additional functional studies to validate some of the new hypotheses generated from the GRN and will present the latest results from these studies. Supported by NSF (IOS 0546503).

**379 Hydro-patterning: Moisture induced Polarity in Lateral Root Initiation**Pooja Aggarwal, Jose Dinneny**Temasek Life Sciences Laboratory Limited, Singapore**

Adaptations to ever-changing environment are key to an organism's survival and act as the mechanistic tool for a species evolution as elegantly explained by Charles Darwin in his famous book 'Origin of Species'. Besides mounting an effective response against biotic pathogens, plants have also learned to adapt to several abiotic stresses such as extreme temperatures, oxidative stress, radiations, chemical toxicity, drought, salinity and submergence. Interestingly, plants demonstrate another kind of growth adaptation that need not depend on the presence of a stress factor. Instead, plants modify their growth and architecture taking cues from natural agents like water, light and air in order to maximize their nutrient harnessing capacity. For instance, when shadowed by canopy of bigger trees plants are known to elongate their stem/hypocotyls to increase their light exposure, a phenomenon known as shade avoidance. Other examples include phototropism, hydrotropism and gravitropism.

On similar lines, we have identified water as an important patterning agent for the *Arabidopsis* root architecture. *Arabidopsis* roots initiate lateral roots preferentially towards the water-rich agar surface, when grown vertically in MS agar plates. We have coined the term 'Hydro-patterning' to describe such phenomenon. Hydro-patterning is also observed in rice roots, suggesting it may be a wide spread mechanism in the plant kingdom. Our preliminary analysis revealed that major clues for hydro-patterning originate at root tip and one such clue may be the preferential synthesis of a major plant growth hormone 'Auxin' towards the wet side and ABA response towards the dry side. Exogenous application of auxin partially overrides the hydro-patterning and thus confirms the involvement of auxin in its regulation. We have scored hydro-patterning in various mutants defective in Auxin/ABA synthesis, transport and signaling and found that both Auxin and ABA regulates hydro-patterning in plant roots by taking cues from their local water environment.

**380 ABSCISIC ACID INSENSITIVE 4 (ABI4) Mediates ABA and Cytokinin Inhibition of Lateral Root Formation****by Reducing Auxin Polar Transport**Doron Shkolnik-Inbar, Dudy Bar-Zvi**Ben-Gurion University of the Negev, Beer-Sheva, Israel**

Lateral roots (LRs) formation is an essential process in plant's development and adaptation to the environment. LR development is controlled by a balance between three phytohormones: auxin is the key hormone promoting LR formation, whereas cytokinin and ABA inhibits this developmental process. We present here direct evidences for *ABSCISIC ACID INSENSITIVE 4 (ABI4)* encoding an ABA-regulated AP2-domain transcription factor role in root branching. *ABI4* is intensively studied in ABA and glucose signaling in seed germination. Mutation in *ABI4*, results in an increased number of LR and its overexpression impairs LRs development. Root expression of *ABI4* is enhanced by ABA and cytokinin and repressed by auxin. *ABI4* also affects the profiles of the auxin and cytokinin hormones in the root, as determined by the activities of the respective hormone-response promoters *DR5* and *ARR5*. LRs are initiated in xylem-pole pericycle cells accumulating threshold level of auxin, leading to a series of divisions, resulting in the LR primordia formation. *ABI4* is expressed in phloem companion cells, and its expression reduces the level of the auxin-efflux carrier PIN1, abrogating auxin accumulation, and thus, LR initiation. We therefore suggest that *ABI4* plays a key inhibitory role in LR development by affecting auxin polar transport, in a mechanism regulated by ABA and cytokinin. *abi4* mutants also display an increased tolerance to salt-stress and osmotic-stress. The involvement of *ABI4* in plant response to abiotic stress will also be discussed. [Shkolnik-Inbar and Bar-Zvi, (2010) Plant Cell 22, 3560–3573; Shkolnik-Inbar and Bar-Zvi (2011) Plant Signaling & Behavior (in press)].

**381 The roles of SHORT INTERNODES/STYLISH during leaf vein development in *Arabidopsis thaliana***Tammy Baylis<sup>1</sup>, Izabela Cierlik<sup>2</sup>, Eva Sundberg<sup>2</sup>, Jim Mattsson<sup>1</sup>**<sup>1</sup>Simon Fraser University, Burnaby, BC, Canada, <sup>2</sup>Swedish University of Agricultural Sciences, Uppsala, Sweden**

Leaves depend on a highly developed venation system to collect fixed carbon in the form of sugar for transport to other organs and also to distribute water throughout the leaf blade. Although it is well known that auxin can induce vascular differentiation, the mechanism behind leaf vein patterning is still veiled in some mystery. Here we have assessed the roles of members of the *SHORT INTERNODES/STYLISH (SHI/STY)* gene family in this process. Members of the family encode transcription factors linked to auxin signalling primarily in gynoecium development. We found that *SHI/STY* genes are primarily expressed in the apex, developing marginal serrations, and base of *Arabidopsis* developing leaf primordia, with little or no expression at sites of vein formation. Mutant analysis nevertheless revealed reproducible effects on leaf venation in single mutants with increasing severity in multiple mutant combinations. Taken together, our data imply that *SHI/STY* genes play a role in leaf vein patterning perhaps through local regulation of auxin synthesis and transport at sites in the margin of the leaf blade.

**382 The Molecular Basis of Natural Variation in Root Development**Wolfgang Busch, Mónica Meijón Vidal, Radka Uhliřová**Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Austria**

Key to understanding development is the characterization of the regulatory networks that govern developmental processes. The root of *Arabidopsis thaliana* has proved to be an excellent model system for studying such complex phenomena and elucidating their genetic bases. Using forward genetic approaches, remarkable progress has been made in understanding how root development is regulated at the molecular level. Typically, it appears that development is governed by regulatory networks, rather than single genes, and that these networks exhibit robustness due to redundancy and complex regulatory loops. However, it is difficult to identify multiple components of

these complex networks using forward genetics methods. A promising experimental avenue is to exploit natural variation and conduct genome wide association studies to identify genes and gene networks that regulate development.

We are using high throughput image acquisition and analysis to monitor root growth and quantify novel, dynamic traits for root development in several hundred natural *Arabidopsis* accessions. We determine those traits at different resolutions from whole roots to the cellular level. We have identified variation at a broad scale among *Arabidopsis* accessions during development at the scale of both the whole organ and the cellular level. We are currently using those quantified traits to conduct genome wide association mapping, thereby making use of the available sequencing and genotyping data. The genomic regions associated with specific traits will be globally analyzed to infer biological pathways and functional categories that are involved in natural variation of root development. Furthermore, the involvement of specific genes in the phenotypic variation will be studied. Our long-term goal is to understand the molecular mechanisms of development and their patterns of adaptive variation.

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### **383 *Arabidopsis thaliana* Small Auxin-Up RNA 63 (SAUR63) functions in plant growth by regulating basipetal auxin transport**

*Keun Chae<sup>1</sup>, Cameron Issacs<sup>1</sup>, Paul Reeves<sup>1</sup>, Greg Maloney<sup>2</sup>, Esther Park<sup>1</sup>, Gloria Muday<sup>2</sup>, Jason Reed<sup>1</sup>*

<sup>1</sup>**University of North Carolina, Chapel Hill, NC, USA, <sup>2</sup>Wake Forest University, Winston Salem, NC, USA**

Auxin governs plant growth and development by regulating both gene expression and polar auxin transport. *Small Auxin-Up RNAs (SAURs)* are a family of early Auxin-responsive genes with yet-unknown functionality, present in both angiosperms and moss. We have found that plants expressing an artificial microRNA that targets a *SAUR* subfamily (*SAUR61 - 68, 75*) had a slight defect in hypocotyl elongation of the seedling, and mature flowers of these plants had short stamen filaments, which decreased pollination. In contrast, our transgenic plants expressing SAUR63 with C-terminal fusion of GFP or GUS (*SAUR63:GFP* or *SAUR63:GUS*) had long hypocotyls and stamen filaments, and twisty inflorescence stems, suggesting that these protein fusions caused a gain of function. SAUR63:GUS seedlings also had higher rates of basipetal transport of exogenous IAA in the hypocotyl, compared to the wild-type. Accordingly, the *SAUR63:GUS* transgene could restore both hypocotyl and stamen filament elongation to *pgp1 pgp19* plants, which have a decreased basipetal auxin transport rate. A *SAUR63:HA* transgene did not cause these phenotypes. Confocal microscopic analysis showed that *SAUR63:GFP* was localized to the plasma membrane of epidermal cells in the hypocotyl and cotyledons. Our microsomal fractionation showed that *SAUR63:GFP* was found mostly in the membrane fraction, whereas *SAUR63:HA* was in both soluble and membrane fractions. With cycloheximide treatment, *SAUR63:GFP* showed higher stability with delayed protein turn-over, compared to that of *SAUR63:HA*. Taken together, we propose a model that *Arabidopsis* *SAUR63* localizes to the plasma membrane and regulates homeostasis of intercellular basipetal auxin transport for plant growth.

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### **384 ATHB12, a homeodomain-leucine zipper (HD-Zip) class I protein, controls the stem elongation through the regulation of GA20ox1 level.**

*Yoon-Sun Hur, Sunghan Kim, Choong-Ill Cheon*

**Department of Biological Science, Sookmyung Women's University, Seoul, Korea**

Plant homeobox genes have been studied to be critical in various developmental processes such as the formation of embryos and vascular bundles. *Arabidopsis thaliana* *homeobox 12* (*ATHB12*) belongs to the HD-Zip class of plant homeobox genes, and was shown to be induced to abiotic stresses such as drought, cold, salt, and ABA treatments, and also to biotic stresses such as *Pseudomonas syringae* and geminivirus. *ATHB12* is highly expressed in inflorescence stem, and its T-DNA mutant has thin and longer stem. T-DNA insertion mutant also had a higher germination rate on ABA-containing media. In contrast to the *athb12* mutant, *ATHB12* overexpressor showed the growth retardation at early stem developmental stage. Moreover, ABA treatment induced *ATHB12* expression in inflorescence stem and inhibited the stem growth, similarly to the phenotype of *ATHB12* overexpressor. We found that expression of *gibberellins 20-oxidase 1* (*GA20ox1*) was increased in the stems of *athb12*, whereas *ATHB12* overexpressor had reduced level of *GA20ox1* in stems. On the other hand, the stems of *ATHB12* overexpressor grew rapidly after first three weeks. We also found changes of *GA20oxs* and *GA3oxs* expression and the increased  $GA_4$  levels in the stems of A12OX, which appear to result from feedback regulation. Interestingly, transient expression of *ATHB12* reduced the transcript level of *GA20ox1* in *Arabidopsis* protoplasts. All these findings suggest that an ABA-inducible gene, *ATHB12*, negatively regulates the expression of *GA20ox1* in inflorescence stems.

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### **385 Meristematic Growth Zones are Altered in Exocyst Mutants to Affect *Arabidopsis* Root Growth**

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The exocyst is an eight protein complex that is believed to act as a tether at the plasma membrane, serving in certain specialized secretory processes to help target vesicles to sites of exocytosis in yeast and mammalian cells. The exocyst also exists as a complex in plants, where it is involved in the tip growth of pollen tubes, cytokinesis, seed coat development, and hypocotyl elongation (e.g., Hala et al. 2008; Zarsky et al., this conference). We noted that mutant *Arabidopsis* plants defective in four different exocyst proteins are dwarfed, with shorter roots due to growth rates significantly slower than wild-type. Based on cellular measurements and *CYCB1::GUS* assays, the basis for the slower growth rate is a combination of reduced cell elongation and shorter meristems (i.e., fewer dividing cells). An analysis of exocyst mutants with confocal microscopy provided a more detailed characterization of the alterations in the growth zones of the mutant root tips. Surprisingly, the cell file configuration appears unaltered, and the meristematic cell cycle length does not appear to be longer in exocyst mutants, indicating that the root growth defect is not primarily due to a problem with cytokinesis. Instead, the altered lengths of the mutant root growth zones suggest that the exocyst may play a role in one or more of the hormone signaling pathways (e.g. auxin,

brassinosteroids, gibberellins, cytokinins, etc.) that determine the size of these growth zones. This hypothesis was tested by evaluating root growth of exocyst mutants after the application of exogenous hormones or their inhibitors, testing for genetic interactions between brassinosteroid and exocyst mutations, and performing selective gene expression analysis. The results reveal that the role of the exocyst in root growth is complex, and may involve a combination of interacting hormone signaling pathways.

**386 ER-localized PIN8 Modulates Cell And Plant Development by Regulating Intracellular Auxin Homeostasis**

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The phytohormone auxin regulates many processes that are critical for plant growth, development, and environmental responsiveness. Typically auxin is produced at specific cellular locations within the plant body, then released and transported to target cells where it elicits responses. Although our knowledge of the pathways that trigger auxin biosynthesis and of intercellular auxin transport has progressed, little is known about the mechanisms regulating its intracellular homeostasis. *Arabidopsis thaliana AtPIN8* encodes a functional auxin transporter which resides in the endoplasmic reticulum and is expressed preferentially in the male gametophyte. Overexpression of AtPIN8 resulted in dramatic developmental abnormalities in both systems used, whole plants and single developing cells. By combining co-localization, genetic, physiological and biochemical analysis in tobacco and *Arabidopsis* plants and cells we showed that AtPIN8 affected intracellular auxin homeostasis. This further suggests an essential role of the ER in regulation of intracellular auxin transport.

**387 Differential Root Growth Is Regulated By Auxin-Mediated Interaction of PIN2 And The Cell Wall**

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Plants explore environment by growth and organ development. Their capacity to reorient organ growth is tightly connected with auxin-regulated differential cell elongation and divisions. PIN proteins, a family of auxin efflux facilitators, are polarly localized in cells and thus regulate direction of polar auxin transport as well as generation of local auxin gradients essential for differential growth. Little is known on how PIN polarity is maintained, what prevents lateral diffusion of this protein and how exactly PIN-modulated local auxin gradients are established. PIN2 proteins form unique clusters of a very low mobility at the cell periphery, indicating a novel role of PINs in differential root growth. Here we show that a direct interaction of PIN2 with cellulose components of the cell wall is crucial for maintenance of PIN2 polarity in root epidermis cells of *Arabidopsis* and this interaction could be mediated by auxin. By using chemical and genetic tools in combination with various imaging techniques and electron microscopy we developed a data-based model of how auxin, PINs and cell wall are interconnected to achieve differential growth of cells within the root tip.

**388 Altered Auxin Transport and Gravitropic Response in the *scd1* Mutant**

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The mechanisms that control the localization of auxin transport proteins that are needed to establish auxin gradients are poorly understood. We are exploring the role of RAB dependent vesicle targeting in this process by examining the *scd1-1* mutant, which has a defect in a gene with sequence similarity to RAB interacting and regulatory proteins. We asked if IAA transport, the distribution of IAA efflux proteins, and gravitropic responses are altered in *scd1* mutants. We are using the temperature sensitivity of the *scd1-1* mutant, which at the nonpermissive temperature of 25°C, exhibits a reduced root elongation rate and delayed gravitropic curvature relative to wild-type, but not at the permissive temperature of 18°C. Both acropetal and basipetal IAA transport polarities were reduced in the *scd1-1* mutant relative to the wild type at the nonpermissive temperature, but were equivalent at 18 °C. Transfer of *scd1-1* from 18 ° to 25 °C for as short as 6 hours is sufficient to reduce the gravitropic response and auxin transport. In addition, *scd1-1* is impaired in formation of asymmetric auxin induced gene expression after gravitropic reorientation, as judged by DR5-GFP fluorescence at 25°, but not 18 °C. We have used laser scanning confocal microscopy to examine the tissue level and sub-cellular distribution of six auxin transport proteins,

PIN1, PIN2, PIN3, PIN7, AUX1, and MDR1 (ABCB19) fused to GFP or YFP. When *scd1-1* was grown at 25°C, the expression of PIN1, PIN2, and PIN3 reporters was restricted to fewer cells, reduced in intensity, and accumulated in endomembrane structures. However, the expression of the other reporters was unchanged compared to wild type. Together these experiments suggest that SCD1 may play an important role in targeting selected IAA transport proteins to the plasma membrane, thereby regulating root gravitropism. (This work is supported by NASA grant NNX09AK82G to GKM).

### **389 Characterization of second-site enhancer mutations of the auxin-resistant4 mutations**

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The auxin-resistant4 (*axr4*) mutations of *Arabidopsis* have been previously found to alter localization of the AUX1 auxin import protein (Dharmasiri et al., 2006, *Science* 312, 1218-20), but the molecular function of AXR4 itself is unclear. An extensive screen for genetic enhancers of the auxin-resistant root elongation of the *axr4* mutations resulted in identification of two such enhancers that are auxin sensitive in the absence of the *axr4* mutation. Characterization of the root response of these enhancers to diffusible and actively-imported auxins support the hypothesis that these enhancers affect auxin uptake rather than response. Characterization of different aspects of the phenotypes of the *axr4* enhancer double mutants shows that the enhancer mutations do not simply increase auxin resistance in all auxin-related processes. Additional genetic and physiological characterization of the enhancers will be presented.

### **390 The Arabidopsis OSR1 Regulates Organ Growth and Final Organ Size in Orchestration with ARGOS and ARL**

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The growth of an organ in plants up to its characteristic size entails coordinative regulation of cell proliferation and expansion, but the mechanism underlying such regulation remains largely elusive. We previously reported that *Arabidopsis* ARGOS and ARL that function in the regulation of organ growth and final organ size by promoting cell proliferation and expansion respectively. Here, we identified another *Arabidopsis* gene, *ORGAN SIZE RELATED1 (OSR1)*, that regulates cell proliferation and expansion during organ growth in orchestration with *ARGOS* and *ARGOS-LIKE (ARL)*. Ectopic expression of *OSR1* in *Arabidopsis* leads to enlarged organs, due to an increase in both cell number and cell size, and disruption of *OSR1*, *ARGOS* and *ARL* results in smaller lateral organs. *OSR1* shares a conserved OSR domain with ARGOS and ARL, which is essential and sufficient for their functions in promoting organ growth. Similar to that of *ARGOS*, the promotive role of *OSR1* in cell proliferation is *AINTEGUMENTA (ANT)*-dependent. Three OSR proteins are localized to endoplasmic reticulum and appear to function in a redundant manner. In addition, *OSR1* is induced by ethylene but repressed by ABA and brassinosteroid (BR). These results, together with the previous work on *ARGOS* and *ARL*, suggest that three *OSR* members may act as co-evolved factors that integrate plant hormone signals to fine tune cell proliferation and cell expansion, thereby affecting organ growth during plant development.

### **391 RABBIT EARS Regulates microRNA164 Genes in Arabidopsis Sepal and Petal Development**

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The establishment and maintenance of organ boundaries are vital for animal and plant development. In the *Arabidopsis* flower, three *microRNA164* genes (*miR164a*, *b* and *c*) regulate the expression of *CUP-SHAPED COTYLEDON1 (CUC1)* and *CUC2*, which encode key transcriptional regulators involved in organ boundary specification. These three *miR164* genes are expressed in distinct spatial and temporal domains that are critical for their function. Here we show that C2H2 zinc finger gene *RABBIT EARS (RBE)* coordinately regulates all three *miR164* genes. Furthermore, we demonstrate that *RBE* directly interacts with the promoter of *MIR164c* and negatively regulates its expression. These results indicate *RBE* functions as a rheostat to fine-tune *miR164* expression to regulate developmental events required for sepal and petal organogenesis.

### **392 Analyses of the effect of exogenous polyamines on the stem growth of Arabidopsis thaliana**

*Jun-ichi Kakehi<sup>1</sup>, Wurina Tong<sup>1</sup>, Kaori Yoshimoto<sup>1</sup>, Hiroyasu Motose<sup>1</sup>, Masaru Niitsu<sup>2</sup>, Taku Takahashi<sup>1</sup>*  
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Polyamines are low-molecular-weight cations present in all organisms. Loss-of-function mutants of *ACAULIS5 (ACL5)* in *Arabidopsis thaliana* show excessive differentiation of xylem tissues and severe dwarf phenotype. *ACL5* encodes thermospermine synthase. Exogenously supplied thermospermine suppresses the dwarf phenotype of *acl5* mutants but spermine, which is a structural isomer of thermospermine, has no such effect. To identify the genes responsive to exogenous thermospermine, we performed microarray and real-time RT-PCR experiments using wild-type and *acl5* seedlings, and found that a number of the key genes involved in xylem differentiation are up-regulated in the mutant seedlings and are down-regulated by exogenous thermospermine. These genes are also responsive to norspermine. Norspermine (C3C3C3) and thermospermine (C3C3C4) has the same arrangement of carbon chains (C3C3), which is not present in spermine (C3C4C3). We further examined whether the other minor polyamines containing the C3C3 arrangement could substitute for thermospermine or not. Our result revealed that C3C3C2 and C3C3C5 suppress xylem overproliferation and down-regulate the genes related to vascular development in *acl5* seedlings while C3C3C3C4 has little or no effect. Based on the results, we conclude that tetramines containing the C3C3 arrangement are structurally crucial for the repressive control of xylem development.

### 393 The *Arabidopsis* NGATHA transcription factors act as negative regulators of cell proliferation in lateral organ growth

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The *Arabidopsis* NGATHA genes that belong to the B3-type transcription factor superfamily have been shown to regulate style growth and development. They are also known to be involved in lateral organ growth. We have characterized in detail the role of NGA genes in lateral organ growth. Overexpression of NGA1 through NGA4 all resulted in markedly reduced size of lateral organs compared to the wild type: lateral organs, such as leaves, flowers, and cotyledons, were small and distinctively narrow, and their root growth was severely inhibited as well. The NGA overexpressors have reduced number and size of leaf and petal cells. Kinematic analysis on leaf growth revealed that both the rate and duration of cell proliferation declined during organogenesis, which was accompanied by reduced expression of cyclin genes. On the other hand, combinations of *nga* mutants increased lateral organ size, especially petal size, because of increased number of petal cells. Taken together, these data indicate that *NGA* genes act as a negative regulator of cell proliferation.

### 394 An apical root growth program directed in the vascular stem cells

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*Arabidopsis* primary root develops from the root apical meristem (RAM). The RAM harbors the stem cell niche that consists of pluripotent stem cells encircling few (2-4) mitotically inactive cells, called quiescent centre (QC). The QC maintains the stem cell niche and prevents stem cells from premature differentiation, thereby ensures root growth.

Over the years, several studies enhanced our knowledge about the genetic networks and other signaling modules that govern the development and function of RAM. One of them is the pathway directed by *SHORT ROOT* (*SHR*) and *SCARECROW* (*SCR*). When this pathway is perturbed, the apical root growth is significantly retarded. This has been thought to be resulted from the loss of QC identity. Recently we and colleagues reported the bidirectional cell signaling process that involves the movement of *SHR* and microRNA (miR) 165/6. This mechanism ensures a proper spatial distribution of HD-ZIP III transcription factors.

We found that *SHR-SCR-miR165/6* regulation is important for the apical root growth and that *PHABULOSA* (*PHB*) plays a critical role as a downstream regulator. In *phb shr* and *phb scr* mutants, the roots grew significantly longer than in *shr* and *scr*. This root growth recovery was not resulted from the restoration of QC identify. *PHB* was found to regulate the apical root growth in the procambium, a part of the proximal meristem of the root stem cell niche. A high dose of *PHB* expressed in the root procambium inhibited the apical root growth. Furthermore, it affected the QC identity. Interestingly, the *SHR-PHB* pathway seems to regulate the apical root growth via the crosstalk with the cytokinin signaling cascade. This intricate regulatory program points vascular stem cells as an important place in the RAM activity.

### 395 PHYTOCHROME INTERACTING FACTOR 4 regulates auxin biosynthesis at high temperature

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When grown at high ambient temperature, plants display considerable stem elongation and elevated levels of the plant hormone auxin. *Arabidopsis* mutants deficient in the bHLH transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4) show severely attenuated elongation responses to high temperature, suggesting a key regulatory role for this protein. PIF4 also acts redundantly with its homologue, PIF5, to regulate diurnal growth rhythms and elongation responses to the perceived threat of vegetational shade. It has previously been reported that PIF4 activity is limited, in part, by binding to both the bHLH protein LONG HYPOCOTYL IN FAR RED 1 (HFR1) and the DELLA family of growth-repressing proteins. Despite the importance of PIF4 in integrating multiple environmental signals, the mechanisms through which PIF4 controls growth are unknown. Here we demonstrate that PIF4 directly regulates levels of the plant hormone auxin, through temperature-dependent binding to key biosynthesis gene promoters. We additionally identify auxin-regulated genes which promote elongation growth in a PIF4-dependent manner. Together, our data provide a direct molecular link between temperature and growth, via PIF4 and the plant hormone auxin. Understanding the mechanisms through which plants direct growth and allocate resources in response to changes in ambient temperature will be important in enhancing crop yield in a changing climate.

### 396 Lateral Root Development Associated with a Polyadenylation Factor in *Arabidopsis*

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Alternative polyadenylation plays an important role in gene expression regulation in eukaryotes. We previously reported that an *Arabidopsis* cleavage and polyadenylation specificity factor 30 (AtCPSF30) is involved in posttranscriptional processing to control the responses of plants to oxidative stresses, and the mutation of *AtCPSF30*, called *oxt6*, can affect the poly(A) site selection (Zhang et al., PLoS One, 3:e2410). We further found that this *oxt6* mutant has drastically reduced lateral roots. Upon examination of the lateral root primordium development, the results showed that the reduction is mainly on stage I to III in *oxt6*. How AtCPSF30 regulates root development, however, is not known. Here, we have developed a deep sequencing method for the identification of polyadenylated RNA termini with poly(A) tags (PAT-seq), and applied this method to investigate alternative polyadenylation in the root development. PAT-

seq identified 283 genes showed different alternative polyadenylation patterns in WT and *oxt6*. Among them, 25 are known genes that are involved in lateral root development. These findings provide candidate genes for studying post-transcriptional regulation in lateral root development.

### **397 Class II HD-ZIP Proteins Are Oppositely Regulated by Ad/abaxial Regulators and Control Meristem Size, Leaf Blade Development and Proliferation of Stem Tissue**

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The ad/abaxial regulatory network controls fundamental aspects of plant development including the formation and growth of shoot apical meristems. We have identified seven of ten members of the class II HD-ZIP family as targets of the adaxializing REVOLUTA HD-ZIP III transcription factor. In addition, a subset of these is regulated by KANADI – some in the same direction and some in the opposite direction. Previous work on HD-ZIPII proteins has implicated them in shade avoidance and as targets for red light regulation. Our studies extend their roles to include regulation of meristem size, leaf blade polarity and outgrowth, and control of growth in basal stem tissues. Preliminary data suggest the possibility that class II and class III HD-ZIP proteins might interact physically. This would be the first case of an inter-class interaction. An inspection of the class II HDZIP phylogeny indicates that control of class II HDZIPS by class III HD-ZIPs is an ancient regulatory link. Our current experiments are directed at understanding the interaction between the class II and III HD-ZIP genes as well as their interaction with other factors that control shoot development.

### **398 NIMA-related Kinases Redundantly Regulate Directional Cell Expansion in *Arabidopsis thaliana***

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NIMA-related kinases (NEKs) are a family of Ser/Thr protein kinases in eukaryotes. In fungi and animal cells, NEKs regulate various mitotic events including G2/M transition, centrosome separation, and spindle formation. To elucidate plant NEK functions, we analyze seven NEK members encoded in the genome of *Arabidopsis thaliana*. The promoter GUS analysis shows that NEKs are expressed in specific tissues including apical meristems, vascular system, and stomatal guard cells. All of the seven NEKs tagged with GFP colocalize with NEK6 and microtubules. NEK6 interacts with NEK4 and NEK5, phosphorylates tubulin and armadillo-repeat containing kinesin 1 (ARK1), and regulates epidermal cell expansion through suppression of excessive microtubule stabilization. Triple mutant analysis indicates that *NEK1*, *NEK2*, and *NEK3* regulate directional cell expansion in root epidermal cells. To identify signaling component downstream of NEKs, we isolate several proteins interacting with NEK6 by using immunoprecipitation and yeast two-hybrid analysis. These results suggest that plant NEKs interact with each other and redundantly regulate directional cell expansion. The functional redundancy and diversification of plant NEKs will be discussed.

### **399 UNHINGED Controls Leaf Vein Pattern in *Arabidopsis***

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Polar distribution of auxin controls many aspects of plant development including leaf vein pattern formation. Asymmetric localization of PIN proteins, the auxin efflux carriers, mediate the nature and directionality of auxin transport. The dynamic nature of PIN localization is regulated by vesicle cycling to and from the plasma membrane through the plant endomembrane system. The dynamic changes in PIN localization have been shown to be especially important in the formation of leaf vein pattern. We have identified an *Arabidopsis* recessive mutant, *unhinged (unh)*, having a simpler vein network with distal non-meeting of the secondary and tertiary veins when compared to wild type. Expression of the auxin response marker *DR5::GUS* is reduced throughout early leaf development as well as in the roots suggesting global defects to auxin response in *unh*. *UNH* encodes a putative member of the *Arabidopsis* GARP/VFT complex. The GARP complex is an important component of the plant endomembrane system. It is involved in the process of retrograde transport from the plasma membrane to the Trans Golgi Network (TGN). The GARP complex tethers vesicles derived from the endosome and Pre-Vacuolar complexes to the TGN through interaction with cognate SNAREs (t-SNAREs and v-SNAREs). We will provide genetic evidence for the identity of UNH as a component of the GARP complex as well as evidence that UNH and hence the GARP complex is important for PIN1 localization within developing veins.

### **400 PLA- I γ1 and PLA- I γ2 Proteins Are Required for Shoot Apical Meristem Development and Leaf Polarity in *Arabidopsis***

*Jong-Yoon Park<sup>1</sup>, Mijin Oh<sup>2</sup>, Ilha Lee<sup>1</sup>*

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Phospholipid-derived products generated by phospholipase A (PLA1 and PLA2) play important roles in plants as signaling molecules that mediate various cellular processes. Based on amino acid sequence analysis, *Arabidopsis* PLA1 family have been classified into three

groups, class I, II and III. AtPLA1 class I contains seven proteins: PLA-I $\alpha$ 1 (DGL), PLA-I $\alpha$ 2, PLA-I $\beta$ 1 (DAD1), PLA-I $\beta$ 2, PLA-I $\gamma$ 1 (DAF4), PLA-I $\gamma$ 2 (DAF3) and PLA-I $\gamma$ 3. Most of these proteins have not yet been characterized for their function in *Arabidopsis*. During embryogenesis and organogenesis, the class III HD-ZIP genes have key roles in the development of shoot apical meristem (SAM) and determination of leaf polarity. Such morphogenesis might be regulated by altering auxin polar transport and *WUSCHEL* expression. The presence of a START domain in HD-ZIP III proteins suggests that their activity is regulated via interaction with a lipophilic ligand. Serendipitously, we found that the loss-of-function mutations in two PLA-I $\gamma$  genes cause similar phenotypes with HD-ZIP III mutants.

To explore genetic functions of PLA-I $\gamma$ 1(*DAF4*) and PLA-I $\gamma$ 2(*DAF3*) during plant development, we generated double mutant, RNAi-DAF3 *daf4*. This mutant designed through expressing *DAF3* coding sequence in antisense orientation under the control of the CaMV 35S promoter in *daf4*, transposon inserted knock-out mutant background. These RNAi-DAF3 *daf4* mutants showed pleiotropic phenotypes in embryogenesis and organogenesis, such as abnormal formation of SAM, cotyledons and defects in leaf polarity-some of them were lethal. In adult plants, these mutants showed various phenotypes such as dwarfism, twisted and fasciated inflorescence, abnormal flower, and reduced fertility. Such phenotypes are similar to those of HD-ZIP III mutants. Furthermore, auxin polar transport was also altered in RNAi-DAF3 *daf4* mutants. In future study, functional mechanism of DAF3 and DAF4 proteins will be investigated.

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#### **401 A Role for CSLD3 During Cell Wall Synthesis in Apical Plasma Membranes of Tip-Growing Root Hair Cells**

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In plants, cell shape is determined by the cell wall. Cellulose is a major component of cell walls, and synthesis of this polysaccharide is accomplished by CESA of glycan synthases. *Arabidopsis* contains ten CESA genes that form a subfamily within the larger group of processive glycan synthases, the cellulose synthase-like gene family (CSL). While functions for most CSL subfamilies remain unknown, CSLD subfamily members are highly similar to CESAs, showing conserved domain organization and higher overall sequence conservation with CESA proteins than with any other CSL subfamily. Mutation of *CSLD3* disrupts root hair growth, resulting in cell rupture upon initiation of root hair tip-growth. While controlled deposition of cellulose is known to affect asymmetric expansion during diffuse growth, it was unclear if cellulose synthesis occurred during tip-restricted expansion in root hairs. We examined if de novo cellulose synthesis was required during root hair tip-growth. Using a combination of cellulose synthase inhibitors, a novel root hair growth assay utilizing cell wall hydrolytic enzymes, and cellulose-specific probes, we show root hairs deposit cellulose, or cellulose-like (1 $\rightarrow$ 4)-beta-glucan polymers during tip-restricted expansion. Interestingly, while neither EYFP-CSA6 nor EGFP-CSA3 localized to apical plasma membranes, EYFP-CSLD3 selectively accumulated in this apical plasma membrane domain during root hair growth. High-resolution confocal microscopy and fluorescence recovery after photobleaching (FRAP) experiments revealed that EYFP-CSLD3 was non-uniformly distributed within the apical plasma membrane, and these localized regions of higher fluorescence were motile, suggesting that CSLD3 may organize into higher order complexes within the plasma membrane. Finally, we show that a chimeric EYFP-CSLD3 protein in which the catalytic domain is replaced with a CSA6 catalytic domain restores root hair growth in a *csld3* null mutant. These results indicate that CSLD3 synthesizes (1 $\rightarrow$ 4)-beta-glucan polysaccharides during root hair tip-growth.

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#### **402 Imprinted Expression of Polarizing Genes in the Seed Endosperm is Subject to Natural Variation**

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The endosperm is a polarized structure in the seed that provides maternal resources to the developing embryo. The chalazal (posterior) pole of the endosperm is defined, in part, by a multinucleate cyst. In seeds lacking the *FIS* Polycomb histone-methyltransferase complex (*FIS* P<sub>c</sub>G) the multinucleate cyst is enlarged and ectopic cyst structures are apparent. The formin *AtFH5* is also involved in chalazal cyst establishment and *AtFH5* mutants can suppress the enlarged cyst phenotype in *FIS* mutant backgrounds. In other eukaryotes, formins are localized and activated by their association with Rho GTPases. We asked whether plant formins might be similarly regulated and found that GFP-Atfh5 localization is perturbed in pollen lacking *ROP2*, a Rho-related GTPase which controls actin-dependent pollen tube growth. Regulation of *AtFH5* expression involves a paternal silencing established by maternal *FIS* P<sub>c</sub>G. We hypothesized that *AtFH5* regulators might also demonstrate parental bias in gene expression. Using polymorphisms between Landsberg (Ler) and Cape Verde Island (Cvi) strains of *Arabidopsis*, we found that *ROP2* is maternally expressed in Ler but biallelically expressed in Cvi. This suggests that either Ler ecotypes possess a 'passive' paternal imprint or that Cvi might actively silence paternal genes from 'foreign' pollen. We are currently testing whether Ler fathers silence *ROP2* with other mothers or if this is specific to Cvi. In either case, *ROP2* silencing is possibly determined by either a *cis*-DNA sequence element or a *trans*-factor in the Ler or Cvi background. F1 and F2 populations of Cvi x Ler crosses are being used to examine this. Finally, our results suggest that imprinting programs might reflect an adaptive trait influencing loci involved in basic cellular mechanisms of development.

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#### **403 Control of Stomatal Polarity by a Peripherally-Localized LRR Receptor-Like Kinase**

*Sandra Keerthisinghe<sup>1</sup>, Jeannette Nadeau<sup>2</sup>, Jessica Lucas<sup>3</sup>, Tsuyoshi Nakagawa<sup>4</sup>, Fred Sack<sup>1</sup>*

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Stomata consist of two guard cells around a pore, function in shoot gas exchange, and display a highly polarized, mirror-like symmetry in the distribution of cell wall thickenings and intracellular components. Mutations in *MUSTACHES* (*MUS*), which encodes an atypical LRR Receptor-Like Kinase, disrupt these wall thickenings as well as stomatal bilateral symmetry. In addition to regulating cell wall development, *MUS* is also required for coordinating the alignment and opposite placement of radial microtubule arrays in

each guard cell as well as for the net outward polarity of microtubule growth in these arrays. To determine where MUS is localized, a translational GFP fusion was constructed which complements the *mus* mutant phenotype. GFP signal was absent from the majority of cells except, in those that were dividing where MUS was centrally located. However, division appeared normal in *mus* loss-of-function mutants. Unlike other types of dividing cells throughout the plant, the guard mother cell (which divides symmetrically to produce the two guard cells), displayed a peripheral MUS localization pattern during division as well as early morphogenesis. These findings indicate that MUS enforces guard cell wall and cytoskeletal polarity at the center of the developing stoma (near pore cell walls) via signaling from the vicinity of the outer guard cell membrane.

#### **404 LAZY1 and ARG1 define two genetic pathways of gravitropism in inflorescence**

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Generally, shoots of higher plants bend to vertically upward by negative gravitropism, which is a fundamental adaptation response. Recent progress in the study of shoot gravitropism using *Arabidopsis* has revealed that it proceeds through three major conserved steps: gravity perception in statocytes, signal transduction and asymmetric growth (Morita, 2010). Although a number of mutants that have defects in shoot gravitropism have been reported, genetic factors and its pathways that are involved in signal transduction are still largely unknown. In rice, *LAZY1* gene is suggested to function in the signaling process (Li et al., 2007; Yoshihara and Iino, 2007). But the molecular function of *LAZY1* is still unknown. In previous Conference in 2010, we reported that a novel *Arabidopsis* mutant of *LAZY1* ortholog (*AtLAZY1*) showed diagravitropism (bending to horizontal direction) in shoots and that the defects were partially recovered under dark condition, suggesting that shoot gravitropism was composed of multiple pathways, such as those dependent on or independent from light. We also showed that *AtLAZY1* protein was localized in plasma membrane. Here, we will show that *AtLAZY1* is specifically expressed in the endodermal cell layer (statocytes of *Arabidopsis* shoot) in inflorescence and hypocotyl, consistent with the shoot-specific defects of the mutant and its suggested gene functions in intracellular signaling process in statocytes. Next, we have investigated genetic interactions between *AtLAZY1* and other gravitropism-related genes by making multiple mutants. We have found that a double mutant of *ARG1* (*ALTERED RESPONSE TO GRAVITY 1*), which encodes DnaJ protein (Sedbrook et al., 1999), and *AtLAZY1* shows almost complete loss of gravitropic response in inflorescence under both light and dark conditions, whereas a single mutant of *ARG1* appears to be normal. This result suggests that the gravity signal of statolith could be divided in two major pathways: *AtLAZY1*-dependent and *ARG1*-dependent one.

#### **405 How to Grow Straight? *tortifolia 2*, an $\alpha$ -Tubulin Point Mutation Links Helical Expansion of Single Cells and Torsional Organ Growth**

*Henrik Buschmann<sup>1</sup>, Malay Das<sup>2</sup>, Dierk Niessing<sup>2</sup>, Clive Lloyd<sup>1</sup>, Tony Schaeffner<sup>2</sup>*

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Division and expansion of individual plant cells has to be tightly coordinated to establish the final form of an organ. A fundamental problem is the *de novo* generation of a straight structure. The analysis of *Arabidopsis* helical growth mutants has revealed important factors controlling the polarity of organ growth. We had identified three *TOR(TIFOLIA)* loci, whose mutations led to consistent right-handed (*tor1*, *tor2*) and left-handed (*tor3*) torsional growth of petioles and hypocotyls. All mutations affected microtubular components. *tor2* shows a mutation in the  $\alpha$ -tubulin TUA4 replacing the absolutely conserved arginine-2 (R2) with lysine. Based on a published tubulin structure TUA4-R2 forms intermolecular contacts with the GTPase domain of  $\beta$ -tubulin; structural modeling suggests that these contacts are interrupted in *tor2*. Consistent with this interpretation microtubule dynamicity was reduced in *tor2*, which coincided with net left-handed cortical microtubules that would direct rightward-oriented cellular expansion. We also employed the *tor2* mutant to investigate the developmental origin of the helical growth phenotype. Helical growth in *tor2* arose independently of cell division and was based on cell elongation defects only. Interestingly, right-handed twisting also occurred in isolated, single *tor2* suspension cells or trichomes. Thus, torsional organ growth can be a higher order expression of the helical expansion of individual cells<sup>1</sup>. The genetic interaction of *tor2* with the left-handed *tor3* turned out to be complex with a dominant, dose-dependent influence of the *tor3* allele.

<sup>1</sup>Buschmann et al. (2009) Plant Cell 21, 2090

#### **406 JAGGED LATERAL ORGANS controls auxin signalling and organ development**

*Madlen Rast, Rüdiger Simon*

**Heinrich Heine University, Düsseldorf, Germany**

The *Arabidopsis thaliana* *JAGGED LATERAL ORGAN* (*JLO*) gene, a member of the *LATERAL ORGAN BOUNDARY DOMAIN* (*LBD*) gene family, is required for coordinated cell division during embryogenesis. *JLO* controls expression of several *PINFORMED* (*PIN*) genes during embryonic and root development, and regulates all known auxin responses in the root. We have identified the partner proteins that *JLO* requires for its activity, and have studied their molecular interactions and complex formation. This allows us to outline how transcription factors controlling meristem cell fate interact with the auxin localization and signaling machinery.

**407 STIMPY Modulates Cytokinin Signaling in Meristem Development in *Arabidopsis thaliana***Anna Skylar, Xuelin Wu**University of Southern California, Los Angeles, CA, USA**

The establishment of the primary meristems through proliferation after germination is essential for plant's post-embryonic development. Cytokinins have long been considered a key regulator of plant cell division. Here we show that cytokinins are essential for early seedling development of *Arabidopsis*. Loss of cytokinin perception leads to a complete failure of meristem establishment and growth arrest after germination. We also present evidence that cytokinin signaling is involved in activation of the homeobox gene STIMPY (STIP or WOX9) expression in meristematic tissues, which is essential for maintaining the meristematic fate. Cytokinin-independent STIP expression is able to partially compensate for the shoot apical meristem growth defects in mutants that cannot sense cytokinin. These findings identify a new branch of the cytokinin signaling network, linking cytokinin to the process of meristem and seedling establishment.

**408 Linking the genetics and biochemistry of ROP signaling cascades to the mechanics of leaf epidermal morphogenesis**Chunhua Zhang, John Mason, John Roesel, Eileen Mallory, David Umulis, Dan Szymanski**Purdue University, W. Lafayette, (IN), USA**

The leaf epidermis is a biomechanical shell that modulates the overall architecture of the organ. The biological control of its morphogenesis spans from nanoscale protein machines (Szymanski and Cosgrove, 2009) to the macroscopic growth properties of a polarized tissue (Coen et al., 2004). Understanding the dynamics of protein and organelle functions across these spatial scales is a major challenge in biology. In the leaf epidermis, one set of challenges stem from the complexity of the morphogenesis process itself. For example, pavement cell development is asynchronous, terminal cell sizes and shapes are extremely variable, and the process occurs in the context of an expanding planar tissue. One aspect of our work uses long-term time-lapse analyses of cell shape and the microtubule cytoskeleton to better understand the geometric details of the this shape change as a function of organ development (Zhang et al., 2011). New live cell imaging assays, mutants, and computational models are being developed to learn more about how the diverse types of cytosolic and extracellular machineries coordinate growth. The results are providing new insights into how signaling proteins, the cytoskeleton and auxin interact during distinct waves of symmetry breaking and isotropic expansion. We will present our most recent work that attempts to define plausible mechanisms by which growth anisotropy occurs in the context of an intact tissue.

Coen, E., Rolland-Lagan, A.-G., Matthews, M., Bangham, J.A., and Prusinkiewicz, P. (2004). The genetics of geometry. *PNAS USA* **101**, 4728-4735.

Szymanski, D.B., and Cosgrove, D.J. (2009). Dynamic coordination of cytoskeletal and cell wall systems during plant cell morphogenesis. *Curr. Biol.* **19**, R800-R811.

Zhang, C., Halsey, L., and Szymanski, D.B. (2011). The development and geometry of shape change in *Arabidopsis thaliana* cotyledon pavement cells. *BMC Plant Biol.*, 10.1186/1471-2229-1111-1127.

**409 Plastid Signal Is Involved In The Dynamic Regulation of FIL Expression Pattern**Toshiaki Tameshige<sup>1</sup>, Maki Kondo<sup>1</sup>, Keiro Watanabe<sup>2</sup>, Koichi Toyokura<sup>1</sup>, Ryuji Tsugeki<sup>2</sup>, Kiyoshi Tatehashu<sup>1</sup>, Mikio Nishimura<sup>1</sup>, Kiyotaka Okada<sup>1</sup>**<sup>1</sup>NIBB, Okazaki, Japan, <sup>2</sup>Kyoto Univ., Kyoto, Japan**

Many land plants have leaves with expanded lamina and asymmetric structure along adaxial-abaxial axis. Such features are the structural basis for efficient photosynthesis. While several transcription factors regulating leaf lamina growth and asymmetric cell differentiation have been identified, the regulatory mechanisms of the spatio-temporal pattern of their expression have yet to be fully understood.

An *Arabidopsis* gene *FILAMENTOUS FLOWER(FIL)* encodes one of such transcription factors. Though the abaxial-specific expression of *FIL* in developing leaf was well known, our detailed observation revealed that its expression is gradually restricted from whole leaf expression to the abaxial specific pattern. And finally, the expression is restricted to the abaxial epidermis.

A novel *Arabidopsis* mutant, *enlarged fil expression domain2 (enf2)*, which we isolated, shows larger *FIL* expression domain than that of wild type. However, the expression is finally restricted to abaxial epidermis indicating that the dynamic change of *FIL* expression is delayed in this mutant.

Interestingly, the responsible gene, *ENF2*, encodes a chloroplast-targeted protein. Chloroplast development of *enf2* was found to be impaired in the developing leaf by electron microscopy observations. It is known that some nuclear genes' expression are tightly linked to plastid condition via GUN1 protein dependent signaling. Mutation in *GUN1* resulted in relatively normal pattern of *FIL* expression even in *enf2* mutant background. These results indicate that plastid signal affect the dynamic regulation of *FIL* expression.

**410 AKIN10 and FUSCA3 Interact to Control Lateral Organ Development and Phase Transitions in *Arabidopsis***Allen Tsai, Sonia Gazzarrini**University of Toronto, Toronto, Canada**

The Snf1/AMPK/SnRK1 kinases act as sensors of energy status in eukaryotes. Although alterations of the SnRK1 kinase expression lead to transcriptional changes at the global level in *Arabidopsis*, only few SnRK1 substrates have been identified to date. Using yeast-two hybrid screens, we have identified the *Arabidopsis* SnRK1 kinase homologue 10 (AKIN10) as an interactor of the B3-domain

transcription factor FUSCA3 (FUS3), an essential regulator of late embryogenesis in Arabidopsis. We show that AKIN10 interacts with FUS3 *in vitro* and *in planta* by pull-down and BiFC assays, respectively. AKIN10 is shown to phosphorylate FUS3 by in-gel kinase assay; while the N-terminal domain of FUS3 is required for AKIN10 phosphorylation, the C-terminal domain of FUS3 negatively regulates phosphorylation by other kinases. AKIN10 positively regulates FUS3 stability, as over-expression of AKIN10 delays FUS3 degradation in a cell-free system. Loss- and gain-of-function mutant analysis indicates that both *FUS3* and *AKIN10* positively regulate seed dormancy, while antagonizing vegetative and reproductive phase transitions. Furthermore, both *FUS3* and *AKIN10* mutants display altered number, symmetry and phyllotaxy of cotyledons, siliques and floral organs, suggesting *FUS3* and *AKIN10* regulate lateral organ development. Genetic interaction studies show that the loss-of-function *fus3-3* mutation partially rescues the phase transition and organ development defects caused by over-expressing *AKIN10*, but it also enhances the manifestations of various novel phenotypes. These findings indicate that *FUS3* and *AKIN10* act in both overlapping and parallel pathways to regulate developmental phase transitions and lateral organ development. We propose a model for separate and overlapping functions of AKIN10 and FUS3 during embryogenesis in Arabidopsis.

#### **411 The MADS-domain Factors AGL15, AGL18, AGL24 and SVP Act Redundantly to Prevent Premature FT Expression and Leaf Curling**

Chieh-Ting Wang, Donna Fernandez

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Flowering time is tightly regulated by floral activators and repressors, including several members of the MADS domain family of transcription factors. We found that the *agl15 agl18* double mutant combination accelerates the floral transition and enhances leaf curling in *agl24 svp* double mutants. Teper-Bamnolker and Samach (Plant Cell 17: 2661-2675, 2005) previously showed that leaf curling results from FT-dependent activation of *SEP3* in leaf tissues. We find that *FT* expression is increased at least 2-fold relative to wild type in 10 day seedlings and rosette leaves of *agl15 agl18 agl24 svp* quadruple mutants, while *SEP3* expression is boosted as much as 20-fold. In addition, in lines carrying *FT* promoter-GUS reporters, increased GUS activity was observed in the first rosette leaves of the quadruple mutant, confirming the direct analysis of *FT* expression. Introduction of *ft* or *sep3* mutant alleles into the quadruple mutant background resulted in complete suppression of leaf curling. Introduction of *soc1* alleles into quadruple mutants resulted in a delayed floral transition and a larger number of curled rosette leaves, as well as production of bracts and terminal flowers. Our data indicate that AGL15, AGL18, AGL24, and SVP all contribute to negative regulation of *FT* and are necessary to maintain normal leaf morphology during the vegetative phase. Supported by NSF #IOS-0718598.

#### **412 Polyadenylation Factor PCFS4 and Arabidopsis Development**

Denghui Xing, Qingshun Li

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Arabidopsis polyadenylation factor, PCFS4, an ortholog of human and yeast PCF11, functions in Arabidopsis flowering time control, leaf development and phyllotaxy determination. The role of PCFS4 in flowering time control is partially mediated by *FLC* and correlated with the regulation of alternative polyadenylation (APA) of *FCA*. PCFS4 forms an *in vivo* complex with FY, another polyadenylation factor and a regulator of the APA of *FCA*, suggesting a polyadenylation machinery acting on the APA of *FCA*. Since the role of PCFS4 in leaf development and phyllotaxy determination could not be explained by the APA of *FCA*, we speculated that there could be additional genes, other than *FCA*, being targeted by PCFS4. To identify these targets, we employed a tiling microarray assay and developed a program, RADPRE (Ratio-based Analysis of Differentially Processed and Expressed genes), for tiling data analysis. Using RADPRE, we compared the transcriptomes of *pcfs4-1* mutant and wild type Col, and identified 68 Differentially Processed Genes (DPG) and 114 Differentially Expressed Genes with estimated false discovery rate of 1% and 2%, respectively. GO analysis of those targets revealed a highly enriched GO term "regulation of flower development", verifying the efficacy of the RADPRE pipeline. To further explore whether PCFS4 specifically regulates the target mRNA processing by directly targeting on the loci of those genes, we performed a ChIP assay for one of the DPG targets, ATIM, using PCFS4-TAP recombinant protein. We found PCFS4-TAP was indeed enriched on the 3' end of ATIM locus. To address the same question for other DPG targets, we are carrying on a ChIP-Seq assay using the PCFS4-TAP recombinant protein.

#### **413 MACCHI-BOU 2 involved in bract suppression in *Arabidopsis thaliana***

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Higher plants produce leaf-like structures, called bracts, at the base of flowers or inflorescences, while most *Brassicaceae* species do not. Previous studies showed that cryptic bracts are found at the base of flower primordia in *Arabidopsis*, a genus of the *Brassicaceae* family, suggesting the existence of a mechanism of bract suppression. Recently, *BLADE ON PETIOLE 1 (BOP1)/BOP2* and *PUCHI* have been shown to coordinately function in *Arabidopsis* bract suppression. Both *bop1 bop2* and *puchi* mutations induce the outgrowth of bract primordia at the base of flower primordia. However, the bract primordia cease to grow, leading to immature bracts at the base of pedicels in these mutants.

Here, we report *MACCHI-BOU 2 (MAB2)* as a novel factor involved in bract suppression. *MAB2* encodes AtMED13, a component of Cyclin-dependent kinase 8 (Cdk8) complex. In yeast and *Drosophila*, Cdk8 complex is reported to act as a transcriptional factor. In *mab2* mutants, bract primordia developed coincidentally with floral meristem in the inflorescence meristem and then grew up to bracts. To elucidate the relationship between *MAB2* and other factors involved in bract suppression, we constructed *mab2 bop1 bop2* and *mab2 puchi* mutants. Bract primordia of the *mab2 bop1 bop2* mutants outgrew as *mab2* single mutants, indicating that *mab2* was genetically epistatic to *bop1 bop2*. By contrast, bract primordia of *mab2 puchi* mutants were more enlarged than those of respective single mutants.

These results from the genetic studies indicate that *MAB2* functions in the same pathway as *BOP1/BOP2* while it is different from *PUCHI* related pathway in bract suppression. In addition, we found that *BOP2* could interact with HUA ENHANCER 3, which is a subunit of Cdk8 complex, in yeast and *Arabidopsis* protoplasts. Based on these results, we will present the molecular mechanism of bract suppression in *Arabidopsis*.

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#### 414 Control of Multiple Organ Development by the miR160-regulated Auxin Signaling

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MicroRNAs (miRNAs) have emerged as key regulators of gene expression at the post-transcriptional level in both plants and animals. However, the specific functions of *MIRNA (MIR)* genes and the mechanisms regulating their expression are not fully understood. So far, the functions of a few miRNAs have been analyzed by loss-of-function mutations in *MIR* genes. Here, we report our characterization of *floral organs in carpels (foc)*, an *Arabidopsis* mutant with a *Ds* transposon insertion in the 3' regulatory region of the *MIR160a* gene. *foc* plants exhibited a variety of intriguing phenotypes, including serrated rosette leaves, irregular flowers, floral organs inside siliques, reduced fertility, aberrant seeds, and viviparous seedlings. Further analysis showed that abnormal cell divisions in both apical and basal regions of the embryo led to various defects during embryogenesis in *foc* plants. Detailed expression analysis demonstrated that the 3' region was required for the expression of *MIR160a*. Previous studies showed that miR160 negatively regulates three genes that encode AUXIN RESPONSE FACTORS (ARF10, 16 and 17). Our Northern blot results showed that the accumulation of mature miR160 was greatly reduced in *foc* plants, while RT-PCR and quantitative RT-PCR results demonstrated that expression of *ARF10*, 16 and 17 were increased. Furthermore, *in situ* hybridization showed that the expression pattern of *ARF16* and *17* was altered during embryo development in *foc* plants. *foc* plants were also deficient in auxin responses. Moreover, auxin was involved in regulating the expression of *MIR160a* through its 3' regulatory region. Our study not only provides insight into the molecular mechanism of embryo development via *MIR160a*-regulated ARFs, but also reveals the mechanism regulating *MIR160a* expression.

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#### 415 Function and Phylogeny of Cytokinin Response Factors

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Leaf development is highly regulated from initiation, through differentiation, to senescence. Cytokinin (CK) is involved in this regulation; however, the mechanism by which it acts is unclear. Our work focuses on linking CK to the molecular mechanisms of leaf development through analysis of CRFs. Cytokinin Response Factors (CRFs) are AP2/ERF transcription factors defined by a conserved "CRF domain" ubiquitous in land plants. Phylogenetic analyses reveal five distinct CRF clades (I-V) in angiosperms, defined by C-terminal regions. Whereas all CRF proteins interact with each other and some components of the CK signaling pathway via the CRF domain, specification of clades is evident as expression of only two clades (I and III: CRF1,2 and 5,6) shows induction by CK. To identify possible processes regulated by CRFs, we generated CRF-promoter:GUS lines showing CRFs 1-8 are expressed predominately in leaf vasculature, verified with qPCR. Using these same tools, we are examining CRF expression during developmental and diurnal time points as well as changes in response to conditions altering leaf development. Leaf GUI analysis shows altered vascular patterning in *crf* mutant leaves as compared to WT, suggesting CRFs are required for normal patterning. Microscopic examination of *crf 1* and *2* (clade I) leaves shows retention of excessive starch, confirmed using Lugol's staining. Also, *crf6* mutants are insensitive to the senescence delaying effect of CK on WT plants and CRF6 (clade III) is highly expressed in senescing leaves. Together with phloem localized expression this implicates a role for CRFs in regulation of carbon partitioning, with each clade regulating distinct yet, related processes. These processes, known to be CK regulated, are developmentally integral as a leaf transitions from sink to source, and ultimately relinquishes its resources to other organs through senescence.

**416 A set of mutants defective in Caspary Strip formation**Julien Alassimone, Niko Geldner**DBMV university of Lausanne, Lausanne, Switzerland**

Every single cell in higher plants is made from mineral compounds drawn from the soil by roots and distributed to the aboveground organs via the vasculature tissues. To set up an efficient selective sorting of nutrients, a diffusion barrier between the soil and the vascular tissues is established by the endodermis. It does so by means of a differentiation feature "the Caspary Strip" (C.S), a highly localized hydrophobic cell wall impregnation, that seals the extracellular space. Recently, we have described the developmental sequence of events leading to a differentiated endodermis (1).

So far, mutants defective in C.S integrity have not yet been identified. We initiated a forward genetic screen to find actors of C.S establishment using a stably-expressed, secreted GUS line that allowed us to evaluate the presence and the integrity of the C.S. This approach led to the identification of 11 endodermis permissive mutants, which we named *schengen* (*sgn*) mutants. Genetic analysis indicates that those mutations are recessives and fall into 5 complementation groups. We could demonstrate that *sgn3* and *sgn4* were actually allelic to two genes that we have independently identified, by reverse genetics, encoding for an LRR-RLK and an NADPH oxidase, respectively. *sgn5* is allelic to *scr*, an intensively investigated gene involved in endodermal specification. This new *scr* allele induces extra divisions of cortical cells, strongly affecting the cell identity, but also leading to incorrect differentiation. *sgn1* and *sgn2* are currently being identified.

This new set of Caspary Strip defective mutants will allow the dissection of mechanisms involved in C.S establishment. In addition our mutants might be very useful to investigate nutrient uptake processes in roots of higher plants and should be relevant to understand plant behaviour under environmental stresses and plant survival in general.

1. Alassimone,J., et al., Proc Natl Acad Sci U S A, 2010. 107 (11)

**417 Functional analysis of regulators of STM in Arabidopsis**Jose Antonio Aguilar Martinez, Neelima Sinha**Department of Plant Biology, University of California, Davis, CA, USA**

The activity of the shoot apical meristem (SAM) greatly determines plant architecture. *SHOOT MERISTEMLESS (STM)*, a KNOX1 gene from *Arabidopsis thaliana*, has a key role in the regulatory network that controls the activity of the SAM. STM maintains part of the cells of the SAM as undifferentiated and the local repression of STM is required to allow cell differentiation and formation of lateral organs. We are analyzing the regulation of STM. We previously showed well conserved regions between species in the 5' regulatory region of STM, named the K-box and the RB-box. The K-box is necessary for the permanent STM downregulation in leaf development. To determine factors that control the regulation of STM we performed yeast one hybrid assays. In the screening we identified TCP7 (At5g23280) as a factor binding to the K-box region. EMSA experiments also show that this factor binds the K-box region. We perform a functional analysis of this and the related genes TCP15, TCP23, TCP22, TCP8. The T-DNA insertions available for TCP7 and RNAi lines show no obvious phenotype. As single mutants for the other genes show no obvious phenotype, we are developing double and triple mutants. Transgenic lines ectopically expressing TCP7 show plants with altered leaf morphology and a reduction in STM expression levels. Also, transgenic lines overexpressing TCP15 show a phenotype similar to STM mutants and reduced expression levels of STM. Analysis of transgenic reporter lines indicate that these genes are specifically expressed in young leaves. The role of these genes in repressing STM expression and in general leaf development will be discussed.

**418 A Forward Genetic Approach to Identify Paternal Effects on Early Embryogenesis in *Arabidopsis thaliana***Yashodar Babu, Agnes Henschen, Martin Bayer**Max-Planck-Institute for Developmental Biology, Tuebingen, Germany**

Sperm cells of mature pollen possess a distinct transcriptome and it is therefore conceivable that some of these transcripts might be delivered to the zygote during fertilization.

The impact of such paternal factors on fertilization and early seed development, however, is poorly understood. Recently, we described the Pelle/IRAK-like cytoplasmic kinase SHORT SUSPENSOR that links the onset of embryogenesis with the fertilization event by a novel parent-of-origin effect.

To identify novel mutants with similar effect on early seed development, we conducted a forward genetic screen for paternal effect mutants. Individual M2 plants of an EMS mutagenized plant population were used as pollen donor, while a conditionally male-sterile mutant was used as female crossing partner to circumvent manual emasculation. Seed set was recorded ~72h after pollination and immature seed were dissected and cleared for visual inspection of embryo and endosperm development by DIC microscopy.

In total, over 3000 M2 plants were screened this way and so far 34 candidates showed reproducible defects in seed and/or embryo development after crossing. The majority of mutants seems to affect the fertilization process itself and comprises presumably gametophytic mutants. Four of the candidates, however, show defects in embryo or endosperm after fertilization.

Here, we report first results for some of these candidates.

**419 FUN Is Where It's At: RNA Profiling Of The Arabidopsis Funiculus**

*Mark Belmonte<sup>1</sup>, Sara Kost<sup>1</sup>, Ryan Kirkbride<sup>2</sup>, Julie Pelletier<sup>2</sup>, Robert Goldberg<sup>3</sup>, Edward Yeung<sup>4</sup>, John Harada<sup>2</sup>*

<sup>1</sup>**University of Manitoba, Winnipeg Manitoba, Canada, <sup>2</sup>UC Davis, Davis California, USA, <sup>3</sup>UCLA, Los Angeles California, USA, <sup>4</sup>University of Calgary, Calgary Alberta, Canada**

The ovule is structurally simple but harbors many different domains that are highly differentiated, each representing a unique developmental pathway leading up to the development of the seed. The ovule can be divided into three parts during early stages of development. First, the nucellus will go on to form the female gametophyte, second, the chalaza will form the integuments and finally, the funiculus (FUN) will anchor the developing ovule and later the seed to the parent plant. While our understanding of the nucellus and the chalaza is becoming clearer, there is remarkably little information available for the funiculus, especially during seed development. Moreover, we know nothing about how this structure is specified at the genetic or cellular level. Therefore we have taken an initiative to identify all of the genes expressed in the funiculus and compared them to our seed compartment dataset (seedgenenetwork.net) using laser capture microdissection and DNA microarray technology. This work provides the first and only comprehensive profile of gene activity of the *Arabidopsis* funiculus over time. Data reveal novel biological roles for hormone metabolism, transport and metabolic activities of processes that have never been described in this maternal seed compartment. We have complemented the genetic data with a complete histological analysis of the funiculus from the earliest stages of development through to seed maturation at the light and electron microscopy levels. Finally, we studied how the funiculus is transcriptionally regulated when compared to other seed compartments over time. Using newly designed *in silico* algorithms, we identified a number of transcriptional networks hypothesized to be responsible for biological processes found specifically within the funiculus compared to other seed compartments. Taken together, patterns of gene activity and histological observations reveal putative functions of understudied seed compartments like the funiculus and identify novel mechanisms for the transcriptional regulation of biological processes.

**420 DORNRÖSCHEN-LIKE (DRNL) Activity Marks *Arabidopsis* Floral Organ Founder Cells, Precedes Auxin Response Maxima and Initiates Organogenic Competence.**

*John Chandler; Ingo Seeliger; Bianca Jacobs, Melanie Cole, Petra Comelli, Wolfgang Werr*

**University, Cologne**

Floral organogenesis depends on the migration of pluripotent stem cells from the central meristem zone into the peripheral zone, where they gain competence to differentiate. However, the mechanisms by which cells perceive positional information and how they become specified as organ founder cells are largely unknown, partly due to a lack of early molecular markers, and since specification conceptually must be inferred from subsequent developmental events. Auxin maxima correlate with sites of lateral organ initiation but their causal role in founder cell specification remains unclear.

*DRNL* expression via 3D live imaging marks groups of floral meristem cells that correspond exactly to estimated founder cell numbers by clonal analysis and as such, *DRNL* is the earliest floral organogenesis marker. *DRNL* transcription precedes auxin response maxima as measured by the *DR5* reporter by at least one floral stage and identifies a defined temporal series of organ initiation in the four floral whorls, including pairs of sepal anlage, two morphogenetic fields that pre-pattern petals and lateral stamens and a ring-shaped field giving rise to the medial stamens before carpel primordium specification.

Functionally, *drnl* mutants are affected in floral organ outgrowth and boundary specification, that is highly sensitized by loss of *CUC* gene activity. Ectopic expression of *DRNL* or its parologue *DRN* in the L1 layer causes supernumerary cell divisions, alters cell identity and results in hyperplasia and patterning defects such as stomatal clustering. *DRNL* apparently provides novel molecular access to the interplay of founder cell specification and organogenesis in the peripheral zone, whereas *DRN* delivers a similar function in the L1 layer of the central stem cell zone.

**421 Genome-wide Direct Target Analysis Reveals A Role For SHORT-ROOT In Root Vascular Patterning Through Cytokinin Signaling**

*Hongchang Cui, Yueling Hao*

**Florida State University**

*SHORTROOT* (SHR) is a key regulator of root growth and development. Made in the stele, SHR moves into an adjacent cell layer, where it specifies endodermal cell fate. SHR also plays a pivotal role in apical meristem maintenance, ground tissue patterning, vascular differentiation, and lateral root formation. Much has been learned about the mechanism by which SHR controls radial patterning, but how it regulates other aspects of root morphogenesis is still unclear. To dissect the SHR developmental pathway, we have determined the genome-wide locations of SHR direct targets using a ChIP-chip method. By K-mean clustering analysis we not only identified additional SHR targets that are probably involved in stem cell renewal, but also uncovered a direct role for SHR in gene regulation in the pericycle and xylem. Using cell-type-specific markers, we showed that the pattern of pericycle and vascular tissue was indeed altered in *shr*. We further showed that *shr* had an elevated level of response to cytokinin and that exogenous cytokinin caused a *shr*-like vascular patterning phenotype in wild-type root. Interestingly, cytokinin also repressed the expression of *miR165A* and *miR166B*, which were recently shown to play a critical role in protoxylem differentiation. Based on these results, we suggest that one mechanism by which SHR controls vascular patterning is through regulation of cytokinin signaling. Our study also revealed distinct roles for SHR in the maintenance of root apical meristem and vascular patterning, because the short-root defect of *shr* was not alleviated when cytokinin level was reduced by genetic manipulation.

**422 Peptide Hormones During Root Development And Branching In Arabidopsis**

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Asymmetric cell division is essential in many organisms to generate cell diversity and tissue patterns and to maintain pools of stem cells. But especially in plants, the tight coordination of asymmetric division of cells fixed within cell walls is of particular importance as their post-embryonic growth is based on *de novo* formation of cell types, tissues and even entirely new organs (De Smet and Beeckman, 2011, *Nat Rev Mol Cell Biol* 12:177-188). In the *Arabidopsis* root, exchange of molecules (such as transcription factors) via plasmodesmata and active directional transport of phytohormones (such as auxin) have been studied extensively, but only lately the involvement of membrane-associated receptor-like kinases in registering and conveying (positional) information has become more apparent in this aspect of plant development (De Smet *et al.*, 2009, *Nat Cell Biol* 11:1166-1173; De Smet *et al.*, 2008, *Science* 322:594-597). Here, we will present data on the expression pattern and the role of various peptide hormones involved in cell-to-cell communication during asymmetric cell division, maintenance of stem cell pools, and *de novo* lateral root organogenesis. Our results show that members of several small signalling peptide families are involved in lateral root initiation, and that they act on pericycle cell division in both cell-autonomous and non-cell autonomous ways. In addition, (lateral) root mutant phenotypes for these putative peptide ligand genes resemble *acr4* phenotypes, with ACR4 being the first membrane-associated receptor-like kinase with a decisive function in maintenance of the root apical stem cell niche and during differentiation upon pericycle cell division towards lateral root development (De Smet *et al.*, 2008, *Science* 322:594-597). These genetic data together with partial insensitivity of *acr4* to peptide treatments suggest that one or more of these putative signalling peptides might form a peptide ligand-ACR4 pair during root development and branching. It further suggests that several peptide ligand-receptor kinase pairs involved in asymmetric cell divisions in the root await identification.

**423 amiGO RBR is a useful tool for network dissection**

*Sara Diaz-Trivino, Alfredo Cruz-Ramirez, Yujuan Du, Ikram Blilou, Hongtao Zhang, Yuchen Long, Ben Scheres*

**Molecular Genetics Department- Utrecht University**

Retinoblastoma protein (RBR) is involved in root stem cell maintenance. RBR interacts with many different proteins, such as the cell cycle regulator E2F and the stem cell identifier SCR. Existing alleles that could be used to dissect the function of RBR are either gametophytic lethal (*rbr-1-1*, *rbr1-2*, *rbr1-3*) or non complementable (*RBRi*). We have developed a new tool, the Artificial MicroRNA for Gene Overcome (*amiGO*) that phenocopies *RBRi* but is fully complementable. Domain-specific complementation of *amiGO RBR* shows cell-autonomous rescue. We are using these plants to obtain the RBR protein-protein interaction profile in a domain-specific manner. In order to dissect the role of each interaction in the stem cell maintenance, we have generated point mutations in RB that disrupt specifically some of these interactions in the *amiGO RBR* background. Some of these RBR mutants can only partially complement the *amiGO RBR* phenotype, showing specific functions for each interaction.

**424 A Putative Leucine Zipper Protein Essential for the Activation of FLC and Delay of Flowering Time by FRI**

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Many naturally occurring *Arabidopsis* accessions are late flowering winter annuals, due to the presence of *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*). *FRI* upregulates expression of *FLC*, which encodes a MADS-box transcription factor that acts as a potent floral repressor. To date, a number of suppressors of *FRI* have been identified by genetic screens, including *FRI-LIKE 1* (*FRL1*)/*FRL2*, *FRI-ESSENTIAL 1* (*FES1*), *SUPPRESSOR OF FRI 4* (*SUF4*), and *FLC EXPRESSOR X* (*FLX*). In our further effort to decode the mechanism by which *FRI* activates the *FLC* expression, a new mutant that suppressed late flowering of *FRI*, named *frigida mediator1* (*fme1*), was isolated. *fme1* affected neither the expression level of *FRI* nor that of the other *FRI*-specific *FRI* suppressors. *fme1* completely suppressed *FLC* activation and consequently abolished the late flowering phenotype of *FRI*, but only weakly suppressed the late flowering phenotype of the autonomous-pathway mutants (which are also late flowering due to elevated *FLC* expression). *FME1* encodes a putative leucine zipper containing protein. Like *FLC*, *FME1* is highly expressed in the shoot meristem of *Arabidopsis* seedlings, which is consistent with its function in suppression of the *FLC* expression. *FME1* physically interacts with *FRI*. A mutation in the putative leucine zipper region of *FME1* disrupts this interaction and abrogates the ability of *FME1* to rescue *fme1* mutants. *FME1* also interacts with another *FRI* suppressor *FLX* and together showed synergistic transcription activation activity in yeast. A model for the activation of *FLC* by *FRI* will be discussed.

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**425 An Analysis of Vascular Phenotypes in ADP-RIBOSYLATION FACTOR A1 Mutant Cotyledons**

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ADP-ribosylation factors (ARFs) are GTP binding proteins essential for intracellular membrane trafficking in Eukaryotes. ARF-GTP is associated with donor membranes where it sorts cargo and recruits coat proteins required for vesicle budding. Following hydrolysis, ARF-GDP is cytosolic and triggers coat dissociation prior to docking and fusion of vesicles with target membranes. There are 19 different

ARFs in *Arabidopsis*, six of which are Class I ARF family members, ARFA1a-ARFA1f. The ARFA1 family exhibits >80% sequence similarity and overlap in tissue expression pattern, indicating potential redundancy. Previous RNAi knockdown of ARFA1 family expression performed by Gebbie et al. (2005) revealed global defects in plant development, but no analysis of vein pattern was presented.

During initial screens for mutant abnormalities cotyledons of single gene insertion mutants in *ARFA1a-ARFA1e* were compared to wild type to reveal a subtle mutant phenotype characterized by increased vein pattern variability, increased number of gaps, increased distal non-meeting, and in some cases increased vein number. Values were not statistically significant, which supports the idea of redundancy between ARFA1 proteins. We are further testing this idea through the generation of double mutants and analysis of their vascular phenotypes.

## 426 A Chemical Genomics Approach to Identify Enhancers and Repressors of Somatic Embryogenesis in *Arabidopsis*

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Embryogenesis in plants is normally restricted to zygotic embryo development however embryogenesis can also be induced *in vitro* from individual cells or explants in the absence of fertilization. Somatic embryogenesis (SE), in which embryos are derived from vegetative cells, is most commonly induced by exposing explants to stress conditions and/or exogenous growth regulators. In *Arabidopsis*, 2,4-dichlorophenoxyacetic acid (2,4-D) treated immature zygotic embryos are highly embryogenic, while only a low percentage of mature or germinating embryos produce SEs under the same culture conditions.

We used a chemical genomics approach in *Arabidopsis* to identify compounds that enhance cell competence for *in vitro* embryogenesis. For this purpose we made use of the 3600 compound LATCA library of small molecules in a high-throughput screen to identify compounds that induce somatic embryogenesis in *Arabidopsis* seedlings cultured in the presence of 2,4-D. From the initial screen 27 compounds were identified as putative inducers of somatic embryogenesis of which 2 were chosen for further analysis based on their ability to strongly induce SE.

At present we are performing microarray analysis of compound induced versus control seedlings to identify the genes upregulated by the compounds. This work is combined with testing different marker lines (auxin, embryo). Using this strategy we aim to identify the pathway(s) activated or repressed by the compounds resulting in the formation of somatic embryos.

## 427 Long-distance Regulation of Cambium Activity

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Long distance cell-to-cell communication is critical for the development of multicellular organisms. In this respect, plants are especially demanding as they constantly integrate environmental input to adjust growth processes to different conditions. One example is lateral growth of shoots and roots mediated by the cambium, a stem cell-like tissue whose activity depends on long-distance regulation by the plant hormone auxin. How auxin signaling is integrated on the level of cambium cells and how cambium activity is coordinated with other growth processes is unknown. Here, we provide physiological, genetic, and pharmacological evidence that strigolactones (SLs), a group of plant hormones recently described to be involved in the repression of shoot branching, positively regulate cambial activity, and that this function is conserved among species. We show in *Arabidopsis* that SL signaling in the cambium itself is sufficient for cambium stimulation and that it acts downstream of the auxin signaling pathway and upstream of the *WOX4* transcription factor, an essential regulator of cambium activity. Our results provide a model of how auxin-based long distance signaling is translated into cambium activity, and suggest that SLs act as general modulators of plant growth forms linking the control of branching and lateral growth of growth axes.

## 428 TFL1 Controls Flowering Transition and Vesicle Transport in *Arabidopsis*

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Homologous proteins TFL1 and FT are flowering regulators, however, their actions are antagonistic. FT is known as a florigen and induces flowering, whereas TFL1 represses flowering through controlling the activity of the shoot apical meristem. To uncover the molecular nature of the antagonistic actions of TFL1 and FT, we conducted amino acids swapping experiments between FT and TFL1. This attempt, together with domain swapping conducted by another group, identified a residue that is responsible for the TFL1 repressor action and a C-terminus domain that is responsible for the FT inducer action, suggesting that these regions are key to the TFL1 and FT antagonism. To clarify the molecular actions of the TFL1 and FT specific regions, we performed a yeast two hybrid screen and identified 9 different classes of interactors that bind to TFL1, and named TFL1 IN LOVE (TIL). We demonstrated that TIL3 showed the specific binding to TFL1 depending upon the TFL1 key residue.

*TIL3* encodes an inositol polyphosphate 5-phosphatase (5PTase) and is a member of a small gene family that consists of 15 5PTases. Some of these 5PTases, including TIL3, are known to be involved in the regulation of intercellular vesicle transport during organ development. The *til3/5ptase13* mutant shows enhanced root gravitropism due to its elevated vesicle transport. We found that *tfl1-1* and *35S::TFL1* also showed an effect in root gravitropism, suggesting that TFL1 and TIL3/5PTase13 may act in the same pathway. Our flowering time measurements showed that the *5ptase12 til3/5ptase13* double mutant was slightly early flowering and that a *5ptase12* mutation completely suppressed the late flowering phenotype of *35S::TFL1*, indicating that 5PTases may share a redundant function in the TFL1 action in flowering control. The function of TFL1 and 5PTases in flowering control will be further discussed.

**429 Finding Meristemoid-Specific Genes: Fuel for Stomatal Development***Robin Horst<sup>1</sup>, Kylee Peterson<sup>1</sup>, Lynn Pillitteri<sup>2</sup>, Keiko Torii<sup>1</sup>*<sup>1</sup>**University of Washington, Seattle, <sup>2</sup>Western Washington University, Bellingham**

The leaf epidermis is composed of different cell types. While pavement cells minimize uncontrolled evaporation of water, stomata -microscopic valves in the epidermis formed by a pair of guard cells- mediate the controlled exchange of water vapor and CO<sub>2</sub>. Transition through the intermediate cell stages of stomatal development in *Arabidopsis* (protodermal cell, meristemoid mother cell, meristemoid, guard mother cell, guard cells) is controlled by combinatorial and sequential action of five bHLH transcription factors, while a complex receptor-ligand system controls proper spacing and orientation of stomata on the leaf surface. The low-density and transient nature of stomatal precursor cells has hampered their molecular profiling. We took advantage of specific mutant combinations to enrich stomatal precursor stem cells, called meristemoids. We performed a transcriptome analysis of *spch*, *scream-D; mute* and *scream-D* mutants, which develop epidermis solely composed of pavement cells, meristemoids and their sister cells, and guard cells, respectively. Pairwise comparison of transcriptomes in these mutants revealed molecular signatures associated with the meristemoid state. We found known stomatal lineage markers as well as novel putative regulators of stomatal development, i.e. receptors, ligands, transcription factors, cell-cycle genes and phytohormone-responsive genes and verified their cell type-specific expression by GFP and GUS fusions. We compared our data of meristemoid-enriched seedlings with transcriptome studies performed on the shoot and root apical meristem and found that genes involved in auxin metabolism and receptor-like kinases were significantly enriched in meristemoids and the meristems. This allowed us to identify a set of commonly regulated genes that may regulate the maintenance of the stem-cell state.

**430 Signalling Components of BABY BOOM-induced Somatic Embryogenesis***Anneke Horstman<sup>1</sup>, Hiroyuki Fukuoka<sup>2</sup>, Mieke Weemen<sup>1</sup>, Gerco Angenent<sup>1</sup>, Richard Immink<sup>1</sup>, Kim Boutilier<sup>1</sup>*<sup>1</sup>**Plant Research International, Wageningen, The Netherlands, <sup>2</sup>National Institute of Vegetable and Tea Science, Ano, Mie, Japan**

Embryogenesis in plants is normally restricted to zygotic embryo development, which takes place in the seed after fertilization. Embryogenesis can also be induced *in vitro* from both gametophytic and somatic cells. Somatic embryogenesis (SE) is induced by stress conditions and/or exogenous growth regulators, however ectopic expression of the AP2/ERF domain transcription factor BABY BOOM (BBM) is also sufficient to induce SE in the absence of growth regulators. To gain more insight into the BBM signalling pathway, BBM target genes were identified in a microarray experiment by overexpressing BBM:GR in seedlings. ChIP-Seq experiments are being performed using somatic embryo cultures to validate these genes as direct BBM targets and to identify those that are expressed during early SE development.

In addition, we are investigating whether BBM-interacting proteins are important components of the SE pathway. We have shown that BBM interacts with a chromatin remodeler PICKLE-RELATED1 (PKR1) and at least three HOMEO DOMAIN GLABROUS (HDG) transcription factors. Mutant analysis has shown that the HDG proteins are essential for initiation of BBM-mediated somatic embryogenesis, but do not play a major role in 2,4-D-induced somatic embryogenesis. We have also shown that a subset of these HDG proteins induce regeneration when over-expressed.

**431 The AP2/ERF Transcription Factor WIND1 Controls Cell Dedifferentiation in *Arabidopsis****Akira Iwase<sup>1</sup>, Masaru Ohme-Takagi<sup>2</sup>, Keiko Sugimoto<sup>1</sup>*<sup>1</sup>**RIKEN Plant Science Center, Yokohama, Japan, <sup>2</sup>National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan**

Many multicellular organisms have a remarkable capability to regenerate new organs after wounding. As a first step of organ regeneration, adult somatic cells often dedifferentiate to reacquire cell proliferation potential and pluripotency but the mechanisms underlying this control remain unknown in plants. We have recently shown that an AP2/ERF transcription factor WOUND-INDUCED DEDIFFERENTIATION 1 (WIND1) participates in the regulation of cell dedifferentiation in *Arabidopsis* (Iwase et al. Current Biology, 2011). *WIND1* is rapidly induced at the wound site and it promotes formation of callus, mass of pluripotent cells. We further demonstrate that ectopic overexpression of *WIND1* is sufficient to establish and maintain the dedifferentiated status of somatic cells without exogenous auxin and cytokinin. An *in vivo* imaging of a synthetic cytokinin reporter reveals that wounding upregulates the B-type ARABIDOPSIS RESPONSE REGULATOR(ARR)-mediated cytokinin response and that WIND1 acts via the ARR-dependent signaling pathway to promote cell dedifferentiation. We will discuss possible functions of WIND1 in reprogramming plant somatic cells to less differentiated states by transcriptional control.

**432 The COP9 signalosome regulates cell division rates and root meristem function in *Arabidopsis* embryos and seedlings***Nahill Matari<sup>1</sup>, Laila Moubayidin<sup>2</sup>, Sabrina Sabatini<sup>2</sup>, Giovanna Serino<sup>2</sup>, Pablo Jenik<sup>1</sup>*<sup>1</sup>**Franklin & Marshall College, Department of Biology, Lancaster, PA, USA, <sup>2</sup>Sapienza Università di Roma, Dipartimento di Biologia e Biotecnologie, Rome, Italy**

The COP9 signalosome (CSN) complex is involved in many aspects of plant life, including photomorphogenesis, hormone response and development of lateral organs. The complex is composed of eight subunits (CSN1 to CSN8). Null mutations in any subunit lead to the loss of the complex. *csn* mutants are seedling lethal, with a characteristic *fusca* phenotype (anthocyanin accumulation, small cotyledons, short roots). It has been suggested that *csn* seedlings arrest after germination as a consequence of not being able to progress past the

G2 phase of the cell cycle, possibly due to the activation of DNA damage pathways (Dohmann et al., 2008, *Development* 135: 2013). However, *csn* mutants are able to complete embryogenesis and to germinate. We sought to better characterize *csn* mutant seedlings. All *csn* mutant seedlings have a smaller than normal root apical meristem (RAM). The RAM differentiates and gets consumed by six days after germination. This arrest is characterized by reduced QC function, differentiation of columella initials and cortex cells, and reduced auxin response. We decided to trace the origins of these phenotypes during embryogenesis. We focused on the *csn2* mutant (also known as *fus12*). *csn2* embryos and endosperm develop at a slower rate compared to wild type siblings, indicating a slower cell cycle. Embryonic morphology is for the most part normal, and auxin transport and distribution appear unaffected. However, there are defects in the cells that generate the RAM: the hypophysis divides abnormally during the globular stage, leading to an abnormal RAM in which QC function is partially lost. Therefore, the seedling phenotypes have their genesis during embryonic development. The defects observed might be secondary to alterations in the speed of progression through the cell cycle.

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#### 433 Developmental Profiling of Gene Activity in *Arabidopsis* Seed Compartments Identifies Significant Differentiation in Endosperm Domain Identities

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Seeds directly account for approximately 75% the calories consumed by humans, and represent a highly successful evolutionary innovation. Inherently complex in angiosperms, this key structure consists of three major regions that differ in ploidy and genotype: the embryo, endosperm and seed coat. Using laser capture microdissection and microarray analysis, we have developed a high-resolution map of seed mRNA populations by dividing the three seed regions of *Arabidopsis* into seven distinct compartments: the embryo proper and suspensor; the micropylar, peripheral, and chalazal endosperm; and the distal and chalazal seed coat. Covering five stages of seed development, this dataset provides insight into the occurrence and distribution of biological processes and identifies compartment differences during development. Our data indicate that in some cases, ontogenetic origin is a predictor of mRNA population similarity, for example the distal and chalazal seed coat are characterized by sets of mRNAs that show a high degree of similarity overall. Surprisingly, the zygotic tissues of the embryo proper, suspensor, micropylar and peripheral endosperm domains show more similarity in their mRNA populations than is found within the three endosperm domains themselves. This is largely due to the unique nature of the chalazal endosperm mRNA populations, with many genes expressed there uniquely within the plant. Genes active in the antipodal cells of the female gametophyte are known to be specifically active the chalazal endosperm, despite significant differences in the developmental origin of these tissues. This indicates that conserved positional cues may be key in determining cell specification at the chalazal pole of both the female gametophyte and seed. We are investigating the role of localized phytohormone biosynthesis as a possible source of this positional information.

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#### 434 AINTEGUMENTA-LIKE6 (AIL6) regulates cellular differentiation in flowers

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During flower development, pluripotent stem cells within floral meristems give rise to proliferative precursor cells whose progeny acquire specialized functions within each floral organ. The regulatory mechanisms by which cells transition from a proliferating state to a differentiated state are still not clear. Several members of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) transcription factor family, including ANT and AIL6/PLT3, are important regulators of cell proliferation in flowers. ANT and AIL6 act redundantly to promote floral meristem patterning and floral organ growth. To further investigate the role of AIL6 during flower development, we have characterized transgenic plants in which the coding region of *AIL6* was expressed under the control of the constitutive 35S promoter (35S:*cAIL6*). These plants display changes in floral organ size and morphology that are associated with alterations in the pattern and duration of cell divisions within developing organs. In addition, we find that very high levels of *AIL6* expression have negative effects on cellular differentiation. In a 35S:*cAIL6* line with 50 fold higher *AIL6* mRNA levels as wild type, floral organs were found to lack characteristic epidermal surface morphologies. Furthermore, we find that *ant ail6* double mutants display premature differentiation of floral meristem cells. These results indicate that these two transcription factors are critical for controlling not only proliferation but also differentiation in flowers.

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#### 435 A WUSCHEL-like Gene Controls Stem Secondary Growth in Trees

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Wood formation starts with cell division and differentiation in a secondary meristem called vascular cambium, which forms a continuous cylinder of meristematic cells in the stem. Although many anatomical studies have been performed on the cambial zone and its derivatives, very little is currently known about the molecular and genetic mechanisms regulating the maintenance and differentiation of these stem cells as well as the patterning during the secondary growth of the woody plants. Here we investigate the role of a *WUSCHEL*-like gene *PtHB3* during secondary growth in Poplar. In the transgenic plants expressing an RNAi construct targeting the *PtHB3* gene, the width of the vascular cambium was severely reduced and the secondary growth was severely diminished, showing that *PtHB3* controls the cell identity and division activity in the vascular cambium. Moreover, ectopic expression of a Poplar *CLE41/44-like* (*CLAVATA3/ESR-RELATED 41/44*) gene in trees caused defects in the establishment of cambial cell divisions and the patterning of the vascular

tissues. Based on the transcriptional data, a positive feedback loop involving *PtHB3*, *PtCLE41* and the receptor-like kinase gene *PtRLK3* is suggested to regulate the identity and activity of the vascular cambium.

#### **436 Dynamic Regulation of H3K27 Trimethylation during *Arabidopsis* Differentiation**

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Polycomb-group (Pc-G) proteins are widely conserved and maintain gene repression and epigenetic memory by regulating trimethylation of histone H3 tails at Lysine 27 (H3K27me3). Developmental expression changes of Pc-G target genes like *FLOWERING LOCUS C* (*FLC*) and *AGAMOUS* (*AG*) suggest that despite its nature as epigenetic mark H3K27me3 is dynamically regulated. To reveal if gene expression changes of Pc-G target genes are generally correlated with alterations in H3K27me3 genome wide H3K27me3 analyses of different tissue samples were performed. The comparison of undifferentiated meristematic tissue with differentiated young leaves uncovered alterations in H3K27me3 coverage of about 600 genes. Thus H3K27me3 is indeed dynamically reset or set up during somatic development. Bioinformatic analyses further revealed meristem- and leaf-specific targeting of individual gene families including known but also likely novel regulators of differentiation and stem cell regulation. Interestingly, H3K27me3 directly represses only specific transcription factor families, but indirectly activates others through H3K27 me3-mediated silencing of microRNA genes. Furthermore, H3K27me3 targeting of genes involved in biosynthesis, transport, perception and signal transduction of the phytohormone auxin demonstrates the control of an entire signalling pathway. Although western blot analyses of weak Pc-G mutants uncovered a global reduction of H3K27me3, ChIP-qPCR and RT-qPCR analyses revealed that H3K27me3 reduction occurs gene specifically but does not lead to mis-expression of all deregulated target genes. Thus Pc-G regulation is generally correlated with gene repression but its loss is not sufficient for gene activation.

#### **437 Interplay of GRAS Transcription Factors in the *Arabidopsis* Shoot System**

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SHORT-ROOT (SHR) and SCARECROW (SCR) are key regulators of stem cell maintenance and radial patterning in the *Arabidopsis* root. It is relatively well characterized on the molecular basis of root radial patterning processes by the SHR/SCR regulatory module. In the shoot system, however, little is known about the SHR/SCR pathway, even though *shr* and *scr* mutants show evidently obvious phenotypes in the shoot. Recent study demonstrated that SHR and SCR play important roles in proliferative divisions, regulating cell cycle in the leaf. In an attempt to elucidate the role of the SHR/SCR pathway in the shoot system, we have isolated a SHR-interacting protein, SCARECROW-LIKE 23 (SCL23), which also belongs to the GRAS transcription factor family. SCL23 is most closely related to SCR, which is also known to interact with SHR, among the GRAS members. *SCL23* is expressed only in the shoot system, not in the root. Our genetic analysis reveals that *SHR* is epistatic to *SCL23*, and *SCL23* expression is reduced in the *shr* mutant shoot. Similarly to the relationship between SHR and SCR, SHR is also associated with the promoter region of *SCL23*. In addition, overexpression of *SCL23* exhibits smaller rosette leaves, suggesting its involvement in the leaf development in conjunction with the SHR/SCR regulatory module.

#### **438 Misexpressed CPC Affects the Cell Fate Specification in the *Arabidopsis* Root Epidermis**

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The multicellular organisms are composed of different types of cells originated from a single zygote cell. The appropriate cell fate specification of these cells is an important developmental process. The specification of a hair cell and a non-hair cell in *Arabidopsis* root epidermis is a good model for explaining the cell fate specification. The epidermal cell fate is controlled by the complex transcription events, and the CAPRICE(CPC)-mediated lateral inhibition mechanism has been suggested. CPC, a small R3 single-repeat MYB protein, is expressed in N position cells. However, it functions only in H position cells and directs the hair cell specification. To investigate how CPC induces the hair cell fate, we examined effects of misexpressed CPC in the epidermal cell fate specification. Here we show that CPC moves easily within the root epidermis and the CPC movement is not crucial for its proper function.

#### **439 Gating of sperm entry to the Central Cell during double fertilization is mediated by GLAUCE**

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Double fertilization in flowering plants results in formation of the embryo from the egg cell and the endosperm, from the central cell. We have previously described an *Arabidopsis* female gametophytic deletion mutant named *glouce* (*glc*), in which a globular embryo develops without any endosperm (Ngo *et al* 2007, *Development* 134, 4107-4117). We have now molecularly identified *GLC*, and found that it has an unusual gene structure with two redundant exons related to acyl transferases, and is predominantly expressed in the central cell prior to fertilization. To track the double fertilization process in *glc* ovules, we used fluorescent markers for the sperm cells and the embryo sac cells. In the *glc* ovules, both sperm cells successfully entered the synergid cell. One sperm effectively fertilized the egg cell, however the second sperm did not fertilize the central cell and remained excluded from this cell. Double fertilization is a hallmark of flowering plant evolution, and it is interesting that fertilization of *glouce* mutant embryo sacs resembles that of gymnosperms which have two sperm cells, of which only one fertilizes an egg cell and the other eventually degenerates. Our results suggest a "gate" for female fertilization which governs sperm entry into the central cell independently of entry into the egg cell, and which requires *GLAUCE* for function.

**440 Genome-wide Analysis of SCL3-responsive Transcriptome in the *Arabidopsis* Root**Jun Lim, Shin Ae Lee, Kwang Suk Chang, Jung-Ok Heo**Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Korea.**

Recently, we have reported that SCARECROW-LIKE 3 (SCL3), a GRAS transcription factor, acts as a tissue-specific integrator of GA signaling in the root endodermis. Our genetic analysis reveals that RGA, a major repressor of GA signaling, is the direct upstream of SCL3. Interestingly, SCL3 acts as a positive regulator in GA signaling to maintain GA homeostasis by attenuating the master growth DELLA repressors. The endodermis-confined GA signaling controls coordinated cell elongation, and also the timing and extent of formative divisions to fine-tune post-embryonic root development. To gain more insights on SCL3 function in the root, we analyzed SCL3-responsive transcriptome in the root with Affymetrix ATH1 microarrays. Among SCL3-responsive transcription factors, we are currently focusing on a novel C2H2 zinc finger, named CZF. Expression of CZF is primarily observed in the vasculature of the root transition zone (TZ). Interestingly, CZF expression is upregulated by ABA, suggesting that there is a possible cross-talk between GA and ABA in the root. Furthermore, expression of CZF is upregulated by loss of SHR function in the stele, suggesting that the mobile SHR transcription factor regulates two different direct targets, SCL3 and CZF, in endodermis and stele, respectively. Currently, we are focusing on the elucidation of the interplay of these transcription factors in root development.

**441 HYL1 mediates patterning of the *Arabidopsis* root stem cell niche by regulating PLETHORA**Jinxin Liu, Yuke He**National Key Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China**

In plant root, stem cell are organized around the quiescent centers (QC) to form the root stem cell niche where PLETHORA (*PLT*) genes encoding AP2-domain transcription factors act to maintain the activity of stem cell. MicroRNAs (miRNAs) guide the post-transcriptional regulation of target genes and play an important role in developmental processes including the organization and maintenance of stem cell niche. Here, we report that HYL1, one of the major regulators of miRNA biogenesis, maintains root stem cell niche by regulating *PLTs*. Mutation of *HYL1* leads to defective maintenance of the root stem cell niche, lost meristematic activity, stunted root growth, and significantly impairs the expression of *PLETHORA* (*PLT*) transcription factor genes in root stem cell niche. Because HYL1 is required for miRNA biogenesis, the patterning defects in *hyll* root stem cell niche were likely due to the loss of specific miRNAs and the consequent up-regulation of their target genes. The accumulation of miR165/166 is significantly reduced in *hyll* root tips, while the expression of miR65/166-targeted genes, *HD-ZIP III* family genes, is increased more than 3 folds compared with wild type. The antagonistic interaction between *HD-ZIP III* family and *PLTs* in the embryogenesis is essential for the proper apical-basal patterning. To investigate the role of *HD-ZIP III* genes in HYL1-mediated *PLTs* pathway, we constructed the *hyll rev* double mutant and *hyll phb phv* triple mutant. We observed significant restoration of the root length, the maintenance of root stem cell niche and the transcript level of *PLTs* in these multiple mutants. Together, these results suggest that HYL1 mediates the antagonism between the *HD-ZIP III* and *PLT* gene family in root stem cell niche through miR165/166.

**442 Detection of Transcriptome Landscape in *Arabidopsis* Male Meioocytes Using High-throughput Sequencing**Pingli Lu<sup>1</sup>, Hongxing Yang<sup>2</sup>, Yingxiang Wang<sup>2</sup>, Hong Ma<sup>1,2</sup>**<sup>1</sup>The Pennsylvania State University, University Park, PA USA, <sup>2</sup>Fudan University, Shanghai China**

Meiosis is essential for eukaryotic sexual reproduction, with two consecutive rounds of nuclear divisions, allowing the production of haploid gametes. Information regarding meiotic transcriptome should provide valuable clues about global expression patterns and detailed gene activities. Here we employed RNA sequencing to explore the transcriptome of a single plant cell type, the *Arabidopsis* male meiocyte, detecting the expression of ~20,000 genes. Transcription of introns of >400 genes were observed, suggesting previously unannotated exons. >800 genes may be meiocyte-preferentially expressed, including known meiotic genes. Among the 3,378 Pfam gene families in the *Arabidopsis* genome, 3,265 matched meiocyte-expressed genes, and 18 gene families were overrepresented in male meiocytes, including transcription factor and other regulatory gene families. Expression was detected for many genes thought to encode meiosis-related proteins, including MSHs, kinesins, and ATPases. We identified >1,000 orthologous gene clusters that are also expressed in meiotic cells of mouse and the fission yeast, including 503 single-copy genes across the three organisms, with a greater number of gene clusters shared between *Arabidopsis* and mouse than either to yeast. Interestingly, ~5% transposable element genes were apparently transcribed in male meiocytes, with a positive correlation to that of neighboring genes. In summary, our RNA-seq transcriptome data painted a portrait for gene expression in male meiocytes and provide invaluable information for future functional studies.

**443 Exploring Two Ethylene Biosynthetic Enzymes as Potential Targets of *Arabidopsis* RING E3 Ligase, XBAT32, During Lateral Root Production.**Wendy Lyzenga, Sophia Stone**Dalhousie University, Halifax, (NS), Canada**

Ubiquitin-mediated proteolysis is a widespread mechanism used by plants to respond to environmental stimuli and to regulate hormone signals that influence development. XBAT32 is a RING type E3 ligase which functions as a substrate recruiting component of the ubiquitination pathway. Studies indicate that XBAT32 regulates the abundance of ethylene biosynthetic enzymes (ACS4 and ACS7). Loss of *xbat32* results in ethylene overproduction and reduced lateral root production. Our current model suggests that overproduction of

ethylene in the *xbat32* root disrupts auxin transport and blocks essential auxin uploading into pericycle cells, preventing specification of lateral root founder cells. We are currently investigating whether auxin transport in the *xbat32* mutant is altered, and if loss of *acs4* and/or *acs7* can rescue the lateral root phenotype of *xbat32* mutants. ACS family members are regulated by ubiquitin-mediated proteolysis, and the C-terminal extensions of these proteins are required for degradation. However, it was thought that ACS7, which has the shortest C-terminal extension, is not regulated by ubiquitin-mediated degradation. Using a cell free degradation assay and transgenic plants expressing HA-ACS7, we demonstrate that ACS7 is turned over in a proteasome dependent manner. In addition, we have shown *in planta* that HA-ACS7 is stable in *xbat32* mutant seedlings and treatment with proteasome inhibitors does not increase ACS7 protein levels. This suggests that XBAT32 is indeed responsible for ubiquitin-mediated degradation of ACS7. We are currently investigating if the single conserved lysine in the C-terminal extension of ACS7 plays a role in its turnover.

#### **444 C2H2 Factors Regulate Cell Identity and Asymmetric Divisions in the Arabidopsis Root**

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**Duke University**

Organ patterning and growth are key aspects of normal development in many multicellular organisms. Patterning requires that certain cells adopt a specific fate while other cells adopt different fates. During postembryonic developmental patterning in the root, stem cells located at the root tip divide asymmetrically to produce the different cell lineages. We have found that different C2H2 transcription factors (TFs) regulate cell identity and asymmetric division in the Arabidopsis root. Combining different insertion line mutants in these TFs results in an abnormal radial pattern and cell lineages show altered cell identity as indicated by cell-type marker lines and histological stains. Ectopic expression of these TFs results in extra radial layers, and can rescue the asymmetric division defect in *shr* and *s cr* mutants. In addition, microarray analyses suggest a connection with other genes either directly or putatively involved in asymmetric cell division in the root. Taken together, our results suggest that these C2H2 TFs regulate both asymmetric cell division and identity in the Arabidopsis root stem cells.

#### **445 SCARECROW Sustains Stem Cell Activity Inhibiting Cytokinin Dependent Cell Differentiation Input**

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Understanding the molecular mechanisms through which plant meristems are maintained is a central question in developmental biology. In the root of *Arabidopsis thaliana*, stem cells in the apical region of the meristem self-renew and produce daughter cells that differentiate in the distal meristem transition zone. To ensure root growth, the rate of cell differentiation must equal the rate of generation of new cells. Cell differentiation takes place in the transition zone that is localized in the distal part of the root meristem, but must be synchronized and balanced with division of the stem cells that are localized in the apical part of the meristem. We have previously shown that maintenance of the *Arabidopsis* root meristem size - and consequently root growth - is controlled by the interaction between two hormones at the meristem transition zone: cytokinins, which promote cell differentiation, and auxin, which promotes cell division, but it is still unknown how the cytokinin/auxin interaction maintains a balance between cell differentiation at the transition zone and cell division in the stem cell niche. Here we show that SCARECROW (SCR) maintains stem cell activity repressing cytokinin-mediated differentiation input in the stem cell niche through down-regulation of the cytokinin-responsive transcriptional regulator ARR1 thus controlling root meristem size.

#### **446 Three AIL/PLT Transcription Factors Function Together in Regulating Shoot Apical Meristem Activity**

*Janaki Mudunkothge, Beth Krizek*

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The shoot apical meristem (SAM) is the tiny dome shaped structure at the shoot tip that initiates lateral organs and provides cells to the growing stem. We have identified three novel regulators of SAM activity: *ANT*, *AIL6* and *AIL7*. They encode related proteins belonging to the AIL/ PLT family of transcription factors. They exhibit contrasting expression patterns within the SAM with *ANT* and *AIL6* expressed in the peripheral region of the SAM and *AIL7* expressed in the central region. Loss of function of all three genes together results in plants that initiate 3-6 abnormally shaped leaves followed by termination of SAM activity. At early seedling stages, we observed reduced cell division in the SAM region of the triple mutants and differentiation of meristem cells. At 7 days post germination expression of the meristem regulators *WUS*, *CLV3* and *STM* is reduced, however expression of these genes was similar to the wild type in embryos. Using a genetic approach, we determined that the AILs do not act in either the WUS/CLV or STM pathway. Furthermore our results indicate that *ANT*, *AIL6* and *AIL7* have distinct functions within the meristem, consistent with the different expression patterns of these genes. This study reveals three new members of the complex SAM regulatory network that are likely to be required post-embryonically for maintenance of meristem function.

**447 Dissecting Receptor Function in Shoot Stem Cell Maintenance in Living Meristematic Tissue***Zachary Nimchuk, Paul Tarr, Carolyn Ohno, Vijay Chickarmane, Qu Xiang, Elliot Meyerowitz***Caltech, Pasadena, California, USA**

The regulation of the shoot stem cell niche in *Arabidopsis* depends upon the perception of the glycopeptide CLAVATA3 (CLV3) by a partially redundant series of transmembrane receptor kinase complexes including CLV1 and CLV2/CORYNE, among others. It is thought that the CLV3 pro-peptide is secreted from apical meristem cells where it is processed into its active form which diffuses into the rib meristem. Current models predict that CLV3 binds target receptors triggering kinase activation which limits the expression of the stem cell promoting homeodomain transcription factor WUSCHEL (WUS). CLV1 has been proposed to signal independently of the CLV2/CORYNE dimer in this process, with CORYNE providing the critical kinase activity. Recent work has suggested that CLV3 signaling is highly buffered in meristems. There are several outstanding questions remaining as to how CLV1 is activated in meristems, how the different receptors signal and how CLV3 perception is buffered. Here we present recent work demonstrating that CLV3 alters trafficking of the CLV1 receptors using living meristematic tissue for the first time. In *clv3* plants, CLV1 is localized to the plasma membrane and is trafficked to the lytic vacuole in response to CLV3. This process is reminiscent of receptor mediated endocytosis and trafficking in animal cells. The trafficking behavior of CLV1 allows us to estimate the diffusion area of CLV3 in the meristem. In addition we demonstrate that CLV2 is not essential for CLV1 stability or trafficking. Despite this, the CLV2/CORYNE dimer is unlikely to signal independently of CLV1 as several lines of evidence suggest CORYNE is a conserved pseudokinase. Our work suggests that CLV1 trafficking may contribute to buffering of CLV3 perception and provides a glimpse at the complexity underlying receptor mechanisms in stem cell maintenance.

**448 Abstract Withdrawn****449 The role of CORYNE in root development.***Helge Pallakies, Rüdiger Simon***Heinrich-Heine-Universität, Düsseldorf, Germany**

The *CLAVATA3 (CLV3)* signaling pathway is proposed to comprise the receptor kinase *CLAVATA1 (CLV1)* and the receptor-like protein *CLAVATA2 (CLV2)*. *CORYNE (CRN)* has been identified in a screen for suppressor mutants of *CLV3* overexpression. We previously showed that it encodes a membrane associated kinase which acts together with *CLV2* and is able to perceive the *CLV3* signal in parallel with *CLV1*. *CRN* has additional functions during plant development, that are shared with *CLV2*. The interaction of both proteins is further made possible by a widely shared expression pattern in the shoot and the root. Although *crn* and *clv2* mutant roots are phenotypic, a function of both genes in the root was found in a *CLV3* overexpression experiment. Additional experiments involving the treatment with other *CLAVATA3/ESR-RELATED (CLE)* peptides, that are expressed in the root, suggest a role of *CRN* in the perception of the CLE peptide signal in the root.

**450 Local Auxin Biosynthesis Is A Key Step In The Patterning Of The *Arabidopsis* Female Gametophyte***Aneesh Panoli<sup>1</sup>, Monica Alandete-saez<sup>1</sup>, Yunde Zhao<sup>2</sup>, Venkatesan Sundaresan<sup>1</sup>***<sup>1</sup>University of California, Davis, USA, <sup>2</sup>University of California, San Diego, USA**

During plant evolution, gametophytes have undergone an extreme reduction in size from the macroscopic thali of mosses to the few-celled, highly specialized embryo sac, which is the female gametophyte of the flowering plants. Recently, the phytohormone auxin has been shown to be implicated in the patterning of the embryo sac, through an auxin gradient within the developing embryo sac, that specifies the female gametes (egg cell and central cell) and accessory synergid cells. However, it was unclear whether auxin synthesized by the gametophyte is essential for embryo sac development. Here, we studied the expression pattern and the roles of auxin biosynthetic genes in the developing embryo sac. We find that genes from both the YUCCA pathway (YUC8), and alternate TAA pathway (TAA1, TAR2) for auxin biosynthesis are expressed in the micropylar (distal) end of the gametophyte around second post-meiotic mitosis (4-nuclear stage), this expression pattern is retained until cellularization. There is no expression in the adjacent sporophytic tissues at these stages. Mutant analysis revealed that in the *yuc8* mutant, there are defects in the cell specification and cell fate. A significant fraction of the embryo sacs arrested at the 4-nuclear stage with a very small or no central vacuole, indicating a likely role for auxin in vacuole formation and cellularization. We also detected similar defects in *taa1/+; tar2/+*, and an additive effect in the *taa1/+; tar2/+; yuc8/yuc8* plants, suggesting a combined role for the two auxin pathways in regulating auxin maxima in the micropylar end of the gametophyte. Taken together the results show that local synthesis of auxin within the gametophyte is a key determinant in the development of *Arabidopsis* female gametophyte. Previous studies on mosses and these studies on female gametophytes of *Arabidopsis* point to the importance of local auxin biosynthesis in gametophytic development, vs. polar auxin transport, which plays a vital role in sporophytic development.

## 451 RETINOBLASTOMA-RELATED Protein and Cytokinin Interaction during Root Meristem Cell Differentiation

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Continuous root growth is ensured by the establishment of a balance between cell division and cell differentiation within the root meristem. It has been recently demonstrated that this balance results from the antagonistic interaction between two plant hormones, cytokinins (promoting differentiation) and auxin (promoting division) through a regulatory circuit whereby the ARR1 and ARR12 cytokinin-responsive transcription factors activate the SHY2 gene, which in turn negatively regulates the PIN genes encoding auxin transport facilitators (Dello Ioio et al., 2008).

Although the basic molecular framework of the cytokinin/auxin interaction controlling root meristem size has been unveiled, several evidences suggest that other genes and regulatory networks are involved in root growth, and must interact with the ARR<sub>s</sub>/SHY2/PINs circuit. One of these candidate genes, the plant RETINOBLASTOMA-RELATED (RBR) protein, has emerged as a key player in the control of stem cell identity, affecting cell differentiation and not cell cycle in the root stem cell niche (Wildwater et al., 2005). In this work, by means of physiological and pharmacological approaches as well as tissue- and cell-specific gene expression techniques and classic genetic analyses, we show that the RBR protein triggers cell differentiation in the root meristem through the ARR12 cytokinin-dependent transcription factor.

## 452 Capturing the Dynamics of Stomatal Cell Specification in Growing Leaves

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The mechanisms that generate dynamic spatial patterns within proliferating tissues are poorly understood, largely because of difficulties in unravelling interactions between cell specification, polarity, division, and growth. Here we address this problem for stomatal spacing in the *Arabidopsis* leaf epidermis. By using time-lapse confocal imaging to track lineages and gene activities over extended periods we were able to capture the dynamics of the system. We show that stomatal precursor identity depends on the maintenance of the SPCH (SPEECHLESS) transcription factor in one of the two daughter cells through a sequence of divisions. The observed lineages tended to internalise the SPCH-expressing daughter and thus reduce contact with other precursors. We used modelling to investigate mechanisms of controlling which daughter maintained SPCH expression. Our study revealed that the observed stereotypical stomata lineages can be re-created if SPCH maintenance is controlled by a polarity factor positioned away from new division walls. We propose that BASL (BREAKING OF SYMMETRY IN THE STOMATAL LINEAGE) is acting as the polarity determinant and displaces the new division wall. We validated the model by predicting the location of BASL and the resulting cellular arrangements over multiple divisions. Comparing the model to tracked BASL-expressing lineages showed a good match. Thus, complex patterning dynamics can be accounted for by the interplay of cell specification, division and polarity in a growing tissue.

## 453 Endoreduplication Represses Small Cell Identity

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Coordination of cell division with cell specification is a fundamental process in organogenesis. The sepal epidermis is a model system for understanding this link because the cells have diverse sizes and have undergone different cell cycles: the highly elongated giant cells enter endoreduplication and replicate their DNA without dividing, whereas the small cells divide (1). We first asked whether these cells have different identities. One enhancer trap line is generally expressed in giant cells (2) and another is expressed in small cells within the sepal epidermis suggesting that these cell types have different identities at the level of enhancer activity. To determine how endoreduplication correlates with giant and small cell identity, we changed the proportion of endoreduplicating cells by altering cell cycle inhibitor activity. In *loss of giant cells from organs (lgo)* mutants, large cells are absent due to a reduction in endoreduplication (1); however, the giant cell marker is expressed in many small cells indicating that endoreduplication is not required for giant cell identity. Likewise, forcing cells to endoreduplicate by ectopically expressing the cell cycle inhibitor *KRP1* (1, 3) does not cause the cells to express the giant cell marker. We conclude that giant cell identity is established upstream of endoreduplication. Surprisingly, we also find that overexpression of *KRP1* inhibits the small cell marker expression suggesting that endoreduplication represses small cell identity. Likewise, more cells adopt small cell identity when endoreduplication is reduced in *lgo* mutants. Taken together, we conclude that cell identity influences the cell cycle, and that the cell cycle also feeds back to influence cell identity.

1. A. H. K. Roeder et al., *PLoS Biol* 8, e1000367 (2010).
2. Y. Eshed, et al., *Development* 131, 2997 (2004).
3. S. M. Bemis, K. U. Torii, *Dev Biol* 304, 367 (2007).

**454 What Makes a Root Hair? Integrated Transcriptomic and Proteomic Analysis of *Arabidopsis*****Trichoblasts***Ping Lan<sup>1</sup>, Wenfeng Li<sup>1</sup>, Ya-Yun Liao<sup>1</sup>, Simonetta Santi<sup>2</sup>, Wolfgang Schmidt<sup>1</sup>*<sup>1</sup>**Academia Sinica, Taipei, Taiwan, <sup>2</sup>Universita degli Studi di Udine, Udine, Italy**

Mapping diverse omics data sets on a given phenotype or cell type provides a way to understand physiological or developmental processes at a systems level. Parallel profiling of transcripts and proteins was conducted on root hair-forming cells (trichoblasts) in protoplasts isolated from plants carrying a GFP reporter coupled to the trichoblast-specific EXP7 protein. GFP-expressing cells were separated from non-active cells by FACS equipped with a cooling device. RNA collected from several runs was pooled and analyzed by RNASeq using the Solexa II Genomic Analyzer platform without amplification. In total, 23 million uniquely mapped 150-bp paired-end reads were generated, matching to 20,890 transcripts. As anticipated, genes coding for cell wall hydrolytic enzymes and enzymes involved in cell extension were highly expressed in root hairs. Expression of genes encoding the negative regulators of the root hair cell fate GL2 and WER was < 1 RKMB, while CPC, a positive regulator showed high transcript abundance. Transcripts of RHD6 and RSL4, encoding bHLH-type transcription factors that control early stages of root hair differentiation, were also highly abundant. A total of 1600 proteins were identified in trichoblasts by LC-MS/MS on a LTQ Orbitrap Velos. The GO categories 'intracellular protein transport', 'glycolysis' and 'amino acid biosynthesis' were strongly enriched at the protein level. The corresponding transcript for a subset of proteins was not detected. Some of these proteins carry secretion signals, indicative of their possible lateral or radial movement into trichoblasts. In summary, through correct confrontation of deep transcriptomic and proteomic data sets, we provide a systems view into the metabolism of a single cell type that undergoes highly dynamic developmental changes.

**455 A Novel Semi-dominant Allele of *MONOPTEROS* Reveals Pleiotropic Functions for *MONOPTEROS* during Plant Development***Jasmine Garrett<sup>1</sup>, Miranda Meents<sup>1</sup>, Hongwei Hou<sup>1</sup>, Kamran Kaviani<sup>1</sup>, James Meservy<sup>1</sup>, Michael Blackshaw<sup>1</sup>, LeeAnna Tavernini<sup>1</sup>, Danielle Styranko<sup>2</sup>, Elizabeth Schultz<sup>1</sup>*<sup>1</sup>**University of Lethbridge, Lethbridge, AB, Canada, <sup>2</sup>Department of Biology, University of Western Ontario, London, ON, Canada**

The plant hormone auxin controls a variety of plant developmental processes including embryonic polarity, root patterning, formation of lateral roots, phyllotaxis, gravitropism, floral development and leaf vein formation. The distribution of auxin within the plant is controlled by directional auxin transport. The resulting auxin distribution alters transcription of auxin responsive genes whose expression is controlled by the ARF family of transcription factors. ARFs are inactive when dimerized with members of the AUX/IAA proteins. Auxin influences ARF activity by targeting AUX/IAA proteins for degradation. Large numbers of both ARF and AUX/IAA genes exist, suggesting significant developmental specificity in their partnerships. One partnership that has been extensively characterized in *Arabidopsis* is that between ARF5 (*MONOPTEROS*, MP) and IAA13 (*BODENLOS*, BDL). This ARF-AUX/IAA pair has been shown to be important during embryonic root development, and also during formation of leaves and veins. We have identified a semi-dominant allele of *MONOPTEROS* that has a pleiotropic phenotype including proliferation of leaf veins, altered phyllotaxis, changes to floral organ arrangement and male sterility. Our analysis suggests that the product of the mutant allele is unable to dimerize with the BDL repressor, resulting in an irrepressible auxin response. The implications of this phenotype to our understanding of MP function will be discussed.

**456 Phenotypic Analysis of an Embryo-Aborted Mutant in *Arabidopsis****Jiao Shi, Jingjing Liu, Li-Jia Qu***State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University, Beijing, People's Republic of China**

The plant life cycle alternates between a diploid sporophytic phase and a haploid gametophytic phase. The gametophytic phase is a plant specific process in reproduction. In order to identify the mutations that play key roles in plant gametogenesis and embryogenesis, we performed a distorted mendelian segregation screen of a T-DNA activation tagging mutant collection in our lab. We identified a mutant, *rms2*, in the siliques of which 19.70% of the embryos were aborted. We analyzed the selfing progeny of the *rms2* by genotyping and found that there were no homozygous lines, with the segregation ratio of heterozygotes vs. wild type 1.3: 1, deviated from the typical Mendelian segregation ratio. The reciprocal crosses between the heterozygous and wild-type plants showed that the female transmission was normal in *rms2*, but the male transmission was greatly reduced. The observation of ovules in *rms2* selfing siliques showed that the embryos were arrested at variable developmental stages. Meanwhile, the embryos of the reciprocally crossed plants were basically normal, no matter the *rms2* mutant were used maternally or paternally. Therefore, these data suggest that the *rms2* mutation resulted in defective male gametophyte development and aborted embryogenesis. *RMS2* gene was expressed specifically in the anthers, and the protein was localized in cytoplasm. We are currently working on the function of this gene.

**457 Molecular Genetic Analysis of *Arabidopsis TSO1*, a Regulator of Cell Proliferation and Differentiation During Flower Development***Paja Sijacic, Charles Hawkins, Zhongchi Liu***University of Maryland, College Park, MD, USA**

In multicellular organisms, cell division and cell differentiation processes need to be tightly controlled to ensure proper organ development. Although the genetic mechanism of floral organ specification is well established, little is known about how floral organs

grow and differentiate into their final shape and morphology. The accumulation of callus-like cells in *tso1-1* mutant floral organs and the presence of ectopic stem cells in *tso1-1* shoot apical meristems (SAM) indicate that *Arabidopsis* TSO1 is a key coordinator of cell proliferation and cell differentiation during flower development.

*TSO1* encodes a putative transcription factor that belongs to an eight-member gene family. TSO1 contains two CXC motifs that may serve as the DNA-binding domains. In animals, TSO1 homologs form higher order complexes, named dREAM complex, to regulate cell cycle progression together with Retinoblastoma. Using artificial microRNA and T-DNA insertional lines, we determined the *tso1* null phenotype, which is much weaker than *tso1-1*, a missense allele. Further genetic analysis indicated that *tso1-1* is an antimorphic allele that not only inactivates TSO1 itself but also other members of the TSO1 family, including SOL2.

To identify target pathways regulated by TSO1, we conducted a microarray analysis comparing *tso1-1* mutant and wild type inflorescences. Several genes known to play an important role in the cell cycle control and meristem initiation and maintenance are found to be significantly upregulated in *tso1* mutant plants. Chromatin immunoprecipitation (ChIP) and an inducible TSO1-GR system are being used to test these putative target genes and to distinguish direct from indirect targets.

#### 458 Characterizing LEAFY Transcriptional Complexes in *Arabidopsis thaliana*

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Flowers, as the reproductive structure of angiosperms, are arguably the most important organs of these plants. The *LEAFY* gene of *Arabidopsis thaliana* is a floral meristem identity gene; it is both necessary and sufficient for the formation of flowers. *LFY* encodes a plant specific transcription factor that regulates a complex set of developmental events during flower development. Although *LFY* has been a subject of intense research interest, relatively little is known about the complexes in which it acts to regulate transcription. Our project is aimed at identifying and characterizing *LFY*-containing transcriptional complexes and how these regulate target genes. *LFY* functions as a homodimer and we have identified a conserved region within the N-terminal portion of the protein that functions as a dimerization domain. This region contains leucine residues in a  $7 \times 8$  pattern, reminiscent of a leucine zipper. Replacement of these leucines by alanines disrupts homodimerization of *LFY* and in planta function, suggesting that dimerization is essential. In addition to examining *LFY* dimerization, we have taken biochemical and genetic approaches to identify proteins that function with *LFY* in transcriptional complexes. Here we present our work on a previously uncharacterized C2H2 transcription factor, FOL1 (Friend of *LFY*) that binds to *LFY* and functions during floral development.

#### 459 Radial patterning in the *Arabidopsis* root: transcriptional effect of SHR at cell-type resolution

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The development of multicellular organisms relies on the coordinated control of cell divisions that lead to proper organ patterning and growth. The molecular mechanisms underlying pattern formation are still poorly understood, in particular how developmental pathways regulate genes involved in formative divisions. In the *Arabidopsis* root, the formative cell divisions that give rise to the cortex and endodermis are controlled by the transcription factors, SHORTROOT (SHR) and SCARECROW (SCR). In this study, the cell-type specific transcriptional effects of SHR and SCR induction combined with ChIP-chip data revealed that SHR regulates the spatial and temporal activation of specific genes involved in cell division. Coincident with the onset of a specific formative division, SHR and SCR directly activate a D-type cyclin. Altering its expression resulted in formative division defects in both loss-of-function and gain-of-function plants. Our results indicate that proper pattern formation is achieved through transcriptional regulation of specific cell cycle genes in a cell-type and developmental stage-specific context. Taken together we provide evidence for a direct link between developmental regulators, specific components of the cell cycle machinery and organ patterning.

#### 460 The Folypolyglutamate Synthetase Plastidial Isoform is Required for Postembryonic Root Development in *Arabidopsis*

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A recessive *Arabidopsis* mutant with short primary roots was identified from a forward genetic screen. The disrupted gene in the mutant encoded the plastidial isoform of folypolyglutamate synthetase (FPGS) previously designated as AtDFB, an enzyme that catalyzes the addition of glutamate residues to the folate molecule to form folypolyglutamates. The short primary root of *atdfb* was associated with a disorganized quiescent center (QC), dissipated auxin gradient in the root cap, bundled actin cytoskeleton, and reduced cell division and expansion. The accumulation of monoglutamylated, and reduction of polyglutamylated forms of some folate classes in *atdfb* was consistent with impaired FPGS function. The observed cellular defects in roots of *atdfb* underscore the essential role of folypolyglutamates in the highly compartmentalized one carbon transfer reactions (C1 metabolism) that lead to the biosynthesis of compounds required for metabolically active cells found in the growing root apex. Indeed, metabolic profiling uncovered a depletion of several amino acids and nucleotides in *atdfb* indicative of broad alterations in C1 metabolism. Methionine and purines, which are synthesized *de novo* in plastids, were particularly depleted. The root growth and QC defects of *atdfb* were rescued by exogenous application of 5-formyl-tetrahydrofolate (5-CHO-THF), a stable folate that we showed was readily converted to metabolically active folates. Exogenous methionine partially rescued

the root defects of *atdfb*. Collectively, our results indicate that AtDFB is the predominant FPGS isoform that generates polyglutamylated folate cofactors to support C1 enzymatic reactions required for meristem maintenance and cell expansion during postembryonic root development in Arabidopsis.

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**461 Abstract Withdrawn**

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**462 RUL3, a Novel Regulator of Auxin-dependent Root Patterning and Differentiation**

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We identified a family of four genes encoding for nuclear-localised proteins with a conserved plant-specific DUF domain (domain of unknown function). Here we report on one of the members, the so far uncharacterised *RUL3* gene (*REDUCED UNLOADING OF GFP-LIKE3*). Analyses of p*RUL3::RUL3-GUS* lines showed that the protein is expressed mainly in meristematic cells of the primary root, in developing lateral roots and to a lesser extent in the vascular tissue of all organs. Loss of RUL3 function dramatically affects development and differentiation leading to extremely short roots, disorganized root patterning and severe vascular defects. In addition the mutant shows altered leaf shape. A transient expression assay suggests that *RUL3* might act as a transcriptional activator. Analysis of the auxin-response reporter p*DR5::erGFP* in the *rul3* mutant background indicates a reduced accumulation of the phytohormone auxin in the quiescent centre (QC), where an auxin maximum is essential for stem cell maintenance and tissue patterning in wildtype plants. Furthermore the promoters of the auxin-dependent QC marker gene *WOX5* (*WUSCHEL-RELATED HOMEOBOX5*) and of the auxin efflux transporter gene *PIN1* (*PINFORMED1*) show ectopic activity in the *rul3* mutant background as compared to wildtype. Our results characterize RUL3 as a new factor involved in auxin-regulated processes affecting root patterning and differentiation.

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**463 Nuclear Size Matters; The Role of Chromatin Organization in Seed Maturation, Dormancy and Germination in *Arabidopsis thaliana***

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Seeds have low moisture contents and strongly reduced metabolic activities. During the maturation phase, they accumulate storage reserves and become desiccation tolerant and dormant. Growth is resumed after release of dormancy and the occurrence of favorable environmental conditions. We employ two independent approaches to study chromatin organization and chromatin remodeling in the important phase transitions from embryo to mature seed and germination in *Arabidopsis thaliana*.

Using cytogenetic approaches, we found that ripe seeds have a strongly reduced nuclear size, which is established at the beginning of seed maturation. Nuclei revert to their normal size upon germination. The reduction in nuclear size is controlled by ABSCISIC ACID INSENSITIVE3 and the increase during germination requires LITTLE NUCLEI1 and LITTLE NUCLEI2. Interestingly, genes encoding proteins associated with elongating RNA polymerase II, are upregulated during seed maturation and have reduced seed dormancy. Our data strongly suggests that RNAPII associated factors are required to maintain expression of dormancy genes in nuclei with reduced size towards the end of seed maturation.

In a second approach we study the role of epigenetic modifications, *i.e.* histone acetylation in the control of seed dormancy. Treatment with the HISTONE DEACETYLASE (HDA) inhibitor Trichostatin-A releases dormancy and *histone deacetylase9* knock-out mutants (*hda9*), but not of homologous HDAs, show reduced seed dormancy. Using Chromatin-Imunoprecipitation (ChIP) we are identifying HDA9-target genes to characterize the mechanisms by which HDA9 controls seed dormancy.

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**464 The GATA-type Transcription Factors HAN-LIKE1 and HAN-LIKE2 are Required for Apical-Basal Pattern Formation During *Arabidopsis thaliana* Embryogenesis**

*Matthew Volny, Wolfgang Lukowitz*

**University of Georgia**

The GATA-type transcription factor HANABA TARANU (HAN) is a key regulator of transcriptional programs during *Arabidopsis thaliana* embryogenesis. Previous work has shown that HAN functions to maintain the inductive boundary between proembryo and suspensor cell lineages at which the root apical meristem (RAM) originates. Loss of HAN redirects auxin transport at the base of the

embryo causing an apical shift in the fate map. However, the mutants eventually recover to form complete seedlings. To better understand the contribution of HAN to early embryonic patterning we are assessing the functional contribution of closely related GATA transcription factors in the HAN sub family. I have determined by both gene swap experiments and multiple mutant analysis that two closely related genes, HAN-LIKE1 (HANL1) and HAN-LIKE2 (HANL2) are biochemically equivalent to HAN but, by themselves, not necessary for normal embryogenesis. In contrast, triple mutant embryos (han hanl1 hanl2) are more severely affected than han single mutants, as they never recover from their early defects and arrest as oblong structures with abnormally enlarged cells along their periphery. No apical meristems are recognizable by anatomical criteria. I am in the process of analyzing mutant development with fluorescent reporter genes to determine whether the defects of han hanl1 hanl2 mutants in embryonic patterning are reflected in the loss of specific cell fates. In addition, I am characterizing the expression of HAN-like genes in the embryo. We hope that this work will contribute to understanding the transcriptional networks regulating pattern formation in the *A. thaliana* embryo.

#### **465 Trehalose 6-phosphate acts at the shoot apex to induce flowering in *Arabidopsis thaliana***

*Vanessa Wahl<sup>1,2</sup>, Jathish Ponnu<sup>2</sup>, Armin Schlereth<sup>1</sup>, Stéphanie Arrivault<sup>1</sup>, Annika Franke<sup>1</sup>, Regina Feil<sup>1</sup>, John Lunn<sup>1</sup>, Markus Schmid<sup>2</sup>, Mark Stitt<sup>1</sup>*

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The floral transition is a major developmental change in a plant's life cycle and is triggered by environmental and endogenous cues. Among the potential endogenous cues is sucrose, which could link flowering to the metabolic and energy status of the plant. However, the molecular mechanisms by which sucrose and other internal signals are integrated into the complex network of flowering time regulation are not understood. The level of trehalose 6-phosphate (T6P) is closely linked to the level of sucrose, which supports the hypothesis that T6P acts as a signal of sucrose status. *TREHALOSE 6-PHOSPHATE SYNTHASE 1 (TPS1)* is expressed in the shoot apical meristem. We found that T6P levels rise in the shoot apex over time, increasing more than two-fold during the floral transition in long day conditions as well as in short days. Plants over-expressing an artificial microRNA against *TPS1* flower much later than wild type plants. Further, increased *TPS1* expression in the stem cells alone is sufficient to induce precocious flowering, while reducing T6P content by overexpression of *TREHALOSE 6-PHOSPHATE PHOSPHATASE (TPP)* in the stem cells delays flowering. The miRNA156 and its target genes were recently found to integrate plant age into flowering time regulation. Our data show that sucrose availability, signalled via T6P, modulates the plant's competence to flower, and that the T6P signal is integrated into the miR156 node of the flowering network.

#### **466 Identification of Targets of the *Arabidopsis* B3 Domain Protein FUSCA3**

*Fangfang Wang, Sharyn Perry*

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Embryogenesis in higher plants encompasses the developmental processes by which the single-celled zygote proliferates, differentiates, and produces a mature, quiescent embryo. AGAMOUS-like (AGL15) is a MADS-domain transcription factor that promotes somatic embryogenesis by binding DNA and regulating gene expression. Global analysis of *Arabidopsis* AGL15 targets using a ChIP-chip approach and the Affymetrix tiling and expression arrays have identified *LEAFY COTYLEDON2 (LEC2)*, *FUSCA3 (FUS3)* and *ABA INSENSITIVE3 (ABI3)* as directly expressed targets, which encode B3 domain transcription factors that are key regulators of embryogenesis. *FUSCA3* is a member of the *LEC* gene family (*LEC1*, *LEC2*, *FUS3*), which have major effects on embryogenesis when defective, both at early and late stages of development. *ABI3* is not a member of the *LEC* family, but is a major regulator of programs during maturation. Cross- and auto-regulation have been demonstrated for the *LEC* genes and *ABI3*, both among these genes and in terms of the downstream programs that they control.

Here, our goal is to identify direct and indirect targets of *FUS3*, which includes mapping *in vivo* *FUS3* binding sites using ChIP-chip, assessing gene expression in response to *FUS3* using microarrays, and identification and prioritization of *FUS3* targets. We have obtained a stable embryonic culture (ECT) accumulating *FUS3:FUS3-cMYC* in the *fus3-3* mutant with *35S:AGL15*. We can detect *FUS3* protein accumulation by Western blot and are optimizing ChIP. We have generated transgenic plants with a *35S:FUS3-GR* transgene for microarray analysis and found that 9-10 day developing seeds of Col and *fus3-3* have most obvious changes in gene expression.

#### **467 RUG3 is a New Mediator of Auxin Response During Specific Developmental Processes**

*Magdalena Weingartner, Benjamin Weller, Norbert Sauer*

**Molecular Plant Physiology, University of Erlangen, Germany**

A gene coding for a so far unknown nuclear-localized protein, which we named RUG3 (REDUCED UNLOADING OF GFP), was identified in a genetic screen originally designed to identify mutants with reduced cell-to-cell movement of soluble GFP in post-phloem tissues of the root meristematic zone. The *rug3* mutation revealed to be a recessive loss of function mutation leading to several defects usually associated with altered auxin response such as short roots with a reduced number of meristematic cells, loss of quiescent center identity, and impaired formation of lateral roots. In addition, *rug3* mutants display a disorganized venation pattern in leaves. Using pRUG3::RUG3-GUS reporter lines, we show that the RUG3 protein specifically accumulates at sites of auxin maxima such as the apical root and shoot meristem, at lateral root initiation sites and in the vasculature of leaves. Ectopic expression of RUG3 leads to drastic shortening of inflorescence stems and severe defects in leaves where vascular tissue becomes progressively disorganized and loses functionality. We analyzed auxin response in the *rug3* mutant background using the auxin response reporter pDR5::GUS. pDR5::GUS staining was dramatically reduced in the vascular tissue of *rug3* leaves as compared to wildtype. In *rug3* roots pDR5::GUS activity was observed in the apical meristem but it was absent from xylem-pole pericycle cells, which show a strong pDr5::GUS response at lateral

root initiation sites in wild type plants. Collectively our data identified RUG3, which has no homology to any known plant protein, as a new factor critical for specific auxin dependent patterning events.

**468 The Vegetative Transcriptome of *Arabidopsis thaliana***

*Matthew Willmann<sup>1</sup>, Yeonjong Koo<sup>1</sup>, Kevin McCormick<sup>2</sup>, Blake Meyers<sup>2</sup>, R. Scott Poethig<sup>1</sup>*

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Plants grow continuously, and undergo numerous changes in their vegetative morphology and physiology during their life span. The molecular basis of these changes is largely unknown. To provide a more comprehensive picture of shoot development in *Arabidopsis*, microarray analysis and deep sequencing were used to profile the mRNA and small RNA content of shoot apices of different ages, as well as leaf primordia and fully-expanded leaves from 6 different positions on the shoot, in early-flowering and late-flowering genotypes. This extensive dataset provides a new and unexpectedly complex picture of shoot development in *Arabidopsis*. At any given time, the pattern of gene expression is different in every leaf on the shoot, and reflects the activity at least 6 developmental programs. Three of these are specific to individual leaves (leaf maturation, leaf aging, leaf senescence), two occur at the level of the shoot apex (vegetative phase change, floral induction), and one involves the entire shoot (shoot aging). Our results demonstrate that vegetative development is a much more dynamic process than previously imagined, and provide new insights into the underlying mechanism of this process.

**469 Mutations in the GW-Protein SUO Reveal a Developmental Function for MiRNA-mediated**

**Translational Repression in *Arabidopsis***

*Li Yang, R. Scott Poethig*

**University of Pennsylvania, Philadelphia, PA, USA**

Plant microRNAs (miRNAs) typically mediate RNA cleavage, but examples of miRNA-mediated translational repression have also been reported. The functional significance of this latter process for plant development is unclear. We identified SUO in a screen for *Arabidopsis* mutations that increase the expression of the miR156-regulated gene, SPL3. *suo* has a loss-of-function phenotype characteristic of plants with reduced AGO1 activity. An analysis of RNA and protein levels in *suo* mutants demonstrated that this phenotype is a consequence of a defect in miRNA-mediated translational repression; the effect of *suo* on vegetative phase change is specifically attributable to a reduction in miR156/miR157 activity. SUO encodes a large protein with N-terminal BAH and TSF2N domains and two C-terminal GW repeats. SUO is present in the nucleus, and co-localizes with the Processing-body (P-body) component DCP1 in the cytoplasm. Our results suggest that SUO is a functional homolog of the translational repressor GW182, and demonstrate that translational repression is important for the biological function of miRNAs in plants.

**470 A Gene Encoding an Auxin Receptor *TIR1* is a Direct Target of the MADS-domain Protein *AGL15* and Impacts on *Arabidopsis* Somatic Embryogenesis**

*Qiaolin Zheng, Yumei Zheng, Whitney Burnie, Sharyn Perry*

**University of Kentucky, Lexington (KY), USA**

Many of the regulatory processes occurring during higher plant embryo development are still unknown. *AGL15* (for AGAMOUS-like 15) is a member of the plant MADS domain family of transcriptional regulators that preferentially accumulates during embryo development. To better understand *AGL15*'s role in promotion of somatic embryogenesis, direct target genes were identified by ChIP-chip and expression arrays. One potential directly down-regulated target is the gene encoding the auxin receptor *TIR1*, an F-box protein that mediates Aux/IAA degradation and the consequent ARF activation. Enrichment tests and qPCR on ChIP experiments were used to verify that *TIR1* is a direct target of *AGL15*. The results of expression arrays hybridized with probe generated from Col wild type tissue, *35Spro:AGL15* or *agl15 agl18* double mutant showed that *TIR1* is down-regulated by *AGL15* in a shoot apical meristem somatic embryo (SAM SE) system. Additionally, qRT-PCR indicates that the transcripts of *TIR1* increased in the *agl15 agl18* double mutant in developing seeds. A knockout allele of *tir1* increased the occurrence of somatic embryos in SAM SE. Meanwhile, treatments of both AVG (an inhibitor of ethylene biosynthesis) and GA significantly decreased the frequency of SAM SE, which suggests that *TIR1*-mediated pathways might interact with other hormones, such as ethylene and GA, in *Arabidopsis* embryogenesis.

**471 Mobile Transcription Factors AHL3/4 Regulate Xylem Development**

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One major question in biology is how different cell types are organized and specified during the multicellular organ development. In plants, this process is thought to be determined primarily by positional information. Local cell communication between cells provides the fine-tuned positional information through signal exchange, by which specifies cell types and defines boundaries between them.

To understand the pattern formation mediated by cell-cell communication, we study *Arabidopsis* root vascular tissues as model system. Plant vascular system consists of two specialized tissues, xylem and phloem. These tissues are generated from a group of stem cells, procambium/cambium. Xylem, phloem, and procambium/cambium together form a highly organized structure. Studies including ours showed that such patterning occurs long before each tissue matures, by determining cell fates very early in the root meristem. During this process, transcription factors (TFs) which are commonly studied in the context of genetic regulatory networks have been underestimated as intracellular signals for the boundary definition. To gain a better understanding of how TFs regulate patterning events,

we characterized a list of TFs enriched in xylem initial cells based on genome-wide expression profiling data in root. While screening their mutant phenotype, we found two closely related AT-hook family TFs AHL3/4 whose knockout mutants form ectopic xylem in the root. Our results indicated that these two proteins physically and functionally interact with each other. Interestingly, a loss-of-function mutant changed the boundary between xylem and procambium which is very likely to be the reason for the formation of ectopic xylem. In addition, AHL3 moves from procambium cells to xylem precursors in the root. Current efforts have been put to find the link between their mobility and the functionality in defining the cell type boundaries.

Our findings will provide new insights into the importance of the cell-cell movement of TFs as positional information in the vascular tissue patterning.

**472 XAP5 CIRCADIAN TIME KEEPER (XCT): A Global Player in Plant Growth, Development, and Stress****Signaling?***Shajahan Anver<sup>1</sup>, Assen Roguev<sup>2</sup>, Nevan Krogan<sup>2</sup>, Stacey Harmer<sup>1</sup>*<sup>1</sup>**Dept. of Plant Biology, UC Davis, <sup>2</sup>Department of Cellular and Molecular Pharmacology, UCSF**

To uncover new components of the *Arabidopsis thaliana* circadian system, seeds were mutagenized with EMS and plants were screened for altered free-running rhythms. One short period mutant identified in this screen has an alteration with in the *XAP5 CIRCADIAN TIME KEEPER (XCT)* gene, resulting in both circadian clock and photomorphogenic phenotypes. *xct* mutants also have a subtle early flowering phenotype in long days. The XCT protein is nuclear localized and is conserved in many eukaryotes, including *Schizosaccharomyces pombe*. However the function of XCT is not known in any organism. *S. pombe* *Δxap5* (*XCT* ortholog) mutant has a temperature dependent slow growth phenotype which is rescued by the *Arabidopsis XCT* gene expressed under the control of the yeast *nmt1* promoter. In turn, yeast *xap5<sup>+</sup>* gene expressed under the control of the *Arabidopsis XCT* promoter rescues the photomorphogenic and flowering phenotypes of *Arabidopsis xct-2* mutants. However, the *S. pombe* gene did not rescue the *xct-2* circadian phenotype. These results indicate that *XCT* orthologs are functionally conserved between *S. pombe* and *A. thaliana*. We are therefore using *S. pombe* and *A. thaliana* model systems to characterize the biochemical function of XCT orthologs in eukaryotes. High throughput genetic interaction studies in *S. pombe* suggest a possible link between *xap5<sup>+</sup>* and chromatin remodeling, which is also indicated by co-expression analysis of *XCT* using publicly available microarray data. We also are identifying proteins that physically interact with Xap5 and XCT using tandem mass spectrometry. Both genetic and physical interaction analyses suggest an involvement of *xap5<sup>+</sup>* and *XCT* in stress response pathways. These two potential functions of *XCT* and *xap5<sup>+</sup>*, in chromatin remodeling and stress signaling, could be independent or interdependent.

**473 Poly(A) in the 5' Untranslated Regions of a Large Family of *Arabidopsis* mRNA Suggests a Broad Role for Cap Independent Translation in Plant Stress Responses***Raymond Moore, Kim Mogen, Scott Ballantyne***University of Wisconsin, River Falls, USA**

Most eukaryotic mRNA have a 3' poly(A) tail. In animals, changes in poly(A) tail length control such diverse processes as germ cell formation, embryo development, learning and memory, and stem cell maintenance. We are using *Arabidopsis thaliana* to study the biological roles of poly(A) mediated mRNA regulation in plants. Here, we report that poly(A) sequences are present in the 5'untranslated regions (UTR) of many *Arabidopsis* mRNA. We propose that this signature represents a conserved, cis-acting, mRNA regulatory element that is important for plant adaptation.

We analyzed the distribution of 15-20 nucleotide homo-polymers in the *Arabidopsis* genome. Poly(A) occurs over ten times more frequently in transcribed DNA relative to intergenic sequences, and poly(A) is the preferred homo-polymer in transcripts. We show that poly(A) occurs more frequently in 5'UTRs than other portions of the transcript and identify over 100 *Arabidopsis* mRNA with this feature. Many of these mRNA encode regulatory proteins with known roles in plant stress responses. Gene ontology comparisons between this family and the total proteome reveal a significant over-representation of protein kinases and proteins that localize to the cell periphery.

Imperfect poly(A) stretches were first reported in the 5' UTR of poly(A) binding protein (PAB) mRNA, where they function as part of an autoregulatory negative feedback loop that controls PAB protein levels (1). More recently, poly(A) rich sequences in the 5' UTR of two yeast mRNA were implicated in the switch to cap-independent translation that occurs when yeast respond to nutritional stress (2). Our findings suggest that this process is conserved and expanded in plants.

(1) De Melo Neto, O.P., 1995 Nucl. Acids Res. 23: 2198-2205. (2) Gilbert, W. et al., 2007 Science 317: 1224-1227.

**474 High Resolution Profiling of Small RNAs in *Arabidopsis thaliana* Roots***Natalie Breakfield, Philip Benfey***Duke University, Durham, NC, USA**

Since plants cannot move if conditions are poor, they rely on their roots to explore the local environment and scavenge needed nutrients and water. Root development is controlled through the regulation of gene expression by transcription factors, but it is increasingly apparent that this development is fine tuned through the action of one variety of small RNAs, called microRNAs (miRNAs). Attempts to identify the small RNA populations have been complicated by the presence of multiple cell types and developmental stages within a tissue. The intersection of two technologies, namely cell sorting and high throughput DNA sequencing, revealed small RNA populations in individual cell types. We used Illumina sequencing technology to query the small RNA species in sorted populations of specific cell types and hand dissected developmental zones, and analyzed the expression of miRNAs. Most known miRNAs showed differential expression in the cell types and/or developmental zones, and comparisons with expression of target genes identified regions where miRNAs are likely to be playing an important role. Many novel miRNAs have been identified and experiments are ongoing to validate their predicted targets and functions. Future work will focus on elucidating the functions of these known and novel miRNAs in the regulation of root development.

**475 Histone Methylation Associated with Changes in Gene Expression During Senescence***Judy Brusslan<sup>1</sup>, Ana Rus-Alvarez<sup>1</sup>, Judd Rice<sup>2</sup>, Michael Hitchler<sup>3</sup>, Matteo Pellegrini<sup>4</sup>*<sup>1</sup>**California State University Long Beach, Long Beach (CA), USA, <sup>2</sup>USC, Los Angeles, CA, USA, <sup>3</sup>Kaiser Permanente, Los Angeles, CA, USA, <sup>4</sup>UCLA, Los Angeles, CA, USA**

Major changes in gene expression accompany leaf senescence. In an effort to understand how epigenetic mechanisms contribute to these gene expression changes, two histone modifications were measured on a genome-wide basis using ChIP-Seq. Nuclei were isolated

from fully-expanded green leaves from 1) non-senescence plants that were 23 days old and 2) senescent plants that were 52 days old. Antibodies that recognize H3K4me3, a histone methylation mark associated with gene activation, or H3K27me3, a histone methylation mark generally associated with gene silencing, were used for chromatin immunoprecipitation. Input DNA sequences were found to be highly similar to general histone H3 sequences, and were used for background subtraction. Significant peaks in the two samples were compared over 1 kb regions that surrounded transcription start sites in order to quantitatively compare histone marks between the two samples. An increase in H3K4me3 was found in many senescence up-regulated genes, although a smaller number showed the opposite trend. Surprisingly, *SAG12* (At5g45890), the standard gene expression marker for senescence, had no H3K4me3 in either mature or senescent leaves despite a 30,000-fold increase in mRNA levels. *SAG12* did, however, show a decrease in H3K27me3 in senescent leaves. 40 senescence down-regulated genes were also analyzed, and a strong correlation between decreased gene expression and reduced H3K4me3 was observed in 27/40 genes, while no correlation to changes in H3K27me3 could be established. Our results suggest that a reduction in H3K4me3 for genes that are down-regulated as leaves progress into senescence may be an important general mechanism for the final stage of leaf development.

#### **476 Transcriptome Profiling Indicates The Existence Of Post-Transcriptional Control In Response To Abscisic Acid And Glucose In *Arabidopsis thaliana***

*Gustavo Duarte<sup>1</sup>, Cleverson Matiolli<sup>1</sup>, Delphine Gey<sup>2</sup>, Sandra Pelletier<sup>2</sup>, Jean-Pierre Renou<sup>2</sup>, Renato Vicentini<sup>1</sup>, Michel Vincentz<sup>1</sup>*

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Abscisic acid (ABA) is one of the major plant hormones involved in abiotic stresses responses. Moreover, ABA interacts with sugars to adjust plant development and growth. Sugars are essential energy sources and also act as signaling molecules controlling gene expression. Evidences for the existence of post-transcriptional control in response to ABA and glucose stimuli have been reported. More specifically, we have found that a synergistic repression in the expression of *AtbZIP63* by the combination of glucose and ABA may be partly explained by a post-transcription control. In order to unravel the importance of ABA- or glucose-mediated mRNA stability control, we defined the RNA profile of *Arabidopsis thaliana* in response to these signals after transcription inhibition. An experimental model which optimizes the conditions for transcription inhibition was established and used for transcriptome profiling with CATMA microarrays. A total of 962 genes were found to be differentially expressed after the treatments, suggesting a possible post-transcriptional control in 204 genes in response to glucose, 245 to ABA and 512 to the combination glucose + ABA. The genes were classified by their functions according to Gene Ontology, suggesting a close relation with adaptative response to stress conditions. ABA- and glucose-mediated control of mRNA stability follows two opposite strategies which are likely related to the regulatory needs of these signals.

#### **477 Intraspecific *Arabidopsis* Hybrids Have Altered Levels Of sRNA and DNA Methylation**

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Hybrid vigour, or heterosis, is the superior performance of F1 heterozygous progeny in a number of traits such as growth, seed yield and resistance to pests and diseases. Crop species such as canola, maize, wheat and rice rely heavily on hybrid seed to enhance productivity. While extensively used in agriculture, the mechanism(s) underlying hybrid vigour is poorly understood. One possible mechanism contributing to hybrid vigour is the alteration of epigenetic systems as a consequence of "genomic shock" following the hybridization of two different genomes. To test this we have used next generation sequencing to study the siRNA profiles and methylomes of intraspecific hybrids between *Arabidopsis thaliana* accessions C24 and Landsberg erecta. We show that both reciprocal hybrids show a decrease in 24nt siRNAs when parental expression levels of the siRNAs are markedly different. Similarly we show significant amounts of non-additive DNA methylation in the hybrids at loci which have markedly different parental methylation levels. The pattern of non-additive DNA methylation is dependent on the cytosine sequence context, highlighting that different mechanisms and pathways that set up the epigenetic effects. We suggest that such epigenetic differences may alter the levels and patterns of gene activity which in turn contribute to hybrid vigour. The epigenetic diversity between ecotypes may provide increased allelic (epi-allelic) variability contributing to heterosis.

#### **478 The Histone Acetyltransferase GCN5 Affects Trichome Patterning**

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In this genomics era, a fundamental challenge that remains is to discern how gene action is regulated to promote precise development of form and function. While it is established that chromatin structure helps to ensure gene expression in appropriate temporal and spatial patterns, questions remain about how this occurs genome-wide and why specific chromatin modifiers are required for certain developmental pathways. We have chosen to begin to address these questions by initiating research aimed at identifying targets of GCN5 that play a role in floral and trichome development in *Arabidopsis thaliana*. GCN5 is a histone acetyltransferase that has been shown to participate in regulating developmental gene expression in several metazoan species. In *Arabidopsis*, plants with T-DNA insertions in *GCN5* (also known as *HAG1*) display a variety of pleiotropic effects including dwarfism, loss of apical dominance, and floral defects affecting fertility. Previously we demonstrated that GCN5 targets genes involved in *Arabidopsis* floral development. Our most recent work employs scanning electron microscopy to provide a detailed characterization of rosette leaf trichomes, single-cell epidermal structures that exhibit a unique developmental pathway controlled by a well-characterized set of genes. Our initial results demonstrate that *gcn5-1/hag1-1* mutants display

a reduced number of trichome branches, leading us to hypothesize that GCN5 impacts the expression of genes important in the initiation of trichome branch formation. Our current work will extend these results by examining a second allele (*hag1-6*) and assessing stalk and branch dimensions, branching angles, and formation of papillae. The resulting morphological data coupled with a rich genetic literature should permit us to identify specific GCN5 target genes involved in this developmental transition.

**479 An siRNA Pathway Controls Transposition in Plants Subjected to Stress**

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Transposon is a major genomic component of many organisms and is an important factor for genome evolution. However we still poorly understand what controls the transgenerational transpositions. We report that a copia-type retrotransposon ONSEN ("hot spring" in Japanese) was activated with heat stress in *Arabidopsis thaliana*. ONSEN became not only transcriptional active but also synthesize their extrachromosomal DNA copies. It was found that high frequency of retrotransposition in the progeny of stressed siRNA mutant plants indicating that siRNAs were required to control the transposition of an active transposon. It was surprising that the memory of stress applied to seedlings has been maintained throughout the entire plant development allowing ONSEN to transpose during change of plant generations. Our result suggested the new mechanism of maintenance of active retrotransposon and also demonstrated the epigenetic control of retrotransposition as post-transcriptional machinery.

**480 The AtJmj12 encoding JmjC Domain-Containing Protein Represses the Expression of FLC in Arabidopsis**

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Histone modifications often impose an epigenetic layer of regulation on genes that affect diverse biological processes. Methylation of histone is one of the important histone modifications and diverse histone methyltransferases and demethylases are involved in the regulation of gene expression. Jumonji C (JmjC) domain-containing proteins have been reported as histone demethylases and among twenty-one predicted members in *Arabidopsis*, only a few members were characterized. Here, we show that mutation of *Arabidopsis thaliana* *Jumonji12* (*AtJmj12*) causes late flowering phenotype both in long-day and short-day conditions. The mutants showed increased expression of *Flowering Locus C* (*FLC*), the floral suppressor. *flc* mutant suppressed the late flowering phenotype of the *atJmj12* mutant, indicating that *AtJmj12* regulates flowering time through *FLC*. *atJmj12* mutant responded vernalization, suggesting that *AtJmj12* is a member of the autonomous pathway. The double mutant between *atJmj12* and *relative of early flowering 6* (*ref6*), a JmjC domain containing-protein that represses *FLC* expression, was substantially late-flowering when compared to the single mutants. Correspondingly, the *FLC* expression level was additively increased in the double mutant. Chromatin immunoprecipitation result suggested that *AtJmj12* and *REF6* repress *FLC* expression through distinct mechanisms.

**481 Arabidopsis RbAp46/48-Like Proteins Associate with a Histone Deacetylase to Act Redundantly in Chromatin Silencing**

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RNA molecules such as small-interfering RNAs (siRNAs) and antisense RNAs (asRNAs) trigger chromatin silencing of target loci. In the model plant *Arabidopsis*, RNA-mediated Chromatin Silencing (RmCS) involves repressive histone modifications including histone deacetylation, histone H3 lysine-9 methylation and H3 lysine-27 methylation. Here, we report that two *Arabidopsis* homologs of the human histone-binding proteins RbAp46/48, function in partial redundancy in transcriptional silencing of RmCS target loci. We show that these two genes acts in partial redundancy to silence *FLOWERING LOCUS C* (*FLC*) which is a crucial floral repressor subject to RmCS, *FLC* homologs, and other loci including transposable and repetitive elements which are targets of siRNA-directed DNA Methylation (RdDM). In addition, we found that these two proteins can bind histones and associate with histone modifiers to form co-repressor-like complexes. Our findings indicate that these two genes play an important role in RmCS in plants.

**482 Cytokine pathway plays as cross node of IKU genetic controlling and epigenetic regulation in endosperm growth**

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In flowering plants, the seed is produced by a double fertilization event, and is composed of the endosperm and the embryo, surrounded by maternal integuments. The endosperm controls the supply of maternal nutrients to the embryo and plays a central role in embryo growth and seed size determination. Endosperm growth is controlled genetically by the IKU pathway (Garcia et al., 2003) and by the activity of imprinted genes including genes encoding subunit of the chromatin modifier Polycomb Group FIS (Berger and Chaudhury, 2009).

We found that cytokinin synthesis and degradation are localized at opposite poles in endosperm. The cytokine degradation pathway is under the control of the IKU pathway and the Polycomb Group FIS. Our data show the cytokinin pathway could play central role in controlling the morphogen gradient in endosperm and decide the final seed size. In addition to metabolic genes of cytokinin pathway, by transcription profiling analysis combined with bioinformatics analysis we found that a group of genes might share similar characters controlled by both IKU pathway and epigenetic regulation. Further efforts are needed to identify the functions of these group genes and their relationship with phytohormone, cytokinin.

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### 483 Interactions Between TOPLESS and Histone-Modifying Enzymes

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The transcriptional co-repressor TOPLESS (TPL) is required for maintaining apical-basal polarity during *Arabidopsis* embryogenesis. Similar to co-repressors in other organisms, such as Groucho in flies, TPL requires additional factors for its repressive function, including histone-modifying enzymes. Genetic interactions have shown a role for both a histone acetyltransferase and a histone deacetylase in TPL-dependent regulation. Recently, we have also identified a jumonji domain-containing protein, Jumonji8 (JMJ8), as a TPL-interacting partner. JMJ8 is a putative histone demethylase, and, based on homology to proteins in other organisms, it is predicted to be a transcriptional repressor. There are 21 jmj domain-containing proteins in *Arabidopsis*, and JMJ8 belongs to a small clade containing four highly similar members. JMJ8 has been shown to interact with TPL in a yeast-two-hybrid system. Mutation of JMJ8 enhances the *tpl-1* double root phenotype, suggesting that this interaction is biologically relevant. We confirmed that JMJ8 can repress transcription in an *in planta* repression assay, and we are currently testing if this repression requires histone demethylase catalytic activity and/or interaction with TPL.

### 484 Decapping Proteins Are Involved in miRNA Pathway in *Arabidopsis thaliana*

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Decapping enzyme 1 (DCP1), Decapping enzyme 2 (DCP2), and Varicose (VCS) are the components of a decapping complex that is necessary to remove m<sup>7</sup>GDP from the 5' end of mRNA in plants. It has been shown that the DCP1, DCP2, and Heds/Ge-1, a human homolog of VCS, are involved in an miRNA-mediated translational repression in animals, whereas the roles of decapping proteins in the miRNA pathway in plants are unclear. Here, we demonstrated that the decapping proteins were important for the accumulation of miRNAs in *A. thaliana*. The amount of miR158, miR161, miR162, miR164, miR166 and miR167 decreased in *dcp1*, *dcp2* and *vcs* mutants. The decrease of the miRNAs was not due to the seedling lethal phenotype in the mutants. Our results indicate that decapping proteins are involved in the biogenesis and/or the stability of the miRNAs in *A. thaliana* and suggest that the decrease of the miRNAs in the *dcp1*, *dcp2*, and *vcs* mutants may cause the seedling lethal phenotype.

### 485 Cytosolic Electron Transfer Component-like Protein Deficiency Impaired Expression of Imprinted Gene *FWA* in the Endosperm

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In *Arabidopsis* fertilization, one sperm fertilizes the egg cell, and the other fuses with the central cell derived from female gametophyte, producing both the diploid embryo and the triploid endosperm. Endosperm tissue is essential for healthy embryo growth. It has been known that endosperm has a unique transcriptional feature that is parent-origin-specific expression referred as imprinting. *FWA* is an imprinted gene and expresses specifically in the endosperm. The *FWA* gene has the heavily methylated transcriptional start site and is transcriptionally silent in vegetative tissues. However, the mechanism underlying this endosperm-specific transcription remains unknown. To address this, we screened a mutagenized population of the *FWA* promoter-GFP transgenic plants for mutants that defect in the expression of the transgene in the endosperm. The identified mutant was designed as *alarm clock for FWA imprinting (alac)*, because activation of sleeping *FWA* was impaired. In the *alac2* mutant, about half of ovules in siliques showed decreased expression of the endogenous *FWA* and other imprinted *FIS2* gene but not the imprinted *MEA* gene. The *ALAC2* gene encodes a homolog of the yeast protein that is reported to be involved in cytosolic electron transfer system. A mutant of the other component in the same pathway also showed impaired *FWA*-GFP expression in the endosperm. These data suggest that the electron transfer system plays important roles in the expression mechanism of the endosperm-specific genes. We will discuss a possible link between electron transfer system and the *alac2* phenotypes.

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### 486 Initiation and Maintenance of Epigenetic Transposable Element Silencing in *Arabidopsis*

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Eukaryotic organisms defend the stability and integrity of their genome by suppressing the activity of transposable elements (TEs). In the plant, fungal and animal kingdoms, slightly different classes of small RNAs (sRNAs) target repressive chromatin modifications, such

as DNA and histone tail methylation to TEs. The activity of these sRNAs renders the TE chromatin compacted and silenced. However, each cell and subsequent daughter generation does not determine "de novo" which sequences are TEs and should be silenced. Rather, this information is passed down from the progenitor cell or generation in a process termed *epigenetic inheritance*. The objective of our laboratory is to discover and understand how TE silencing is initiated and maintained across generations, as well as to characterize new forms of epigenetic gene regulation that have evolved from the evolutionarily arms race between TEs and the host genome. We have categorized where and when the epigenetic regulation of TEs are lost in the reference plant *Arabidopsis*: in mutants that lose symmetrical DNA methylation, long-term cultured cells, and nurse cells. In this last category, wild-type plants have a programmed activation of TEs in nurse cell types that are adjacent to much more important cells, such as gametes or meiocytes. Research projects in our laboratory focus on how TE silencing is initiated, the role of nurse cells in the propagation of epigenetic inheritance, and how TEs can regulate non-TE gene expression.

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#### 487 Genomes, Transcriptomes, Methylomes and smRNAomes of the *Arabidopsis* Accessions Col-0, Cvi-0 and Ler-1

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In collaboration with Roche 454, we generated a de novo assembly of a Cape Verde Island (Cvi-0) accession. A comparison to Col-0 shows greater than 3% sequence difference between these accessions in the form of SNPs, indels, and larger structural variations. Using the Cvi-0 assembly as a reference, we have mapped the Cvi-0 transcriptome, methylome and smRNAome. A comparison of methylomes between Cvi-0, Col-0 and the Landsberg *erecta* (Ler-0) accession reveals that Cvi-0 lacks half the methylation of the other accessions primarily due to a decrease in the methylated cytosines at CG dinucleotide (mCG). The highest mCG conservation is found immediately upstream of the transcriptional start site suggesting a possible role in gene regulation. While there is significant conservation of smRNA loci between the accessions, hundreds of accession-specific epialleles have been identified, some of which silence expression and are reversible in the DNA methyltransferase mutant, *met1-1*. Many epialleles can be explained by transposition events, and direct and inverted repeats of genes. About a third of epialleles are found to occur in closely related gene families suggesting a role for RNA directed DNA methylation occurring in *trans*.

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#### 488 Divergent Roles For the Two PolI-like Organelle DNA Polymerases of *Arabidopsis thaliana*

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DNA polymerases play a central role in the process of DNA replication. Yet, the proteins in charge of the replication of plant organelle DNA have not been unambiguously identified. There are however many indications that a family of proteins homologous to bacterial DNA polymerase I is implicated in organelle DNA replication. Here, we have isolated mutant lines of the *PollA* and *PolIB* genes of *Arabidopsis thaliana* to test this hypothesis. We find that mutation of both genes is lethal, thus confirming an essential and redundant role for these two proteins. However, the mutation of a single gene is sufficient to cause a reduction in the levels of DNA in both mitochondria and plastids. We also demonstrate that *polIb*, but not *polla* mutant lines, are hypersensitive to ciprofloxacin, a small molecule that specifically induces DNA double-strand breaks in plant organelles, suggesting a function for PolIB in DNA repair. In agreement with this result, a cross between *polIb* and a plastid *Whirly* mutant line yielded plants with high levels of DNA rearrangements and severe growth defects, indicating impairments in plastid DNA repair pathways. Taken together, this work provides further evidences for the involvement of the plant *Poll-like* genes in organelle DNA replication and suggests an additional role for PolIB in DNA repair.

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#### 489 Genetic Analysis of *Arabidopsis phyA'* Epiallele – A case of Transcriptional Silencing Associated with Exonic Methylation

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DNA methylation is a major epigenetic mark of gene silencing in eukaryotes. Methylation of cytosines in CG contexts and its association with transcriptional silencing is common to plants and vertebrates. However, the role CG methylation in gene regulation is not well understood. We isolated an epi-allele of *A. thaliana* Phytochrome A gene termed *phyA'*, which contains methylation in CG sites resident to transcribed region, and is transcriptionally suppressed. These exonic modifications confer a strong *phyA* mutant phenotype, characterized by elongated hypocotyls in seedlings grown under continuous far-red light (FRc). Demethylation of *phyA'* in the DNA methyltransferase I mutant (*met1*) background resulted in restoration of the WT expression level and phenotype, confirming the pivotal role of the mCG in *phyA'* silencing. Genetic analysis revealed that a number of chromatin modification and RNAi genes have no significant role in *phyA'* silencing. This analysis covered DNA methylation genes (*CMT3* and *DRMs*), histone methylation gene (*KYP*), RNAi genes (*RDR2*, *RDR6*, *AGO1*, *AGO4* and *SGS3*), and chromatin modification factors (*DDM1* and *MOM1*). To identify the novel genes involved in *phyA'* silencing we took the approach of suppressor screening. Seeds of *phyA'* epimutant were mutagenized by EMS, and M2 populations were screened for the WT phenotype. Phenotypic screening of M2 populations resulted in detection of the suppressor-mutation, *sps-1*. Molecular and genetic analysis of *sps-1* revealed that *sps-1*, a second-site mutation reactivates *phyA* locus in spite of *phyA'* hypermethylation. Ongoing work on further characterization of *sps-1* line will be presented.

**490 Characterization of the microRNA miR396 regulatory network in plants.***Ramiro Rodriguez, Juan Debernardi, Martin Mecchia, Javier Palatnik***IBR, Rosario, Argentina**

Leaf primordia are initiated by recruiting cells from the peripheral zone of the shoot apical meristem. Then, extensive mitosis proceeds until cells begin to enlarge. This process is regulated by the concerted action of various transcription factor networks, among them the *GROWTH REGULATING FACTORS* (*GRFs*). Mir396 regulates seven out of the nine transcription factors of the *GRF* family in Arabidopsis. Overexpression of miR396 leads to downregulation of *GRFs* and reduced leaf cell number. Conversely, high levels of *GRF2* activity increases cell proliferation in leaves. Using different reporter constructs, we determined that miR396 is essential to establish a dynamic spatio-temporal pattern of *GRF2* expression, which in turn coincides with the activity of cell proliferation markers. Analysis of miR396 and its targets in various plants species revealed the existence of novel miRNA-target pairs. First, particular miR396 variants are expressed only in certain species. Expression of these versions in Arabidopsis indicated that they are stronger repressors of the *GRFs* than the Arabidopsis miR396 variants. Second, a search for novel miR396 targets revealed that a basic-helix-loop-helix transcription factor is regulated only in a group of *Brassicaceae*. The importance of miR396-mediated repression of this *bHLH* for normal Arabidopsis development will be discussed.

**491 Abstract Withdrawn****492 In Association with AGAMOUS or Polycomb Group Proteins, KNU Promoter Status Determines the Timing of Arabidopsis Floral Stem Cell Termination***Bo Sun, Zemiao He, Toshiro Ito***Temasek Life Sciences Laboratory**

Flowers among the same species normally have similar sizes and fixed number of floral organs. This is contributed by the precise regulation of floral meristems. In Arabidopsis, the floral meristem is first maintained to generate four different types of floral organ primordia with fixed numbers. While after the rising of carpel primordia, the floral meristem is rapidly terminated. The precisely timed-repression of the homeobox gene *WUSCHEL* (*WUS*) by the floral homeotic protein AGAMOUS (AG) plays an essential role in this process.

We have shown that *KNUCKLES* (*KNU*), which encodes a C2H2-type zinc finger protein with a C-terminal EAR-like repression motif, mediates the repression of *WUS* by AG for the floral meristem determinacy control. AG directly binds *KNU* promoter and induces the transcription of *KNU*. In turn, *KNU* represses *WUS* transcription to abolish stem cell activity.

The timing of *KNU* induction is the key in balancing proliferation and differentiation in flower development. Delayed *KNU* expression results in an indeterminate meristem, whereas ectopic *KNU* expression prematurely terminates the floral meristem. Furthermore, *KNU* induction by AG is preceded by changes in repressive histone modification at the *KNU* locus, which occurs in an AG-dependent manner.

Interestingly, TERMINAL FLOWER2 (TFL2), a Polycomb Group protein which functions to maintain the repressive mark H3K27me3, also associates with *KNU* promoter at the same promoter region where AG binds. This result leads to our hypothesis of a competition model that the landing of AG may inhibit the association of TFL2 on *KNU* locus, thus release *KNU* from a silent status.

This competition model is supported with our several experiments. First, insertion of a 6-bp NdeI restriction site in this region leads to strong ectopic expression of *KNU*, suggesting that binding of TFL2 requires a PRE-like motif. Second, artificial *KNU* promoter bound by LhG4 protein at the same region leads to *KNU* ectopic expression as well. We also notice that *KNU* timing is cell-cycle dependent. With altered cell cycle length by treating the plants with cell cycle inhibitors or accelerators, obvious changes of *KNU* timing are observed. With these evidence, we will discuss a new mechanism how AG controls histone modification.

**493 Characterization of a Novel Mutation in the PHD Finger Implicates HSI2 in Chromatin-mediated Epigenetic Repression of Seed-specific Genes in Arabidopsis Seedlings***Vijaykumar Veerappan<sup>1</sup>, Jing Wang<sup>2</sup>, Huazhong Shi<sup>3</sup>, Randy Allen<sup>1</sup>*

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In *Arabidopsis*, two related B3 domain transcriptional repressors high-level expression of sugar-inducible gene 2 (HSI2) and HSI2-LIKE 1 (HSL1) function redundantly to repress key transcriptional regulators of seed maturation genes during seed germination and seedling development. However, the molecular mechanism by which HSI2 and HSL1 mediate transcriptional repression is unknown. We isolated a novel mutant *hsii2-10* harboring a point mutation that disrupts the plant homeodomain (PHD) of HSI2 by map-based cloning of an *Arabidopsis* mutant showing constitutively elevated *GSTF8-LUC* transgene expression. The *hsii2-10* mutant shows constitutively elevated luminescence without any inducing treatments. Interestingly, in the *hsii2-10* mutant, the luciferase (*LUC*) transgene transcripts are up-regulated whereas the endogenous *GSTF8* transcripts are not affected. Microarray analysis of *hsii2-10* and *hsii2-10/hsl1* mutants indicate that HSI2 PHD domain represses seed maturation genes during seedling development in an unequally redundant manner. In *hsii2-10* mutant seedlings, 20% of the up-regulated genes are seed-specific. Seed-specific genes up-regulated in *hsii2-10* and *hsii2-10/hsl1* mutants include well-known seed maturation genes such as those that encode cupin proteins, late embryogenesis abundant proteins, oleosins, 12S globulins and 2S albumins. Several HSI2 PHD domain target genes are also affected in *pkl* and *fie* mutants. Recently, PKL and FIE were reported to be involved in the deposition of trimethylation marks on the histone H3 at lysine 27 residues (H3K27me3) of the

target gene chromatin. Based on the characterization of a novel mutation in the PHD domain of HSI2 protein, we hypothesize that HSI2 represses a subset of seed-specific genes during seed germination and seedling development by chromatin-based epigenetic mechanisms.

**494 The use of high-throughput sequencing technologies to identify the substrates of RNA-dependent RNA polymerases in Arabidopsis.**

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One recently identified mechanism that regulates mRNA abundance is RNA silencing, and pioneering work in *Arabidopsis thaliana* and other genetic model organisms helped define this process. RNA silencing pathways are triggered by either self-complementary fold-back structures or the production of double-stranded RNA (dsRNA) that gives rise to small RNAs (smRNAs) known as microRNAs (miRNAs) or small-interfering RNAs (siRNAs). These smRNAs direct sequence-specific regulation of various gene transcripts, repetitive sequences, viruses, and mobile elements via RNA cleavage, translational inhibition, or transcriptional silencing through DNA methylation and subsequent heterochromatin formation. Early genetic screens in Arabidopsis were instrumental in uncovering numerous proteins required for these important regulatory pathways. Among the factors identified by these studies were RNA-dependent RNA polymerases (RDRs), which are proteins that synthesize siRNA-producing dsRNA molecules using a single-stranded RNA (ssRNA) molecule as a template. Recently, a growing body of evidence has implicated RDR-dependent RNA silencing in many different aspects of plant biology ranging from reproductive development to pathogen resistance. Here, we focus on the specific functions of the six Arabidopsis RDRs in RNA silencing, their ssRNA substrates and resulting RDR-dependent smRNAs, and the numerous biological functions of these proteins in plant development and stress responses.

**495 An Arabidopsis AT-hook motif protein is required for silencing of transposable elements and the developmental transition to flowering**

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Transposable elements (TEs) are silenced by epigenetic mechanisms. Here we show that knockdown of the AT-hook DNA binding protein TRANSPOSSABLE ELEMENT KILLER (TEK) results in robust activation of *FLOWERING LOCUS C* (*FLC*) that contains a TE in the Arabidopsis Landsberg *erecta* (*Ler*) background. The derepression is associated with chromatin conformation change, reduction of the silencing mark histone H3K9 dimethylation and increased levels of histone acetylation, even causing TE jumping. TEK directly binds to an *FLC*-repressive region and furthermore interacts with FVE which participates in histone deacetylation complexes. Microarray analysis shows robust derepression of various TEs, suggesting that TEK maintains genome stability partly through histone deacetylation.

**496 Multiple cis-elements Regulate The Vernalization-induced Expression of VERNALIZATION INSENSITIVE 3**

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Vernalization, a long term cold inducing acceleration of flowering, is one of the major environmental factors determining flowering time in plants. In *Arabidopsis*, mitotically stable repression of floral repressor, *FLOWERING LOCUS C* (*FLC*), is a crucial mechanism for the vernalization-induced flowering. A PHD finger domain protein VERNALIZATION INSENSITIVE 3 (*VIN3*) is known to induce the repression of *FLC* chromatin. Differently with cold-induced genes, whose expressions are increased by a short period of cold, a transcription of *VIN3* is slowly activated by the long period of cold. Despite intensive research, the question how plants can distinguish winter signal from cold signal are still elusive.

To elucidate this, we have generated transgenic lines of a series of deletion of *VIN3* promoter, including 5'-UTR and 1<sup>st</sup> intron, fused with GUS. Our results showed that -0.2kb of promoter and 1<sup>st</sup> intron sequences were sufficient to induce *VIN3* by vernalization treatment. Interestingly, in the absence of intron, GUS expression was greatly reduced in both vernalization and non-vernalization condition. But 1<sup>st</sup> intron itself has not shown vernalization response. We found that 1<sup>st</sup> intron of *VIN3* had a transcriptional enhancer activity and the activity was dependent on a distal region of intron. In contrast, a proximal region of intron had a role for *VIN3* repression in warm temperature.

We suggest that a gradual increase of *VIN3* transcription during vernalization was caused by multiple *cis*-elements. In warm temperature, *VIN3* expression is repressed by chromatin modification. When winter cold signal relives the repression, the enhancer activity of intron is slowly activated.

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**497 ARABIDOPSIS TRITHORAX-RELATED3/SET DOMAIN GROUP2 Is Required for Repression of *Arabidopsis thaliana* Flowering in Non-Inductive Photoperiods and in Non-Vernalized Winter Annuals**

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The winter-annual habit of *Arabidopsis thaliana* requires active alleles of *FLOWERING LOCUS C* (*FLC*), which encodes a potent flowering repressor, and *FRIGIDA* (*FRI*), an activator of *FLC*. *FLC* activation by *FRI* is accompanied by an increase in specific histone modifications, such as tri-methylation of histone H3 at Lys 4 (H3K4me3). H3K4 methylation at the *FLC* locus requires members of two known classes of H3K4 methyltransferases, the Drosophila Trithorax-class ARABIDOPSIS TRITHORAX1 (ATX1) and yeast Set1-class ARABIDOPSIS TRITHORAX-RELATED7 (ATXR7). Here, we show that ARABIDOPSIS TRITHORAX-RELATED3, which is a member of a novel class of SET domain proteins, also contributes to H3K4 methylation and the activation of *FLC* and other *FLC* clade members, such as *FLOWERING LOCUS M/MADS AFFECTING FLOWERING1* (*FLM/MAF1*) and *MAF5*. An *ATXR3* lesion suppresses the delayed flowering caused by either non-inductive photoperiods or an active allele of *FRI* in non-vernalized plants. The decrease in *FLC* expression in *atxr3* mutants was accompanied by reduced H3K4me3 levels at *FLC* chromatin. Rapid flowering of *atxr3* was epistatic to that of *atxr7*, suggesting that ATXR3 functions in the *FLC* activation together with ATXR7. Our results indicate that the novel-class H3K4 methyltransferase, ATXR3, is a transcriptional activator that plays a key role in the photoperiod response and in establishing the winter-annual habit.

**498 Characterization of the *DAWDLE* Gene in *Arabidopsis***

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Protein-protein interaction is an essential component of many biochemical and cellular functions in plant growth and development. Proteins with a Fork Head-Associated domain (FHA) mediate interactions with phosphorylated proteins. FHA domain is one of the phosphobinding domain identified in plants. In addition, FHA domain is present in proteins from all kingdoms including bacteria, animals, and plants. FHA domain's phosphorylation-dependent interactions play a role in many processes critical for cell proliferation, such as DNA damage responses, DNA repair, mitotic progression, cell cycle events, and signal transduction. In *Arabidopsis thaliana*, 18 genes encode a protein with a predicted FHA domain. One of these genes is the *DAWDLE* (*DDL*) gene. A mutation in the *DDL* gene causes plants to be developmentally delayed and display pleiotropic phenotypes such as defective roots, shoots, and flowers and reduced seed set. Also, the levels of several micro RNAs and small interfering RNAs are reduced in *ddl* compared to the wild type. The *DDL* gene has a FHA domain spanning the gene from exon six through nine in the C terminus, and contains several nuclear localization signals in the N terminus. However, it is not completely known whether the FHA domain and N terminus of the *DDL* gene are necessary for its function. Furthermore, genetic interactors involved in the same signaling pathway as *DDL* have not been identified yet. To determine which domain of the gene is necessary for its function, we screened a TILLING population to identify point mutations in both domains of the *DDL* gene. To identify genetic interactors involved in the same signaling pathway as *DDL*, we isolated two suppressors of a weak allele of *ddl*.

**499 Extensive Gene Regulatory Networks Utilizing Trans-Acting siRNAs in Legumes**

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Trans-acting siRNAs (tasiRNAs) negatively regulate target transcripts and are characterized by siRNAs spaced in 21-nucleotide "phased" intervals. This pattern is triggered by miRNA-directed cleavage and the phasing is formed by DICER-LIKE 4 (DCL4) processing. To date, tasiRNAs have not been extensively described in many plant species. To investigate miRNAs, tasiRNAs and other classes of small RNAs in legumes, we sequenced 21 libraries from tissues of Medicago, soybean, common bean and peanut. We identified dozens of new miRNA candidates and variants of known miRNA families. Cleavage of 119 miRNA targets was confirmed by PARE analysis. A search for phased tasiRNA-like small RNAs ("phasiRNAs") found at least 114 Medicago and 41 soybean loci. The majority of the Medicago phasiRNA loci were NBS-LRR encoding genes. We identified several 22-nt miRNA families with unique characteristics in legumes (miR1507, miR1509, miR2109, miR2118), which predominantly target conserved domains in NBS-LRR-encoding RNAs to trigger phasiRNA production. RNAs for *DCL2* and *SGS3*, components of the RNA silencing pathway, are also cleaved by some of these same 22-nt miRNAs and generate phased small RNAs, suggesting synchronization between silencing and pathogen defense pathways. In addition, a pair of miR156-miR172 binding and cleavage sites was found to initiate phasiRNA processing at an AP2-like transcription factor locus, representing only the second known example after *TAS3* of tasiRNA production that fits the "two-hit" model. We propose that these loci comprise a complex regulatory circuit possibly modulating signaling in plant-microbe interactions in legumes. Our data extend a model for tasiRNA biogenesis via new "two-hit" loci, and indicate diversity in their functional roles in plants.

## 500 Towards Defining Roles for the *Arabidopsis* GOLDEN2-LIKE genes, AtGLK1 and AtGLK2, in the Regulation of Core Circadian Clock Components and Physiological Outputs

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Transcriptional regulation is a major regulatory motif in circadian biology. In both plants and animals, circadian clock component regulation at the promoter involves binding to particular sequences by various transcription factors at a specific time. Identification of these transcriptional elements can begin to unravel how the clock regulation at the transcriptional level is controlled. For the gene *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), our group has found a 92 bp region that confers rhythmicity of expression, identified through analyses of promoter constructs. Subsequently, a "promoter hiking" strategy was employed to identify transcription factors that are bound to the 92 bp sequence. We identified several proteins that appear to bind within this region and among them was the GARP-domain containing protein GOLDEN2-LIKE1 (AtGLK1). Here, we describe our characterization of the roles of *GLK1* as well as its homolog GOLDEN2-LIKE2 (*GLK2*) in *Arabidopsis*. In addition, some of the physiological and molecular effects observed in the mutant accessions *glk1*, *glk2* and *glk1 glk2* with emphasis on clock-controlled phenotypes and core component/output expression levels, will be presented.

## 501 COP1-Mediated Degradation of BBX22/LZF1 Optimizes Seedling Development in *Arabidopsis*

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Light regulates multiple aspects of growth and development in plants. Transcriptomic changes govern the expression of signaling molecules with the perception of light. Also, the 26S proteasome regulates the accumulation of positive and negative regulators for optimal growth of *Arabidopsis* in the dark, light or light-dark cycles. *BBX22*, whose induction is both light regulated and HY5 dependent, is a positive regulator of de-etiolation in *Arabidopsis*. We found that during skotomorphogenesis, the expression of *BBX22* needs to be tightly regulated at both transcriptional and post-translational levels. During photomorphogenesis, the expression of *BBX22* transiently accumulates to execute its roles as a positive regulator. *BBX22* protein accumulates to a higher level under the short-day condition and functions to inhibit hypocotyl elongation. The proteasome-dependent degradation of *BBX22* protein is tightly controlled even in plants overexpressing *BBX22*. An analysis of *BBX22* degradation kinetics shows that the protein has a short half-life under both dark and light conditions. COP1 mediates the degradation of *BBX22* in the dark. Although dispensable in the dark, HY5 contributes to the degradation of *BBX22* in the light. The constitutive photomorphogenic development of the *cop1* mutant is enhanced in *cop1BBX22ox* plants, which show a short hypocotyl, high anthocyanin accumulation and expression of light-responsive genes. Exaggerated light responsiveness is also observed in *cop1BBX22ox* seedlings grown under short-day conditions. Therefore, the proper accumulation of *BBX22* is crucial for plants to maintain optimal growth when grown in the dark as well as respond to seasonal changes in day length.

## 502 The LRB1 and LRB2 Pair of *Arabidopsis* BTB E3s Modify Red Light Signaling by Regulating Phytochrome Levels

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Plants measure the wavelength, intensity and duration of their light environment and regulate developmental regimens in accordance with continuously changing light conditions. Here, we describe two genes, *LIGHT REGULATED BTB (LRB) 1* and *LRB2*, that modulate red-light (R) responsiveness in *Arabidopsis thaliana*. They encode a subfamily of highly conserved broad complex/tramtrack/bric-a-brac (BTB) proteins that assemble into ubiquitin E3 ligase complexes. Whereas the single *lrb1* and *lrb2* mutants are phenotypically indistinguishable from wild-type plants, the *lrb1 lrb2* double mutant is hypersensitive to R, but not far-red or blue light. This R hypersensitivity can be inhibited through the inactivation of *PHYB*, indicating that *LRB1* and *LRB2* are epistatic to the *PHYB* locus. Whereas the *phyB lrb1 lrb2* plants retain some sensitivity to R, the quadruple *phyB phyD lrb1 lrb2* seedlings are completely insensitive to R, implying that PhyD signaling is affected by *LRB1* and *LRB2* as well. We find that *lrb1 lrb2* seedlings have higher levels of PhyB and PhyD mRNA and protein relative to wild type and that turnover of PhyB and PhyD is dampened in the *lrb1 lrb2* background. Although several previously identified R-hypersensitive mutants (*pif3,4,7*, and *cop1*) accumulate PhyA protein similar to or higher than wild-type levels, the *lrb1 lrb2* mutants accumulate less PhyA mRNA and protein in response to R, suggesting LRB1 and LRB2 also positively regulate *PHYA* expression through R signaling. Together, these data show that the family of LRB1 and LRB2 BTB ubiquitin ligases negatively regulate R signaling by controlling phytochrome levels.

## 503 Automated Analysis of Hypocotyl Growth Dynamics During Shade Avoidance in *Arabidopsis*

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Plants adapted to environments where light is abundant are especially sensitive to competition for light from neighboring vegetation. As a result, these plants initiate a series of changes known as the shade avoidance syndrome, during which plants elongate their stems and

petioles at the expense of leaf development. While the developmental outcomes of exposure to prolonged shade are known, the signaling dynamics during the initial exposure of seedlings to shade is less well studied. To aid dynamic studies of shade-regulated processes, we report the development of a new software-based tool, called HyDE (Hypocotyl Determining Engine) to measure hypocotyl lengths of time-resolved image stacks of *Arabidopsis* wild-type and mutant seedlings. Using this tool, we show that *Arabidopsis* grows rapidly in response to the shade stimulus, with measurable growth after just 45 minutes of shade exposure. Similar to other mustard species, this growth response occurs in multiple distinct phases, including two phases of rapid growth and one phase with slower growth. Using mutants affected in shade avoidance, we demonstrate that most of this early growth requires new auxin biosynthesis via the indole-3-pyruvate pathway. When activity of this pathway is reduced, the first phase of elongation growth is missing and this is correlated with reduced activity of auxin-regulated genes. We further show that varying shade intensity and duration can affect the shape and magnitude of the growth response, indicating a dynamic spectrum of elongation response to shade. Thus, we demonstrate imaging-enabled dynamic assays to be informative in dissecting complex morphological processes, and will continue to utilize this technology to further probe physiological responses to shade in *Arabidopsis* and other species.

#### **504 Investigation Of DNA-Protein Interactions At The Promoter Of The Circadian Clock Gene *LHY* In *Arabidopsis thaliana***

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The circadian clock is the endogenous mechanism by which a wide variety of biological and physiological processes are regulated in anticipation of daily changes in the external environment. The clock comprises a number of complex gene and protein interactions, involving multiple regulatory feedback loops. In *Arabidopsis*, the *LHY* gene is central to these regulatory loops, encoding a transcription factor which upregulates morning-expressed genes such as *PRR7* and 9 and downregulates evening-expressed genes such as *TOC1*. The *PRR7* and 9 proteins repress *LHY* transcription during the day, whereas *TOC1* upregulates it in the morning. Of these proteins, none is known to contain a DNA-binding domain; they are believed to function as transcriptional co-regulators. In order to identify transcription factors which mediate their effects on *LHY* transcription, we have undertaken a detailed analysis of the *LHY* promoter.

A number of evolutionarily conserved *LHY* promoter motifs have been identified, two of which have been shown to contribute to circadian expression. To investigate whether mutation of specific motifs abolishes the effects of upstream regulators such as *TOC1* and *PRR7/9*, expression of *LHY:luciferase* reporter constructs is being analysed in various *Arabidopsis* clock mutants.

In order to identify transcription factors which bind the *LHY* promoter, a Yeast-One-Hybrid screen was performed. This identified several proteins involved in light or abscisic acid signalling pathways. As some of these proteins are known to interact, we are currently examining whether they act antagonistically or whether they target the promoter in complex using a modified Yeast-One-Hybrid assay. Similarly, we are testing whether any of the transcription factors identified are capable of recruiting *TOC1* or other known clock proteins to the *LHY* promoter.

#### **505 Phytochromes Regulate HEMERA Accumulation Via Direct Interaction**

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Phytochromes A and B (*phyA* and *phyB*) are prominent plant photoreceptors responsible for sensing far-red and red light cues and regulating every aspect of plant development. Our laboratory recently identified a novel light-signaling component named HEMERA (HMR). HMR is required for both *phyA*- and *phyB*-mediated responses; in particular, it is involved in early phytochrome signaling mechanisms such as phytochrome nuclear body formation and the degradation of *phyA* and phytochrome interacting factors (PIFs). In previous studies, we showed that HMR protein accumulates to higher levels in the light compared to the dark, suggesting that HMR protein abundance is directly regulated by light. However, the mechanism controlling HMR accumulation and its significance for phytochrome signaling were unclear. Our recent results show that HMR accumulation in the light is dependent on phytochromes. HMR failed to accumulate in both *phyB* mutants (*phyB-9*) growing in red light and *phyA* mutants (*phyA-211*) growing in far-red light. In addition, dark-grown seedlings expressing the constitutively active form of *phyB*, *PHYB<sup>Y276H</sup>*, had an elevated HMR level comparable to that of wild-type seedlings grown in the light. Collectively, these results suggest that HMR accumulation is regulated by both *phyA* and *phyB*. We further tested whether this intimate relationship between HMR and phytochromes could be due to a direct interaction between them. Indeed, *in vitro* binding assays and characterization of *hmr* mutants with altered HMR levels supported the notion that HMR and *phy* interact directly and that this interaction is required for HMR accumulation in the light. Taken together, these studies have uncovered a novel early phytochrome signaling mechanism by which phytochromes mediate light responses by interacting with and stabilizing HMR in the light.

#### **506 Cell Autonomous and Cell-Type Specific Circadian Rhythms in *Arabidopsis***

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The circadian system of plants regulates a wide range of rhythmic physiological and cellular output processes with a period of about 24 hours. The rhythms are generated by an oscillator mechanism that, in *Arabidopsis*, consists of interlocking feedback loops of several components including *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)*, *LATE ELONGATED HYPOCOTYL (LHY)*, and *TIMING OF CAB EXPRESSION 1 (TOC1)*. Over the past years, researchers have gained a detailed picture of the clock mechanism at the resolution of the whole plant, but far less is known about the specificities of the clock mechanism in individual cells. We have developed a technique using

transgenic plants with fluorescence-tagged CCA1 to measure rhythmicity in individual cells in intact living plants. We show that stomatal guard cells have a different period from surrounding leaf cells. An examination of guard transcript accumulation from key circadian oscillator genes identified a molecular mechanism that may explain the circadian differences in guard cells. Finally, we demonstrate that the oscillators of individual cells in the leaf tend to be robust in constant conditions, but become partially desynchronized. Taken together our results suggest that at the level of individual cells there may be differences in the canonical oscillator that has been described for plants.

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## 507 Assessment of *Arabidopsis* Phytochrome phyABCDE Null Mutant

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An *Arabidopsis* *phyABCDE* quintuple mutant in the Col accession was recently characterized; this mutant required both gibberellin treatment and the *ft* mutation for efficient germination (Strasser *et al.* 2010). We have independently constructed new *phyABCDE* quintuple mutants in the Ler accession that were derived from a previously described *phyABDE* mutant. Quintuple mutants are viable in full spectrum white light, like the parent quadruple mutant line, both of which exhibit very early flowering under long- and short-day growth conditions. Western blot analyses indicate that the new quintuple mutant lines are authentic nulls while only trace amounts of phyC protein could be detected in *phyABDE*. Despite the absence of phytochromes, quintuple mutant seed germination proved similar to that of the quadruple mutant. GA4 treatment does improve germination of lines with very low germination rates, while some quintuple mutant lines appear to germinate well even in the absence of GA. Quadruple and quintuple mutant seedlings are both blind to red light and impaired in blue light signaling. The number of genes under red light control in these mutants is reduced to only a handful, all of which appear to be stress-related. Both mutants are etiolated under red light, yet retain the ability to synthesize some chlorophyll. Grown under red light for prolonged period, a small number of plants developed rudimentary leaves at moderate fluence rates and some even could initiate reproductive development at higher fluence rates. Taken together, these studies indicate that phytochromes are not indispensable for plant development and support the earlier hypothesis that phyC accumulation/function depends upon the presence of phyB, phyD and/or phyE.

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## 508 ESD6/HOS1 Participates In The Control Of Photoperiodic Flowering In *Arabidopsis* Negatively Regulating CONSTANS Abundance

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Flowering time must be tightly regulated as it is essential for reproductive success in plants. Both promotive and repressive factors are involved in the control of the floral transition. We have isolated the *early in short days 6* (*esd6*) mutant in a screening for mutations that accelerate flowering time in *Arabidopsis*. *esd6* displays early flowering in both long and short day conditions among other developmental alterations. Fine mapping of the mutation showed that *esd6* was affected in the *HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1)* locus, which encodes a RING finger-containing protein that works as an E3 ubiquitin ligase. *esd6/hos1* mutation causes decreased expression of the *FLOWERING LOCUS C (FLC)* gene and shows a strong requirement of a functional *CONSTANS (CO)* gene for its early flowering phenotype under long days. Besides, CO and HOS1 physically interact *in vitro* and *in vivo*, and HOS1 is regulating CO abundance, particularly during the daylight period. Accordingly, the *hos1* mutation causes a shift in the typical long day pattern of *FT* transcript, starting to rise four hours after dawn. In addition, *HOS1* interacts synergistically with *CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)*, another regulator of CO protein stability, in the control of flowering time. These results indicate that HOS1 is involved in regulating CO abundance, ensuring that CO activation of *FT* occurs only when the light period reaches a certain length and preventing precocious flowering in *Arabidopsis*.

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## 509 *Gulliver2* Suppresses the Dwarfism of the *brassinosteroid insensitive 1-5* mutant

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Brassinosteroids (BRs) are essential plant hormones supporting normal growth and development such as photomorphogenesis and skotomorphogenesis. Because severe BR mutants exhibit extremely short hypocotyl regardless of light conditions, it was thought that BRs are not a light- or dark-specific regulator of hypocotyl elongation. To expand our understanding on the mechanisms of interaction between various light conditions and BRs, we isolated and investigated *Arabidopsis gulliver2* (*gul2*) mutants that were isolated from a genetic screen for resistance to the BR biosynthetic inhibitor, Brassinazole (Brz) and the white light condition. We isolated two independent alleles from two different mutant populations. *gul2* mutants displayed longer hypocotyl phenotypes in the presence of white light and Brz than their corresponding wild types. Double mutant analyses of *gul2* with known BR signaling mutants revealed that *gul2* can suppress the dwarf phenotype of a weak *BRI1* allele *bri1-5* but not a severe allele *bri1-1*, suggesting that GUL2 acts upstream of BRI1. However, the level of the BR biosynthetic gene expression was not elevated in the *gul2*, suggesting that the suppression of *bri1-5* by *gul2* is not through upregulation of BR biosynthesis. The underlying mechanisms of interaction between *bri1-5* and *gul2* have been extensively explored. The most possible model will be presented.

**510 Phytochrome-Regulated Arabidopsis BPG2 Binds to Plastid Ribosomal RNAs and Regulates****Ribosomal RNA Processing***Byung-Hoon Kim<sup>1</sup>, Przemyslaw Malec<sup>2</sup>, Albrecht von Arnim<sup>3</sup>*<sup>1</sup>**Albany State University, Albany, GA, USA, <sup>2</sup>Jagiellonian University, Krakow, Poland , <sup>3</sup>University of Tennessee, Knoxville, TN, USA**

*BPG2* (*brassinazol insensitive pale green 2*) is a dark-repressible and light-inducible gene that is required for the greening process in *Arabidopsis*. Light pulse experiments suggested that light-regulated gene expression of *BPG2* is mediated by phytochrome. The T-DNA insertion mutant *bpg2-2* exhibited a reduced level of chlorophyll and carotenoid pigmentation in the plastids. The  $F_{v/m}$  measurements as well as 77K fluorescence emission spectra indicate defective photosystem functions in *bpg2* mutants. BPG2 is localized in the plastid stroma fraction. The protein binds with specificity to chloroplast 16S and 23S ribosomal RNAs. The direct physical interaction with the plastid rRNAs supports an emerging model whereby BPG2 provides light-regulated ribosomal RNA processing functions, which are rate-limiting for development of the plastid and its photosynthetic apparatus.

**511 Bimolecular Fluorescence Complementation Studies Support An *in vivo* Interaction Between The****F-BOX Protein COLD TEMPERATURE GERMINATING10 And PHYTOCHROME INTERACTING FACTOR 1***Santosh Kumar, Nihar Nayak, Kathleen Martin, Kim Schafermeyer, Taylor Lloyd, Randy Dinkins, Michael Goodin, Bruce Downie***University of Kentucky, Lexington, (KY), U.S.A.**

The *Arabidopsis thaliana* F-BOX protein COLD TEMPERATURE GERMINATING10 (CTG10) was identified from an activation tagged mutant screen as causing seeds to complete germination faster than wild type at 10°C when its expression was increased (Salaita et al. 2005. J. Exp. Bot. 56: 2059). Our unpublished data suggest its role in germination is affected by light. There is substantial *in vitro* and circumstantial evidence of CTG10s interaction with the bHLH transcription factor PHYTOCHROME INTERACTING FACTOR 1 (PIF1) known to be a negative regulator of germination in darkness. To further prove their interaction *in vivo*, we have used a technique called Bimolecular Fluorescence Complementation (BiFC). Transient expression of CTG10 fused with the amino-terminal portion of the eYFP reporter (CTG10:Y) and PIF1 fused to the carboxy-terminal moiety (PIF1:FP) led to YFP fluorescence in the nucleus of *Nicotiana tabacum* (tobacco) leaf cells 24 hours following bombardment with gold particles carrying these two plasmids. The development of fluorescence due to protein:protein interaction between CTG10:Y and PIF1:FP was similar to that of two nuclear localized and interacting proteins of the nucleorhabdovirus, Sonchus Yellow Net Virus (SYNV): the phosphoprotein (P), and the HLH-containing nucleocapsid protein (N) (Deng et al. 2007. J. Virology 81: 5362). Negative controls included CTG10:Y or PIF1:FP alone, the two viral proteins alone, CTG10:Y+SYNV-N:FP, and SYNV-P:Y:PIF1:FP. Cells receiving gold were identified, and their transcriptional/translational capacity confirmed, by including a plasmid constitutively expressing nuclear localized DsRED or the tubulin-targeting MICROTUBULE ASSOCIATED PROTEIN65-1 (MAP65-1:DsRED) on the particles for each bombardment.

**512 An Approach To Identify PHYTOCHROME INTERACTING FACTOR1 (PIF1) Interacting Proteins From*****Arabidopsis* Seeds***Rekha Kushwaha, Santosh Kumar, Bruce Downie***University of Kentucky, Lexington, (KY), U.S.A.**

Many biological processes include a hierarchy of protein-protein interactions that together orchestrate overall physiological pathways. Therefore, the identification of interacting protein partners with which a protein associates can be an informative way to pursue an understanding of the protein's function under a variety of physiological conditions. There are a number of physical-, molecular biological- and genetic-approaches that have been used to detect protein-protein interactions. In this study we have designed a system to identify interacting partners of PHYTOCHROME INTERACTING FACTOR1 (PIF1), one of the negative regulating factors preventing completion of germination in darkness of *Arabidopsis thaliana* seeds. For this we have expressed recombinant PIF1 in *E. coli*. Upon expression, we have standardized a means of purification of large quantities of hexahistidyl-tagged PIF1 from inclusion bodies. Purified protein was further tested for its retention on micotiter (ELISA) plate wells. After overcoming this hurdle we ascertained that the purified, bound protein retains one of its biologically activities, that of binding an approximately 500 bp DNA fragment known to be a direct target of PIF1 binding *in vivo* and containing one hexameric G-BOX 'CACGTG' motif (PIF1 canonical binding site). This observation was tested further by using two negative controls, BOVINE SERUM ALBUMIN (BSA) to which the DNA fragment did not bind, and the DNA fragment containing a mutated (m)G-BOX with two non-canonical base pairs (CAAGGG) to which neither BSA nor PIF1 bound. In all instances, PIF1-containing wells were washed, blocked, and washed again, before DNA was introduced, incubated, and extensively washed. The DNA remaining in the wells (if any) was released by heating the last wash to 95°C prior to liquid recovery. Presence or absence of the G-BOX-containing- or mG-BOX-containing-DNA was determined by PCR. We are now using seed phage display libraries and biopanning to discover interacting proteins of the bound, biologically active PIF1.

**513 Coordinated Transcriptional Regulation Underlying the Circadian Clock in *Arabidopsis***

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The circadian clock controls many metabolic, development and physiological processes in a time of day specific manner in both plants and animals. The photoreceptors involved in the perception of light and entrainment the circadian clock have been well characterized in plants. However, how light signals are transduced from the photoreceptors to the central circadian oscillator, and how the rhythmic expression pattern of a clock gene is generated and maintained by diurnal light signals remain unclear. Here, we show that in *Arabidopsis thaliana*, FHY3, FAR1 and HY5, three positive regulators of the phytochrome A signaling pathway, directly binding to the promoter of *ELF4*, a proposed component of the central oscillator, and activate its expression during the day, while, the circadian controlled CCA1 and LHY proteins directly suppress *ELF4* expression periodically at dawn through physical interaction with these transcription promoting factors. Our findings provide evidence that a set of light and circadian regulated transcription factors act directly and coordinately at the *ELF4* promoter to regulate its cyclic expression, and establish a potential molecular link connecting the environment light dark cycle to the central oscillator.

**514 PIL1: A Negative Regulator of the Shade Avoidance Transcriptional Network of Plants**

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Plants grown under dense canopies or in close proximity become limited for light, and re-allocate energy resources from storage organs to stem-like organs. This response is called the shade avoidance syndrome (SAS). Upon detection of a change in spectral quality (a lowering of the ratio of red to far-red light) by the phytochrome photoreceptors, a rapid growth response is initiated that involves the synthesis of plant hormones and a transcriptional cascade that leads to sustained growth. Previous studies indicated that the bHLH transcription factor, *PIL1*, is the most dramatically shade-induced gene (up to 100-fold within 15 minutes of exposure to shade light); however, little is known of *PIL1*'s function. Here we describe the phenotype of *pil1* mutant seedlings, which display enhanced shade avoidance phenotypes. Overexpression of *PIL1* leads to shade insensitivity, indicating that *PIL1* is a negative regulator of shade avoidance responses. We also show that *PIL1* protein accumulates after shade treatment and represses *PIL1* promoter activity, indicating a negative feedback mechanism. Results from RNA sequencing experiments suggest that *PIL1* overexpression strongly attenuates both shade-induced or repressed expression of many shade-regulated genes. *PIL1* requires both its bHLH and phytochrome-interaction domain (APB) to function. Our results indicate that *PIL1* acts as a negative regulator of the shade-induced transcriptional response in *Arabidopsis*.

**515 Rapid, Organ-Specific Transcriptional Responses to Light Regulate Photomorphogenic Development in Dicot Seedlings**

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The dicotyledon seedling undergoes organ-specific photomorphogenic development when exposed to light. The cotyledons open and expand, the apical hook opens and the hypocotyl ceases to elongate. Using the large and easily dissected seedlings of soybean (*Glycine max* cv. Williams 82), we show that genes involved in photosynthesis and its regulation dominate transcripts specific to the cotyledon, even in etiolated seedlings. Genes for cell wall biosynthesis and metabolism are expressed at higher levels in the hypocotyl, while examination of genes expressed at higher levels in the hook region (including the shoot apical meristem) reveals genes involved in signaling. The early transcriptional events in these three organs in response to a one-hour treatment of far-red light are highly distinctive. Not only are different regulatory genes rapidly regulated by light in each organ, but the early-responsive genes in each organ contain a distinctive subset of known light-responsive *cis*-regulatory elements. We detected specific light induced gene expression for the root phototropism gene *RPT2* in the apical hook, and also phenotypes in *Arabidopsis rpt2* mutants demonstrating that the gene is necessary for normal photomorphogenesis in the seedling apex. Significantly, expression of the *RPT2* promoter fused to a GUS reporter gene shows differential, light responsive expression across the hook region. We conclude that organ-specific, light-responsive transcriptional networks are active early in photomorphogenesis in the aerial parts of dicotyledon seedlings.

**516 Utilization of ChIP-Seq to Identify PSEUDO RESPONSE REGULATOR 7 Transcriptional Targets in the *Arabidopsis* Circadian Clock**

*Tiffany Liu*

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The circadian clock is comprised of a central time keeping mechanism that coordinates biological processes through regulating output pathways at appropriate times in order to optimize growth and development. The circadian clock regulates outputs such as leaf movements, stomatal opening, hypocotyl elongation, shade avoidance, floral transitioning, volatile emissions, and photosynthesis. PSEUDO RESPONSE REGULATOR 7 (PRR7) plays a fundamental role in the central circadian clock by repressing core clock components *LATE ELONGATED HYPOCOTYL* (LHY) and *CIRCADIAN CLOCK ASSOCIATED 1* (CCA1); and in turn, LHY and CCA1 activate PRR7

transcription. In order to elucidate the downstream targets of PRR7, chromatin immunoprecipitation combined with high through-put sequencing (ChIP-seq) was conducted. The PRR7 ChIP-Seq experiments identified enriched binding sites associated with 127 genes in common between two biological replicates. These results reveal that PRR7 is involved in regulating processes such as light signaling and cold response, thereby providing insight on the role of PRR7 in orchestrating the circadian regulated transcriptional network.

### **517 Differential Expression Of PIF1-Targeted Genes In Various PIF1 And CTG10 Mutants**

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Successful completion of germination of *Arabidopsis thaliana* seeds is contingent upon the actions of PHYTOCHROME (Phy) and PHYTOCHROME INTERACTING FACTOR 1 (PIF1). Previous studies have shown that PIF1, a basic helix-loop-helix (bHLH) transcription factor, regulates genes through preferential binding to G-box motifs in their regulatory regions. A polyubiquitin- 26S proteasome mediated pathway has been identified as a regulator of PIF1 amounts. One aspect of this pathway is hypothesized to be the binding of the kelch beta-propeller of the COLD TEMPERATURE GERMINATING 10 (CTG10) F-BOX protein to PIF1 following a PIF1 phosphorylation event caused by the movement of active Phy into the nucleus. The binding of CTG10 leads to the polyubiquitination of PIF1 and its subsequent degradation by way of the 26S proteasome. This experiment investigated the interaction of PIF1 and CTG10 indirectly through a study of the relative expression of PIF1 direct-target genes using quantitative Real Time – Polymerase Chain Reaction (qRT-PCR). Over-expressing and knockdown mutants of *PIF1* along with over-expressing mutants and an RNAi line of *CTG10* were verified as affecting transcript abundance for *PIF1* and *CTG10*, respectively. Additionally, indirect evidence supporting, in some instances, the hypothesized interaction of PIF1 and CTG10 was acquired from two up- and two down-regulated PIF1 direct target gene transcripts using qRT-PCR. The preponderance of these results indirectly corroborate the interaction of the two proteins, PIF1 and CTG10, which can lead to the degradation of PIF1 thus allowing the completion of germination in the presence of light of the seeds from the positively photoblastic model plant.

### **518 Towards the modelling of the shade avoidance response of the *A. thaliana* hypocotyl**

*Séverine Lorrain<sup>1</sup>, Micha Hersch<sup>2,3</sup>, Sven Bergmann<sup>2,3</sup>, Christian Fankhauser<sup>1</sup>*

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Plants constantly monitor their environment and integrate different incoming parameters (such as temperature, light) to adapt to the surrounding conditions. This will ultimately optimize the light capture and plant development. In the frame of a system biology project we plan to model plant growth in such a changing environment. We first focuss on plant growth in response to changes in light quality during neighbour detection in the shade-intolerant plant *A.thaliana*. Such neighbouring is detected through a reduction in the red to far-red ratio by the phytochrome photosensors and triggers the so-called Shade Avoidance Syndrome characterized by reallocation of energy resources to growth mechanisms in order to reach unfiltered sunlight. We use hypocotyl elongation as a read-out for SAS that combines easiness of study and numerous molecular data present in the literature. Especially reduction of the R/FR ratio triggers phytochrome B inactivation leading to auxin production through the Tryptophan Aminotransferase (TAA1) as well as the stabilisation of Phytochromes Interacting Factors 4 and 5 (PIF), which are required for a full SAS. After prolonged exposure to shade a negative feedback loop limits the response of plants through formation of heterodimer between PIF4 and 5 and another transcription factor called HFR1 (long Hypocotyl in Far Red light). New molecular data will be presented in this poster concerning the regulation of the neighbour detection as well as a computational model developed to better understand this regulation

### **519 A Transcription Factor Overexpression Screen for Novel Regulators of the *Arabidopsis* Circadian Clock**

*Jeffrey Nelson, Steve Kay  
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The circadian clock is an endogenous molecular oscillator that is responsible for anticipating daily alterations in light and temperature, aligning an organism's physiology with the appropriate time of day, and thereby providing an adaptive advantage. While much progress has been made in understanding the transcriptional architecture of the circadian clock in *Arabidopsis thaliana*, current models indicate that gaps still exist. In order to uncover new clock associated transcription factors (TFs), we have generated a high quality cDNA library of all TFs and transcriptional regulators in the *Arabidopsis thaliana* genome. By systematically screening transgenic TF overexpression lines in a clock promoter::luciferase reporter background, we have identified a number of novel genes, including members of the B-Box zinc finger family, that seem to play a role in the maintenance of appropriate clock rhythmicity. Ongoing work aims to characterize the biochemical activity of these candidate genes and position them within the framework of the clock genetic network.

### **520 Regulation of Seedling De-etiolation And Seed Development By Transcription Co-activator SHB1**

*Yun Zhou, Wei Li, Xiaojun Kang, Min Ni  
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We have previously identified SHB1 as a critical regulator of *Arabidopsis* seedling de-etiolation. SHB1 is localized to the nucleus and is homologous to the SYG1 protein family. SHB1 contains an N-terminal SPX domain and a C-terminal EXS domain. Over-accumulation of the SPX domain caused a long hypocotyl phenotype similar to that of *shb1-D*, again-of-function over-expression allele, under red, far-red, or blue light. By contrast, over-accumulation of the C-terminal EXS domain led to a short hypocotyl phenotype similar

to that of *shb1*, a loss-of-function allele, under blue light. The N-terminal SPX domain was associated with a smaller protein complex than the native protein complex associated with endogenous SHB1. The EXS domain was associated with a slightly smaller protein complex than the native protein complex, but it largely displaced endogenous SHB1 from its native protein complex. In addition, all six mis-sense mutations that we identified from a suppressor screen were clustered within or close to the SPX domain, and these mutations impaired the assembly of the SHB1-containing protein complex. We propose that both SPX and EXS domains likely anchor SHB1 to a protein complex, and the SPX domain is critical for SHB1 signaling. We have recently found that SHB1 also acts as a positive regulator of *Arabidopsis* seed development. *shb1-D* increases seed size and *shb1* reduces seed size. The increase in *shb1-D* seed size is associated with endosperm cellurization, chalazal endosperm enlargement, and embryo development. SHB1 regulates the expression of *MINI3* and *IKU2*, a WRKY transcription factor gene and an LRR receptor kinase gene. Mutation in either *MINI3* or *IKU2* retards endosperm development and reduces seed size. SHB1 associates with *MINI3* and *IKU2* promoters in vivo and exists in a 305 kDa protein complex. Therefore, SHB1 may be a putative transcriptional co-activator in both light signaling and seed development.

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## 521 *Arabidopsis* shade avoidance strategy is temperature-dependent and involves the receptor-like kinase ERECTA

*Dhaval Patel, Keara Franklin*

**University of Bristol, Bristol, U.K.**

Vegetative shading resulting in light limitation is a common problem for plant survival. Light reflected from and transmitted through living vegetation is depleted in photosynthetically active red (R) and blue wavelengths and enriched in green and far-red (FR) wavelengths. Plants detect the presence of neighbouring vegetation through monitoring the ratio of R to FR wavelengths (R:FR) in ambient light. In crowded environments with a low R:FR, plants display a suite of characteristic responses including increased stem and petiole elongation, reduced leaf area and thickness, decreased leaf chlorophyll content, leaf hyponasty and accelerated flowering. Collectively, these responses are termed the shade avoidance syndrome and are regulated by phytochromes. Shade avoidance has largely been studied at growth temperatures higher than 20°C. Using the Landsberg *erecta* (*La-er*) accession of *Arabidopsis*, we studied shade avoidance at a cooler temperature (16°C) and found a strikingly different response. In cooler conditions, plants responded to low R:FR by dramatically increasing leaf area and thickness, rather than elongating petioles. To explore natural genetic variation in temperature-dependent light foraging strategy, we screened common lab *Arabidopsis* accessions and found that Cape verde island (*Cvi*) plants displayed conventional shade avoidance responses, irrespective of growth temperature. Comparative analysis using *La-er* X *Cvi* NILs resulted in mapping of the *ERECTA* gene. I have established that *ERECTA* is a major regulator of petiole elongation in low R:FR at cool temperatures. Further molecular characterisation of the role of *ERECTA* in temperature-regulated light foraging strategy is underway.

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## 522 Screening of Mutants in the BBX24/STO Light Signaling Pathway

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BBX24/STO (Salt Tolerance) is a negative regulator of photomorphogenesis (Indorf et al. 2007). It belongs to the recently described BBX-family of proteins that contain one or two B-box Zn finger motives at the N-terminus (Khanna et al. 2009). *Arabidopsis* seedlings constitutively over-expressing BBX24 display longer hypocotyls than wild-type seedlings when germinated and grown under different fluences of monochromatic red, far-red or blue light. Expression of BBX24 is light-regulated, and the protein accumulates in the nucleus of cells during seedling de-etiolation. In darkness, degradation of the protein is controlled by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) (Indorf et al. 2007). To identify components of the BBX24 signaling pathway, we performed a forward genetic analysis using an EMS mutagenized population of seedlings over-expressing the GFP:BBX24 translational fusion. Screening of the M2 generation was carried out in short-day light cycles, since seedlings over-expressing BBX24 display longer hypocotyls also in those conditions. From a total of 30.000 M2 seedlings, we selected around 2.700 plants, from which approximately 2.300 set seeds. M3 populations of about 500 putative mutants have been re-screened to-date. From those, approximately 30 lines which displayed hypocotyl lengths different from the over-expressor are being characterised in the following generation. Light fluence response analysis in different monochromatic light conditions, as well as, dynamics of the BBX24:GFP accumulation during seedling de-etiolation will be used for classification of the different mutants. Preliminary data describing the phenotype of selected mutants will be presented.

\* Authors contributed equally to this work.

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## 523 Tuning of circadian period by micronutrients availability in *Arabidopsis*

*Patrice Salomé<sup>1</sup>, Michele Oliva<sup>2</sup>, Ute Krämer<sup>2</sup>, Detlef Weigel<sup>1</sup>*

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The circadian clock controls the expression of thousands of genes, many of which are involved in photosynthesis and metabolism. The proper daily timing of physiological and cellular processes by the circadian clock confers a fitness advantage, as a disruption of circadian period results in plants with lower photosynthetic capacity and lower biomass. In animals, the circadian clock and the metabolic state of the cell are intimately interconnected: one of the mammalian clock proteins acts as a sensor of intracellular heme, and modulates the expression of other clock genes accordingly. To determine whether changing heme levels could also impact the plant circadian clock, we tested how clock parameters (circadian period, phase and amplitude) were affected when seedlings are grown under limited iron supply. We found that the phase and amplitude of several luciferase reporters (for clock genes and output genes) remained unchanged between 0-100 µM Fe-HBED. However, the clock responded to a lowering of available iron by lengthening the period of all reporters tested by up to 3 hours over iron replete conditions. This response was specific to iron, as conditions of low copper, manganese or zinc

did not affect period length. Loss of function in the clock genes *CCA1*, *LHY*, *TOC1*, *ZTL* or *GI* all responded to lower available iron by lengthening their fre-running period, indicating that the encoded clock proteins do not require heme as a cofactor. However, a mutant in the chloroplast-localized heme oxygenase *HO1* failed to adjust its circadian period to available iron levels, suggesting that, just like in animals, heme might play a critical signaling role in connecting the circadian clock and the cell metabolic state. We will discuss our efforts to characterize how the circadian clock responds to changing iron levels, and how this might impact plant fitness.

## 524 PRMT5, a Piece Connecting the Circadian Clock and Alternative Splicing

*Sabrina Sanchez<sup>6</sup>, Petrillo Ezequiel<sup>1</sup>, Xu Zhang<sup>2</sup>, Matias Rognone<sup>6</sup>, Carlos Hernando<sup>6</sup>, Micaela Godoy Herz<sup>1</sup>, Craig Simpson<sup>3</sup>, John Brown<sup>3,4</sup>, Justin Borevitz<sup>2</sup>, Paloma Mas<sup>5</sup>, Alberto Kornblihtt<sup>1</sup>, Marcelo Yanovsky<sup>6</sup>*

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Circadian clocks are endogenous mechanisms which allow organisms to adjust and to anticipate physiological and developmental responses to environmental changes. Transcriptional and posttranslational processes are known to be crucial regulatory steps for proper functioning of biological rhythms. Alternative splicing is a process that generates multiple mRNA products from a single gene enhancing proteome diversity. Until now, several examples of co-regulation of these mechanisms have been demonstrated in different organisms, but a direct link was missing. We have conducted a genetic screen using *Arabidopsis thaliana* as a model system to identify novel components of the circadian signalling network and we isolated a new mutant allele of *PRMT5* gene, which encodes for an arginine methyltransferase. Analysis of *prmt5*, a long period mutant, allowed us to determine that *PRMT5* is involved in the proper functioning of the central core of the circadian clock and that *PRMT5* expression follows itself a circadian pattern, suggesting that it is part of a feedback loop controlling clock function. We also found that *PRMT5* is responsible for alternative splicing defects detected in the mutant plant and it modulates the recognition of donor or 5' splicing sites, being specially important for the weak ones. Moreover, this protein seems to be, at least in part, the link between alternative splicing and circadian network. This connection seems to have physiological relevance, since *Rubisco Activase* alternative splicing is regulated both, by biological rhythms and *PRMT5*. Finally, we detected in the mutant an altered photomorphogenic development suggesting a defect in light perception or signalling. These data suggest that *PRMT5* could be a useful tool to fine-tuning the timing and place of expression of different gene targets and would be the first example identified of a protein linking circadian clocks to alternative splicing.

## 525 Regulation of Flowering Time by a bHLH Transcription Factor in *Arabidopsis*

*Nidhi Sharma, Enamul Hug  
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Flowering in plants is a very dynamic and synchronized process where various cues including age, day-length, temperature and endogenous hormones fine-tune the timing of flowering for reproductive success. *Arabidopsis thaliana* is a facultative long day plant where long-day (LD) photoperiod promotes flowering. *Arabidopsis* still flowers under short-day (SD) conditions, albeit much later than LD conditions. Although, factors regulating the photoperiodic LD pathway have been extensively investigated, the SD pathway is much less understood. Here we identified a critical transcription factor called bHLH93 (basic Helix-Loop-Helix 93) that is essential to induce flowering specifically under SD conditions in *Arabidopsis*. *bhlh93* mutants do not flower from primary meristem under SD conditions, but flowers similar to wild type under LD conditions. The late flowering phenotype is rescued by exogenous application of GA, suggesting that bHLH93 acts upstream of GA pathway to promote flowering. Double mutant studies showed that *bhlh93* is epistatic to *phyB* and *soc1* genes under SD conditions. *bHLH93* is expressed at the meristematic regions and its expression peaks at 8 hours after dawn under SD conditions. As expected, the bHLH93 is localized in the nucleus. Taken together, these data suggest that bHLH93 is a key transcription factor necessary for *Arabidopsis thaliana* to evolve as a facultative plant.

## 526 FHY3 and FAR1 mediate clock adaptation to the light environment in *Arabidopsis thaliana*

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The circadian clock controls many metabolic, developmental and physiological processes in a time-of-day-specific manner in both plants and animals. The photoreceptors involved in the perception of light and entrainment of the circadian clock have been well characterized in plants. However, how light signals are transduced from the photoreceptors to the central circadian oscillator is only now becoming clear. Recently we showed that in *Arabidopsis thaliana*, FHY3, FAR1 and HY5, three positive regulators of the phytochrome A signaling pathway, directly bind to the promoter of ELF4, a proposed component of the central oscillator, and activate its expression during the day, whereas the circadian-controlled CCA1 and LHY proteins directly suppress ELF4 expression periodically at dawn through physical interactions with these transcription-promoting factors (Li *et al.*, 2011). Our new findings provide us further insight that FHY3, FAR1 and HY5 play an important role in adaptation to day length. In doing so they validate this connection between environmental light perception and the central oscillator of the plant circadian clock, and show that this has significant consequences on the overall physiology and developmental performance of plants. Our latest results in this regard will be presented.

**527 A Genetic Screen Identifying Mutations Which Suppress or Enhance the Phenotype of a Red Light****Hypersensitive Mutant***Gavin Sunde<sup>1</sup>, Timothy Lauer<sup>1</sup>, Matthew Christians<sup>2</sup>, Richard Viestra<sup>2</sup>, Derek Gingerich<sup>1</sup>***<sup>1</sup>University of Wisconsin-Eau Claire, Eau Claire, WI, USA, <sup>2</sup>University of Wisconsin-Madison, Madison, WI, USA**

A plant's ability to assess light quantity and quality is fundamental to maintaining healthy growth. One way that plants sense changing light conditions is via the perception of red (~670 nm) and far red (~730 nm) wavelengths by a group of light receptors called the phytochromes (phy). We have found that two closely related genes, *POB1* (*POZ/BTB-CONTAINING PROTEIN 1*) and *POB2* act as negative regulators of the phy-dependent red light response pathway. *POB1* and *POB2* encode putative BTB/Cullin3 E3 ubiquitin-ligase target adapters. Plants with disruptions of these two genes are red light hypersensitive, exhibiting reduced elongation of hypocotyls and petioles, increased greening, and increased cotyledon size under red light treatment when compared to wild-type. In order to identify the target of these putative E3s, or other components of the red light signaling pathway, we have conducted a genetic suppressor/enhancer screen, identifying mutations which relieve or enhance the exaggerated red light inhibition of hypocotyl elongation seen in a *pob1/pob2* double mutant. We screened ~30,000 M2 progeny of EMS-treated *pob1/pob2* individuals and identified 102 individuals with putative suppressor phenotypes and 33 individuals with putative enhancer phenotypes. We have been conducting detailed characterization of seedling red light responses in the suppressor lines in the M3 generation, and have found a range of phenotypes in the population. We have identified lines with differing degrees of suppression of *pob1/pob2*-enhanced red light inhibition of hypocotyl elongation, as well as lines with possible selective alteration of specific red-light responses. Of particular interest is one line where hypocotyl elongation appears to have been largely decoupled from light regulation.

**528 Genetic Dissection of Early Phytochrome Signaling Mechanisms***Elise Van Buskirk<sup>1</sup>, He Wang<sup>1,2</sup>, Meina Li<sup>1</sup>, Rafaelo Galvao<sup>1</sup>, Tao Ma<sup>1</sup>, Detlef Weigel<sup>sup>3</sup>, Meng Chen<sup>1</sup>***<sup>1</sup>Department of Biology, Duke University, Durham, NC 27708, USA, <sup>2</sup>Dalian Institute of Biotechnology, Liaoning Academy of Agricultural Science, Dalian 116024, China, <sup>3</sup>Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany**

During the plant life cycle, arguably the most critical environmental signal is light, which is perceived by a suite of wavelength-specific photoreceptors including the red/far-red sensing phytochrome (phy) family. At the cellular level, one of the earliest light responses is the translocation of active phy from the cytoplasm to the nucleus. In the nucleus, phys interact with and are colocalized with a group of phy-interacting bHLH transcription factors (PIFs) in subnuclear foci termed phy nuclear bodies (phyNBs). The function of phyNBs in relation to other phy signaling events is still enigmatic. However, the identification of a novel phy signaling component, HEMERA (HMR), links phyNBs to protein degradation. Light-grown *hmr* mutants are impaired in their ability to form large phyNBs, and they are defective in all phy-mediated responses including hypocotyl growth inhibition and chloroplast differentiation, suggesting that phyNBs are essential for normal phy signaling. Additionally, light-grown *hmr* seedlings accumulate light-labile proteins, including phyA, PIF1, and PIF3. HMR is structurally similar to the yeast protein RAD23, which shuttles ubiquitylated proteins to the proteasome. In accordance with this, HMR can partially complement the *rad23Δ* mutant in yeast. Together, these data suggest that phyNBs are required for normal phy signaling, and that they are sites for protein degradation.

To identify other components of the HMR-mediated phy signaling pathway, we performed a *hmr* suppressor screen on a weak *hmr* allele. This screen led to the identification of three dominant missense alleles of the same gene, *SUPPRESSOR OF HEMERA (SOH)*. *SOH* encodes an unknown protein. Remarkably, loss-of-function *soh* mutations lead to a *hmr*-like phenotype, including aberrant phyNB formation and impaired phy signaling responses. These results suggest that both HMR and SOH work in concert as part of the early phytochrome signaling mechanisms involving phyB localization to phyNBs, as well as subsequent light-dependent proteolytic events.

**529 LIGHT-REGULATED WD 1 and PSEUDO-RESPONSE REGULATOR 9 Form a Positive Feedback****Regulatory Loop in the Arabidopsis Circadian Clock***Ying Wang<sup>1</sup>, Jing-Fen Wu<sup>1</sup>, Norihito Nakamichi<sup>2</sup>, Hong-Gil Nam<sup>3</sup>, Shu-Hsing Wu<sup>1</sup>***<sup>1</sup>Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, <sup>2</sup>Plant Productivity Systems Research Group, RIKEN Plant Science Center, Yokohama, JAPAN, <sup>3</sup>Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang, Korea**

The *Arabidopsis* circadian clock is formed by several negative feedback loops composed of oscillator genes expressing at specific time during a day. The identification of additional clock genes will help to better dissect the complex nature of the circadian clock. *LIGHT-REGULATED WD REPEATS PROTEIN 1 (LWD1)* and LWD2 proteins share 91.4 % identity in amino acid sequence, and they are new clock genes regulating photoperiodic flowering and circadian period length.

Here we show that LWD1/2 plays dual functions in the light input pathway and the regulation of the central oscillator. Promoter:luciferase fusion studies showed that transcriptional activities of *LWD1/2* are rhythmic and depend on functional PRR9 and PRR7. LWD1/2 is also needed for the expression of PRR9, PRR7 and PRR5. LWD1 is preferentially localized within the nucleus and associates with promoters of PRR9, PRR5 and TOC1 *in vivo*. Our results support the existence of a positive feedback loop within the *Arabidopsis* circadian clock. Further mechanistic studies of this positive feedback loop and its regulatory effects on the other clock components will further elucidate the complex nature of the *Arabidopsis* circadian clock.

**530 Circadian Clock-Regulated Phosphate Transporter PHT4;1 Plays an Important Role in Arabidopsis Defense**

*Guoying Wang, Jiangli Shi, Gina Ng, Stephanie Battle, Chong Zhang, Hua Lu  
University of Maryland Baltimore County*

The *Arabidopsis accelerated cell death 6-1* (*acd6-1*) mutant shows constitutive defense, cell death, and extreme dwarf phenotypes. In a screen for *acd6-1* suppressors, we identified a mutant that was disrupted by a T-DNA in the *PHOSPHATE TRANSPORTER 4;1* (*PHT4;1*) gene. The suppressor mutant *pht4;1-1* is dominant, expresses truncated *PHT4;1* transcripts, and is more susceptible to virulent *Pseudomonas syringae* strains but not to several avirulent strains. Exogenous salicylic acid (SA) treatment induced a similar level of resistance in Col-0 and *pht4;1-1*, suggesting that *PHT4;1* acts upstream of the SA pathway. Genetic analysis further indicates that *PHT4;1* contributes to *SID2*-dependent and -independent pathways. Transgenic expression of the DNA fragment containing the *PHT4;1-1* region or the full-length *PHT4;1* gene in wild type conferred enhanced susceptibility to *Pseudomonas* infection. Interestingly, expression of *PHT4;1* is regulated by the circadian clock. Together, these data suggest that the phosphate transporter *PHT4;1* is critical for basal defense and also implicate a potential role of the circadian clock in regulating innate immunity of *Arabidopsis*.

**531 The conserved aspartate residue is important for both phytochrome photoconversion and photomorphogenesis in *Arabidopsis thaliana***

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Phytochrome (Phy) is a group of dimeric red/far red light [R/FR] responsive proteins, which evolve as an essential indicator of plant neighboring environments. The light sensing hub of Phy is a deeply buried tetrapyrrole chromophore directly converting the light energy into chemical signals, which are further tuned within the photosensing module to initiate conformational changes in the Phy dimer. Subsequent movements likely include interdomain re-orientation and dimerization re-arrangement. These events eventually result in a light-activated Phy triggering downstream signal transduction pathways, and accordingly re-adjusting the plant's developmental strategies. To dissect the intramolecular photoconversion process of Phys, I have expressed mutant plant PhyA and PhyB proteins with a single Asp-to-Ala substitution (*PhyA<sup>D207A</sup>*, *PhyB<sup>D207A</sup>*) in *Arabidopsis* *PhyA* null and *PhyB* null plants, respectively. The resulting *PhyA<sup>D207A</sup>* and *PhyB<sup>D207A</sup>* transgenic plants exhibit loss-of-function phenotypes in several photomorphogenic pathways, including defects in FR (*PhyA<sup>D207A</sup>*) and R (*PhyB<sup>D207A</sup>*) induced inhibition of the hypocotyl growth. Further spectral characterizations have shown R is unable to photoconvert phycocyanobilin chromophore assembled, recombinant *PhyA<sup>D207A</sup>* and *PhyB<sup>D207A</sup>* PAS-GAF-PHY truncation mutants to active forms, though the ground states are normal. These data indicate that 1) this central Asp residue is required to complete the light-induced chromophore movements, re-confirming its essential role in photoconversion across Phy superfamily. 2) The chromophore movement(s) perturbed by the D207A mutation is essential for Phy-associated phenotypic responses.

**532 Changes in Plant Physiology and Development Induced by Green Light**

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Light quality and quantity affect plant adaptation to changing light conditions. Various wavelengths in the visible and near-visible spectrum have discrete effects on plant growth and development, and the effects of red, far-red, blue and UV light have been well described. In this report the effect of green light on rosette architecture was tested, using a narrow-bandwidth LED-based lighting system. Upon addition of green light to a background of unchanging red and blue, plants exhibited elongation of petioles, upward leaf reorientation, and decreased anthocyanin accumulation—symptoms consistent with a shaded light environment. The phenotypes persist in phytochrome and cryptochrome mutant backgrounds. To further probe the molecular mechanism of the response, the accumulation of shade-induced transcripts was measured in response to enriched green environments. The addition of green light does not change, or even decreases, the expression levels of genes normally induced by supplemental far-red light. However, far-red light-associated transcript accumulation patterns are observed in cryptochrome mutants when green light is added, indicating that the green light triggers cryptochrome to actively gate at least facets of the transcriptome response normally induced by far-red light. The results indicate that shade symptoms can be induced by addition of shade-abundant wavebands other than far-red, and that cryptochrome blue-light receptors assist in separating green responses from those induced by far-red light.

**533 VAS1 Negatively Modulates Auxin Biosynthesis to Inhibit the Shade Avoidance Responses in Plants**

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For many plants including *Arabidopsis*, shade avoidance ensures survival to reproductive stages of development. This competitive developmental response is manifested by exaggerated stem and petiole growth, but at a cost of decreased biomass and seed yield. Stem and petiole elongation results from increased growth rates in response to the plant hormones such as auxin. Previously, our laboratory reported the *sav3* mutant (*shade avoidance 3*) that is defective in shade-induced hypocotyl elongation due to the impaired auxin biosynthesis. Here, we describe the *vas1* (*reversal of sav3-1*) mutant that was identified from a genetic screen for *sav3* suppressor based on hypocotyl elongation growth in response to shade. The *vas1* mutant fully rescues the *sav3* mutant short hypocotyl phenotype under shade and shows moderately constitutive shade avoidance syndromes (SAS) under white light. On the other hand, constitutive overexpression of *VAS1*

compromises the shade avoidance responses and causes other phenotypes typical of auxin biosynthetic mutants. In addition, we provide several lines of evidence suggesting that the auxin biosynthetic defect of *sav3* mutant is restored by *vas1* mutation. Our data demonstrate that VAS1 plays a vital role in slowing down shade avoidance responses by negatively modulating auxin biosynthesis.

## **534 Combining Nested Association Mapping and Correlated Genome Associations to a Quantitative Trait Network to Unravel the Genetic Basis of Fitness in *Arabidopsis thaliana***

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In natural environments, plant populations are confronted with multiple selective pressures, leading to selection for an optimal multi-trait phenotype. Identifying the genetic basis of fitness and its underlying components therefore remains a major goal in evolutionary biology. Statistical estimation of correlated genome associations may provide insights into the process of adaptation by unraveling the origin of genetic correlations among phenotypic traits. The goal of this project is to unravel the genetic basis of fitness in *Arabidopsis thaliana*. To understand which phenotypic traits contribute substantially to fitness, we'd like to identify the SNPs associated with seed production and with many other phenotypes (germination, vegetative growth, phenology, height, branching). To achieve this objective, we phenotyped nearly 12,400 plants under field conditions, including 184 worldwide natural accessions genotyped for 216,509 SNPs and a Nested Association Mapping population consisting of 4,366 RILs derived from 13 independent crosses chosen to maximize genetic and phenotypic diversity. Initial results from the joint analysis of natural accessions and the NAM population will be discussed and applied to the phenotypic networks described by path analyses. The natural accessions and the NAM population were also phenotyped for the same phenotypic traits in a greenhouse experiment, allowing us to identify the genetic basis of Genotype x Environment interactions.

## **535 Identification of key genes underlying quantitative resistance to *Xanthomonas campestris* in *Arabidopsis thaliana* by Genome Wide Association mapping**

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A major challenge in plant breeding and evolutionary biology is to identify the genetic and molecular bases for natural variation in resistance in plant species. The identification of genes underlying resistance might have enormous practical implications by increasing crop yield and quality, and give fundamental insights into evolutionary trajectories of natural populations. We aimed to identify key genes underlying quantitative resistance in *Arabidopsis thaliana* to a pathogen species of the bacterial foliar community, i.e. *Xanthomonas campestris* pv. *campestris* (*Xcc*). Black rot of crucifers caused by *Xcc* is possibly the most important disease of crucifers worldwide. Performing Genome Wide Association (GWA) mapping at different spatial scales using 384 worldwide natural accessions genotyped for 216,509 SNPs, the detection of QTLs associated with *Xcc* quantitative resistance was carried out with different strains representative of pathovar diversity in *Xcc*. One major association peak was detected at the worldwide scale for each *Xcc* strain. Association peaks specific to local populations were also detected for each *Xcc* strain, highlighting the need to include both worldwide accessions and local populations in GWA mapping studies. To identify ecological and evolutionary forces acting on *Xcc* quantitative resistance, we looked at frequencies of top SNPs associated with *Xcc* quantitative resistance in a panel of 49 natural French stands ecologically characterized for climate and soil conditions.

## **536 Implementation of Large-Scale QTL Studies of the Seedling Root Gravitropic Response Using Scanner Technology and Automated Image Processing in an Undergraduate Setting**

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Doane College

Genomic tools have become widely available for *Arabidopsis* research, yet most gene mutations do not result in readily-observable phenotypes. Identifying functional roles for genes within the intact organism is important for understanding genetic underpinnings of physiological processes. In previous studies that characterized mutations within a gene related to mammalian ionotropic glutamate receptors (*AtGLR3.3*), a set of tools consisting of high-resolution image capture coupled with custom computational algorithms were used to detect subtle, yet robust effects of single-gene mutations at the organismal level. This approach is being expanded to the genome scale at Doane College in collaboration with the University of Wisconsin and the University of Nebraska. The study focuses on a single physiological response, gravitropism, in a morphologically simple organ, the seedling root. The gravitropic responses of 108 seedlings are captured simultaneously using six flatbed scanners. Images are captured every few minutes at 4800 dpi over a period of nine hours. The total dataset will be over 32 TB in size and will consist of the responses of 162 recombinant inbred lines in six conditions varying in seed size and seedling age. A Condor array has been installed at Doane College in collaboration with the University of Nebraska to process the raw data (collected at 480 GB/day) before sending it out to the University of Wisconsin for feature extraction. Root tip angle will be extracted from each image and a map of genetic loci correlated with tip angle development during the progression of the gravitropic response will be constructed. Additionally, loci responsible for mediating response plasticity in different developmental contexts will be determined. These results will provide a wealth of information about how the genome is used throughout the root gravitropic response and will form a framework from which similar genomic studies can be launched. The study has provided multiple directions for the development of independent undergraduate research projects.

**537 Combining Nested Association Mapping and ecology-phenotype relationships to identify ecological and evolutionary forces acting on vegetative growth in *Arabidopsis thaliana***

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Vegetative growth-related traits are key components of plant performance. Rapid early development of leaf area and above-ground biomass may be tightly linked to fitness in natural populations by increasing seedling competitiveness against conspecifics. Relative growth rate (RGR), a proxy for estimating plant performance and productivity, is a very complex integrative trait whose quantitative variation is related to ecological settings both at the inter- and intra-species level. The goal of this project is to unravel the genetic basis of RGR and to identify the ecological factors acting as selective pressures on this trait in *Arabidopsis thaliana*. In order to greatly increase our power to finely map genomic regions associated with phenotypic variation, our first experiment involved phenotyping nearly 18,700 plants, including 184 worldwide natural accessions genotyped for 216,509 SNPs and a NAM population consisting of 4,366 RILs derived from 13 independent crosses chosen to maximize genetic and phenotypic diversity. We will present the first results from the joint analysis of data sets from natural accessions and the NAM population. To identify the selective agents that act on RGR, we set up a second experiment that entailed phenotyping of 800 accessions collected from 49 French natural stands ecologically that were characterized for climate and soil conditions. RGR relationships with ecological factors appeared stronger at the within-region than at the among-region scale.

**538 An Adaptive Model for Parental Genomic Imprinting**

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Rhodes College

Seed size is an agriculturally important trait as it relates to plant vigor and fitness. Simple genetic studies on seed size are confounded, however, by the complexity of seed development. The seed itself is composed of three genetically distinct structures, and the final size of the seed is biased by non-additive parental effects. For example, Landsberg *erecta* (Ler) produces smaller seeds compared to Columbia (Col) and C24, but Ler pollen on Col or C24 mothers promotes a seed size significantly larger than self-fertilized Col or C24 or the reciprocal crosses. Both maternal and paternal models have been presented to explain differential parental contributions among ecotypes, but without knowing the genes involved it is difficult to specify a precise mechanism. In 10 independent crosses between C24 and a selection of *Arabidopsis* ecotypes additive parental effects on seed size were only observed when the crosses involved a tetraploid parent, suggesting that the differential effects observed are possibly due to parental genomic imprinting (PGI). PGI is a mechanism of gene silencing whereby an offspring will express only one copy of a gene depending on whether that copy was inherited from the mother or the father. We hypothesized that PGI pathways or targets might differ in natural populations. Although both Col and Ler pollen increase seed size from C24 mothers, ColxLer recombinant inbred lines sort into 3 categories of phenotypes; those that are consistent with the Col or Ler strains, those that produce large seeds as mothers to C24 fathers, and a third class where the paternal enlargement of seed size is increased. QTL mapping identified separate Col and Ler paternal loci that are associated with both an increase seed size and a down-regulation of maternal gene expression. This suggests that pathways or genes that control PGI potentially differ in natural populations of *Arabidopsis*. These QTL might represent polymorphism in imprinted genes, differentially imprinted genes or regulators of PGI themselves.

**539 Ribosome Number is Negatively related to Biomass Accumulation in *Arabidopsis* in a Stable Diurnal Growth Regime**

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Intuitively, we would expect higher growth rates to be associated with a higher ribosome amounts. In unicellular organisms, ribosomal RNA and proteins amounts linearly increase with the growth rates under unlimited nutrition. In fast growing yeast cells, for instance, they represent over 80 % and 30–50% of the total RNA and protein, respectively (Warner, 1999). As a consequence, ribosomes represent a major investment which requires large amounts of C, N, and energy. In plants, all energy and materials necessary for the production of ribosomes come from photosynthesis, and are then limited. Thus, we could expect that a plant which has developed mechanisms to use its ribosomes efficiently might grow faster. Pointing to that, in multicellular organisms like plants, high amounts of ribosomes are only encountered in young growing tissues (Eilam et al., 1971). To test this hypothesis, we determined the ribosome amounts in 20 *Arabidopsis thaliana* accessions displaying large differences in biomass. Absolute quantification of the ribosome numbers per unit fresh weight was performed by spiking plant material with artificial RNA (Piques et al. 2009). In addition, leaf morphological parameters and metabolite levels were measured. The results indicated that the ribosome number at the End of the Night correlates negatively to biomass, as well as with the rate of leaf initiation and the initial rate of leaf growth, two important traits contributing to biomass accumulation. Interestingly, only weak correlations between the ribosome numbers at the End of the Day and the other traits were found. The correlation between ribosome abundance at End of the Night and biomass was linked with a diurnal oscillation in ribosome abundance in young growing leaves. Thus, our results suggest that controlling ribosome number during the night could be a promising approach for improving plant biomass.

**540 HSP90-facilitated Divergence of Transcription Factors in Brassinosteroid Signaling**Jennifer Lachowiec, Jennifer Nemhauser, Christine Queitsch**University of Washington, Seattle, WA, USA**

In plants, steroid hormones regulate essential processes in growth and development: cell expansion, cell division, differentiation, and reproduction. Plant steroid hormones, brassinosteroids (BRs), bind membrane-associated receptors that signal downstream to a set of evolutionarily conserved kinases. These kinases regulate plant-specific transcription factors that alter target gene expression. Mammalian steroid hormone signaling requires HSP90, a protein chaperone. Despite extensive study of plant steroid hormone signaling in plants, it is unknown whether it too requires HSP90. Using pharmacological approaches, we determined that BR signaling requires the chaperone HSP90. Genetic analyses revealed that HSP90 interacts with BR signaling at the level of the transcription factors, unlike mammalian steroid hormone signaling, in which the nuclear receptors interact with HSP90. The transcription factor BES1, but not its closely related transcription factor BZR1, is a substrate of HSP90. We show that *BES1* and *BZR1* are recent gene duplicates, and *BES1* has evolved faster than *BZR1*. Moreover, in natural populations, *BES1*, but not *BZR1*, encodes nonsynonymous polymorphisms. The faster evolutionary rate and nonsynonymous polymorphisms associated with *BES1*, but not *BZR1*, are consistent with the idea that HSP90 promotes *BES1* stability, allowing *BES1* to tolerate increased amino acid changes, thus altering the evolutionary trajectory of *BES1* relative to its paralog. Almost one-third of the *A. thaliana* genome encodes duplicated genes, and the proteins encoded by these genes tend to be kinases and transcription factors. Interestingly HSP90 substrates tend to be of the same classes. We suggest that HSP90 may play a broader role in promoting the evolution and maintenance of duplicate genes.

**541 Genetic Mapping Of Broad Resistance To Downy Mildew In Arabidopsis C24**Dmitry Lapin<sup>1</sup>, Rhonda Meyer<sup>2</sup>, Guido van den Ackerveken<sup>1</sup><sup>1</sup>**Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands, <sup>2</sup>Heterosis, Department of Molecular Genetics, Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466 Gatersleben, Germany**

The downy mildew oomycete *Hyaloperonospora arabidopsisidis* (*Hpa*) is an obligate biotrophic pathogen of *Arabidopsis*. Broad resistance to all tested isolates of this pathogen was identified in *Arabidopsis* accession C24. Segregation analysis in F<sub>2</sub> and backcross (BC) populations from a cross between Col-0 *flc3* and C24 suggests that the resistance is genetically complex. To identify loci underlying resistance against downy mildew we performed QTL mapping using recombinant inbred lines and introgression lines derived from a cross between Col-0 and C24. The level of susceptibility to *Hpa* was quantified by scoring the intensity of sporulation and by determining the relative *Hpa* DNA content in the infected plants. We identified 3 major loci explaining >45% of phenotypic variation with mostly additive effects. Interestingly, C24 carries not only resistance loci but also a susceptibility locus. The F1 progenies of crosses between Col-0 and introgression lines show intermediate level of susceptibility to *Hpa* indicating that the major QTLs are co-dominant. To fine map *Arabidopsis* QTLs affecting *Hpa* development we use traditional mapbased cloning and a BC approach in which resistance loci are eliminated from the C24 genome. The identification of the molecular mechanisms underlying C24 resistance may reveal novel aspects of plant immunity or host genes involved in susceptibility to downy mildew.

**542 Binding Site Divergence Between a Pair of Recently Duplicated AP2 Transcription Factors in Two *Arabidopsis* Species**Melissa Lehti-Shiu, Kelian Sun, Cheng Zou, Shin-Han Shiu**Michigan State University, East Lansing, MI, USA**

Expansion of plant transcription factor families by gene duplication is thought to have played a critical role in plant morphological diversification and adaptation to stress. However it is unclear how gene functions diverge after duplication. We are studying a pair of paralogous AP2 domain transcription factors, *DWARF AND DELAYED FLOWERING1* (*DDF1*) and *DDF2* that function in abiotic stress response. These genes are derived from the most recent whole genome duplication event in the *Arabidopsis* lineage and are present in both *A. thaliana* (*At*) and *A. lyrata* (*Al*). We hypothesize that *DDF1* and *DDF2* have been retained because their functions have diverged due to changes in binding site preference and/or changes in expression pattern. To test this hypothesis, we have performed Protein Binding Microarray (PBM) experiments to identify the DNA sequences bound by *DDF1* and *DDF2* in both *At* and *Al*. Although *DDF1* and 2 were derived from a duplication event that took place 25-40 million years ago, and the *At* and *Al* lineages diverged ~5 million years ago, *AtDDF1*, *AlDDF1*, *AtDDF2*, and *AlDDF2* all bind the same DRE-like binding site with the highest affinity. However, considering other high affinity sites, the binding preferences of *AtDDF1* and *AlDDF1* are different from those of *AtDDF2* and *AlDDF2*. We are currently confirming the PBM findings using *in vitro* binding assays and will perform *in vivo* chromatin precipitation assays to determine whether differences in affinity are reflected by differences in binding site occupancy. We are also characterizing the expression patterns of *DDF1* and *DDF2* in detail and exploring the functional divergence of *AtDDF1* and *AtDDF2* through loss of function analysis. By combining detailed knowledge of binding sites, expression patterns and loss of function phenotypes, we will gain a better understanding of how the functions of duplicate transcription factors have diverged in a cross-species context.

**543 Automated Measurements of Root Gravitropism Add the Time Domain to Quantitative Trait Loci Analysis***Candace Moore, Logan Johnson, Miron Livny, Edgar Spalding***University of Wisconsin, Madison, (WI), USA**

One method of gaining insight into the action of a genetic locus is to measure a response or trait in members of a recombinant inbred line (RIL) population and then subject the data to statistical modeling that attributes cause of variation to specific genetic loci. Typically, the precision and throughput of the phenotyping process determines the quality of such quantitative trait loci (QTL) analyses. We designed a high-throughput platform that acquires high resolution digital images of seedling roots at 2 min intervals for 8 h as they perform gravitropism. A custom algorithm run by the Condor distributed computing system extracts and quantifies the root tip angle at each time point. We treated each of the 241 tip angles as a trait for QTL mapping. This required large-scale permutation testing and QTL model selection which was also accomplished by running the R-based algorithms on the Condor-managed grid at the UW Center for High Throughput Computing and off campus on the Open Science Grid. The result was a type of QTL map that included the time domain. The y-axis of the resulting heat map is genome position, the x-axis is time, and color represents the significance of the effect as determined by the multiple interval mapping technique employed. The results show that several loci play a major role in the phenotypic variation in tip angle, but none of the loci contributes all the time. Instead, the effects of loci are time-dependent, with some loci affecting the root tip angle earlier and others hours after the gravitropic stimulus. With this collaboration between biologists and computer scientists, we aim to enable the scientific community's study of the timing of action of temporal QTL, and the study of many phenotypes at once, by creating a feasible workflow for automating the phenotyping and model selection.

**544 Genetic Analysis of Seed Longevity In *Arabidopsis thaliana****Thu-Phuong Nguyen<sup>4,3</sup>, Leónie Bentsink<sup>3,4</sup>***<sup>3</sup>Molecular Plant Physiology Group, Utrecht University, Utrecht, the Netherlands, <sup>4</sup>Laboratory of Plant Physiology, Wageningen University, Wageningen, the Netherlands**

Seed longevity is one of the most important factors for seed resource conservation and for crop success. It is defined as seed viability after a long time of dry storage (storability) and represents a quantitative trait. During storage seeds age, deteriorate and loose vigour, which ultimately leads to germination failure even under favourable conditions. Six *Arabidopsis thaliana* recombinant inbred line (RIL) populations, derived from crosses between Landsberg *erecta* and other accessions, show natural variation for seed longevity after natural storage at room temperature. A mix model quantitative trait loci (QTL) analysis reveals a number of loci for three parameters that could be extracted from the germination curve, the germination rate, the maximum germination and the area under the curve. The two major QTLs have been confirmed by near isogenic lines (NILs) after both natural and artificial ageing (controlled deterioration test). Results indicate that natural variation for seed longevity is regulated by additive genetic pathways.

**545 Ovule Patterning and Development in *Arabidopsis****Dunia Pino Del Carpio, Cornelia Gieseler, Rüdiger Simon***Heinrich Heine University, Duesseldorf, Germany**

In *Arabidopsis* the total number of ovules per plant (ONP) is controlled by the flower number and by the ovule number per flower (ONF). The ONF is set by the maximum silique size and by the patterning mechanism that regulates the spacing of ovules in the placenta. In order to dissect this complex trait an initial survey of ovule number was conducted among 15 *Arabidopsis* ecotypes. The trait variation was evaluated over three environments and as a result significant differences were found among the ecotypes. Based on this initial result we selected the Cvi x Ler mapping population to conduct a QTL study on siliqe length, the total number of ovules per siliqe and the ovule ratio (fruit length/ONF) data. Three replicates per line were grown in a growth chamber at 16°C under continuous light. The QTL results based on Haley-Knott regression indicate a genomic region in Chromosome 2, which regulate all phenotypic traits. This genomic region corresponds to the ERECTA locus, which is known to influence morphological characteristics. In parallel, we are currently phenotyping a larger population of 96 ecotypes under two different growth chamber conditions. Phenotypic data will be used for Association mapping analysis and candidate regulatory genes will be selected based on low p-value and high-explained variance. Additionally within the present study we aim to identify key regulatory genes involved in ovule patterning and development through the expression profiling of specialized meristematic regions that generate the ovules (placenta) in selected mutants and ecotypes.

**546 Structure/Function Analysis of the NF-YB family in *Arabidopsis****Jan Risinger, Rod Kumimoto, Ben Holt***University of Oklahoma**

One of the most fundamentally important plant developmental processes is the transition from vegetative to reproductive growth. Nuclear Factor Y (NF-Y) are heterotrimeric transcription factors with known regulatory roles in photoperiodic flowering, acting upstream of *FLOWERING LOCUS T (FT)*. NF-YB2 and NF-YB3 were shown to be positive regulators of flowering and *FT* expression under inductive long-day photoperiods, and *nf-yb2/nf-yb3* double mutants flower significantly later than wild type (WT, Kumimoto et al., 2008. 228:709-723). *Arabidopsis* encodes 13 unique NF-YB proteins that all share a conserved domain, consisting of a NF-YC interacting domain, a DNA binding domain, and a NF-YA interacting domain. Due to this high level family conservation, we are interested in investigating whether NF-YB family members, other than NF-YB2 and NF-YB3, can rescue the *nf-yb2 nf-yb3* late flowering phenotype. By ectopically overexpressing all of the *Arabidopsis* NF-YB in both WT and *nf-yb2/nf-yb3* backgrounds, we will be able to detect structural

and functional differences between NF-YB family members. Combining this data with targeted amino acid substitutions, we will identify specific residues that are necessary for positively regulating photoperiodic flowering. Preliminary analyses demonstrate that there will be significant variability between the unique NF-YB subunits, as will be discussed at the ICAR meeting. Using these tools, along with known crystal structures, we will perform a complete structure/function analysis of the NF-YB family in *Arabidopsis*.

#### **547 Molecular Evolution and Selection Patterns of Plant F-Box Proteins with C-Terminal Kelch Repeats**

*Nadine Schumann, Aura Navarro-Quezada, Kristian Ullrich, Carsten Kuhl, Marcel Quint*

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In eukaryotes the Ubiquitin/26S proteasome pathway is responsible for selective degradation of most intracellular proteins. Proteins destined for degradation are polyubiquitinated, whereby an E3 ubiquitin ligase catalyzes the transfer of activated ubiquitin to the target protein. The most prevalent E3 ubiquitin ligases in plants are the Skp1-Cullin-F-box (SCF) complexes, in which target specificity is mediated by the F-box subunit.

The F-box protein superfamily represents one of the largest families in the plant kingdom. F-box proteins phylogenetically organize into numerous subfamilies characterized by their C-terminal protein-protein interaction domain. Among the largest F-box protein subfamilies in plant genomes are those with C-terminal kelch repeats. While the N-terminal F-box motif mediates the binding to the rest of the SCF complex, the C-terminal kelch repeat domain most likely facilitates the interaction with the target protein.

In this study, we analyzed the phylogeny and evolution of FBK proteins/genes in seven completely sequenced land plant genomes including a bryophyte, a lycophyte, monocots, and eudicots.

The construction of a phylogenetic tree based on the full-length amino acid sequences of the FBKs that we identified in the seven species enabled us to classify *FBK* genes into unstable/stable/superstable categories. In contrast to superstable genes, which are conserved across all seven species, kelch domains of unstable genes, which are defined as lineage specific, showed strong signatures of positive selection, indicating adaptational potential. We found evidence for conserved protein features such as binding affinities toward *A. thaliana* SKP1-like adaptor proteins and subcellular localization among closely related FBKs. Pseudogenization seems to occur only rarely, but differential transcriptional regulation of close relatives may result in subfunctionalization.

#### **548 Towards genome-wide association genetics to identify loci involved in responses to Potyviruses in plants**

*Valerie Schurdi-Levraud<sup>1</sup>, Patrick Cosson<sup>1</sup>, Zofia Nehr<sup>1</sup>, Melodie Caballero<sup>1</sup>, Fabrice Roux<sup>2</sup>, Frederic Revers<sup>1</sup>*

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The aim of the project is to identify loci involved in *Arabidopsis thaliana* responses to Potyviruses using genome-wide association (GWA) mapping.

The project will allow designing a map of loci involved in quantitative responses to Turnip mosaic virus (TuMV) in the species *Arabidopsis thaliana*. It will be an important first step towards the identification of quantitative traits loci (QTL) involved in host's responses and the cloning of the most important of them. It will also contribute to an initial setting in understanding plant/virus co-evolution through the comparison of loci involved in the responses to the challenged virus.

In first steps, robust phenotyping protocols are developed on a narrow range of genotypes known to reveal a large variability of responses to TuMV. We optimized the standardization of plant culture, growth and inoculation conditions. We defined the position of the leave to sample through the following of TuMV-GusGFP movement. We determined scoring classes for symptoms, sampling periods and optimized high-throughput RT-QPCR. Based on these first data, we will measure on the whole plant set: (1) symptoms severity, (2) virus accumulation, (3) infection kinetics, (4) length of floral hamps 21 days after inoculation, (5) weight of total plant 28 days after inoculation. Association between the phenotype and the genotype will then be performed using a 250k SNPs database.

#### **549 Tetraploidy in natural populations of *Arabidopsis thaliana* is a transient character state**

*Elisabeth Svedin, Tena Graham, Brian Dilkes*

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Polyplody, the condition of having three or more complete chromosome sets, is broadly distributed in the flowering plants. In many species, established autotetraploid populations exist as a result of tetraploid derivatives outcompeting the diploid parent or adapting to a new environment or niche. *Arabidopsis thaliana* has been used to study molecular mechanisms affecting polyplodization; however, it is not clear if polyplodity plays a role in the distribution of *A. thaliana*. *A. thaliana* is a paleopolyploid—the most recent polyplodity event occurred ~23.3 million years ago—that exhibits infrequent spontaneous tetraploidy in the lab, and naturally-occurring tetraploids have been collected. It is unclear if the collected tetraploids come from established populations in the wild or if they result from stochastic, new formations of tetraploid *Arabidopsis*. To test this we used genetic information available from a worldwide collection of *Arabidopsis*. We used a phylogenetic tree containing 6418 genotypes, and the SNP data for those genotypes to compare four known tetraploids to their closest relatives. If tetraploidy persists, the closest relatives should also be tetraploids, and divergence should indicate relative isolation from the remainder of the species and estimate the time since polyplodity establishment. We found that two tetraploids are sisters to tetraploids; however, they are genetically identical to each other. The other two tetraploids are sisters to diploids, and at the level of information available the genotypes are identical, indicating no divergence from the diploid parents. These data strongly suggest that established tetraploid populations do not exist in *A. thaliana* and that tetraploidy does not persist in the wild. This may indicate that *A.*

*thaliana*, while useful for the study of genetic mechanisms and the consequences of polyploidy for plant development, is not an appropriate model organism for the study of mechanisms governing the establishment of polyploid populations in wild plants.

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## 550 Incidence and Pattern of Nonrandom Mating in *Arabidopsis thaliana* Across the Genetic Spectrum

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The pollen dusted across flower stigmas is often a mixture, whose proportions do not often match proportions within progeny. In other words, some pollen have greater mating success—a phenomenon called nonrandom mating. Our lab has been studying nonrandom mating in the model plant *Arabidopsis thaliana* (*Arabidopsis*). In previous work, we have: (1) developed a system to investigate nonrandom mating in *Arabidopsis*, (2) demonstrated that *Arabidopsis* accessions from geographically distinct regions mate nonrandomly, and (3) used quantitative trait locus (QTL) mapping to construct genetic maps of loci responsible for both female and male-mediated nonrandom mating in accessions. To expand our understanding of the genetic architecture of this trait in *Arabidopsis*, here we report a study intended to identify accessions to act as parental lines for a genome-wide association mapping study. We systematically competed pollen from a group of seven *Arabidopsis* accessions with pollen from the Col-0 accession on females from each accession using a factorial mating design (NC Design II). The accessions, Cvi-0, Kz-1, NFA-10, An-1, Ws-2, Col-0 and Bur-0, were chosen to represent major groups previously identified through hierarchical clustering of relative kinship. The results and analysis of this data set will be presented.

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## 551 Intragenic Tandem Repeats Confer Phenotypic Variability

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Tandem repeats (TR) are hypervariable genetic elements. In order to study the role of intragenic TR in complex developmental traits, we conducted a genome-wide computational survey of *Arabidopsis thaliana* and *Oryza sativa*. TR-containing genes tended to function in key environmental responses and developmental pathways. To establish that TR variation modulates phenotype, we selected the gene *ELF3* in *A. thaliana* based on its TR sequence and its predicted TR copy number variability across divergent *Arabidopsis* accessions. *ELF3* encodes a transcription factor that is a core component of the circadian clock and a floral repressor. The *ELF3* TR encodes a poly-glutamine tract at its C-terminal domain. As predicted, *ELF3* TR copy number is highly variable with copy numbers ranging from 7 to 29 units, and 16 being the most frequent allele. We found that accessions that contain 16 Q's tend to flower about 5 days later than all other accessions, suggesting that this allele might be more active than its counterparts. To establish a causal relationship between TR copy number and phenotype, we generated constructs with *ELF3* alleles that only differed in TR copy number. Alleles varying from 0 to 29 TR copy number were used to transform *elf3-4* (Ws background) and *elf3-200* (Col-0 background) loss-of-function mutants. We analyzed homozygous *T<sub>3</sub>* and *T<sub>4</sub>* transgenic plants with normalized expression levels for flowering time, hypocotyl length, and circadian period phenotypes. Certain TR alleles result in separation of the pleiotropic phenotypes observed in the *elf3*-mutants. Further, TR copy number effects differ between the two tested genetic backgrounds. Our results indicate that altering TR copy number likely disrupts complex interactions among the proteins integrating light signals with different phenotypic outputs. We propose that unstable TR in key regulators of growth and development may facilitate rapid adaptations to local environments due to the high TR mutation rate and compensatory mutations in their interaction partners.

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## 552 The scale of adaptation in *Arabidopsis thaliana*: identifying the ecological factors that act on phenology.

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Despite the increasing number of genomic tools available for the model species *Arabidopsis thaliana*, the lack of data on selection patterns may hamper the identification of the genetic bases underlying adaptive complex traits. The aim of this study was to gain insight into both the spatial scale of adaptation and the selective agents acting on phenology in *A. thaliana*. Forty-nine natural stands from four climatically contrasted French regions were (1) phenotypically characterized for four phenological traits (bolting time, flowering interval, flowering and reproductive periods); (2) ecologically characterized for climate and soil conditions; and (3) genetically characterized using 135 SNP markers. Population demographic history had little or no effect on phenology-ecology relationships, supporting adaptive phenological differentiation. The adaptation for phenological traits appeared stronger at the within-region than at the among-region scale, demonstrating the importance of local selective agents in shaping the phenotypic variation. In addition, the relationships between phenological traits and ecological conditions varied with the trait and the region considered, highlighting the complexity of selection patterns. Although climatic conditions have often been suggested as the main selective agents acting on phenology in *A. thaliana*, we demonstrate here that edaphic conditions were predominant in the patterns of local adaptation, explaining up to 80% of phenological variation in some regions. The observed pattern of local adaptation for phenological traits emphasizes the necessity to study local polymorphic populations in GWA mapping studies in *A. thaliana*.

**553 AaTFL1 Regulates Multiple Aspects of Perennial Flowering in *Arabis alpina***

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Flowering of many plants is induced by environmental signals, but these responses can depend on the age of the plant. Young perennial plants are normally insensitive to floral induction of environmental signals so that they can accumulate enough energy before flowering, to sustain their survival through many years. Exposure of annual species *Arabidopsis thaliana* to vernalization (winter temperatures) at germination induces flowering, whereas a close perennial relative *Arabis alpina* only responds if exposed when at least 5 weeks old. We show that vernalization of these older *A. alpina* plants reduces expression of the floral repressor *PEP1* and activates the orthologues of the *A. thaliana* flowering genes *SOC1* (*AaSOC1*) and *LFY* (*AaLFY*). In contrast when younger plants are vernalized *PEP1* and *AaSOC1* mRNA levels change as in older plants, but *AaLFY* is not expressed. We demonstrate that *A. alpina TFL1* (*AaTFL1*) blocks flowering and prevents *AaLFY* expression when young plants are exposed to vernalization. In addition, in older plants *AaTFL1* increases the duration of vernalization required for *AaLFY* expression and flowering, and therefore function synergistically with *PEP1* to restrict flowering to spring after a long cold winter. *AaTFL1* has similar functions in axillary shoots thus ensuring that following a flowering episode vegetative branches are maintained to continue the perennial life cycle. We propose that *AaTFL1* blocks flowering of young plants exposed to vernalization by setting a threshold for a flowering pathway that is increased in activity as the shoot ages, thus contributing to several perennial traits.

## 554 The Cyber-Language of Flowers: PhytoCognito as Fusion of Omic Data Analysis and Natural Language Processing of the *Arabidopsis* Literature

*Amir Assadi, Noah Larsen*

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The literature in biology is vast and rich with valuable empirical, heuristic and theoretical information. In the last few decades, articles concerning *Arabidopsis thaliana* constitute a large portion. Systematic organization and knowledge mining of research articles play a major role in helping with large-scale project formulation, making groundbreaking discoveries and forming novel hypotheses. The following is a preliminary report on development of Natural Language Processing (NLP) tools tailored for plant systems biology, such as dynamic models of –omic networks, systems-level perturbation of pathways etc. For example, we demonstrate utility of NLP in discovery of hidden and implicit correlations among pairs of genes in massive gene expression data for diurnal and circadian rhythms in wild type *Arabidopsis thaliana* (provided by the Chory Lab). A theory of Collective Intelligence in Plant Biology (PhytoCognito) is under development that provides the synthesis of cognitive, computational and informatics that will sustain collaborative efforts that aim at continuing the heritage of the past into the major successes of the future and scientific breakthroughs. We demonstrate through PhytoCognito tools how new hypotheses could be formulated from explorative analysis of the Chory Lab genomic data, and the odds of discovering such hypotheses without NLP of a very large group of articles. New-Topic-Formation in *Arabidopsis* research is among other notable applications of PhytoCognito tools; e.g. as in iPlant research on new cyber-enabled technologies for team-based research.

## 555 Targeted protein aggregation in *Arabidopsis thaliana* plants as a tool to specifically knock-out protein function

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Neurodegenerative diseases, such as Alzheimer, are caused by a protein aggregation occurring through the formation of intermolecular beta-sheets resulting in functional knockouts. A similar targeted aggregation strategy was applied to *Arabidopsis thaliana* plants in order to develop a technology complementary to known mutagenesis approaches in plants. Good targets were identified in players of brassinosteroids (BRs) signaling pathway regulating plant growth. Mutations in those components affect plant size resulting in clear phenotypes. Transgenic knock-out *Arabidopsis* plants for the BRs negative regulator, Brassinosteroid Insensitive 2 (BIN2) were generated by over-expressing predicted BIN2 aggregating baits, designed by the TANGO algorithm, fused to a Green Fluorescent Protein (GFP). The presence of GFP aggregates was visualized by both Confocal and electron microscopy and by Native-PAGE and FT-IR spectroscopy in the transgenic lines. The *in vivo* physical interaction between the aggregating baits and BIN2 was further assessed by co-immunoprecipitation experiments. Functional analyses of BIN2 aggregating peptides-expressing plants demonstrated clear effect of BIN2 aggregation on BRs signaling and plant growth.

## 556 Temporal EIN3 transcription factor binding reveals role of protein-DNA interactions in hormone crosstalk

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Ethylene plays an integral role in plant growth, development and disease resistance among other important processes, by regulating the expression of a diverse set of genes. To characterize the transcriptional response mediated by ethylene, we used ChIP-Seq and RNA-Seq to generate genome-wide temporal profiles of transcription factor binding and expression. We found EIN3 (ETHYLENE INSENSITIVE3), a master regulator of the ethylene transcriptional response, targets >1000 genes. Binding by EIN3 increased over time upon ethylene treatment, corresponding with the accumulation of EIN3 protein. A subset (~20%) of EIN3 targets was ethylene-regulated; the majority up-regulated upon ethylene treatment. EIN3 binds three general classes of targets: 1) downstream effectors of the ethylene response, including ETHYLENE RESPONSE FACTOR1 and its family members, 2) ethylene signaling pathway genes, indicating a major role for feedback regulation of ethylene responses, and 3) key signaling pathway genes involved in other hormone responses (e.g. JA, ABA, auxin). Finally, the role of EIN3 in ethylene-auxin crosstalk was confirmed when we found EIN3 targeted HOOKLESS1(HLS1) and two of its homologs. HLS1 is a putative N-acetyltransferase regulating the protein levels of AUXIN RESPONSE FACTOR2; a mutant in HLS1 is deficient in establishing the ethylene response apical hook phenotype.

**557 Abstract Withdrawn****558 High Throughput Automated Imaging and Machine Learning Methods for Quantifying Variation of Morphological Traits of *Arabidopsis Thaliana* Roots and Shoots***Hesam Dashti, Hesam Dashti, James Driver, Amir Assadi***University of Wisconsin, Madison, WI, USA**

Plant biology has greatly benefited from Analysis of Quantitative Traits Loci (QTL) and Systems biology has rapidly advanced to offer a number of powerful methods for discovery of networks of genes/proteins and collective functions of families of genes/ proteins in life processes. QTL and other gene functions discovery methods rely on quantification of phenotypic traits that could be observed and used in a systematic way to distinguish between organisms with differing DNA sequences or epigenetic signatures. To extract phenotypic traits that distinguish genotypic characteristics, biologists make repeated observations of the wild type and the mutants of the same species during growth, behavior or in the course of response to external stimuli. This is a progress report in research on dynamical systems of phenotypic changes, where we focused on quantifying dynamical phenotypic traits of *Arabidopsis Thaliana* seedlings growth, namely hypocotyls and roots, subject to different environmental perturbations (heat, light, gravitropism, etc). The technology combines high performance computation, machine learning, image analysis and other analytic tools for high throughput phenotyping, and a fully automated high throughput image acquisition hardware that has a modular with flexible user-specific requirements (e.g. light-weight portable, programmable etc.)

**559 Plant Methods: an Independent Open Access Journal for Technological Innovation in the Plant Sciences***Brian Forde, Mike Roberts***Lancaster University, Lancaster, UK**

*Plant Methods* ([www.plantmethods.com](http://www.plantmethods.com)) was established in 2005 as an electronic journal specialising in the rapid publication of articles focussing on technological innovation in the plant sciences. *Plant Methods* is one of over 100 independent, Open Access journals published by BioMed Central.

Supported by a prestigious international Editorial Board, the journal's primary aim is to stimulate the development and adoption of new and improved techniques and research tools in plant biology. We have now published over 100 papers describing new techniques or resources of value to the plant biology community. In addition to methodology papers, we publish research papers, reviews and commentaries as well as occasional 'Protocol' papers providing step-by-step descriptions of previously established techniques.

*Plant Methods* is indexed by ISI Web of Science and received its first official Impact Factor of 2.98 in June 2010 - putting us 25th out of 172 in the 'Plant Sciences' category. In 2010 the journal was also awarded an 'A' (excellent) rating in the Excellence in Research for Australia (ERA) list of journals.

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**560 Automated high content screen uncovers factors affecting plant cell proliferation***Guillaume Queval, Rudy Vanderhaeghen, Leen Vermeersch, Pierre Hilson***VIB Department of Plant Systems Biology and Department of Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium**

To complement classical genetic approaches, we are forging alternative methods for the dissection of specific segments in plant molecular pathways. In this context, we have assembled a high-content screening platform designed to study the cellular phenotypes of isolated plant cells. Among other applications, this system enables the tracking of *Arabidopsis* cell proliferation with high-throughput and robotized protocols. For this purpose protoplasts expressing a histone 2B-YFP (H2B-YFP) fusion protein are seeded as a monolayer

in microtiter plates and division events are recorded via microscopic time lapse imaging. In this experimental set-up, the mitotic rate of thousands of individual protoplasts can be measured following treatment in a small volume. Dedicated algorithms automatically recognize specific mitotic phases marked by changes in the nuclear H2B-YFP signal. All steps of the analytical pipeline have been streamlined and the system was implemented to screen compound libraries with the aim to identify chemicals that act as growth regulators or that alter in any way cell proliferation. Such compounds may be used as new tools to probe plant growth and development mechanisms. They may also be valuable for the optimization of in vitro culture media. The characterization of mitogenic molecules identified in such screens will be presented. We anticipate that high throughput and high-content screens based on cultured plant cells will soon be important assets for the study of plant systems.

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## 561 iTILLING: a personalized approach to the identification of induced mutations in *Arabidopsis*

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TILLING (for Targeting Induced Local Lesions IN Genomes) is a well-established method for identifying plants carrying point mutations in genes of interest. A traditional TILLING project requires a significant investment of time and resources to establish the mutant population and screening infrastructure. Here, we describe a modified TILLING procedure that substantially reduces the investment needed to perform mutation screening. Our motivation for developing iTILLING was to make it practical for individual laboratories to rapidly perform mutation screens using specialized genetic backgrounds. With iTILLING, M2 seeds are collected in bulk from the mutagenized population of plants, greatly reducing the labor needed to manage the mutant lines. Growth of the M2 seedlings for mutation screening, tissue collection, and DNA extraction are all performed in 96-well format. Mutations are then identified using high-resolution melt-curve analysis of gene-specific polymerase chain reaction products. Individual plants carrying mutations of interest are transferred from the 96-well growth plates to soil. One scientist can complete an iTILLING screen in less than 4 months. As a proof-of-principle test, we applied iTILLING to *Arabidopsis* (*Arabidopsis thaliana*) plants that were homozygous for the mekk1-1 (for MAPK/ERK kinase kinase 1) mutation and also carried a MEKK1 rescue construct. The goal of our screen was to identify mutations in the closely linked MEKK2 and MEKK3 loci. We obtained five mutations in MEKK2 and seven mutations in MEKK3, all located within 20 kb of the mekk1-1 T-DNA insertion. Using repeated iterations of the iTILLING process, mutations in three or more tandemly duplicated genes could be generated.

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## 562 PCR from Plant Tissue without DNA Extraction

*Maiju Kyllainen, Pia Kuusisto, Pak Yang Chum*

**Thermo Fisher Scientific, Vantaa, Finland**

Plant tissues contain inhibitors, (e.g polyphenols, polysaccharides), which interfere with PCR even when present in minimal amounts. Therefore, PCR amplification from plant tissues traditionally involves an initial DNA isolation step which may require expensive or toxic reagents, and is often time consuming.

Thermo Scientific Phire® Plant Direct PCR Kit allows robust and efficient PCR from different types of plant materials without DNA extraction. Tissues such as plant leaves, flowers and seeds can be used as starting materials. The kit can be used with extremely low amounts of starting materials: a punch disc of only 0.35 to 0.50 mm is required for robust and reliable results.

The kit was used for genotyping of *Arabidopsis* plant individuals with dCAPS (derived cleaved amplified polymorphic sequence analysis) technique directly from leave punches and the results obtained were confirmed by conventional analysis. Similarly, the kit facilitated the detection of an RNAi vector in gerbera plants without any DNA extraction or purification steps.

The Phire Plant Direct PCR Kit is compatible with all kinds of thermal cyclers. When combined with Thermo Scientific Piko® Thermal Cyclers and ultra-thin walled UTW® vessels, PCR protocols can be completed in as little as 45 minutes.

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## 563 Determining Degradation and Synthesis Rates of *Arabidopsis* Proteins Using the Kinetics of $^{15}\text{N}$

**Labeling of 2D Gel Separated Proteins**

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The growth and development of plant tissues is associated with an ordered succession of cellular processes that are dictated by the appearance and disappearance of proteins. The control of the kinetics of these processes is central to how plants can rapidly alter specific protein abundance and thus molecular function to respond to environmental or developmental cues. However, these processes are largely hidden during periods of apparent steady-state protein abundance and even when proteins accumulate it is unclear if enhanced synthesis or decreased degradation is responsible. We have used a  $^{15}\text{N}$  labeling strategy with inorganic nitrogen sources coupled to 2-D Fluorescence Difference Gel Electrophoresis (DIGE) and mass spectrometry analysis of 2D IEF/SDS-PAGE gel spots to define the rate of protein synthesis ( $P_{\text{syn}}$ ) and degradation ( $P_{\text{deg}}$ ) of *Arabidopsis* cell culture proteins. Through analysis of MALDI-TOF mass spectra from 120 protein spots we were able to quantify  $P_{\text{deg}}$  and  $P_{\text{syn}}$  for 84 proteins across 6 functional groups and observe over 20 fold variation in protein degradation rates. These rates are dependent on the subcellular location of the protein, its functional role in the cell, and the time in the cell culture cycle. This method is based on  $^{15}\text{N}$  labeling that appears to be innocuous for the plant, and as it can be used to target analysis of specific proteins through the use of gel spots it has broad applicability.

## 564 CRES-T is a novel gene silencing system, useful for functional analysis of transcription factors and manipulation of plant traits

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Gene silencing is a useful tool not only for functional analysis of genes but also for manipulation of plant traits. For gene silencing, gene knockout mainly by T-DNA tagged lines and RNA interference are often used. However, plant genes are frequently duplicated and a number of crops have multi-ploid genome. Such structural and functional redundancy often interferes with efforts to identify functions of genes. Single gene knockout or RNA interference is less effective to such redundant factors. To overcome such difficulties, we developed a novel system, called Chimeric REpressor Gene-Silencing Technology(CRES-T), in which a chimeric repressor produced by fusion of a transcription factor to the plant-specific repression domain, SRDX (LDLELRL), suppresses the target genes of a transcription factor dominantly over the activity of endogenous and functionally redundant transcription factors. As a result, the transgenic plant that expresses a chimeric repressor exhibits phenotype similar to loss-of-function of the alleles of the gene for transcription factor. The chimeric repressors act not only in *Arabidopsis* but also in various plants, including rice, tall fescue, tobacco, morning glory, cyclamen and hexaploid chrysanthemum. By expressing the AG-SRDX, we succeed to produce cyclamen with multiple petals like rose. We have prepared transgenic *Arabidopsis* lines that respectively express the chimeric repressor for each of the transcription factors encoded in *Arabidopsis* genome. Because the chimeric repressor can induce informative phenotype with high frequency, we are analyzing function of transcription factors in *Arabidopsis* using our chimeric repressor library. In addition, we found that the expression of a chimeric repressor can confer tolerance to various environmental stress to plants and we have isolate CRES-T lines that exhibit various stresses, such as high salt (400 mM NaCl), osmotic(650 mM mannitol), draught (11 days), freezing and heat. We present that the CRES-T system is a powerful tool for functional analysis of redundant plant transcription factors and the manipulation of plant traits.

## 565 Artificial Chimeric Repressors Can Increase Seed Oil Content and Plant Biomass

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Plant seed oils have been recently expected to be potent resources for bio-fuels and bio-plastics. Improving seed oil productivity must be difficult only by one-gene manipulation. However, gene technologies developed in *Arabidopsis* could be workable for a plant of *Brassicaceae* such as rapeseed, one of the most popular oil crops.

To develop useful genes, we chose artificial chimeric repressor silencing technology (CRES-T) that can change phenotypes dominant-negatively and may have potential to change quantitative traits such as seed oil productivity. We measured oil content in *Arabidopsis* T2 seeds from 195 CRES-T lines by <sup>1</sup>H-pulse NMR. The seed oil content were increased by up to 41.3% in an At1g56650 (*PAP1*, *AtMYB75*)-SRDX T2 line with larger oil bodies in seeds. Aerial biomass and seed yield were also increased in this line. Seed oil content and oil yield were averagely increased by 6% and by 32% respectively in At5g07580 (AP2/ERF)-SRDX T3 lines. In transgenic rice, At5g07580-SRDX had a similar effect on oil content.

The following genes fused with nucleotides encoding SRDX domain were effective for increasing oil content in seeds as well; At1g22985, At1g24260, At1g25470, At1g67260, At1g71030, At1g74930, At1g80580, At2g31230, At3g15510, At3g25890, At3g61910, At4g01550, At4g36160, At5g09330, At5g24520, At5g25190, At5g47230 and At5g47390.

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## 566 High-resolution imaging of statolith dynamics under hypergravity conditions by using a new centrifuge microscope

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Hypergravity conditions provide a unique opportunity to clarify potential abilities of organisms. We recently developed a centrifuge microscope that creates hypergravity conditions. All optical devices (e.g., objective lens, light source and CCD camera) and specimens were rotated on a rotor, and acquired images were wirelessly transmitted during centrifugation. This new microscope overcame previous limitations and allowed real-time imaging of thick/dark specimens with high spatiotemporal resolution under any angular velocity. Using this microscope, we observed intracellular sedimentation of a plant statolith, amyloplast, under 10-30g hypergravity conditions, which indicated that amyloplast dynamics are strongly correlated with gravity responsiveness in *Arabidopsis* stems. Furthermore, we revealed

that the *shoot gravitropism* (*sgr2*) mutant, which has nonsedimentable amyloplasts and does not sense gravity at 1g, can sense gravity at 30g, when the amyloplasts moved toward hypergravity. These findings strongly support the long-standing starch-statolith hypothesis that plant gravity sensing is triggered by amyloplast redistribution resulting from directional movement toward gravity.

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**567 Teaching Tools in Plant Biology: A New, Award-Winning, On-Line Educational Resource Published by  
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**568 A Role of LATERAL ORGAN BOUNDARIES DOMAIN 37, 38, and 39 in a Subset of ABA Signaling in *Arabidopsis****Chuloh Cho, Jungmook Kim***Department of Bioenergy Science and Technology, Chonnam National University, Gwangju 500-757, Korea**

The *LATERAL ORGAN BOUNDARIES DOMAIN/ASYMMETRIC LEAVES2-LIKE (LBD/ASL)* genes encode proteins harboring a conserved amino acid domain, referred to as the LOB (for lateral organ boundaries) domain. While recent studies have revealed developmental functions of some *LBD* genes in *Arabidopsis* and in crop plants, the biological functions of many other *LBD* genes remain to be determined. It had been reported that *LBD37*, *LBD38*, and *LBD39* function as negative regulators of anthocyanin biosynthesis and nitrogen response. The plant hormone ABA regulates many aspects of plant developmental processes, such as closing of stomata, root growth, seed maturation and seedling growth. We here report a role of *LBD37*, *LBD38*, and *LBD39* in a subset of ABA response as a negative regulator. We found that the expression of *LBD37*, *LBD38*, and *LBD39* is coordinately reduced by ABA. While *lbd37 lbd38* and *lbd38 lbd39* double mutants exhibited weak sensitivity to ABA, *lbd37 lbd39* doublemutants and *lbd37 lbd38 lbd39* triple mutants exhibited hypersensitivity to exogenous ABA in the germination assays and cotyledon expansion assays. However, we were not able to detect significant effects of these triple mutations in water loss and stomatal movement. These results indicate that *LBD37* and *LBD39* might play an important role as a negative regulator of a subset of ABA signaling at germination and post-germination stages. Moreover, *lbd37 lbd38 lbd39* triple mutants displayed reduction of lateral root density at low concentrations of ABA, suggesting that *LBD37*, *LBD38*, and *LBD39* might be involved in the regulation of lateral root formation via the ABA signaling. *lbd37 lbd38 lbd39* triple mutants have reduced lateral root length compared with wild type, indicating that *LBD37*, *LBD38*, and *LBD39* genes might be involved in lateral root growth. This work was supported by grant from the World Class University project funded by the Ministry of Education, Science, and Technology of Korea (grant no. R31-2009-000-20025-0) to J. Kim.

**569 Suppression of Cell Death of *mod1* by Reducing ROS through Disturbing Mitochondrial Complex I***Jian Wu, Yuefeng Sun, Yonghong Wang, Jiayang Li***State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China**

Programmed cell death (PCD) has long been recognized for its importance in the regulation of plant development and defense, but the mechanism is still elusive. Mitochondria play key functions in mediating apoptosis in animal, but little is known about its action involved in plant PCD. We previously identified an *Arabidopsis mosaic death 1 (mod1)* mutant, which shows premature cell death in multiple organs. The *MOD1* gene encodes an enoyl-acyl carrier protein (ACP) reductase, which is a subunit of the fatty acid synthase complex that catalyzes the *de novo* biosynthesis of fatty acids. Recently, we identified and isolated several suppressors of *mod1* (*soms*) through T-DNA insertion mutagenesis. Among them, *som42* was further characterized. Gene cloning revealed that *SOM42* encodes a mitochondria-located PPR protein. Overexpression of *SOM42* could abolish the cell death of *mod1*. We found that the elevated *SOM42* impeded the splicing of the intron 4 of mitochondrial *NAD7* transcripts and thus affected the assembly of mitochondrial Complex I, leading to a reduction of the ROS accumulation in *mod1*. We also found that other Complex I-defective mutants could inhibit the cell death in *mod1*, our study demonstrated that the mitochondrial Complex I plays a key role in regulating plant PCD.

**570 Comprehensive study of plant transcription factors***Nobutaka Mitsuda<sup>1</sup>, Youichi Kondou<sup>2</sup>, Tomotsugu Koyama<sup>3</sup>, Kyoko Matsui<sup>4</sup>, Takeshi Yoshizumi<sup>2</sup>, Miho Ikeda<sup>1</sup>, Yuko Takiguchi<sup>1</sup>, Shinobu Takada<sup>5</sup>, Miki Fujita<sup>6</sup>, Kazuo Shinozaki<sup>2</sup>, Norihiro Ohtsubo<sup>7</sup>, Minami Matsui<sup>2</sup>, Masaru Ohme-Takagi<sup>1</sup>***<sup>1</sup>AIST, Tsukuba, Japan, <sup>2</sup>RIKEN, Yokohama, Japan, <sup>3</sup>Kyoto Univ, Kyoto, Japan, <sup>4</sup>Green Sogna Inc, Tsukuba, Japan, <sup>5</sup>Osaka Univ, Osaka, Japan, <sup>6</sup>RIKEN, Tsukuba, Japan, <sup>7</sup>National Institute of Floricultural Research, Tsukuba, Japan**

In plants, a number of transcription factors (TFs) have been shown to act as master regulators of various phenotypes, where a single TF regulates expression of many genes at once. The model plant *Arabidopsis thaliana* has ca. 2,000 TFs, which regulate the expression of whole ca. 27,000 genes in the genome. For functional analyses of plant TFs, we developed a novel gene-silencing technology designated CRES-T, which utilizes chimeric repressors derived from the TF. We applied it to almost all TFs in *Arabidopsis* and rice and have revealed various biological functions of a number of TFs in *Arabidopsis* and prepared a pool of T2 seeds for screening of interesting phenotypes. We also applied CRES-T to various horticultural plants to modify their flower traits and constructed "FioreDB" database that stores phenotypic data and information regarding plant TFs. Furthermore, we made Gateway entry clones of ca. 1,500 TFs without stop codon and prepared TF-only yeast-one/two-hybrid library. This library enabled us to isolate TF that interacts with specific DNA/protein efficiently with less effort (1). Using these tools, we are currently attempting to build entire transcriptional regulatory network linked with phenotypic information. We here introduce our activities toward this goal.

**571 Unraveling Gene Regulatory Network in *Arabidopsis thaliana****Mohammad Amin Omidbakhshfar, Bernd Mueller-Roeber***Potsdam University and Max-Planck Institute for molecular plant physiology, Golm, potsdam, Germany**

DNA-binding transcription factors (TFs) are central regulators of essentially all developmental and physiological processes. They bind to cis-regulatory elements in promoters of target genes (TGs) whose expression they regulate, often in concert with other TFs (of the same or other families). Our group studies the role of TFs controlling developmental and physiological processes in leaves and is

particularly interested in unraveling the gene regulatory networks (GRNs) controlled by TFs of selected families. The Growth Regulating Factor (GRF) family represents a plant specific TF family which in *Arabidopsis thaliana* includes nine genes. GRF genes are strongly expressed in actively growing and developing tissues, such as shoot tips, flower buds, and roots, but normally only weakly in mature stem and leaf tissues. GRFs have been reported to play a role in leaf growth and cell proliferation. Currently, however, the direct targets of GRFs are virtually unknown. To identify downstream targets of GRFs, we therefore expressed some of them under the control of an estradiol-inducible promoter in transgenic plants which will be used for Affymetrix ATH1 micro-array-based expression profiling after induction of GRF gene expression. Responding genes (potential candidates) will be analyzed further thereafter, using a suite of technologies developed in the group. Additionally, we optimized a previously described method, called FAIRE, to discover regulatory elements in *Arabidopsis thaliana* at a genome-wide scale. Results will be presented.

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## 572 A Mitochondria Localized PPR Protein Is Required For Embryogenesis In *Arabidopsis*

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One embryo-lethal *Arabidopsis* mutant, named as *maia*, was identified from a T-DNA activation tagging mutant collection in our lab. Most mutant embryos were arrested before the 16-cell stage, and some of the mutant embryos displayed aberrant cell division patterns. TAIL-PCR and sequencing analysis revealed that the T-DNA insertion in *maia* was located in an exon of a PPR domain protein-encoding gene and that the T-DNA insertion was co-segregated with the embryo lethal phenotype. Genetic complementation data confirmed that the embryo-lethal phenotype was caused by loss of function of the *MAIA* gene. Co-localization data showed that the MAIA protein was localized in mitochondria. We further found that the *MAIA* was highly expressed in dry and imbibed seeds. The GUS assay with the *P<sub>MAIA</sub>*-GUS transgenic plants showed that high GUS activity was detected in early endosperms and embryos after heart stage. These data suggest that the MAIA protein might regulate the seed development by controlling the expression of some genes in mitochondria. We are now working on the detailed phenotypes in *maia* siliques and regulatory mechanisms of MAIA in seed development.

## 573 NO CATALASE ACTIVITY 1 (NCA1) is an ancient post-transcriptional regulator of plant catalase activity

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We previously showed that catalase is a direct target of the antineoplastic drug hydroxyurea and that *Arabidopsis* catalase mutants are resistant to hydroxyurea (1). Using our hydroxyurea resistance forward genetic screen, we have now identified the *NO CATALASE ACTIVITY 1 (NCA1)* gene as required for catalase activity in *Arabidopsis*. The protein sequence of NCA1 is highly conserved in the plant kingdom, and expression of *Physcomitrella patens* *NCA1* cDNA can rescue the *Arabidopsis nca1-1* mutant phenotype, indicating that NCA1 is an ancient regulator of catalase activity. In *nca1-1* mutants, we found no change in catalase transcript levels but a marked reduction in catalase protein and activity levels, showing that NCA1 regulates catalase at the post-transcriptional level.

(1) Juul et al. The *in vivo* toxicity of hydroxyurea depends on its direct target catalase. *J Biol Chem* (2010) vol. 285 (28) pp. 21411-5

## 574 Sugar Induced Expression of the PAP1 Gene in *Arabidopsis thaliana*

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Regulation of genes by sugars such as glucose and sucrose allows plants to coordinate gene expression with various metabolic and physiological processes. For example, in sugar beet, sucrose is known to repress gene expression of a phloem-loading sucrose transporter, which serves to help plants balance source production with sink demand. In order to dissect sugar signaling pathways, we have focused on identifying sugar response elements regulating the expression of PAP1 (Production of Anthocyanin Pigment-1). PAP1 is a member of the MYB family of transcription factors and a regulator of anthocyanin accumulation in *Arabidopsis thaliana*. Many studies have shown that anthocyanins accumulate in plants treated with sucrose and PAP1 expression has been previously reported to be sugar responsive. We found that sucrose is the most effective inducer of anthocyanins in 4 d old *Arabidopsis* seedlings; other sugars were less effective. PAP1 transcript is expressed in response to sucrose in 4 d old seedlings and expression is also observed with maltose, glucose, and fructose. In order to further investigate sucrose induced expression of PAP1 we constructed β-glucuronidase (GUS) and luciferase (LUC) reporter fusions and tested sucrose-induced expression of the reporter genes. These results show that PAP1 expression is sucrose responsive. The sucrose response element has been localized to 205 bps and further delineation of the element by mutagenesis is in progress. This region does not include any previously reported sucrose response elements (such as SURE-1 or SURE-2). We have also examined PAP1 expression in our GUS lines and found that PAP1 was expressed in most tissues and organs including seedling hypocotyls, rosette and cauline leaves (most notably along veins), flowers, and pedicels. This staining pattern correlated well with PAP1 transcript as detected by RT-PCR.

## 575 Signaling pathways Controlling Cell Wall Loosening and Cell Separation During Floral Abscission

*Melinka Butenko<sup>1</sup>, Chun-Lin Shi<sup>1</sup>, Bin Liu<sup>2</sup>, Michelle Leslie<sup>3</sup>, Sara Patterson<sup>4</sup>, Sarah Liljegren<sup>3</sup>, Reidunn Aalen<sup>1</sup>*

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Small peptides play an important role in plant growth and development. *INFLORESCENCE DEFICIENT IN ABSCISSION (IDA)* encodes a putative peptide ligand necessary for the regulation of floral organ abscission. IDA is dependent on the receptor-like kinases (RLK) HESA (HAE) and HESA-LIKE 2 (HSL2) to exert its function (Stenvik *et al.*, *Plant Cell*, 2008, Cho *et al.*, *PNAS*, 2008). Recent studies suggest that a short C-terminal conserved region of the IDA protein is capable of interacting with one of the RLKs, substantiating that these proteins form a peptide-receptor system regulating cell separation in abscission zones (AZs). To identify proteins downstream of the IDA signaling pathway, a revertant screen was performed on the *ida* mutant. Lines with mutations in the *KNAT1/BP* transcription factor were discovered, which in addition to rescuing the abscission defect of *ida* and *hae hsl2* mutants, show enlargement of AZ cells. We show that this enlargement is due to precocious cell wall loosening and that *BP* functions as a suppressor of organ separation by restricting AZ cell elongation. A mutation in the RLK *EVERSHED (EVR)* is capable of rescuing the abscission defect of *nevershed (nev)* but not of *ida* (Leslie *et al.*, *Development*, 2010). Phenotypes of the *evr nev* mutants suggest that *EVR* acts as an inhibitor of cell wall loosening. Here we present genetic and morphological data proposing signaling pathways where NEV suppresses premature cell wall loosening by repressing IDA signaling but later in the abscission process functions synergistically with IDA to promote cell separation.

## 576 Genetic dissection of GA regulated root growth

*Ester Cancho<sup>2</sup>, Susana Ubeda-Tomás<sup>1</sup>, Malcolm Bennett<sup>2</sup>*

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Gibberellins (GAs) are plant hormones that regulate growth and developmental processes during the plant life cycle. GA promotes growth by degrading DELLA repressor proteins through the SCF/proteosome machinery (Dill *et al.*, 2004). Targeting expression of a non-

degradable form of DELLA in endodermal cells (using the *SCR:gai-GR* transgene) blocked root elongation, highlighting the importance of this tissue for organ growth (Ubeda-Tomás *et al.*, 2008;2009). In order to analyse the gene regulatory network targeted by GA in this tissue to promote root growth, forward and reverse genetic approaches have been pursued. The *SCR:gai-GR* line has been mutagenized to select mutants that are no longer blocked in their root growth. Several mutant lines have been identified and are currently being characterised. The *SCR:gai-GR* line has also been used in transcriptomic studies and a number of novel downstream targets identified which are currently being functionally characterised. Our latest results will be presented.

Dill A, Thomas SG, Hu J, Steber CM and Sun TP (2004) The Arabidopsis F-box protein SLEEPY1 targets GA signalling repressors for GA-induced degradation. *Plant Cell* **16**: 1392-1405.

Ubeda-Tomás S, Swarup R, Coates J, Swarup K, Laplaze L, Beemster GTS, Heden P, Bhalerao R and Bennett MJ (2008) Root growth in *Arabidopsis* requires gibberellin/DELLA signalling in the endodermis. *Nature Cell Biol.* **10**: 625 – 628.

Ubeda-Tomás S, Federici F, Casimiro I, Beemster GTS, Bhalerao R, Swarup R, Doerner, Hasseloff J and Bennet MJ (2009) Gibberellin Signaling in the Endodermis Controls Arabidopsis Root Meristem Size. *Current Biology* **19**: 1194–1199.

## 577 Abolishing of bZIP Factor Dependent Gene Expression through Phosphorylation-mimicking Serine Mutations in the DNA-binding Domain

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Transcription factors containing a conserved stretch of basic amino acids followed by an alpha-helical leucine zipper domain constitute the bZIP protein family. In plants, these factors have an important function in the regulation of many developmental processes and adaptive responses to the environment. Whereas the leucine zipper domain is involved in protein dimerization, the region of basic amino acids is necessary for both nuclear localization and binding of the transcription factor to specific regulatory DNA sequences. The DNA binding domain (DBD) of bZIP factors is highly conserved throughout plant kingdom. By *in silico* sequence analysis of the DBDs of numerous plant bZIP transcription factors we have revealed a surprisingly high level of positional conservation of the amino acids at positions 15 and 19 of the DBD, which are occupied by serine or occasionally threonine residues (Kirchler *et al.*, 2010, *Eur J Cell Biol.* **89**: 175–183). A structural modelling of the DBD of the *Arabidopsis thaliana* bZIP transcription factor 63 (AtbZIP63) indicates that the serines at these positions mediate the direct contact with a phosphodiester oxygen of the DNA bond. This contact appears to be significantly disturbed upon either serine phosphorylation or substitution by aspartate. By a comprehensive quantitative *in vitro* analysis of the DNA binding capacity of AtbZIP63 protein forms with single and double serine-to-aspartate substitutions we demonstrate that the introduction of a negative charge at positions 15 and / or 19 in the DBD completely abolishes the binding to the cognate DNA sequence. Moreover, it also prevents induction of two target promoters, which otherwise are induced by the heterodimers of AtbZIP63 with the bZIP factors of the S1-group in transiently transformed *Arabidopsis* protoplasts. We propose that the modification of these highly conserved serine residues in the DBD can control the fine-tuning of bZIP factor activity at the DNA-protein interaction level.

## 578 Application of a FRET-based Calmodulin Sensor to imaging Calmodulin-Dependent Signaling in *Arabidopsis*

Alexandra Chanoca, Simon Gilroy

UW-Madison

Calcium represents a widely used second messenger in plant signaling systems with both biotic and abiotic signals leading to transient changes in  $\text{Ca}^{2+}$  concentration, activation of calcium-dependent proteins and the triggering of downstream responses. Of the  $\text{Ca}^{2+}$ -binding proteins involved in such signaling networks, calmodulin is ubiquitous among eukaryotes and is known to play a central role in regulating many cellular events. Calmodulin is thought to control a multitude of cellular functions, such as metabolism, ionic balance, the dynamics of the cytoskeleton and protein modifications such as phosphorylation. Although regulation through calmodulin has been implicated in numerous plant responses, the dynamics of any associated  $\text{Ca}^{2+}$  signal and especially the temporal and spatial dynamics of calmodulin activation (the calmodulin signature of the response) remains unanswered. Defining the signatures of these regulators remains a key limit to our understanding of how these signaling networks operate. Therefore, we have developed a GFP-based sensor that enables us to visualize calmodulin activation in intact plants, in real time, with subcellular resolution. The sensor is based on the CFP:Venus FRET partnership, that is rendered sensitive to calmodulin activation through the M13 calmodulin binding peptide. The sensor responds *in vitro* in the physiological calmodulin concentration range. *In vivo* experiments show that it successfully detects changes in calmodulin activation in *Arabidopsis thaliana*, with versions targeted either to the cytoplasm or to the nucleus. When coupled with information from the YC3.6 GFP-based  $\text{Ca}^{2+}$  sensor, the development of a calmodulin FRET-based sensor is providing important insights into  $\text{Ca}^{2+}$  and calmodulin dynamics and subcellular microdomains of activation of this signaling cassette in *Arabidopsis*. This work is funded by the USDA and NSF.

## 579 Phosphobinding Domain in Plants

David Chevalier

Mississippi State University, Mississippi State

Phosphorylation is an excellent way to transduce a signal between cells and within a cell. Phosphorylation changes the function of a protein. However, some proteins require a two-step activation to change their function: the first step involves their phosphorylation and the second step the binding of a phosphobinding domain. In this case, phosphorylation is required but not sufficient to modify the

function of a protein. Phosphobinding domains are the protein's domains that specifically bind phosphorylated peptides. Several types of phosphobinding domains have been identified including the ForkHead Associated (FHA) domain. FHA domain specifically recognized phosphorylated threonine. They are present in both prokaryotes and eukaryotes. Proteins with an FHA domain are involved in basic cellular functions such as cell cycle progression, RNA stability and DNA repair. The plant model *Arabidopsis thaliana* has 18 genes, which encode a protein with an FHA domain. The function of only six of these genes has been identified. Our goal is to identify the function of the other genes.

## **580 Regulation of auxin response in the *Arabidopsis* hypocotyl**

*Goh Choe, Mark Estelle*

**UCSD, La Jolla, CA, USA**

In *Arabidopsis*, hypocotyl elongation promoted by high temperature or shade conditions requires the phytohormone auxin. In addition, hypocotyl bending towards the light or in response to gravity is also dependent on differential accumulation of auxin in the hypocotyl. Therefore, auxin is an essential molecule controlling hypocotyl growth. Plants regulate cellular auxin levels through multiple processes, including biosynthesis, conjugation, degradation, and transport. To better understand which steps of auxin regulation are involved in this response, we examined the expression levels of the PIN auxin transporters and the group II GH3 enzymes including GH3.2-GH3.6 and GH3.17 which have been shown to conjugate amino acids to IAA in the hypocotyl. We found that most of the PIN and GH3 genes were upregulated in the hypocotyl following auxin treatment. Of these genes, upstream sequences of PIN7 and GH3.4 were further characterized to identify promoter regions required for auxin-mediated gene induction. In addition, the role of the auxin signaling pathway for this transcriptional regulation was examined using auxin receptor and auxin response factor mutants.

## **581 Identification and Characterization of a Novel JA Signaling Component, JAH2**

*KwiMi Chung, Agnes Demianski, Inez Oh, Barbara Kunkel*

**Washington University in St.Louis**

Jasmonate is an important plant hormone that regulates plant responses to biotic and abiotic stresses as well as plant growth and development including root growth and pollen formation.

To date, genetic studies using mutants with alterations in JA biosynthesis or signal transduction have identified key regulators of JA signaling, including COI1 and JIN1/MYC2. However, our knowledge about JA signaling is still limited. In order to identify additional JA-signaling components, we have identified and characterized *Arabidopsis* mutants that exhibit increased sensitivity to methyl jasmonic acid (MeJA) in a seedling root growth inhibition assay. Here, we introduce two novel mutants that are JA-hypersensitive, *jah2-1* and *jah2-2*. Although we believe the *jah2-1* and the *jah2-2* mutations are allelic, they show different characteristics: the *jah2-1* mutation is recessive, whereas *jah2-2* is a semi-dominant mutation and the root growth is more strongly inhibited than in the *jah2-1* mutant on low concentrations (e.g. 2.5 uM) of MeJA.

To clarify the genetic relationship between the *jah2-1* mutant and known JA signaling components, we made and are analyzing *coi1jah2-1* and *jin1jah2-1* double mutants.

We have mapped *JAH2* to a 33kb region of chromosome 4 that does not contain any known JA signaling genes, thus *JAH2* defines a novel JA signaling component. Map-based cloning of *JAH2* is underway.

## **582 Auxin Response: How Does ABP1 Signal to Induce Cell Expansion?**

*Nathan Deed, Karine David*

**The University of Auckland, Auckland, New Zealand**

Most plant cells display a many-fold expansion after cell division, which is mainly caused by the phytohormone auxin. However, the underlying mechanisms remain poorly understood. Due to the central role of auxin-induced cell expansion in plants, developing an understanding of this process is important scientifically and also with respect to the agriculture, horticulture and forestry industries.

The action of auxin is mediated by two receptors. These are TIR1, an F-box protein involved in degrading a repressor of auxin responses, and auxin-binding protein 1 (ABP1) located at the plasma membrane. ABP1 is essential for plants and involved in early auxin responses and cell expansion. The aim of this research was to analyse mechanisms of auxin-induced expansion by focusing on ABP1. This research used transgenic *Arabidopsis thaliana* lines which are conditional knock-outs for ABP1. Upon induction with ethanol, these lines express a 'mini-antibody' called scFv (single chain Fragment variable), which blocks ABP1 activity. To identify components downstream of ABP1, we analysed changes in the transcriptome upon ABP1 inactivation.

The transcriptome was analysed using Affymetrix microarrays, which identified 3696 genes differentially expressed upon ABP1 inactivation. Results indicated that the ABP1 pathway interacts with the TIR1 pathway. There was downregulation of cell wall-associated genes upon ABP1 inactivation, which fits with ABP1's role in cell expansion. In addition, ABP1 was transcriptionally upregulated upon ABP1 inactivation, and signalling components were identified as possible candidates in the ABP1 pathway. Homozygous mutants in these candidates were analysed for cell expansion and auxin sensitivity/resistance phenotypes. Results indicated that some of these genes are involved in auxin responses and may be involved in the ABP1 pathway.

**583 Comparison of ABA-, glucose- and sucrose-mediated gene expression profiles between *Arabidopsis* and rice: insights into ancestral angiosperm signaling pathways***Luiz Del-Bem, Renato Vicentini, Michel Vincentz***State University of Campinas, Campinas - SP, Brazil**

Sugars are key metabolic signals that control gene expression and modulate different developmental phases and processes such as photosynthesis, senescence and stress responses. ABA is a key hormone in the signaling of environmental stress responses and germination control. Glucose-insensitive mutants have provided evidences that ABA interact with glucose in the control of developmental processes as germination. With this in mind, we used microarrays to measure global changes in the transcriptome of rice plantlets submitted to 2 hrs treatments with exogenous ABA, glucose and sucrose. The differentially expressed genes were compared to previously published microarray data from *Arabidopsis* under similar experimental conditions (Li et al, 2006 and Osuna et al, 2007). We used a nd in-house phylogenetic algorithm to compared the responses by finding the most probable orthologs of each differentially expressed gene from *Arabidopsis* and rice. Among the possible groups of orthologs responding to ABA, 131 were up-regulated and 12 down-regulated. We found 112 up- and 90 down-regulated groups of orthologs in response to glucose and 39 up- and 48 down-regulated after sucrose treatment. Among these genes we found several archetypical transcription factor and kinases known to act in these signaling pathways and also a set of unknown genes. We interpreted the conserved regulatory patterns that were uncovered as being ancestral angiosperm features that may represent critical elements in angiosperm's function.

**584 Monitoring reactive oxygen species in the auxin-induced oxidative burst of *Arabidopsis thaliana* roots***Desiree den Os, Gabriele Monshausen***The Pennsylvania State University - University Park, PA, USA**

Long known for their destructive potential, reactive oxygen species (ROS) have in recent years been shown to also function as key messengers in cellular signal transduction pathways in plants. Such ROS are not only formed as byproducts of cellular respiration and photosynthesis, but are generated by a suite of dedicated enzymes that may become activated in response to endogenous or environmental cues. Sequential reduction can then convert one type of ROS into other forms of ROS, all of which possess different reactivity and thus different half-lives and diffusion distances. This provides the cell with a signaling system that is spatially and temporally highly flexible and offers great versatility in that specific types of ROS may be employed to signal specific cellular processes. To effectively study this complexity of ROS signaling, it is vital to utilize sensors that are able to sensitively discriminate between different forms of ROS in real time without negatively impacting cellular responses. Because loading of cell-permeant derivatives of commonly used fluorescent ROS probes into plant cells requires entrapment of the probes within the cytoplasm, over-accumulation of entrapped ROS probes can be problematic. To prevent potential over-accumulation, we are performing short-term incubation experiments and using the red fluorescence of cytosolically targeted E2-Crimson proteins as a reference to mark the location and density of the cytoplasm. This work investigates the application of recently developed fluorescein-based single wavelength ROS probes in combination with E2-Crimson to identify intracellular ROS produced during an auxin-induced oxidative burst in *Arabidopsis* roots.

**585 Genetic Identification of Factors Involved in GLV Secretory Peptide Perception***Sarieh Ghorbani, Andrzej Drozdzecki, Pierre Hilson***Department of Plant Systems Biology, VIB , Ghent University, Ghent, Belgium**

We have identified a plant gene family that codes for small secretory peptides designated GOLVEN (GLV) and shown that GLV peptides act locally to regulate gravitropic growth responses in the root and hypocotyl of *Arabidopsis* plants. Roots treated with GLV synthetic peptides or overexpressing *GLV* genes show altered gravitropism when grown on an inclined impenetrable agar surface. With the aim to dissect the GLV signal perception pathway, we have initiated a forward genetic screen based on an EMS-mutagenized population. M2 seedlings were grown in the presence of a GLV-derived synthetic peptide in gravistimulating conditions to identify lines in which the *GLV* gain-of-function curly-root phenotype was either suppressed or enhanced. We will present the detailed analysis of the confirmed line phenotypes, including their root and shoot growth characteristics.

**586 Candidate GPCR Signaling Systems Integrate Multiple Environmental Signals in G-protein Dependent and Independent Pathways***Timothy Gookin<sup>1</sup>, Xiaofen Jin<sup>1</sup>, Rui-Sheng Wang<sup>1</sup>, Sixue Chen<sup>2</sup>, Sarah Assmann<sup>1</sup>***<sup>1</sup>The Pennsylvania State University, University Park, Pennsylvania, U.S.A, <sup>2</sup>University of Florida, Gainesville, Florida, U.S.A.**

The heterotrimeric G-protein signaling cascade is an evolutionarily conserved mechanism by which eukaryotic cells sense and respond to the environment. Perception of extracellular stimuli is performed via membrane spanning G-protein coupled receptors (GPCR) which transduce the signal to G-protein heterotrimers, which stimulate or repress a myriad of pathways. In contrast to the multitude of subunits in metazoan organisms, the *Arabidopsis* genome is limited to a single canonical G $\alpha$  subunit, one G $\beta$  subunit, and three G $\gamma$  subunits. Our previous computational work identified several candidate GPCRs which bind GPA1 in vivo (Gookin et al., *Genome Biology* 2008, 9:R120). *Arabidopsis* single mutants for two of these candidates GPCRs appear phenotypically wild type; and the double mutant exhibits phenotypic differences under specific growth conditions. Additional developmental and reproductive phenotypes become evident in triple (with G $\alpha$ ) and quadruple (adding G $\beta$ ) mutants. A metabolomic analysis of seeds, seedlings, and mature leaves implicates several

signaling pathways. The receptor systems identified play key roles in G-protein dependent and independent signaling pathways involving multiple hormones, light, humidity, and oxidative damage.

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### **587 The AFB4 Auxin Receptor is a Negative Regulator of Auxin Signaling in *Arabidopsis* Seedlings**

Katie Greenham, Cristina Castillejo, Colleen Doherty, Steve Kay, Mark Estelle

University of California San Diego

The auxin receptor family is comprised of six members; TIR1 and AFB1-5. Phylogenetic analysis reveals that the AFB4/5 clade diverged from the other members before seed plant radiation whereas the TIR1/AFB1 and AFB2/3 clades diverged within the angiosperm lineage (Parry *et al.* 2009). This earlier divergence suggests functional specialization of the AFB4/5 clade. We have taken a variety of approaches to investigate the roles of individual members of the TIR1/AFB family. Genetic studies reveal that *afb4-2* and *afb5-5* are resistant to the synthetic auxin picloram while *tir1-1* is not. Biochemical analysis confirmed that picloram works through AFB4/5 and specifically enhances the affinity of these receptors for Aux/IAA proteins indicating that members of the AFB4 clade are the major targets of the picolinate herbicides in *Arabidopsis*. Further analysis of the *afb4-2* mutant reveals several auxin related defects opposite to the *tir1-1* *afb2-3* mutant suggesting that AFB4 functions as a negative regulator of auxin signaling in seedlings. Among the phenotypes observed is a long hypocotyl at 22°C. At 29°C, a condition that results in increased IAA levels, *afb4-2* hypocotyls do not elongate to the extent as wild-type hypocotyls suggesting a unique role for the AFB4 receptor in regulating hypocotyl elongation. A series of transcriptome analyses using hypocotyl tissue from wild type and *afb4-2* seedlings grown at 22°C and 29°C was performed. Results from this analysis have uncovered circadian clock regulated genes that show abnormal expression patterns in *afb4-2* at 22°C. Current work is aimed at examining the relationship between AFB4 and the circadian regulation of hypocotyl growth.

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### **588 Abstract Withdrawn**

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### **589 RIC7 Negatively Regulates Stomatal Movements via Interaction with Exo70B1**

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Stomatal guard cells determine the stomatal pore size via their volume changes in response to various environmental cues. ROP GTPases play critical roles in the process. Previously we have shown that RIC7 functions as a downstream effector of ROP2 GTPase in the light-induced stomatal opening. In this study, we further investigated how RIC7 functions in the control of stomatal movements. RIC7 was expressed highly in stomatal guard cells in *Arabidopsis thaliana*. The RIC7 over-expression (RIC7OX) slowed down both the light-induced stomatal opening and the ABA-induced stomatal closure in *Arabidopsis*. In contrast, a loss-of-function mutation of RIC7 (*ric7ko*) promoted both the light-induced stomatal opening and the ABA-induced stomatal closure. In a search for the interaction partners of RIC7 by using yeast two hybrid screen, we identified Exo70B1, a component of exocyst complex. Exo70B1 interacted with the RIC7 in vitro and displayed similar cellular localization with ROP2 and RIC7 when expressed in *Vicia faba* guard cells. These results suggest the possibility that RIC7 participates in the ROP2-mediated control of stomatal movement by negatively regulating the speed of exocytic vesicle trafficking.

**590 Characterization of <>in Auxin Signaling may Reveal Novel Functions of the COP9 Signalosome**He Huang, Marcel Quint, William Gray

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The COP9 signalosome (CSN) is an eight-subunit protein complex conserved in all higher eukaryotes. In *Arabidopsis thaliana*, the CSN regulates plant auxin response by removing the ubiquitin-like protein NEDD8/RUB1 from the CUL1 subunit of the SCF<sup>TIR1</sup> ubiquitin-ligase (deneddylate). Previously identified loss of function mutations in any CSN subunit result in the pleiotropic *cop/det/fus* phenotype and cause seedling lethality, which hampers the study of CSN functions in plant development. Here we report a viable *csn* mutant of subunit 3 (CSN3), designated as *csn3-3/eta7*, which was isolated in a previous genetic screen for enhancers of *tir1-1*. Phenotypic characterization and expression analyses of auxin-responsive reporter genes show that the *csn3-3* is a weak auxin mutant. Surprisingly, we did not detect any apparent defects in either CSN mediated CUL1 deneddylate or in SCF<sup>TIR1</sup> mediated degradation of Aux/IAA proteins. This suggests that *csn3-3* is an atypical *csn* mutant that defines a novel CSN function. Consistent with this possibility, we observe dramatic differences in double mutant interactions between *csn3-3* and other auxin signaling mutants compared to other *csn* mutants. Lastly, unlike other *csn* mutants, gel-filtration studies demonstrate that assembly of the CSN holocomplex is unaffected in *csn3-3*. However, we detected a small CSN3-containing protein complex that is altered in *csn3-3* plants. We hypothesize that in addition to its role in the CSN as a cullin deneddylase, CSN3 functions in a distinct protein complex that is required for proper auxin signaling.

**591 The Plant U-box/ARM E3 ligases as potential signalling proteins for S-Domain Receptor Kinases**Emily Indriolo, Pirashaanthi Tharmapalan, Daphne Goring

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ARC1, which is necessary for the rejection of self-pollen in the self-incompatibility response. This recognition event is mediated by the binding of the SCR/SP11 pollen ligand to the stigma-specific S Receptor Kinase (SRK) that then activates a signalling pathway in the stigmatic papilla to cause pollen rejection. ARC1 functions downstream of SRK, binding to SRK through its ARM repeat domain. ARC1 is proposed to promote ubiquitination of Exo70A1 during the self-incompatibility response. In addition to the U-box and the ARM repeat domain, ARC1 contains the U-box N-terminal Domain (UND) that may mediate Exo70A1 binding. Large gene families with sequence similarity to the SRK (S-Domain-1 Receptor Kinases) and ARC1 (UND/U-box/ARM) have been identified in plant genomes. The predicted *Arabidopsis* genes show a variety of expression patterns, and may participate in other regulatory signalling pathways. We have found that several of the ARC1-related proteins can interact with S-Domain-1 Receptor Kinases, and using a number of genomic resources, we are investigating these gene families to determine their potential signalling pathways and biological functions.

**592 A Soluble ABC Protein AtNAP9/AtABCI20 Might Play Important Functions for Early Seedling****Development Regulated by Abscisic Acid and Light**Jun-Young Jin<sup>1</sup>, Sehoon Kwon<sup>1</sup>, Soo Young Kim<sup>2</sup>, Enrico Martinoia<sup>1,3</sup>, Youngsook Lee<sup>1</sup>

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AtNAP9/AtABCI20 is a member of Non-intrinsic ABC Protein (NAP) subgroup of ABC proteins. Here we report many lines of evidence which suggests roles of AtNAP9/AtABCI20 in early growth and development regulated by abscisic acid (ABA) and light. *AtNAP9/AtABCI20* has abundant ACGT-containing elements (ACE) in promoter region which implicates that this gene may be under control of ABA or light. *AtNAP9/AtABCI20* knockout mutant (*nap9-1*) seedlings exhibited retarded growth under normal condition, and this phenotype was aggravated in medium containing ABA. *AtNAP9/AtABCI20* expression complemented the phenotype of *nap9-1* mutants. The expression of abscisic acid-insensitive (ABI) series genes was altered in *nap9-1*; *ABI1* and *ABI2* gene expression decreased but *ABI3*, *ABI4* and *ABI5* gene expression increased. These changes in ABI gene expression levels corresponded well with the increased sensitivity of *nap9-1* mutant to ABA. Results from green fluorescence protein-fused AtNAP9/AtABCI20 expression and membrane fractionation assay indicated that this protein is localized to both cytoplasm and endoplasmic reticulum (ER) membrane. We are investigating the interaction partners of AtNAP9/AtABCI20, which might play an important roles in early developmental stage controlled by light and ABA.

**593 Patatin-Related Phospholipases A In Auxin Signal Transduction: Mis-Regulation Of Early Auxin-Induced Genes In pPLA Knockouts**Corinna Labusch<sup>1</sup>, Maria Shishova<sup>2</sup>, Yunus Effendi<sup>1</sup>, Günther Scherer<sup>1</sup>

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In *Arabidopsis*, a family of ten phospholipase A genes with sequence homology to potato patatins has been identified and are involved in auxin and pathogen signaling (e.g. Rietz *et al.*, 2010). Plant PLA activity is rapidly induced by different external signals and the PLA reaction products (released fatty acids and lysolipids) function as second messengers that regulate distinct proteins or downstream processes (Scherer *et al.*, 2010, TIPS). Here we used the T-DNA insertion mutants of *pPLA-I*, *pPLA-IIα*, *pPLA-IIβ*, *pPLA-IIγ*, *pPLA-IIδ*, *pPLA-IIε*, *pPLA-IIIα* and *pPLA-IIIβ* to test the regulation of early auxin genes. Test genes were *IAA2*, *IAA11*, *IAA13*, *IAA20*, *SAUR9*, *SAUR15*, *SAUR23*, *GH3.5*, genes involved in lateral root formation (*IAA3*, *IAA14*, *IAA19*, *PIN3* (Péret *et al.*, 2009) and *PIN1*, *PIN2*

and *PIN5*. 30 to 60% of the genes tested failed to respond to auxin (10 µM) at t=30 min in the different knockouts. In most mutants the genes *IAA11*, *IAA13* and *IAA20* showed no change in gene expression in comparison to the WT, except in *ppla-I*, *ppla-IIδ* and *ppla-IIβ*. Many of the genes involved in lateral root formation and the SAUR genes showed a strong defect in gene expression in the mutants after auxin application. In comparison, the transcription of pPLA-genes is not auxin regulated within 30 min. The mutants did not show any phenotypes under normal growth conditions or when grown on auxin containing medium. Only *ppla-IIε* showed 50% lateral root formation on low nutrient medium. *ppla-IIγ* reacted less sensitive to ABA and to phosphate deficiency (Rietz *et al.*, 2010). In summary, the pPLA-knockouts show a transient mis-regulation of early auxin regulated genes that mostly disappeared after 3h. Because the *abp1/ABP1* mutant regulated none of early auxin-induced genes properly at 30 min we hypothesize that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (Effendi *et al.*, 2011, Plant J.).

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#### **594 Characterization of *gig1* (glucose insensitive growth 1) Reveals the Involvement of the Plastidic Copper Transporter PAA1 in Sugar-mediated Interorganellar Communication**

*Shin Ae Lee, Jun Lim*

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It is known that high glucose (6%) conditions inhibit growth of the *Arabidopsis* seedlings. Based on the growth-arrested phenotype, we have screened ~1500 activation tagging seedlings for insensitive growth in the presence of 6% glucose. We identified a recessive mutant with strong insensitive growth, named *gig1* (*glucose insensitive growth 1*). Under standard growth conditions, *gig1* mutants exhibited a short-root phenotype. Thus, we characterized developmental defects of *gig1* with tissue-specific markers for the specification and maintenance of stem cell niche. Although no patterning defects were found in *gig1*, root meristem size was drastically reduced and cell division potential monitored by CYCB1;1-glucuronidase (GUS) reporter was also severely reduced. To understand the molecular basis of *gig1* in glucose signaling, we isolated the *GIG1* gene by TAIL-PCR, and found a T-DNA insertion in the *PAA1* locus, which is previously known as a P-Type ATPase that transports copper (Cu) to the chloroplast stroma. We also confirmed that growth of *gig1* was complemented with Cu supplement in MS agar plates, and the genomic clone including the promoter region and ORF rescued *gig1* phenotypes. In addition, another recessive T-DNA insertion allele from the SIGNAL database exhibited the identical phenotype. To elucidate the role of *GIG1* in sugar signaling, we performed genetic analysis with *hexokinase 1* (*hxk1*) and *aba-insensitive 4* (*abi4*). Our findings indicate that *GIG1* is epistatic to both *HXK1* and *ABI4* in glucose signaling, suggesting that there is sugar-mediated interorganellar communication.

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#### **595 Abstract Withdrawn**

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#### **596 Inferring the signaling network of ORE1: a molecular and functional approach to a leaf senescence regulatory pathway**

*Lilian Matallana-Ramirez, Bernd Mueller-Roeber, Salma Balazadeh*

**Max Planck of Molecular Plant Physiology and Potsdam University, Golm, Potsdam, Germany**

Leaf senescence, a developmentally regulated process, contributes to nutrient remobilisation during reproductive growth and finally leads to tissue death. This process is triggered by endogenous factors as well as environmental signals and stresses. Transcriptome analysis of leaf senescence in *Arabidopsis thaliana* indicated a complex network of genes involved in the process. ORE1 is a NAC domain transcription factor that shows elevated expression during senescence. Although the pivotal role of ORE1 in controlling leaf senescence has recently been demonstrated, the underlying molecular mechanisms and the pathways it regulates are currently only vaguely defined. Our aim is to establish the regulatory connections between ORE1 and other cellular factors that control senescence during aging and in response to abiotic stresses. To this end, we have initiated a deletion analysis of the *ORE1* promoter; promoter-GUS reporter constructs are currently being tested in transgenic *Arabidopsis* and tobacco plants. Results will be presented. Additionally, we have selected potential direct target genes of the ORE1 transcription factor using genome-wide expression profiling (based on Affymetrix GeneChips) of transgenic plants over-expressing the NAC TF undercontrol of a chemically inducible promoter. We are currently testing transactivation of these genes in *Arabidopsis* mesophyll cell protoplasts. Additionally, we have initiated ChIP (chromatin immuno precipitation) experiments to confirm the *in vivo* binding of ORE1 to its target genes.

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#### **597 Mutations in the Cytokinin Signaling Pathway Can Alter Auxin Signaling**

*Dennis Mathews<sup>1</sup>, McKenzie Shaw<sup>1</sup>, Eric Schaller<sup>2</sup>*

**<sup>1</sup>University of New Hampshire, Durham, NH, USA, <sup>2</sup>Dartmouth College, Hanover, NH, USA**

The plant growth regulators auxin and cytokinin are known to act coordinately in many aspects of plant growth and development. Cytokinin signaling is initiated by activation of receptors located in the outer membrane. These cytokinin receptors are histidine kinase proteins that initiate a multi-step phosphorelay. In *Arabidopsis* the primary targets of this cytokinin phosphorelay signaling pathway are members of a family of response regulator transcription factors (type-B, ARR). In an attempt to gain insight into the interaction between cytokinin and auxin signaling we have introduced auxin signaling reporter constructs into *Arabidopsis* plants with mutations in one or more type-B ARR. Among the constructs used to monitor auxin signaling were the DR5::GUS and BA3::GUS reporters. Of interest was the effect of altered cytokinin signaling on the location, duration and intensity of auxin signaling. Mutants with reduced ability to respond to cytokinin showed enhanced sensitivity to auxin. To investigate whether cytokinin signaling influences auxin transport, expression of the auxin efflux proteins PIN1,2,3,4, and 7 was monitored.

**598 Silver Ions Block Ethylene Responses In *Arabidopsis* Predominantly Through The ETR1 Ethylene Receptor***Brittany McDaniel, Brad Binder***University of Tennessee, Knoxville, (Tennessee), USA**

In *Arabidopsis*, responses to ethylene are mediated by a family of five membrane-bound receptors: ETR1, ETR2, ERS1, ERS2, and EIN4. Previous work showed that the coordination of Cu(I) in the ethylene-binding domain of these receptors is required for ethylene to bind and elicit a response. Interestingly, although Ag(I) is also able to support ethylene binding to ETR1, the presence of Ag(I) blocks ethylene responses *in planta*. In this study we examined the requirements for specific receptor isoforms in mediating responses to Ag(I) in more detail. Using end-point and time-lapse imaging analyses of growth on receptor loss-of-function mutants, we found that ETR1 has the prominent role in mediating responses to Ag(I). Two other receptor isoforms have a modulatory role in this phenotype. Two hypotheses are currently being considered for why Ag(I) mainly affects ETR1. One is that Ag(I) does not affect the other receptor isoforms because it does not bind to these receptors to support ethylene binding. Alternatively, it is possible that Ag(I) binds to and supports ethylene-binding to all five receptor isoforms but only prevents perception by ETR1. To distinguish between these models we are using radio-ligand, ethylene binding assays of exogenously expressed receptors to assess the ability of silver ions to replace copper ions as a cofactor for ethylene-binding activity. The role of each receptor isoform in mediating Ag(I) responses was explored by transforming receptor loss-of-function mutants with various receptor isoforms and ETR1-EIN4 chimeric receptors.

**599 Function Of The N-end Rule Pathway Of targeted Proteolysis In The Regulation Of Abscisic Acid****Insensitive 5 (ABI5) During Seed Germination in *Arabidopsis thaliana****Nurulhikma Md Isa, Daniel Gibbs, Michael Holdsworth***University of Nottingham, Nottingham, UK**

Seed dormancy and germination are controlled by the antagonistic actions of the plant hormones Abscisic acid (ABA) and Gibberellin(GA). Recent studies have addressed the important role of a ubiquitin degradation pathway termed the N-end rule pathway (NERP) in controlling seed germination in *Arabidopsis*. Mutants of the NERP E3 ligase, *prt6* arehypersensitive to ABA preventing the seed fromgerminating; indicating the enhanced stability of the PRT6 substrate is repressing the germination process through the action of ABA. A transcriptome profiling analysis of *prt6-1* versus Columbia (Col-0) revealed many differentially regulated genes. Interestingly, the ABA- like binding site motif was over represented in the promoter of genes highly expressed in *prt6-1*, suggesting that the NERP substrate maybe regulating the expression of ABA responsive genes. To supplement the global transcript analysis, a targeted analysis of *ABI5* transcription factor expression was carried out together with two other well characterized positive regulators of ABA signaling, *ABI3* and *ABI4*. Expression of *pABI5::GUS*was enhanced in the *prt6-1*background compared to Col-0, while *pABI3::GUS*and *pABI4::GUS* expression did notshow any significant differences. Interestingly, expression of *ABI5* in *prt6-1* is detected throughout the endosperm tissue while in Col-0it is restricted to the micropylar endosperm. This demonstrates that the NERPsubstrate might acts as a positive regulator to the *ABI5* promoter to drive its expression at the transcriptional level; to regulate dormancy and germination.

**600 Modulation of Jasmonate Signaling Through Production of JAZ Splice Variants***Javier Moreno, Gregg Howe***MSU-DOE Plant Research Laboratory, East Lansing, MI, USA**

Jasmonate (JA), a lipid-derived hormone, modulates myriad plant responses involved in growth, development, and defense. JA-induced plant responses are negatively regulated by JASMONATE-ZIM DOMAIN (JAZ) proteins that bind to and inhibit bHLH-type transcription factors such as MYC2. The presence of JA-Ile, the bioactive hormone, is perceived by a receptor complex consisting of JAZ and the F-box protein CORONATINE INSENSITIVE 1 (COI1). In this way, JA-Ile imparts specificity to an E3-ubiquitin ligase complex (SCF<sup>COI1</sup>) that targets JAZ proteins for degradation, thereby unleashing JA responses. JAZ proteins share a conserved region known as the Jas domain. This domain is required for JAZ interaction with both COI1 and MYC2. Interestingly, some JAZ genes undergo alternative splicing events that truncate or remove the Jas domain. These splice variants are resistant to COI1-mediated degradation but still retain the ability to interact with and repress MYC2. Plants that overexpress these splice variants show decreased sensitivity to JA. We are using the JAZ10.4 splice variant to study the physiological relevance of *JAZ* alternative splicing. Although JAZ10.4 lacks the entire Jas domain, this protein retains the ability to interact with MYC2 *in vitro* and *in vivo*. Site-directed and deletion mutagenesis experiments were used to define the MYC2-binding motif in JAZ10.4. Deletion of this domain impaired the ability of JAZ10.4 to repress JA responses when overexpressed in *Arabidopsis*. Given that JAZ10.4 accumulates after JA treatment, we propose a model in which active repression of MYC2 by JAZ10.4 modulates JA responses in the presence of high intracellular levels of JA-Ile. Such a mechanism may serve to maximize the plasticity of JA responses and reduce the deleterious effects of JA on plant growth.

**601 Surface potentials in wounded *Arabidopsis* leaves***Seyed Ali Mousavi<sup>1</sup>, Stephan kellenberger<sup>2</sup>, Edward Farmer<sup>1</sup>***<sup>1</sup>Plant Molecular Biology, University of Lausanne, Biophore, CH-1015 Lausanne, Switzerland, <sup>2</sup>Pharmacology and Toxicology, University of Lausanne, CH-1005 Lausanne, Switzerland**

Jasmonates(JAs) are fatty acid-derived hormones that have a broad range of effects on growth and biotic stress responses including wounding which initiates long-distance jasmonate signalling leading to gene expression indistal leaves. Plants respond to wounding with exponential JA accumulation beginning in the range of 30-45 s in the wounded parts and in less than 2 minin unwounded distal leaves.

The velocity of long distance signalling to JA accumulation in *Arabidopsis* is estimated conservatively to be 3.4-4.5cm/min. A question is whether distal JA accumulation depends on long-distance chemical transfer or on electrical or hydraulic signalling in *Arabidopsis thaliana*. Consistent with the literature, surface potential recordings on *Arabidopsis* leaves show depolarization in the wounded leaf. Surface activity was obtained 10s after wounding with an electrode on the midrib 1 cm from the wound. Recording from four electrodes placed on the lamina, midrib, midrib/petiole and petiole respectively showed that the speed of surface potential movement through the lamina is slower than through the vein. The unwounded leaves with direct vascular connections with the wounded leaves also showed depolarization after wounding. In contrast, non-connected leaves did not show any strong surface potential change. The estimated displacement velocities of surface potentials recorded in the wounded leaves were faster than those measured in the connected leaves. Like surface potential recordings, transcripts for the JA synthesis gene *LIPPOXYGENASE2* (*LOX2*) and the jasmonate response gene *JASMONATE ZIM DOMAIN10.3* (*JAZ10.3*) accumulated to higher levels in interconnected leaves than in non-connected leaves. The *fou2* ion channel mutant affects both jasmonate signalling and surface potential activity.

## 602 Quantitative Analysis of Abscisic Acid Signalling in *Arabidopsis*

*Eric Nam, Akira Endo, Peter McCourt*

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Abscisic acid (ABA) plays important roles in regulating plant stress responses and plant development. So far, ABA signalling has been extensively studied in a qualitative manner. To further expand our understanding of ABA signalling, we believe it should be studied more quantitatively. In this study, we focus on the cell-to-cell variation of ABA signalling activity in guard cells in response to both exogenous ABA and osmotic stresses. We created new ABRE-GFP reporters to measure the ABA signalling outputs at the transcriptional level in guard cells. We found that the variations in ABA signalling activities among the guard cells were prevalent in all experimental conditions. We will investigate how those variations may be generated in guard cells.

## 603 Structural and Functional Characterization of DAWDLE (DDL) in *Arabidopsis thaliana*

*Lakshmi Narayanan, David Chevalier*

**Mississippi State University**

*DDL* is one of the eighteen genes that encode a protein with a Fork-Head Associated (FHA) domain in *Arabidopsis thaliana*. FHA is a phospho-threonine binding domain found in plant and animal proteins that function in DNA repair and cell cycle regulation. *DDL* also has an arginine-rich N terminal domain that has sequence similarity with proteins involved in RNA processing. The *DDL* ortholog, SMAD nuclear interacting protein 1, regulates RNA stability of the Cyclin D1 gene and the level of several microRNAs in humans. Similarly, *DDL* regulates the level microRNA in *Arabidopsis*. Our aim is to understand the function of *DDL* in RNA metabolism. Two *ddl* T-DNA insertion alleles in the WS-2 ecotype exhibit a strong phenotype and pleiotropic developmental defects including short root and hypocotyl, reduced fertility, and distorted organs. In contrast a *ddl* T-DNA insertion allele in the Columbia ecotype exhibits a weak *ddl* phenotype. To study the structure-function of *DDL*, twelve point mutations spanning *DDL*, were isolated and the severity of each point mutation allele is being compared to T-DNA alleles. To identify *DDL* interactors, we have identified several suppressors of *ddl* and a modifier of *ddl* caused by a natural variation between the WS-2 and Columbia ecotypes.

## 604 GID1-dependent GA signaling can stimulate germination of the GA-insensitive mutant *sly1* in the absence of DELLA degradation

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Abscisic acid (ABA) and gibberellins (GA) regulate the balance between seed dormancy and germination in *Arabidopsis thaliana*. In dormant seeds, ABA levels are high and GA levels are low. As those seeds lose dormancy during after-ripening, ABA levels decline and GA levels rise. GA normally induces germination by binding to the GA receptor, GID1 (GA-INSENSITIVE DWARF1), allowing GID1 to bind DELLA protein; this in turn allows that SCF<sup>SLY1</sup> E3 ubiquitin ligase to recognize and target DELLA protein for destruction via the ubiquitin-proteasome pathway. Mutations in *SLY1* disrupt DELLA degradation associated with a GA-insensitive phenotype including increased seed dormancy, decreased stature, and infertility. The increased dormancy of the *sly1-2* mutant can be rescued either by long after-ripening and by overexpression of *GID1b*. To investigate whether *GID1b* overexpression and after-ripening stimulate *sly1-2* germination by similar mechanisms, we have examined the effects of both treatments on gene expression and hormone levels. Rescue of *sly1-2* germination by after-ripening is associated with elevated GA<sub>4</sub> hormone levels, whereas rescue by *GID1b* overexpression is not. Both after-ripening and *GID1b* overexpression are associated with elevated levels of GA responsive gene expression suggesting that both methods for stimulating *sly1-2* germination involve GA signaling.

## 605 Transcriptional Profiling of the Floral Organ Abscission Mutant *hae-3 hsl2-3* by RNA-Seq

*Chad Niederhuth<sup>1,2</sup>, John Walker<sup>1,2</sup>*

**<sup>1</sup>Division of Biological Sciences, University of Missouri, Columbia, Missouri., <sup>2</sup>Interdisciplinary Plant Group, University of Missouri, Columbia, Missouri**

Abscission is the shedding of a plant organ. This is a genetically controlled process that occurs at a specialized cell layer called the Abscission Zone (AZ). In *Arabidopsis* only the floral organs abscise. Floral organ abscission is controlled by a peptide ligand IDA,

two receptor-like protein kinases HAE and HSL2, and a downstream MAP kinase cascade. While single mutants of *hae* or *hsl2* appear phenotypically normal, the double mutant *hae-3 hsl2-3* fails to abscise. To identify potential targets of this pathway RNA-Seq was used to obtain a transcriptional profile from flower receptacles enriched for AZ cells from both *hae-3 hsl2-3* and wild type plants. Altered expression of many cell wall hydrolytic enzymes was observed, including the previously implicated polygalacturonases *ADPG2* and *QRT2*. Failure to produce the necessary hydrolytic enzymes would explain why the *hae hsl2* double mutants, as well as other abscission mutants, fail to abscise and are candidate targets for the IDA-HAE-MPK pathway.

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**606 The Role of the Cytokinin Response Factors (CRFs) in Cytokinin Signaling and Plant Development**

*Tracy Raines, Joseph Kieber*

**University of North Carolina at Chapel Hill**

The Cytokinin Response Factors (CRFs) are a subset of AP2 transcription factors whose expression has been shown to be up-regulated by the addition of exogenous cytokinin and dependent on the presence of the Type-B Response Regulators. To better understand the role of the CRFs within the plant, loss of function and overexpression CRF lines were created and screened for altered phenotypes. We observed that the overexpressing CRF lines show accelerated senescence of the rosette leaves, while loss of function lines show a delay. Mutant lines also display altered response to increasing doses of cytokinin as measured by root elongation, suggesting they may play a role in mediating the cytokinin response. NanoString analysis of mutant tissue showed many primary cytokinin signaling genes are altered in their basal expression level as well as in the degree of induction by cytokinin.

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**607 A Transcriptional Cascade Regulates Cytokinin Signaling in Arabidopsis**

*Yi-Hsuan Chiang<sup>1</sup>, Hyo Jung Kim<sup>1</sup>, Kristine Hill<sup>1</sup>, Rebecca Argyros<sup>1</sup>, Dennis Mathews<sup>2</sup>, Joseph Kieber<sup>3</sup>, G. Eric Schaller<sup>1</sup>*

**<sup>1</sup>Dartmouth College, Hanover, NH, USA, <sup>2</sup>University of New Hampshire, Durham, NH, USA, <sup>3</sup>University of North Carolina, Chapel Hill, NC, USA**

Cytokinins regulate many developmental and physiological processes in plants, such as cell division, root and shoot growth, chloroplast development, and leaf senescence. Cytokinin signal transduction is mediated by a two-component signaling pathway that culminates in regulation of the type-B response regulators (type-B ARR family). Mutational analysis indicates that the type-B ARRs are the primary transcription factors regulating the plant's response to cytokinin, with higher order mutants showing substantial cytokinin insensitivity. Conversely, *Arabidopsis* lines hypersensitive to cytokinin can be generated by modifying the expression characteristics of some type-B ARRs. Among the primary response genes regulated by the type-B ARR's are additional families of transcription factors. We have characterized several of these downstream transcription factor families for their roles in plant growth and development, defining roles in chloroplast biogenesis and callus induction. Our results support a model in which type-B ARRs function at the head of a transcriptional cascade, with type-B ARR regulated transcription factors then regulating subsets of the cytokinin response.

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**608 COI1-JAZ Co-receptor Acts as a Ubiquitin Ligase-based Small Molecule Sensor**

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Jasmonates are a family of plant hormones that regulate plant growth, development and responses to stress. The F-box protein CORONATINE INSENSITIVE 1 (COI1) mediates jasmonate signalling by promoting hormone-dependent ubiquitylation and degradation of transcriptional repressor JAZ proteins. Despite its importance, the mechanism of jasmonate perception has remained unclear. Our structural and pharmacological data show that the true *Arabidopsis* jasmonate receptor is a complex of both COI1 and JAZ. COI1 contains an open pocket that recognizes the bioactive hormone (3R,7S)-jasmonoyl-L-isoleucine (JA-Ile) with high specificity. High-affinity hormone binding requires a bipartite JAZ degron sequence consisting of a conserved α-helix for COI1 docking and a loop region to trap the hormone in its binding pocket. In addition, we have identified a third critical component of the jasmonate co-receptor complex, inositol pentakisphosphate, which interacts with both COI1 and JAZ adjacent to the ligand. Our results unravel the mechanism of jasmonate perception and highlight the ability of F-box proteins to evolve as multi-component signalling hubs. We speculate that differing sequence contribution of JAZ proteins to the COI1-JAZ-IP5 signalling hub may serve to create several distinct jasmonate receptors with a range of sensitivity to hormone.

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**609 Identification of the Ligand Binding Site in a Non-Island-Type Leucine-Rich Repeat Receptor Kinase**

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Leucine-rich repeat receptor kinases (LRR-RKs) form the largest group of receptor kinases in *Arabidopsis*. The leucine-rich motif is a 24-residue repeat sequence with characteristically spaced hydrophobic residues and forms repeated tandem α-helix-β-strand pairs. In some LRR-RKs, such as PSKR1 and BRI1, both of which belong to the LRR X subgroup, the extracellular "island" domain that intercepts the LRR domain serves as a ligand binding site (Shinohara *et al.*, 2007) (Kinoshita *et al.*, 2005). The island domain does not share any primary sequence similarity beyond the family members. However, other LRR-RKs, such as CLV1, TDR/PXY and PEPR1, all of which belong to the LRR XI subgroup, have no obvious island domain despite their direct interaction with specific ligands. We investigated

how the non-island-type LRR-RKs specifically recognize individual ligands. We analyzed the ligand binding sites of LRR-RKs in the LRR XI subgroup using BAM1 as a model. We prepared radio-iodinated photoactivatable CLE peptide and performed photoaffinity cross-linking with BAM1 by UV irradiation. The covalently cross-linked ligand-receptor pair was digested by proteases or chemicals, and the resulting fragments were analyzed by SDS-PAGE and autoradiography. Based on the molecular size of the ligand-cross-linked receptor fragments, we determined the ligand binding site of BAM1 within one of the LRR units. We also predicted the three-dimensional structure of BAM1 by using homology modeling software and found that this LRR unit possibly forms a loop-out structure. Our results indicate that subtle amino acid substitutions within the LRR unit are sufficient for creating a ligand binding pocket.

## 610 Chemical Screen Uncovers Link Between One-Carbon Metabolism and Sucrose Signaling in *Arabidopsis*

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Sugars play an important role throughout plant development, acting as structural components, metabolic intermediates, and sources of energy. Because of this, plants have evolved numerous mechanisms through which cellular carbohydrate abundance can be detected. Forward-genetic screens have proven fruitful in uncovering multiple sugar-perception pathways, but can be limited by functional redundancy and seedling lethality. As a means of circumventing these issues, a chemical genetic approach was employed to uncover novel aspects of plant sugar perception. Over 2100 compounds were screened for the ability to perturb seedling responses to exogenous sucrose. This screen revealed a group of chemicals belonging to the sulfonamide family of compounds, known to inhibit one-carbon (C1) metabolism in plants, to restrict hypocotyl elongation in a sucrose-dependent fashion. Mutant and pharmacogenetic analyses suggest this is a HEXOKINASE1-independent phenomenon related to the synthesis of folates. Microarray-based transcriptome analysis identified a small set of transcripts that show altered responses to the compound when administered in the presence of sucrose, including genes implicated in auxin signal transduction. Complementary reverse- and forward-genetic screens are currently being pursued to unveil the genetic basis for crosstalk between C1 metabolism and sugar signalling in *Arabidopsis*.

## 611 The TOC Complex May Mediate the Plastid Localization of a Gravity Signal Transducer in *Arabidopsis*

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Plant roots navigate their heterogeneous soil environments partly through their abilities to sense and respond to gravity. This process involves the sedimentation of dense amyloplasts in the columella cells of the root tip onto the cortical ER or plasma membrane. A genetic screen identified a preprotein receptor (TOC132) of the Translocon at the Outer envelope membrane of Chloroplasts (TOC) complex, as a contributor to gravity signal transduction. The TOC complex transports nuclear-encoded proteins from the cytosol into plastids and plastid membranes. To determine if TOC132 functions directly as a gravity signal transducer or indirectly by mediating the plastid localization of such a transducer, additional TOC complex components were tested for involvement in gravitropism. Similarly to mutations in *TOC132*, mutations in genes encoding the preprotein receptors TOC120 (which functions redundantly with TOC132 in the import of non-photosynthetically-related proteins into plastids) and TOC34 also enhance the gravitropic defects associated with mutations in *ALTERED RESPONSE TO GRAVITY 1*. Furthermore, the cytoplasmic acidic domain of TOC132 is not required for a gravitropic response. These data suggest that the TOC complex may function in gravitropism indirectly by mediating the localization of a plastid-associated molecule that transduces the signal. I have conducted double mutant analyses to place the TOC complex in a genetic model for gravity signal transduction, and my current work centers on a mutagenesis approach to identify new signal transducers potentially associated with the amyloplast. This work suggests an important role for amyloplasts in gravity signal transduction beyond their simple ability to sediment.

## 612 AtPP2CF1 Encodes a Functional *Arabidopsis* PP2C Which Belongs to Group E

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Plant biomass, an important resource for renewable fuels and materials, is receiving industrial attention, in particular on the technology of improving the growth rate and the yield. Plant growth is influenced by both intrinsic genetic and extrinsic environmental cues, indicating the complexity of genetic and molecular networks underlying them.

Reversible protein phosphorylations regulate various signal transduction pathways, and are achieved by the combined activities of protein kinases and protein phosphatases. Higher plants bear a larger number of type 2C protein phosphatases (PP2Cs, classified in group A-J), compared to other eukaryotes, implying higher plant-specific functional diversity and/or functional redundancy. Although many reports have suggested that group A PP2Cs are the key negative regulators of ABA signaling, little is known regarding enzymatic and signaling roles of other group PP2Cs.

We identified a candidate gene for plant growth regulation by using activation tagging method and revealed that the gene (designated *AtPP2CF1*) encodes one of group E PP2Cs. We confirmed that overexpression of *AtPP2CF1* resulted in increasing biomass for *Arabidopsis* (1,2). An *in vitro* enzymatic assay revealed that AtPP2CF1 had a serine/threonine phosphatase activity typical of classical PP2C. *AtPP2CF1* was expressed in vascular tissues and guard cells, and this expression profile is similar to that of group A PP2Cs, such

as *ABI1*. However, yeast two-hybrid system showed that AtPP2CF1 cannot interact with RCAR/PYR/PYLs and SRK2D/E/I, which both are *ABI1* interactors. We will discuss the physiological significance of AtPP2CF1.

- 1) Int. Pat. Pub. WO2009/113684
- 2) Int. Pat. Pub. WO2010/104092

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### **613 *Arabidopsis ASA1* Is Important for Jasmonate-Mediated Regulation of Auxin Biosynthesis and Transport during Lateral Root Formation**

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Plant roots show an impressive degree of plasticity in adapting their branching patterns to ever-changing growth conditions. An important mechanism underlying this adaptation ability is the interaction between hormonal and developmental signals. Here, we analyze the interaction of jasmonate with auxin to regulate lateral root (LR) formation through characterization of an *Arabidopsis thaliana* mutant, *jasmonate-induced defective lateral root1 (jdl1/asa1-1)*. We demonstrate that, whereas exogenous jasmonate promotes LR formation in wild-type plants, it represses LR formation in *jdl1/asa1-1*. *JDL1* encodes the auxin biosynthetic gene *ANTHRANILATE SYNTHASE a1 (ASA1)*, which is required for jasmonate-induced auxin biosynthesis. Jasmonate elevates local auxin accumulation in the basal meristem of wild-type roots but reduces local auxin accumulation in the basal meristem of mutant roots, suggesting that, in addition to activating *ASA1*-dependent auxin biosynthesis, jasmonate also affects auxin transport. Indeed, jasmonate modifies the expression of auxin transport genes in an *ASA1*-dependent manner. We further provide evidence showing that the action mechanism of jasmonate to regulate LR formation through *ASA1* differs from that of ethylene. Our results highlight the importance of *ASA1* in jasmonate-induced auxin biosynthesis and reveal a role for jasmonate in the attenuation of auxin transport in the root and the fine-tuning of local auxin distribution in the root basal meristem.

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### **614 Over Expression of Fatty Acid Amide Hydrolase in *Arabidopsis thaliana* Alters Flowering Time Under Short Day Conditions Through its Hydrolytic Activity**

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*N*-Acylethanolamines (NAEs) are bioactive lipids derived from the hydrolysis of the membrane phospholipid *N*-acylphosphatidylethanolamine (NAPE). In animal systems this reaction is part of the "endocannabinoid" signaling pathway which regulates a variety of physiological processes including cell proliferation, immune cell signaling and embryo development. The inactivation of NAEs is accomplished through the hydrolysis by fatty acid amide hydrolase (FAAH). In plants, recent work in *Arabidopsis thaliana* had shown that overexpression of *AtFAAH* (*At5g64440*) lowers endogenous levels of NAEs, which produces an accelerated growth phenotype, increased sensitivity to ABA and an increased susceptibility to bacterial pathogens. Here we show another characteristic of *AtFAAH* overexpression in *Arabidopsis*. When grown under short day conditions (8h light/16h dark) the bolting pattern is dramatically altered compared to wild type *Arabidopsis*. AtFAAH over expressors bolted several days earlier than wild type, AtFAAH knockouts or site-directed AtFAAH overexpressing mutants, which lack NAE catalytic activity. Microarray analysis of 2 week old AtFAAH over expressors, confirmed by quantitative (q) RT-PCR, revealed that some gene transcripts involved in flowering were elevated compared to wild type and AtFAAH knockouts. This included the FLOWERING LOCUS T (FT) gene which has a major role in regulating flowering time. The data presented here suggests that this early bolting phenotype is initiated by altered gene expression and in part linked to the hydrolase activity of AtFAAH (supported by Department of Energy grant DE-FG02-05ER15647 to KDC and EBB).

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### **615 Two Plant Glutamate-Like Receptors from *Arabidopsis* are Expressed in Root Phloem Tissue and are Involved Lateral Root Initiation**

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The surprising discovery that plants have glutamate-receptor ion channels has raised intriguing questions about their functions in plant physiology. With the completion of the sequencing of the *Arabidopsis thaliana* genome a large family of 20 glutamate-like receptor genes (GLRs) have been identified. Electrophysiological studies of wild-type *Arabidopsis* and knock-out mutants have indicated that at least some plant GLRs encode channels that permit  $\text{Ca}^{2+}$  influx in response to six different amino acids (ala, asn, cys, glu, gly, and ser). In this study, 2 GLR members from clade 3 (*GLR3.2* and *GLR3.4*) were investigated in detail. By using the native promoters to drive the expression of their respective gene product fused to EGFP, we found that both *GLR3.2* and *GLR3.4* showed concentrated expression in the early stages of phloem development (proto- and meta-phloem) as well as in specific foci in mature root phloem indicating possible sieve plate localization of these two GLR receptors. Inspection of internal root development in the mutants showed that *glr3.2* and *glr3.4* mutants produced twice as many lateral root primordia as wild-type, though the number of emerged lateral roots was not affected. GFP-tagged versions (in the background null mutants) of *GLR3.2* and *GLR3.4* used to visualize the expression pattern, completely rescued the phenotype. Consistent with their mutant phenotype, *GLR3.2* and *GLR3.4* were two genes recently associated with lateral root initiation by a machine-learning analysis of transcriptome. Furthermore, we investigated the function of *GLR3.4* in a heterologous system by

transfected cultured mammalian (HEK 293) cells with its cDNA and then measuring whole cell currents by patch clamping. We show here that GLR3.4 functions as an amino acid-gated channel capable of generating an intracellular  $\text{Ca}^{2+}$  signal. Asparagine and serine were the most effective agonists as predicted by previous studies of *glr3.4* mutants. This set of results supports the hypothesis that plant GLRs are  $\text{Ca}^{2+}$ -permeable, plasma membrane channels that respond to extracellular amino acids are involved in lateral root initiation.

### **616 Identification and characterization of suppressors of the *tir1-1* auxin signaling defect**

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Prior genetic screens for *Arabidopsis* mutants exhibiting auxin resistant phenotypes have identified the  $\text{SCF}^{\text{TIR}1}$  ubiquitin-ligase as a positive regulator of auxin response. To identify potential negative regulators of auxin signaling, we screened EMS-mutagenized *tir1-1* seedlings for second site mutations that restore auxin-dependent expression of the auxin response reporter, BA::GUS. Several suppressors of *tir1-1* auxin resistance (*sta*) mutants were identified and are currently being characterized. We used multiple auxins to classify the specificity of *sta* suppression of *tir1*. While some *sta* mutants suppress the diminished BA-GUS phenotype of *tir1* mutants on a wide variety of auxins, others exhibit specificity for IAA and closely related auxins, suggesting that distinct aspects of auxin action are defined by our *sta* mutant collection. For example, *staJ8* suppresses *tir1* on all auxins tested. *staK6* on the other hand exhibits strong suppression of *tir1* on IAA and 4-Cl-IAA, but no suppression whatsoever on 2,4-D. The two best-characterized *sta* mutants to date include *staJ8* and *staB4*, both of which exhibit hypersensitivity to auxin in a *TIR1*<sup>+</sup> background. Additionally, both of these mutants also display altered lateral root development and slow root growth phenotypes, consistent with roles in the auxin pathway. *staJ8* seedlings also exhibit increased hypocotyl elongation. Our map-based cloning efforts position *staJ8* within a 64 kb interval on chromosome IV and *staB4* on the distal end of chromosome I. Candidate genes encoding *STAJ8* have been identified by sequencing and complementation assays are currently underway.

### **617 Trehalose-6-phosphate and sucrose signalling in *Arabidopsis thaliana***

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Trehalose-6-phosphate (Tre6P) plays an essential role in the regulation of plant metabolism and development, although its precise functions are uncertain. It has been proposed that Tre6P acts as a signal metabolite that reflects the availability of sucrose, and thereby regulates the growth and metabolism of the plant. We tested this hypothesis by investigating the specificity of the response of Tre6P to sucrose and other sugars, using *Arabidopsis thaliana* seedlings. Re-addition of sucrose to C-starved seedlings led to rapid and massive (up to 70-fold) increases in the level of Tre6P. Addition of glucose, fructose or maltose also led to a rise in Tre6P. However, these three sugars also increased sucrose levels in the seedlings, and in all experiments Tre6P showed a stronger correlation with sucrose than with glucose or fructose, irrespective of which sugar was supplied. The *A. thaliana* genome contains a family of eleven genes encoding trehalose-phosphate synthase (TPS), and a family of ten genes encoding trehalose-phosphatase (TPP). Transcript profiling using microarrays and Real-Time RT-PCR revealed strong induction of *AtTPS5* and *AtTPP8* transcripts when sucrose was added to C-starved seedlings, whereas *AtTPS8-11* were even more dramatically repressed. The significance of these changes in transcript abundance is unclear as inhibition of transcription by cordycepin had little effect on the sucrose-induced rise in Tre6P. In contrast, inhibition of protein synthesis by cycloheximide strongly repressed the Tre6P response to sucrose. However, polysome loading analysis indicated that none of the *TPS* or *TPP* genes is translationally regulated by sucrose. The response of Tre6P to sucrose is enhanced by treatment of the seedlings with MG132, which inhibits protein turnover via the ubiquitin-26S proteasomal pathway. Based on the sensitivity of the Tre6P response to various inhibitors, it is postulated that sucrose induces synthesis of short-lived regulatory proteins that either activate TPS to increase the rate of Tre6P synthesis, or inhibit the hydrolysis of Tre6P by TPP.

### **618 Abstract Withdrawn**

**619 Determining the function and subcellular localization of the *Arabidopsis* LAZY1 protein***Takeshi Yoshihara<sup>1</sup>, Edgar Spalding<sup>1</sup>, Moritoshi Iino<sup>2</sup>*<sup>1</sup>**Department of Botany, University of Wisconsin, Madison, (Wisconsin), USA ,**<sup>2</sup>**Botanical Gardens, Osaka City University, Katano-shi, (Osaka), Japan**

Mutations in the *LAZY1* gene of rice cause agravitropic growth. Homologous genes are easily recognized in other grass species but in *Arabidopsis* the most similar gene (at5g14090) is only 21% identical to rice *LAZY1*. RNA interference and T-DNA insertion mutagenesis was used to impair this gene. Each affected line showed greater lateral shoot angles, a phenotype analogous to the rice *lazy1* mutant, indicating that at5g14090 is an ortholog of rice *LAZY1*. Detailed time course analyses of hypocotyl gravitropism performed by computerized image processing and manual measurements of inflorescence stem gravitropism showed that At*LAZY1* affects the initial period (30-90 min) of curvature development. Promoter activity analysis using the β-glucuronidase reporter revealed that At*LAZY1* is expressed predominantly in the shoot, especially in vascular tissues. Constructs that fused green fluorescent protein (GFP) to the N-or C-terminus of At*LAZY1* and expressed under the control of the native promoter could neither complement *lazy1* mutant phenotypes nor produce a detectable GFP signal. To overcome these problems, we inserted GFP within At*LAZY1*. One of these constructs rescued the *lazy1* phenotype. This GFP-tagged protein was localized to plasma membrane and nucleus in epidermal cells of *Nicotiana benthamiana* leaves. The same subcellular pattern was observed in *Arabidopsis* plants expressing GFP-tagged *LAZY1* under control of the *HSP18.2* heat shock promoter, following induction. When the predicted nuclear localization signals (NLS) within the coding sequence were mutated, At*LAZY1* was excluded from nucleus in *N. benthamiana*. We are currently investigating the functional significance of the At*LAZY1* protein residing at the plasma membrane and nucleus. Our approach to this intriguing question involves complementation tests using NLS-mutant versions of At*LAZY1* to determine if nuclear localization is essential for its function.

**620 Type-A response regulators are required for proper root apical meristem function through the post-transcriptional regulation of the PIN auxin efflux carriers***Wenjing Zhang<sup>1</sup>, Jennifer To<sup>1</sup>, Chia-Yi Cheng<sup>1</sup>, G. Eric Schaller<sup>2</sup>, Joseph Kieber<sup>1</sup>*<sup>1</sup>**University of North Carolina at Chapel Hill,** <sup>2</sup>**Dartmouth College, Hanover, NH**

The phytohormones cytokinin and auxin regulate a diverse array of growth and developmental processes, often acting together to modulate plant processes. While much has been learned regarding how each of these hormones acts individually, we are just beginning to understand how these signals interact to achieve an integrated response. Previous studies indicate that exogenous cytokinin has an effect on the transcription of several PIN efflux carriers. Here, we show that disruption of the type-A *Arabidopsis* Response Regulators (ARRs), negative regulators of cytokinin signalling, affects root development by altering the size of the apical meristem, and increases the sensitivity to NPA, an inhibitor of the PIN auxin efflux carriers. Disruption of multiple type-A ARR's alters the level of PIN proteins and exacerbates the effects of exogenous cytokinin on PIN abundance. Further, we show that the effect of cytokinin on PIN abundance primarily at the post-transcriptional level. The alterations of PIN levels in the type-A ARR mutants results in an altered distribution of auxin as measured by a DR5::GFP reporter, and an altered pattern of cell division and differentiation in the stem cell niche in the root apical meristem. Together, these data indicate that cytokinin, acting through the type-A ARR's, alters the level of several PIN efflux carriers, and thus direct the distribution of auxin within the root tip.

**621 *Arabidopsis* Tyrosylprotein Sulfotransferase Acts in the Auxin/PLETHORA Pathway in Regulating Postembryonic Maintenance of the Root Stem Cell***Nich Wenkun Zhou<sup>1</sup>, Lirong Wei<sup>1</sup>, Jian Xu<sup>2</sup>, Qingzhe Zhai<sup>1</sup>, Hongling Jiang<sup>1</sup>, Rong Chen<sup>1</sup>, Qian Chen<sup>1</sup>, Jiaqiang Sun<sup>1</sup>, Jinfang Chu<sup>1</sup>, Lihuang Zhu<sup>1</sup>, Chun-Ming Liu<sup>3</sup>, Chuanyou Li<sup>1</sup>*<sup>1</sup>**Institute of Genetics and Developmental Biology, Beijing, China,** <sup>2</sup>**Department of Biological Sciences, National University of Singapore, Singapore,** <sup>3</sup>**Institute of Botany, Beijing, China**

Recent identification of the *Arabidopsis* tyrosylprotein sulfotransferase TPST and a group of tyrosine-sulfated peptides known as root meristem growth factors (RGFs) highlights the importance of protein tyrosine sulfation in plant growth and development. Here, we report the action mechanism of TPST in maintenance of the root stem cell niche, which in the *Arabidopsis* root meristem is an area of four mitotically inactive quiescent cells plus the surrounding mitotically active stem cells. Mutation of *TPST* leads to defective maintenance of the root stem cell niche, decreased meristematic activity and stunted root growth. We show that *TPST* expression is positively regulated by auxin and that mutation of this gene affects auxin distribution by reducing local expression levels of several *PIN* genes and auxin biosynthetic genes in the stem cell niche region. We also show that mutation of *TPST* impairs basal- and auxin-induced expression of the *PLETHORA* (*PLT*) stem cell transcription factor genes and that overexpression of *PLT2* rescues the root meristem defects of the loss-of-function mutant of *TPST*. Together, these results support that TPST acts to maintain root stem cell niche by regulating basal- and auxin-induced expression of *PLT1* and *2*. TPST-dependent sulfation of RGFs provides a link between auxin and PLTs in regulating root stem cell niche maintenance.

**622 IQD22, a negative regulator of GA response, plays a role in the regulatory network among the GA, calcium and auxin pathways**Xin Zhou, Tai-ping Sun**Duke university,Durham,NC,USA**

The plant hormone gibberellin (GA) controls multiple aspects of plant growth and development throughout the whole plant life cycle. GA derepresses its signaling by binding to its receptor GID1, which induces degradation of GA signaling repressors (DELLA s) via the 26S proteasome pathway. DELLA s are likely nuclear transcriptional regulators, which interact with other transcription factors to modulate expression of GA-responsive genes. However, the molecular mechanisms by which DELLA s restrict growth and development are largely unknown. Previous microarray studies identified IQD22 (IQ-domain 22) as a putative direct target gene of DELLA in Arabidopsis seedlings. The IQ domain was shown to be a  $\text{Ca}^{2+}$ -dependent calmodulin-binding domain. IQD22 expression is induced by DELLA and repressed by GA. Here we report that the *iqd* loss of function mutants (*iqd22 iqd23* double and *iqd22 iqd23 iqd24* triple) are hypersensitive to  $\text{GA}_4$  in hypocotyl length test, while *IQD22* OE lines are insensitivity to  $\text{GA}_4$ , and the transcript level of GA biosynthesis genes are increased in the OE lines. These results suggest that IQD22 is a negative regulator of GA response downstream of DELLA. The *iqd* triple mutant has more lateral branches than wildtype, while *IQD22* OE lines have fewer lateral branches that could be rescued by decapitation, suggesting elevated auxin responses. In addition, the *iqd* triple mutant is hypersensitive to a low concentration of  $\text{Ca}^{2+}$ . Our results indicate that IQD22 may play a role in the regulatory network among the GA, calcium and auxin pathways.

**623 Abstract Withdrawn****624 Genetic Dissection of the Plant/Pest Interaction: Mapping of *Arabidopsis* Resistance Gene to *Tetranychus urticae* (Two Spotted Spider Mite) Feeding**

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In response to herbivore attack, plants have evolved a variety of mechanisms to deter herbivore feeding, which prevent the herbivores from jeopardizing the plant's health, reproduction, and ultimately survival. Understanding the fundamental mechanisms of plant resistance to pest and interactions between plant and herbivores represents the basis for breeding of pest-resistant crops.

In order to dissect the genetic base of plant/Two Spotted Spider Mite *Tetranychus urticae* interaction, we screened *Arabidopsis* accessions by evaluating the damage induced by Spider Mite feeding. We identified two tolerant accessions (Bla2 and Col-0) and two susceptible accessions (Ler and Kondara). A combination of QTL and bulk-segregant analysis is used to determine the genetic bases of observed phenotypic differences toward cloning of genes underlying the resistance to Spider Mite. The preliminary results show that Spider Mite resistance in *Arabidopsis* is a quantitative trait controlled by a single QTL. This approach has the potential to isolate plant resistance genes and identify molecular markers for breeding/biotechnological modification of crop plants for resistance against pests in agriculture.

**625 From *Arabidopsis* to *Camelina*: Translating Our Understanding of Trichome Development**

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The trichome is a useful model for studying cell fate and differentiation. The genetic mechanism controlling trichome development in *Arabidopsis* has been well characterized, with over 50 genes required for normal development identified. As trichomes are a natural defensive plant structure against both biotic and abiotic stresses, we are now positioned to translate our knowledge of trichome development from *Arabidopsis* to an agronomic crop. *Camelina sativa* (Brassicaceae) is an oilseed crop with great potential as a biofuel feedstock. The close evolutionary relationship between *Camelina sativa* and *Arabidopsis* position *Camelina* as an easy yet powerful translation from model to crop species. *Camelina* can be dip transformed with Agrobacterium without the need for tissue culturing. Using this, we are examining if the same pathway for *Arabidopsis* trichome development translates to *Camelina*. Our ultimate goal will be to translate our full understanding of trichome development to *Camelina* in order to increase its defensive capabilities.

**626 High-Level Expression of a Set of Six Thermostable Cell Wall-Degrading Enzymes in Tobacco Chloroplasts: A First Step Towards Development of the Auto-Saccharification System of Bioenergy Crops**

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The cost-effective conversion of lignocellulosic biomass into fermentable sugars is key to the commercial production of biofuels and other renewable chemicals from plants. One possible way to reduce cost of saccharification is to produce cell wall-degrading enzymes within the bioenergy crop itself. In this work, we employed tobacco plastid transformation to express thermostable glycosyl hydrolases from microorganisms. A set of six enzymes (endoglucanase, cellobiohydrolase I, cellobiohydrolase II, β-glucosidase, xylanase and xylosidase), which were required for efficient degradation of lignocelluloses contained in tobacco leaves, were successfully overproduced in transplastomic tobacco plants. The expression levels of each enzyme were so high that the recombinant proteins were easily visible as major bands in SDS-PAGE profiles of total soluble proteins (TSPs) stained with CBB. All of the expressed thermostable enzymes were readily recovered as active forms without majority contamination from plant native proteins, by a short-term heat treatment of TSPs. All of the six transplastomic plants, although a few of which exhibited slight growth retardation, did not show any other defective phenotypes and were fertile. Detached leaves from the six transgenic plants were mixed in a certain fresh weight ratio and subjected to extraction of enzyme mixture. The residual plant materials were alkali pretreated, and used as substrates of saccharification reaction, started with readdition of the pre-extracted enzyme mixture. As a result of the experiment, we showed that the preliminary saccharification process could convert more than 50% of own cellulosic biomass into fermentable sugars, such as glucose and xylose. Taken together, our study on model plant tobacco will open up a new avenue to develop the auto-saccharification system of herbal bioenergy crops.

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**627 Evaluation of *Arabidopsis thaliana* as an Experimental Host for *Xylella fastidiosa***

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Pierce's disease of grapes and almond leaf scorch are agronomic diseases caused by the bacterium *Xylella fastidiosa*. To date, progress determining mechanisms of host plant susceptibility, tolerance or resistance has been slow, due in large part to the long generation time and limited available genetic resources for grape, almond and other known hosts of *X. fastidiosa*. To overcome many of these limitations, *Arabidopsis thaliana* has been evaluated as a host for *X. fastidiosa*. A pin-prick inoculation method has been developed to

infect Arabidopsis with *X. fastidiosa*. Following infection, *X. fastidiosa* multiplies robustly and can be detected by microscopy, PCR and isolation. The ecotypes Van-0, LL-0 and Tsu-1 all allow more growth of *X. fastidiosa* strain Temecula than the reference ecotype Col-0. Various *X. fastidiosa* strains also show differential growth in Arabidopsis. Affymetrix ATH1 microarray analysis of inoculated vs. non-inoculated Tsu-1 reveals gene expression changes that differ greatly from changes seen after infection with apoplast colonizing bacteria. Many genes responsive to oxidative stress are differentially regulated while classic pathogenesis-related (PR) genes are not induced by *X. fastidiosa* infection.

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**628 Targeting Mechanisms of the H3K4 Tri-Methyltransferase SET DOMAIN GROUP Protein 2 in *Arabidopsis***

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Tri-methylation of histone H3 lysine 4 (H3K4me3) is preferentially located at the 5' regions of most actively transcribed genes in *Arabidopsis*. Loss of H3K4me3 leads to severe and pleiotropic developmental abnormalities as well as mis-regulation of numerous genes. However, how H3K4me3 is established at specific genes remains unknown. We have previously shown that the SET DOMAIN GROUP PROTEIN 2 (SDG2) is the major H3K4 tri-methyltransferase in *Arabidopsis*. Interestingly, several blocks of amino acid residues outside the SET domain are highly conserved in plants but share no homology with any known proteins. We hypothesized that these regions may mediate protein-protein interactions between SDG2 and other proteins, which may be involved in targeting SDG2 to the chromatin. We therefore used the yeast two-hybrid experiment to identify SDG2-interacting proteins. Several SDG2-interacting (SIP) proteins have been identified this way and will be described here. These results provided valuable information regarding how H3K4me3 is established in plants.

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**629 Stomatal development: signaling fate and renewal**

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During development, multicellular organisms must create a diverse set of specialized cell types and organize these cells into functional tissues. Asymmetric cell division is an important mechanism for solving these challenges. We use stomata (epidermal structures that regulate carbon dioxide and water exchange in plants) as a model to understand asymmetric divisions during pattern formation; stomata guard cells are created via a stereotyped set of asymmetric cell divisions whose number and orientation are dictated by the interplay of cell-type specific transcription factors and local cell-cell interactions. Our recent interests have been in exploring the connection between cell fate and cell polarity and I will present work from genetic, cell-biological and modeling approaches that identifies and characterizes a nascent polarity-generating module at work in the divisions of the stomatal lineage.

## NOTES

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## NOTES

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# AUTHOR INDEX

(Note: Numbers refer to abstract numbers, not page numbers)

## A

- Aalen B, Reidunn..... 575
- Abadie, Cyril..... 102
- Abdeen M, Ashraf..... 346
- Abe, Hiroshi..... 359
- Acevedo, Gustavo..... 63
- Adamczyk, Ben..... 100
- Adams, Nicolette..... 168
- Affourtit, Jason..... 487
- Aggarwal, Pooja..... 379
- Agronomics Consortium Data
  - Contributors, ..... 92
- Agusti, Javier ..... 427
- Ahmadinejad, Nahal ..... 7
- Ahn, Yulkyun ..... 146
- Ahnert, Sebastian ..... 75, 211
- Akimoto-Tomiyama, Chiharu ..... 232
- Aksoy, Emre..... 103
- Alandete-saez, Monica..... 450
- Alassimone, Julien ..... 416
- Albani, Maria ..... 553
- Albertyn I, Zayed ..... 477
- Albrecht, Catherine..... 28
- Aldon, Didier ..... 240
- Alford R, Shannon ..... 104
- Alix, Andrew..... 33
- Allchin, Lorraine..... 22
- Allen, Randy ..... 493
- Alonso M, Jose..... 27, 35, 186
- Alonso-Peral, Maria..... 290
- Aluru, Maneesha..... 378
- Aluru, Srinivas ..... 378
- Amasino M, Richard..... 480, 497
- Andersen U, Stig..... 573
- Anderson G, Ryan..... 212, 248
- Anderson, Sarah..... 378
- Andriankaja, Megan..... 110
- Angenent, Gerco ..... 430
- Angers, Stephane ..... 236
- Antonio Aguilar Martinez,
  - Jose ..... 417
- Antony, Ginny ..... 278
- Anver, Shahjahan..... 472
- Apel, Klaus ..... 129
- Arabidopsis Interactome
  - Mapping Consortium ..... 20, 364
- Argueso T, Cris ..... 213
- Argyros D, Rebecca ..... 607
- Ariizumi, Tohru..... 604
- Armijo, Grace ..... 214
- Arrivault, Stéphanie ..... 29, 465
- Arsovski A, Andrej..... 344

## B

- Ashfield, Tom..... 215, 257
- Assadi H, Amir..... 558
- Assadi, Amir ..... 554
- Assmann M, Sarah ..... 586
- Atias, Osnat..... 368
- Audenaert, Dominique ..... 422
- Auer, Manfred..... 352
- Austin S, Ryan ..... 88
- Auzina, Aija ..... 573
- Babu, Yashodar ..... 418
- Bachan A, Shawn ..... 216
- Bachmair, Andreas ..... 191
- Badiger G, Bhaskara ..... 160
- Baerenfaller, Katja ..... 92, 93
- Baginsky, Sacha ..... 93
- Bai, Yan ..... 553
- Baidoo, Edward ..... 345
- Bailey E, Kate ..... 217
- Bailey, Kate ..... 259
- Bailey-Serres, Julia ..... 58, 101
- Baisa, Gary ..... 349
- Balazadeh, Salma ..... 596
- Balcon, Dominic ..... 321
- Baldwin L, Katherine ..... 272
- Ballantyne M, Scott ..... 473
- Bao, Yun ..... 79
- Bao, Zhilong ..... 234
- Barbier de Reuille, Pierre ..... 452
- Bargmann, Bastiaan ..... 87
- Barkoulas, Michalis ..... 17
- Barrero-Gil, Javier ..... 128
- Bartel P, David ..... 89
- Bartel, Bonnie ..... 173
- Bartels, Dorothea ..... 156
- Barton, M. Kathryn ..... 68, 397
- Bartos, Christopher ..... 356, 358
- Bar-Zvi, Dudy ..... 380
- Bassel W, George ..... 105
- Bassham C, Diane ..... 15, 311
- Battle L, Stephanie ..... 530
- Baudry, Jerome ..... 182
- Baxter R, Ivan ..... 109
- Bayer, Martin ..... 418
- Baylis, Tammy ..... 381
- Bedair, Mohamed ..... 460
- Bednarek, Paweł ..... 244
- Bednarek, Sebastian ..... 349
- Beeckman, Tom ..... 422
- Beem W, Lance ..... 149
- Begum, Tahmina ..... 163
- Behr, Jonas ..... 22, 375
- Belkadir, Youssef ..... 11
- Belmonte F, Mark ..... 419, 433
- Benavente M, Larissa ..... 35
- Bender, Judith ..... 178
- Benfey N, Philip ..... 127, 242, 444, 459, 474
- Bennett J, Malcolm ..... 576
- Benske, Anika ..... 340
- Bent F, Andrew ..... 21
- Bent, Andrew ..... 238
- Bentsink, Leónie ..... 122, 544
- Berardini Z, Tanya ..... 360
- Berganza, Sara ..... 553
- Bergelson, Joy ..... 534, 535, 537
- Berger, Frederic ..... 482
- Berger, Susanne ..... 106
- Berges, Phillip ..... 389
- Bergmann, Dominique ..... 84, 452
- Bergmann, Sven ..... 200, 518
- Berleth, Thomas ..... 67
- Besnard, Fabrice ..... 86
- Bethke, Gerit ..... 47
- Betti, Camilla ..... 555
- Beveridge A, Christine ..... 427
- Beynon, Jim ..... 20
- Bi, Dongling ..... 237
- Bieck M, Anthony ..... 615
- Biedermann, Sascha ..... 123
- Bilsborough, Gemma ..... 17
- Binder M, Brad ..... 598
- Bolley, Jean-Philippe ..... 102
- Birnbaum D, Kenneth ..... 147, 374
- Birnbaum, Kenneth ..... 87
- Bishnoi, Vikram ..... 120
- Bishopp, Anthony ..... 86
- Blackshaw T, Michael ..... 455
- Blackshaw, Michael ..... 399
- Blaisdel, Brandon ..... 502
- Blancaflor B, Elison ..... 273, 460, 614
- Blanchoin, Laurent ..... 327
- Blanco, Francisca ..... 219, 316
- Blanvillain-Baufume, Servane ..... 88
- Blilou, Ikram ..... 423
- Bolivar, Jenny ..... 340, 354
- Bolívar, Julio ..... 196
- Bolle, Cordelia ..... 366
- Bomblies, Kirsten ..... 124, 235
- Bonner J, Anthony ..... 105
- Borevitz O, Justin ..... 524
- Boruc, Joanna ..... 274
- Bosneaga, Elena ..... 352
- Bosse, Jennifer ..... 486

- Botanga, Christopher ..... 167  
 Bourguignon, Jacques ..... 300  
 Boutilier, Kim ..... 426, 430  
 Boutrot, Freddy ..... 28  
 Bowen, Christopher ..... 317  
 Brachi, Benjamin ..... 534, 537, 552  
 Bradley, Desmond ..... 428  
 Brady M, Siobhan ..... 75  
 Brady, Siobhan ..... 199, 211, 459  
 Bragg, Jennifer ..... 59  
 Brandizzi, Federica ..... 9, 283, 320, 328  
 Braun, Pascal ..... 20  
 Braybrook A, Siobhan ..... 36  
 Breakfield W, Natalie ..... 474  
 Breen, Gordon ..... 395  
 Breit, Robert ..... 209  
 Breitenbach, Heiko ..... 220  
 Breuer, Christian ..... 275  
 Brewer B, Philip ..... 427  
 Brière, Christian ..... 222  
 Briesemeister, Sebastian ..... 577  
 Briggs P, Steven ..... 31, 267, 284  
 Brisson, Normand ..... 488  
 Brkljacic, Jelena ..... 356, 358, 363  
 Brock, MT ..... 73  
 Broeckling, Bettina ..... 574  
 Bron, Emeric ..... 36  
 Brown WS, John ..... 55, 99, 524  
 Browse, John ..... 42, 608  
 Bruessow, Friederike ..... 221  
 Bruley, Christophe ..... 300  
 Brunoud, Geraldine ..... 86  
 Brusslan, Judy ..... 475  
 Bryan, Victoria ..... 357  
 Bryant, Adam ..... 310  
 Bryant, Nicole ..... 276  
 Bucher, Etienne ..... 479  
 Buckler, Edward ..... 91  
 Buessim, Dirk ..... 62  
 Bui Q, Anhthu ..... 433  
 Bulankova, Petra ..... 331  
 Bulgarelli, Davide ..... 7  
 Buono A, Rafael ..... 13  
 Burgess, Diane ..... 268  
 Burkhardt, Hans ..... 387  
 Burnie, Whitney ..... 470  
 Burow, Meike ..... 350  
 Burr, Christian ..... 309  
 Burton, Rachel ..... 347  
 Busch, Wolfgang ..... 127, 382, 459  
 Buschmann, Henrik ..... 405  
 Bush M, Susan ..... 561  
 Bush, Daniel ..... 574  
 Butenko A, Melinka ..... 575  
 Butler, Denise ..... 282  
 Bülow, Lorenz ..... 196  
 Böhmer, Maik ..... 77

**C**

- C, Weinig ..... 73  
 Caballero, Melodie ..... 548  
 Cailliatte, Rémy ..... 170  
 Callis, Judy ..... 194  
 Cameron K, Robin ..... 223, 249  
 Campbell M, Malcolm ..... 610  
 Canard, Bruno ..... 169  
 Cancho, Ester ..... 576  
 Caño-Delgado I, Ana ..... 323  
 Canonne, Joanne ..... 222  
 Cantu, Shane ..... 354  
 Cao, Feng Yi ..... 107  
 Carabelli, Monica ..... 69  
 Carbery, Margaret ..... 185  
 Carella, Philip ..... 223, 249  
 Carlson L, Ann ..... 550  
 Carré, Isabelle ..... 504  
 Carroll, Andrew ..... 367  
 Cartagena, Maria ..... 538  
 Caruana C, Julie ..... 256  
 Casady S, Megan ..... 212  
 Casey, Megan ..... 268  
 Cass, Cynthia ..... 349  
 Casstevens, Terry ..... 91  
 Castillejo, Cristina ..... 587  
 Castillo, Rosa ..... 553  
 Catala, Rafael ..... 148  
 Cazaux, Marc ..... 63, 624  
 Celaya, R Brandon ..... 500  
 Celenza L, John ..... 178, 185  
 Cellier, Coralie ..... 86  
 Chaban, Christina ..... 577  
 Chae, Keun ..... 383  
 Chambrier, Pierre ..... 86  
 Chamovitz A, Daniel ..... 368  
 Champigny, Marc ..... 249  
 Chan, Jenkin ..... 402, 538  
 Chan, Simon ..... 30  
 Chandler, John ..... 420  
 Chandran, Divya ..... 224  
 Chang J, Chiung-swey ..... 501  
 Chang N, Katherine ..... 556  
 Chang, Kwang Suk ..... 440  
 Chanoca, Alexandra ..... 578  
 Chapman A, Laura ..... 338  
 Chapman D, Kent ..... 614  
 Chapman, Jarrod ..... 487  
 Chattopadhyay, Abhishek ..... 610  
 Chaturvedi, Ratnesh ..... 225  
 Chauveau, Carine ..... 535  
 Chen, Charles ..... 91  
 Chen, Ho-Ming ..... 56  
 Chen, Iris ..... 309  
 Chen, Jie ..... 108  
 Chen, Li-Qing ..... 278  
 Chen, Meng ..... 505, 528  
 Chen, Qian ..... 613, 621  
 Chen, Rong ..... 613, 621  
 Chen, Sixue ..... 197, 586  
 Chen, Xiyang ..... 21  
 Chen, Xuemei ..... 3  
 Chen, Yani ..... 209  
 Chen, Yazhou ..... 197  
 Chen, Ying ..... 494  
 Chen, Zhoutao ..... 487  
 Cheng T, Yu ..... 6  
 Cheng, Chia-Yi ..... 620  
 Cheng, Daolin ..... 49  
 Cheon, Choong-Ill ..... 384  
 Chetty, Raymond ..... 360  
 Chetvernin, Vyacheslav ..... 365  
 Cheval, Cecilia ..... 240  
 Chevalier J, David ..... 119  
 Chevalier, David ..... 498, 579, 603  
 Chiang, Yi-Hsuan ..... 607  
 Chickarmane S, Vijay ..... 447  
 Chinchilla, Delphine ..... 28  
 Chiu, Tsan Yu ..... 279  
 Cho, Chuloh ..... 568  
 Cho, Hyung-Taeg ..... 14, 342  
 Cho, Misuk ..... 14, 198, 373  
 Cho, Myeongcheoul ..... 146  
 Choe, Goh ..... 580  
 Choe, Sunghwa ..... 509  
 Choi, Hee-Seung ..... 342  
 Choi, Hyunju ..... 343  
 Choi, Won-Gyu ..... 83, 134, 182, 289  
 Chor, Benny ..... 368  
 Chory, Joanne ..... 2, 11, 81, 503, 514, 533  
 Christians, Matthew ..... 502, 527  
 Christiansen M, Katy ..... 45  
 Chu, Heng-Hsuan ..... 109  
 Chu, Jinfang ..... 621  
 Chuah, Aaron ..... 91  
 Chung, KwiMi ..... 581  
 Chung, Taijoon ..... 307  
 Church, Deanna ..... 365  
 Cierlik, Izabela ..... 381  
 Claeys, Hannes ..... 110  
 Clarin E, Arielle ..... 615  
 Clark M, Richard ..... 22, 624  
 Clark, Richard ..... 375  
 Clarke, Alyssa ..... 399  
 Cline, Sara ..... 179  
 Clover, Charles ..... 111  
 Coaker, Gitta ..... 176, 226, 230, 243  
 Coen, Enrico ..... 452  
 Cohen, Jerry ..... 171, 183, 386, 395, 613  
 Colby, Thomas ..... 220  
 Cole A, Rex ..... 385  
 Cole J, Benjamin ..... 503  
 Cole, Benjamin ..... 514  
 Cole, Melanie ..... 420

Cole, Rex.....	329, 337
Cole, Stephanie .....	227
Coll S, Nuria .....	111
Colón-Carmona, Adán .....	282
Comelli, Petra .....	420
Conn J, Simon .....	347
Contento, Anthony .....	311
Cook R, Doug .....	499
Cooper, Martha .....	71
Coppens, Frederik .....	110
Cormack D, Ryan .....	399
Coruzzi M, Gloria .....	147, 201
Cosson, Patrick .....	548
Costantino, Paolo .....	38, 445
Couch, Daniel .....	170
Coupland, George .....	72, 553
Courtney, Stephanie .....	310
Coute, Yohan.....	300
Coutuer, Silvie .....	555
Covington F, Michael .....	50
Craddock P, Christian .....	168
Crawford, Sara .....	550
Crespi, Martin .....	302
Crete, Patrice .....	169
Crisp, Carolyn .....	178
Crist K, Debbie .....	356, 358
Crist, Deborah .....	363
Cruz-Ramirez, Alfredo .....	423
Cuddy K, Katrina .....	280
Cuguen, Joël .....	537, 552
Cui, Hongchang .....	127, 421
Curie, Catherine .....	170
Cutler, Sean .....	426

**D**

Dabi, Tsegaye .....	11
Dabos, Patrick .....	263
Dai, Mingqiu .....	513
Dal Bosco, Cristina .....	386
Dang, Jonathan .....	53
Dangl L, Jeffery ..	8, 20, 111, 213, 232
Daniels, Mark .....	309
Darracq, Aude .....	537
Das, Malay .....	405
Das, Pradeep .....	86
Dashti, Hesam .....	558, 558
David M, Karine .....	582
Davidson, Rebecca .....	201
Davies, Sian .....	504
Davis I, Jerrold .....	180
Day C, Robert .....	297
Day, Brad .....	327
De Bodt, Stefanie .....	110
De Clercq, Inge .....	112
De Clercq, Rebecca .....	97
De Jaeger, Geert .....	60
de Pater S, B.....	305

De Rop, Gieljan .....	422
De Rybel, Bert .....	422
De Smet, Ive .....	422
de Vries, Sacco .....	28
Dean H, Gillian .....	46
Dean, Caroline .....	312
Deb, Devdutta .....	212, 248
Debernardi M, Juan .....	490
Debieu, Marilyne .....	535
DeClerck, Genevieve .....	91
Deed K, Nathan .....	582
DeFraia, Christopher .....	486
Del-Bem EV, Luiz .....	583
Dello Ioio, Raffaele .....	38, 445
Demianski, Agnes .....	581
den Os, Desiree .....	584
Deng, Xing Wang .....	513
Deng, Yan .....	125
Deng, Youhui .....	195
Dennis S, Elizabeth .....	477
Dennis, Elizabeth .....	290
Desai K, Mintu .....	281, 313
Desany, Brian .....	487
Desveaux, Darrell .....	107, 236
Devert, Anthony .....	169
Devlin F, Paul .....	513, 526
Dewey D, Grant .....	536
Dharmawardhana, Palitha .....	91
Dhingra, Sonia .....	310
Di Mambro, Riccardo .....	38
Di Mambro, Riccardo .....	445
Di Rubbo, Simone .....	323
Díaz de la Garza I., Rocío .....	460
Diaz J, Kristophe .....	282
Diaz-Trivino, Sara .....	423
Diener, Andrew .....	227
Dietrich A, Margaret .....	113
Dilkes P, Brian .....	549
Dinesh-Kumar P, Savithramma .....	144, 216
Ding, Geng .....	49
Ding, Lei .....	424
Dinkins, Randy .....	511
Dinneny R., José .....	79, 379
DiTaccchio, Luciano .....	50
Divi K, Uday .....	344, 348
Divol, Fanchon .....	170
Doerner, Peter .....	331
Doherty, Colleen .....	587
Dolja V, Valerian .....	304
Dong A, Malia .....	80
Dong X, Oliver .....	228
Dong, Kang .....	190
Dong, Xinnian ..	6, 231, 261, 270, 271
Dorn M, Kevin .....	625
Dovzhenko, Alexander .....	386, 387
Downie, Bruce .....	511, 512, 517
Dragomir, Isabelle .....	494

**E**

Eaddy, Marcie .....	434
Eastmond, Peter .....	168
Ebert, Berit .....	45, 345, 351
Ecker R, Joseph ..	4, 20, 33, 100, 241, 318, 514, 556
Ecker, Joseph .....	487
Edwards, CE .....	73
Effendi, Yunus .....	593
Efroni, Idan .....	87
Egholm, Michael .....	487
Eguen E, Tenai .....	284
Eguen, Tenai .....	31
Eismann, Margitta .....	386
Elberse, Joyce .....	159, 247
Elmore M, James .....	226, 230
Eloy, Nubia .....	60, 110
Endo, Akira .....	602
Engineer, Cawas .....	115
Epple, Petra .....	111, 213
Erickson L, Jessica .....	425
Esfandiari, Elahe .....	346
Estelle, Mark .....	580, 587
Ewers, BE .....	73
Ezequiel, Petrillo .....	524

**F**

Fabre, Nicolas .....	169
Falbel, Tanya .....	340, 354, 388
Fan, Kai-Ting .....	171, 616
Fang, Yuda .....	285
Fankhauser, Christian .....	200, 518
Farcot, Etienne .....	86
Farmer E, Edward .....	601
Farmer M, Lisa .....	173
Farooq W, Lujaina .....	82
Farre M, Eva .....	54, 80
Faubert, Jennifer .....	249
Faure, Nathalie ..	534, 535, 537, 552
Fawley, Stephen .....	250
Federico Valverde, Federico .....	508

- |                                 |                    |                                  |                    |                             |               |
|---------------------------------|--------------------|----------------------------------|--------------------|-----------------------------|---------------|
| Fee A, Rachel .....             | 212                | Geldner, Niko .....              | 65, 416            | Gu, Hongya .....            | 355           |
| Feil, Regina .....              | 465, 617           | Genissel, Anne .....             | 535                | Gu, Xiaofeng .....          | 481, 495      |
| Felsensteiner, Corinna.....     | 236                | Gerjets, Tanja .....             | 105                | Gu, Yangnan .....           | 152, 291      |
| Fendrych, Matyas.....           | 329, 337           | Gerken M, Dana .....             | 415                | Guédon, Yann .....          | 86            |
| Feng, Guangping.....            | 390                | Germain, Hugo .....              | 228                | Guenther, Manuela .....     | 29            |
| Ferguson, Alison .....          | 286                | Gey, Delphine .....              | 476                | Guerinot, Mary Lou .....    | 23, 109, 181  |
| Fernandez E, Donna .....        | 411                | Ghazi, Zohaib .....              | 174                | Guerra, Damian .....        | 194           |
| Fernandez, Ana .....            | 97                 | Ghorbani, Sarieh .....           | 585                | Guillaume, Hubert .....     | 534           |
| Fernie R, Alisdair .....        | 29                 | Gibbs J, Daniel .....            | 105, 599           | Gujas, Bojan .....          | 67            |
| Ferreira J, Fernando .....      | 213                | Gibon, Yves .....                | 187                | Gulledge, Alyssa .....      | 120, 135, 207 |
| Ferreira, Paulo .....           | 60                 | Gieseler, Cornelia .....         | 545                | Guo, Dan .....              | 389           |
| Fiehn, Oliver .....             | 167                | Gilliam, Matthew .....           | 347                | Guo, Feng .....             | 292, 401      |
| Fiers, Martijn .....            | 426                | Gilroy, Simon .....              | 289, 578           | Guo, Hongwei .....          | 205           |
| Finnegan, Jean .....            | 290                | Gimenez-Ibanez, Selena .....     | 28                 | Guo, Honqing .....          | 378           |
| Fitz Gerald N, Jonathan .....   | 402, 538           | Gingerich G, Derek .....         | 502                | Guo, Yongxia .....          | 533           |
| Fletcher C, Jennifer .....      | 64                 | Gingerich J, Derek .....         | 527                | Gupta, Rishabh .....        | 21            |
| Floris, Maïna .....             | 116                | Glazebrook, Jane .....           | 47, 61, 167, 264   | Gutiérrez A, Rodrigo .....  | 74, 94, 201   |
| Folta M, Kevin .....            | 532                | Glorieux, Cedric .....           | 534, 535           | Guttman, David .....        | 236           |
| Forde G., Brian .....           | 559                | Glaab, Enrico .....              | 105                |                             |               |
| Foster, Cliff .....             | 354                | Godfrey, Jason .....             | 185                | <b>H</b>                    |               |
| Fournier-Level, Alexandre ..... | 71                 | Godin, Christophe .....          | 86                 | Ha, Tracy .....             | 77            |
| Fowler E, John .....            | 304, 329, 337, 385 | Godoy Herz A, Micaela .....      | 524                | Hackenberg, Thomas .....    | 573           |
| Francis, La'Kesha .....         | 178                | Goel K, Ajay .....               | 232                | Hake, Sarah .....           | 59            |
| Franke, Annika .....            | 465                | Goertzen R, Leslie .....         | 415                | Hala, Michal .....          | 329, 337      |
| Franklin A, Keara .....         | 395, 507, 521      | Goldberg B, Robert .....         | 419, 433           | Halimi, Yair .....          | 368           |
| Frentz, Zak .....               | 374                | González J, Alvaro .....         | 499                | Hall A, David .....         | 24            |
| Friml, Jiří .....               | 323                | Goodin M, Michael .....          | 511                | Hamdoun, Safae .....        | 121           |
| Frohman, Lance .....            | 207                | Goodstadt, Leo .....             | 22                 | Hamel, Patrice .....        | 179           |
| Frommer B, Wolf .....           | 278                | Gookin E, Timothy .....          | 586                | Hamilton E, Elizabeth ..... | 54            |
| Fu, Zhengqing .....             | 231                | Goring R, Daphne .....           | 338, 591           | Han Tan, EK .....           | 261           |
| Fujiki, Yuki .....              | 335                | Goring, Daphne .....             | 324                | Han, Jeungsul .....         | 146           |
| Fujita, Miki .....              | 117, 570           | Gou, Mingyue .....               | 234                | Han, Sang Won .....         | 293           |
| Fujita, Yasunari .....          | 117, 140           | Gouhier-Darimont, Caroline ..... | 221                | Hangarter, Roger .....      | 10            |
| Fukao, Takeshi .....            | 58                 | Gould, Pauline .....             | 389                | Hansen B, Maria .....       | 350           |
| Fukuda, Hiroo .....             | 273                | Grabowski, Stephanie .....       | 98                 | Hanzawa, Yoshie .....       | 428           |
| Fukuoka, Hiroyuki .....         | 430                | Graham, Tena .....               | 549                | Hao, Yueling .....          | 421           |
| Furutani, Masahiko .....        | 413                | Grant R, Sarah .....             | 232                | Harada J, John .....        | 419, 433      |
| <b>G</b>                        |                    | Gray III, Will .....             | 119                | Hardtke, Christian .....    | 67            |
| Galaud, Jean-Philippe .....     | 240                | Gray M, William .....            | 171, 395, 590, 616 | Hark T, Amy .....           | 478           |
| Gallagher L, Kimberly .....     | 287                | Gray, Angel .....                | 167                | Harkins, Timothy .....      | 487           |
| Galvao M, Rafaelo .....         | 505, 528           | Grbic, Miodrag .....             | 63, 624            | Harmer L, Stacey .....      | 50, 472       |
| Gan, Xiangchao .....            | 22                 | Grbic, Vojislava .....           | 63, 299, 624       | Harpaz-Saad, Smadar .....   | 348           |
| Ganpudi L, Ashwin .....         | 118                | Greaves K, Ian .....             | 290, 477           | Harrison R, Benjamin .....  | 294           |
| Gao F, Kimberly .....           | 107                | Greb, Thomas .....               | 427                | Harter, Klaus .....         | 577           |
| Gao, Hong-Bo .....              | 288                | Green J, Pamela .....            | 157, 499           | Hartung L, Mara .....       | 278           |
| Gao, Xin-Qi .....               | 330                | Green, Rachel .....              | 506                | Haruta, Miyoshi .....       | 175           |
| García, Consuelo .....          | 214                | Greenham, Katie .....            | 587                | Hashimoto M, Meryl .....    | 433           |
| Gardiner, Anastasia .....       | 217, 259           | Gregory D, Brian .....           | 494                | Hassfurder, Matt .....      | 310           |
| Gardner, Gary .....             | 183                | Grey H, Paris .....              | 280                | Hassidim, Miriam .....      | 506           |
| Garin, Jérôme .....             | 300                | Grienesen, Veronica .....        | 16                 | Haswell S, Elizabeth .....  | 295           |
| Garrett J, Jasmine .....        | 455                | Griffiths, Jonathan .....        | 46, 346            | Hatsugai, Noriyuki .....    | 252           |
| Gaubert, Hervé .....            | 479                | Grossniklaus, Ueli .....         | 439                | Hattori, Etsuko .....       | 612           |
| Gaudinier, Allison .....        | 75, 199, 211       | Groszmann, Michael .....         | 290, 477           | Haughn W, George .....      | 46, 346       |
| Gayler K, Krystal .....         | 132                | Grotewold, Erich .....           | 206, 356, 358, 363 | Hauser, Felix .....         | 77            |
| Gazzarrini, Sonia .....         | 410                | Gruissem, Wilhelm .....          | 92, 93             | Hautekeete, Nina .....      | 552           |
| Gee, Christopher .....          | 52                 | Grundman, Rachael .....          | 47                 | Havighorst R, Amanda .....  | 296           |
|                                 |                    | Gu, Chen .....                   | 395                | Hawkins, Charles .....      | 457           |
|                                 |                    | Gu, Fangwei .....                | 401                |                             |               |

Hazen P, Samuel.....	514	Holomuzki, Nicholas .....	356, 363
He, Cuiwen .....	188	Holt III F, Ben .....	132, 153, 546
He, Hanzi .....	122	Holton J, Nicholas.....	158
He, Shan .....	355, 572	Holuigue, Loreto .....	214
He, Sheng Yang.....	42, 271, 608	Hon, Gary.....	556
He, Wenrong .....	205	Hong, Daewoong .....	589
He, Yuehui.....	481, 495	Hong, Fang.....	285
He, Yuke .....	441	Hong, Zhi .....	308
He, Zemiao .....	492	Hongo, Hiroaki .....	165
Heazlewood L, Joshua .....	45, 48	Hoogewijs, Kurt .....	97
Heberle-Bors, Erwin .....	386	Hooker, Tanya.....	43
Hegeman D, Adrian .....	171	Hooykaas JJ, P .....	305
Hegermann, Jan.....	386	Horii, Yoko.....	165
Hehl, Reinhard .....	196	Horst J, Robin .....	429
Heidrich E, Katharina .....	233	Horstman, Anneke.....	430
Heidstra, Renze .....	38, 445	Horton, Meredith.....	232
Helariutta, Ykä .....	86	Hothorn, Michael .....	11
Helper, Anne .....	54	Hou, Bi-Huei.....	278
Helft A, Laura .....	21	Hou, Hongwei .....	455
Helft, Laura .....	238	Howe A, Gregg .....	26, 42, 600
Hell, Rüdiger.....	192	Howe, Gregg .....	608
Hellmann, Hanjo .....	123	Howell H, Stephen .....	125
Hennig, Lars.....	93	How-Yew-Kin, Theresa .....	248
Henry M, Elizabeth.....	176	Hsu Y, Polly .....	50
Henschen, Agnes.....	418	Hu, Honghong .....	115
Henty L., Jessica .....	327	Hu, Wei .....	507
Heo, Jung-Ok .....	440	Hu, Yuxin .....	390
Hernando E, Carlos.....	524	Hua, Hongjie .....	154
Herold, Silvia .....	427	Hua, Jian .....	234
Herridge P, Rowan .....	297	Hua, Zhihua.....	369, 370
Hersch, Micha .....	518	Huala, Eva .....	360, 371
Herter, Thomas.....	351	Huang, He .....	590
Herzyk, Pawel .....	55	Huang, Jian .....	414
Hicks, Glenn .....	322	Huang, Shanjin .....	301
Hildebrand, Katie .....	22	Huang, Shuai .....	6
Hilhorst WM, Henk.....	122	Huang, Tengbo .....	68, 391, 397
Hill, Claire .....	504	Huang, Xuan .....	150
Hill, Kristine .....	607	Huang, Yan .....	6, 237
Hillebrand, Katie .....	375	Hubbard, Katharine .....	77
Hillwig S, Melissa .....	311	Hubberten, Hans-Michael .....	254
Hilson, Pierre .....	92, 97, 560, 585	Huber, Steven .....	28
Hinds R, Thomas .....	42	Hudson E, Matthew .....	372
Hinds, Tom .....	608	Hudson, Matthew .....	202, 515
Hirsch-Hoffmann, Matthias .....	92, 93	Huen, Amanda .....	290
Hitchler, Michael .....	475	Huep, Gunnar .....	366
Hniličková, Jaroslava .....	323	Huh, Jung-Hyun .....	189
Hnilova, Marketa .....	96	Huisman, Rik .....	239
Ho, Cheng-Hsun .....	177	Humbert, Sabrina .....	125
Ho, Issac .....	487	Humpert, Fabian .....	99
Hobbie, Lawrence .....	389	Hunt G, Arthur .....	396
Hoefgen, Rainer .....	254	Hunter, Ben .....	235
Hoel, Marah .....	25	Huq, Enamul .....	53, 525
Hoffmann, Thomas .....	269	Hur, Yoon-Sun .....	384
Hogan J, Brad.....	178	Hurley, Brenden .....	236
Hohenstatt, Mareike .....	436	Hutchison E, Claire .....	213
Hohm, Tim .....	200	Hwang, Jae-Ung.....	303, 589
Holdsworth J, Michael .....	105, 599	Hynek, Radovan.....	126
Hollister D, Jesse .....	124	Höfte R, Herman .....	44
<hr/>			
<b>I</b>			
Iakovidis, Michael.....	232	Indriolo, Emily .....	591
Ideker, Trey .....	556	Innes W, Roger .....	255, 257
Iino, Moritoshi .....	619	Innes, Roger .....	152, 215, 229, 291
Ikeda, Miho .....	564, 570	Inzé, Dirk .....	60, 110, 555
Imaizumi, Takato .....	54	Irani G, Niloufer .....	323
Immink, Richard .....	430	Irish F, Vivian .....	391
Imura, Yoko .....	165	Ishiga, Takako .....	265
Indriolo, Emily .....	591	Ishiga, Yasuhiro .....	265
Innes W, Roger .....	255, 257	Ishihara, Hirofumi .....	539
Innes, Roger .....	152, 215, 229, 291	Ishii, Rina .....	402
Inzé, Dirk .....	60, 110, 555	Ishii, Tadashi .....	351
Irani G, Niloufer .....	323	Isley, Jonathan .....	388
Irish F, Vivian .....	391	Issacs, Cameron .....	383
Ishiga, Yasuhiro .....	265	Ito, Hidetaka .....	479
Ishihara, Hirofumi .....	539	Ito, Jun .....	45, 48
Ishii, Rina .....	402	Ito, Toshiro .....	492, 495
Ishii, Tadashi .....	351	Iuchi, Satoshi .....	117, 359
Isley, Jonathan .....	388	Ivakov, Alexander .....	539
Issacs, Cameron .....	383	Iwase, Akira .....	431
Ito, Hidetaka .....	479	Iyer-Pascuzzi, Anjali .....	127
<hr/>			
<b>J</b>			
Jackson, Terry .....	127	Jacksonc, Scott .....	499
Jacob, Yannick .....	481	Jacobs, Bianca .....	420
Jadav, Dipika .....	389	Jaiswal, Pankaj .....	91
Jaiswal, Pankaj .....	91	Jali, Sathya .....	349
James B, Allan .....	55	James B, Allan .....	55
Janakirama, Preetam .....	299	Jalil, Sathya .....	349
Jaqinod, Michel .....	300	Jarillo A, Jose .....	508
Jarillo A, Jose .....	508	Jarno, Nolwenn .....	300
Jarvis E, David .....	128	Jarvis E, David .....	128
Jauneau, Alain .....	222	Jayneau, Alain .....	222
Jayasena, Achala .....	389	Jayasena, Achala .....	389
Jenik D, Pablo .....	432	Jenik D, Pablo .....	432
Jenkins I, Gareth .....	55	Jenkins I, Gareth .....	55
Jensen S, Gregory .....	295	Jensen S, Gregory .....	295
Jensen T, Shane .....	88	Jensen T, Shane .....	88
Jeon, Byeong Wook .....	589	Jeon, Byeong Wook .....	589
Jeong, Dong-Hoon .....	499	Jeong, Dong-Hoon .....	157, 499
Jeong, Sangho .....	70	Jeong, Sangho .....	70
Jeong, Young-Min .....	480	Jeong, Young-Min .....	480
Ji, Lijuan .....	3	Ji, Lijuan .....	3
Jiang, Danhua .....	481	Jiang, Danhua .....	481

**ICAR 2011 University of Wisconsin–Madison**

- Jiang, Hongling ..... 613, 621  
Jiang, Ke ..... 57  
Jimenez-Lopez C, Jose ..... 301  
Jin, Jun-Young ..... 592  
Jin, Rong ..... 19  
Jin, Xiaofen ..... 586  
Jin, Zhaoqing ..... 346  
Johnson S, Logan ..... 543  
Johnson, Cameron ..... 439  
Johnson, Kaeli ..... 237  
Jolivet, Sylvie ..... 30  
Jones A, Matthew ..... 50  
Jones DG, Jonathan ..... 217, 259  
Jones M, Alan ..... 244  
Jones, Alexandra ..... 28  
Jorda, Lucia ..... 220  
Jordans, Charlotte ..... 347  
Jorgensen, Jan-Elo ..... 573  
Jouannet, Virginie ..... 302  
Jun, Ji Hyun ..... 49  
Jung, Hyunju ..... 130  
Juul, Trine ..... 573
- K**
- Kadota, Yasuhiro ..... 28  
Kaiser N, Brent ..... 347  
Kakehi, Jun-ichi ..... 392  
Kamiya, Asako ..... 165  
Kamoun, Sophien ..... 1  
Kang S, Earl ..... 284  
Kang, Hong Gu ..... 317  
Kang, Hunseung ..... 130, 253  
Kang, Xiaojun ..... 520  
Kang, Ye Eun ..... 497  
Kang, Yeon Hee ..... 438  
Kanter, Ulrike ..... 114  
Karamoko, Mohamed ..... 179  
Karthikeyan, AS ..... 91  
Kashima, Yasuhiro ..... 626  
Katagiri, Fumiaki ..... 47, 61, 76  
Katari S, Manpreet ..... 201  
Kato, Naohiro ..... 317  
Kauser, Imrose ..... 82  
Kaviani, Kamran ..... 455  
Kawamura, Ayako ..... 275  
Kawashima, Mika ..... 165  
Kay A, Steve ..... 54, 503, 519  
Kay, Steve ..... 500, 587  
Keasling D., Jay ..... 345  
Keating M, Kathleen ..... 202  
Keerthisinghe, Sandra ..... 403  
kellenberger, Stephan ..... 601  
Kemen, Ariane ..... 217, 259  
Kemen, Eric ..... 217, 259  
Kendig, Ashley ..... 478  
Kenobi, Kim ..... 203  
Kerstetter, Randall ..... 68
- Kerstetter, Randy ..... 34, 397  
Kessens, Ryan ..... 215  
Khan, Safina ..... 526  
Khanna, Kanhav ..... 414  
Khatayevich, Dmitriy ..... 96  
Kieber J, Joseph ..... 41, 213, 348, 353, 606, 607, 620  
Kiefer, Christiane ..... 553  
kim, Bokyung ..... 509  
Kim, Byung-Hoon ..... 510  
Kim, Chanhong ..... 129  
Kim, Hyo Jung ..... 607  
Kim, Jeong Hoe ..... 393  
Kim, Jitae ..... 180  
Kim, Jung-Gun ..... 278  
Kim, Jungho ..... 146  
Kim, Jungmook ..... 568  
Kim, Minkyung ..... 130  
Kim, Sang Yeol ..... 424  
Kim, Sangwoo ..... 303  
Kim, Soo Young ..... 589, 592  
Kim, Sun A ..... 181  
Kim, Sunghan ..... 384  
Kim, Tae-Houn ..... 77  
Kim, YongSig ..... 139  
Kim, Yun Ju ..... 3  
Kim, Yungil ..... 61  
Kim, Yu-Young ..... 343  
King J, Jasmine ..... 54  
Kinoshita, Tetsu ..... 485  
Kinoshita, Yuki ..... 485  
Kirch, Hans-Hubert ..... 156  
Kirchler, Tobias ..... 577  
Kirkbride C, Ryan ..... 419, 433  
Kleinboelting, Nils ..... 366  
Klessig F, Daniel ..... 32  
Klimke, William ..... 365  
Klocko L, Amy ..... 304  
Knee M, Emma ..... 358  
Knee M., Emma ..... 356  
Knee, Emma ..... 363  
Knight, Jim ..... 487  
Knip, Marijn ..... 305  
Knowles, Steve ..... 500  
Kobayashi, Masatomo ..... 117, 359  
Kobayashi, Toshihiro ..... 359  
Kobayashi, Yuriko ..... 117  
Kodicek, Milan ..... 126  
Koester, Tino ..... 99  
Kohlbacher, Oliver ..... 577  
Kohout, Ladislav ..... 323  
Koiwa, Hisashi ..... 103  
Koizumi, Koji ..... 287  
Kojima, Soichi ..... 136  
Koller, Teresa ..... 21, 238  
Kondo, Maki ..... 409  
Kondo, Satoshi ..... 565, 612  
Kondou, Youichi ..... 165, 570
- Konik, Peter ..... 126  
Koo JK, Abraham ..... 26  
Koo, Yeonjong ..... 468  
Kopka, Joachim ..... 151  
Kornblihtt R, Alberto ..... 524  
Korneli, Christin ..... 99  
Korte, Arthur ..... 71, 131  
Kost, Sara ..... 419  
Kotak, Jenna ..... 478  
Kotchoni O, Simeon ..... 339  
Kover, Paula ..... 22, 375  
Koyama, Tomotsugu ..... 565, 570  
Krasnogor, Natalio ..... 105  
Krieger, Uri ..... 57  
Krischke, Markus ..... 106  
Krizek A, Beth ..... 434, 446  
Krogan J, Nevan ..... 472  
Krogan, Naden ..... 67  
Kroll, Phillip ..... 436  
Krouk, Gabriel ..... 87, 147, 201  
Krysan J, Patrick ..... 561  
Krysan, Patrick ..... 210  
Krämer, Ute ..... 523  
Kubic, Jennifer ..... 155  
Kuckova, Stepanka ..... 126  
Kucukoglu, Melis ..... 435  
Kuhl, Carsten ..... 547  
Kuhlemeier, Cris ..... 36  
Kukula, Katarzyna-Lorenc ..... 245  
Kulich, Ivan ..... 337  
Kumakura, Naoyoshi ..... 484  
Kumar, Manoj ..... 331  
Kumar, Santosh ..... 511, 512, 517  
Kumar, Vinod ..... 395  
Kumimoto W, Rod ..... 546  
Kumimoto W, Roderick ..... 132, 153  
Kunkel N, Barbara ..... 250, 581  
Kunst, Ljerka ..... 43  
Kuo D, Paul ..... 556  
Kurihara, Yukio ..... 100  
Kuriyama, Tomoko ..... 165  
Kuroha, Takeshi ..... 96  
Kuromori, Takashi ..... 165  
Kusano, Tomonobu ..... 262  
Kushwaha, Rekha ..... 512  
Kuusisto, Pia ..... 562  
Kwak, Kyungjin ..... 130  
Kwon, Sehoon ..... 592  
Kwon, So Hyun ..... 393  
Kwaaitaal, Mark ..... 239  
Kylliainen, Maiju ..... 562
- L**
- Labusch, Corinna ..... 593  
Lachowiec A, Jennifer ..... 540  
Lafos, Marcel ..... 436  
Lagarias J, Clark ..... 507

Lahner C, Brett.....	109	Lewis R, Daniel .....	306
Laila, Moubayidin.....	38	Lewsey G, Mathew .....	241
Lalonde, Sylvie .....	278	Lewsey, Mathew .....	33
Lam, Patricia.....	43	Leyser, Ottoline.....	40
Lamb S, Rebecca .....	458	Li Q, Qingshun.....	108, 396, 412
Lamesch, Philippe.....	371	Li, Chuanyou.....	613, 621
Lan, Hui .....	105	Li, Donghui.....	360
Lan, Ping .....	454	Li, Fan .....	494
Lanet, Elodie .....	116	Li, Faqiang .....	307
Lanz, Evan .....	389	Li, Gang .....	513, 526
Lapin, Dmitry.....	541	Li, Hai .....	556
Larsen, Noah .....	554	Li, Hongzhe.....	88
Last L, Robert .....	24	Li, Jianming .....	308, 334
Lau, On-Sun.....	513	Li, Jiayang .....	569
Lauer D, Timothy .....	527	Li, Jigang.....	513
Law F, Theresa .....	232	LI, Jing .....	482
Lawrence, Michael .....	207	Li, Lei.....	378, 563
Lazaro, Ana .....	508	Li, Li-Juan .....	330
Le Guillou, Laurent.....	36	Li, Lin .....	514
Le H, Brandon.....	433	Li, Ling .....	25
Leaflight, Ren.....	356	Li, Meina .....	528
Leba, Louis-Jerome.....	240	Li, Shengben .....	3
Lebeurre, Vincent .....	102	Li, Shibai .....	355
Lee Y, Michelle .....	284	Li, Tian .....	182
Lee, Jin Suk.....	96	Li, Wei .....	520
Lee, Byung Ha .....	393	Li, Wenfeng .....	454
Lee, Connie .....	59	Li, Wenyang .....	205
Lee, Eun-Jung .....	589	Li, Xin .....	6, 228, 237
Lee, HwaJung .....	253	Li, Xugang .....	613
Lee, Hyeeun .....	146	Li, Ying .....	372, 515
Lee, Hyoung Yool .....	317	Li, Yingfu .....	174
Lee, Ilha .....	400, 496	Li, Yingzhong .....	6
Lee, Jiwon .....	14	Li, Yuan .....	258
Lee, Ji-Young .....	66, 394, 471	Li, Yupeng .....	499
Lee, Keun Pyo.....	129	Li, Zhaolong .....	34
Lee, Mi-Hyun.....	437	Li, Zhonghai .....	205
Lee, Min Jeong.....	393	Liao, Ya-Yun .....	454
Lee, Minsoo .....	14	Lberman, Louisa .....	242
Lee, Myeong Min .....	438	Liljegren, Sarah .....	309, 575
Lee, Sang Ho.....	395	Lim, Jun .....	437, 440, 594
Lee, Seung Cho .....	58	Lin D, Zuh-Jyh .....	226, 243
Lee, Shin Ae .....	440, 594	Lin, Changfa .....	342
Lee, Stephen .....	77	Lin, Li .....	514
Lee, Young-Jin .....	49	Lin, Rongcheng .....	513
Lee, Youngsook....	303, 343, 589, 592	Lin, Shan-Hua .....	177
Legendre, Matthieu .....	551	Lin, Zhefeng .....	422
Legrand, Jonathan .....	86	Lingard J, Matthew .....	173
Lehti-Shiu D, Melissa .....	542	Lippman B, Zachary .....	57
Lei, Jiaxin.....	133	Liptay, Albert .....	149
Leibler, Stanislas .....	374	Lister, Ryan .....	487
Leigh A, Roger .....	347	Liu L, Tiffany .....	516
Leister, Dario .....	366	Liu, Bao .....	553
Leiva, David .....	214	Liu, Bin .....	575
Lemoine, Rémi.....	102	Liu, Chun-Ming .....	621
Leon, Luis .....	214	Liu, Fang .....	613
Lepage, Etienne.....	488	Liu, Fuquan .....	312
Leshem, Yehoram .....	439	Liu, Guoqin .....	258
Leslie E, Michelle .....	575	Liu, Hao .....	154

**M**

Ma, Hong .....	442
Ma, Shisong .....	144, 216
Ma, Tao .....	528
MacIntosh C, Gustavo .....	311

**ICAR 2011 University of Wisconsin–Madison**

Mackaluso D, Joshua .....	19	McCormick P, Kevin.....	468	Moreno E, Javier .....	600
Mackey, David .....	269	McCouch, Susan .....	91	Moreno, Adrián .....	219, 316
Macknight C, Richard.....	297	McCourt, Peter.....	602	Moreno, Ignacio .....	316
Macrae, Rhiannon .....	483	McCue D., Andrea .....	486	Moreno-Risueno A, Miguel .....	444, 459
Madder, Annemieke .....	97, 323	McDaniel K, Brittany.....	598	Morin, ValerieE .....	86
Maeda, Hiroshi.....	184	McDowell M, John .....	212, 248	Morita T, Miyo .....	566
Maglott, Donna .....	365	McFarlane E, Heather .....	348	Moriyama, Yuji .....	609
Maintz, Jens .....	239	Md Isa, Nurulhikma .....	599	Morohashi, Kengo.....	206
Maizel, Alexis .....	302	Mecchia A, Martin .....	490	Morrissey, Joe .....	109
Makaroff A, Christopher .....	336	Medina, Joaquin .....	148	Mosher, Rebecca .....	5
Malec, Przemyslaw .....	510	Meents J, Miranda.....	455	Motomura, Kazuki .....	484
Maleux, Katrien .....	110	Mehmeti, Vlora .....	162	Motose, Hiroyasu .....	12, 392, 398
Malinovsky, Frederikke .....	28	Mei, Yu .....	288, 428	Mott, Richard .....	22, 375
Mallery, Eileen .....	408	Meier, Iris .....	274, 281, 313	Mottarella, Scott .....	178
Malolepszy, Anna .....	573	Meijón Vidal, Mónica .....	382	Moubayidin, Laila .....	432, 445, 445
Maloney, Greg .....	383	Meinke, David .....	276, 314, 361	Mousavi R, Seyed Ali .....	601
Maloof N, Julin .....	52, 501	Melas I, Marisa .....	249	Muday K, Gloria .....	306, 383, 388
Mann, James .....	356	Melas, Marisa .....	223	Mudgett, Mary Beth .....	278
Mao, Haibin .....	42, 608	Melatti, Carmen .....	69	Mudunkothge S, Janaki .....	446
Maoyin, Li .....	225	Mellgren M, Eve .....	250	Mueller J, Martin .....	106
Marco, Yves .....	263	Melzer, Michael .....	387	Mueller-Roeber, Bernd .....	208, 571, 596
Mari, Stéphane .....	170	Men, Xiao .....	190	Muhammad, Durreshah .....	82
Marimuthu, Mohan .....	30	Mercier, Raphael .....	30	Mukhopadhyay, Aindrila .....	351
Marino, Daniel .....	222	Merithew B, Sarah .....	536	Muller, Bob .....	360
Marks, David .....	625	Meservy L, James .....	455	Muralla, Rosanna .....	314
Marlatt A, Sara .....	188	Meyer C, Rhonda .....	541	Muramoto, Nobuhiko .....	565, 612
Marquardt, Sebastian .....	312	Meyer, Stefan .....	90	Muranaka, Toshiya .....	343
Marr, Sharon .....	268	Meyerowitz M, Elliot .....	37, 447, 453	Mutka M, Andrew .....	250
Marsalova, Lucie .....	126	Meyers C, Blake .....	157, 468, 499	Myers L, Chad .....	61
Marshall, Jacqueline .....	55	Meyers, Blake .....	362	Mylle, Evelien .....	323
Marteaux, Benjamin .....	86	Michael, Todd .....	34	Myouga, Fumiyoishi .....	276
Martens J, Helle .....	350	Michaels D, Scott .....	424, 481	Mysore S, Kirankumar .....	265
Martin, Kathleen .....	511	Millar, A. Harvey .....	563		
Martinec, Jan .....	126	Miller, Nathan .....	198, 210, 373		
Martinoia, Enrico .....	592	Miller, Rita .....	314		
Maruhnich, Stefanie .....	532	Mirouze, Marie .....	479		
Maruyama, Hayato .....	136	Mito, Tomomi .....	564		
Maruyama, Kyonoshin .....	137, 140	Mitsuda, Nobutaka .....	564, 565, 570		
Mas, Paloma .....	524	Mitsukawa, Norihiro .....	565, 612		
Mason, John .....	408	Mizoi, Junya .....	137, 143, 335		
Masson H, Patrick .....	272, 611	Moeder, Wolfgang .....	107		
Masson, Patrick .....	349	Moffatt A, Barbara .....	174		
Matallana-Ramirez P, Lilian .....	596	Moffatt, Barbara .....	192		
Matari, Nahill .....	432	Mogen L, Kim .....	473		
Mathews E, Dennis .....	607	Moghe, Gaurav .....	138		
Mathews, Dennis .....	597	Mohan, Rajinikanth .....	231		
Mathieu, Johannes .....	63	Moller, Isabel .....	347		
Matiolli C, Cleverson .....	476	Monaco, Marcela .....	91		
Matsubayashi, Yoshikatsu .....	609	Monaghan, Jacqueline .....	28		
Matsui, Kyoko .....	564, 565, 570	Monihan M, Shea .....	113, 139		
Matsui, Minami .....	165, 570	Monniaux, Marie .....	88		
Mattox, Cassie .....	388	Monshausen B, Gabriele .....	289, 325, 584		
Mattsson, Jim .....	381	Mooney, Sutton .....	123		
Mayers, Akielia .....	282	Moore R, Candace .....	543		
Mazars, Christian .....	240	Moore, Raymond .....	473		
McAbee M, Jessica .....	96	Moreau, Magali .....	32		
McCain R, Elizabeth .....	478	Morelli, Giorgio .....	69		
McClung, CR .....	73				

**N**

Nadeau, Jeannette .....	403
Nafisi, Majse .....	350
Nahal K, Hardeep .....	209
Nakagawa, Tsuyoshi .....	403
Nakahira, Yoichi .....	626
Nakamichi, Norihitto .....	529
Nakamura, Miyuki .....	485
Nakashima, Kazuo .....	140
Nakata, Masaru .....	564
Nalam, Vamsi .....	225
Nam H, Eric .....	602
Nam, Hong-Gil .....	529
Naramoto, Satoshi .....	273
Narayanan A, Lakshmi .....	603
Nash G, David .....	294
Navarro, Marie .....	63, 624
Navarro-Quezada R, Aura .....	547
Nawy, Tal .....	87
Nayak, Nihar .....	511
Nehr, Zofia .....	548
Nelson D, Jeffrey .....	519

Nelson, Benjamin.....	155
Nelson, Clark .....	563
Nelson, Sven .....	604
Nemhauser L, Jennifer .....	52, 540
Nery, Joe .....	33
Nery, Joseph .....	556
Neuhaus, Gunther .....	522
Newell, Nicole .....	397
Newell, Niki .....	68
Ng, Gina .....	121, 530
Ng, Kian-Hong.....	495
Ngo, Quy .....	439
Nguyen, Anh .....	97
Nguyen, Henry .....	188
Nguyen, Le.....	47
Nguyen, Thu-Phuong .....	544
Ni, Min.....	520
Nie, Xin.....	482
Niederhuth, Chad .....	605
Nielsen L, Kåre .....	573
Nielsen, Erik .....	292, 401
Niessing, Dierk .....	405
Niewohner J, Devon .....	536
Niitsu, Masaru.....	392
Nikolau J, Basil .....	49
Nikoloski, Zoran .....	208
Nilsson, Jeanette .....	435
Nilsson, Ove.....	435
Nimchuk L, Zachary .....	447
Nimmo A, Gillian.....	55
Nimmo G, Hugh.....	55
Nishida, Ikuo.....	303, 335
Nishimura, Mikio .....	252, 409
Nishimura, Noriyuki .....	77
Nito, Kazumasa .....	514
Njo, Maria .....	422
Nodine D, Michael .....	89
Noel P, Joseph .....	11
Noel, Joseph .....	533
Noh, Yoo-Sun .....	480
Nordborg, Magnus .....	71, 131, 534, 537
Normanly, Jennifer .....	185
Norris C, David .....	207
Novacki, Jérôme .....	263
Novokreshchenova, Maria .....	141
Noweder, Ahmad .....	82
Ntoukakis, Vardis .....	28
Nusinow A, Dmitri .....	54
Nuthikattu, Saivageethi .....	486

**O**

O'Connor L, Devin.....	59
Ogasawara, KIMI .....	252
Ogawa, Ken'ichi.....	612
Oh E, Saehong .....	142
Oh, Inez .....	581
Oh, Man-ho .....	28

Oh, Mijin .....	400
Ohama, Naohiko .....	143
Ohme-Takagi, Masaru.....	431, 564, 565, 570
Ohno, Carolyn .....	447
Ohto, Chikara .....	565, 612
Ohtsubo, Norihiro .....	570
Ohyama, Kiyoshi .....	343
Oikawa, Akira .....	110
Okada, Kiyotaka .....	409
Okrent, Rachel .....	268
Olinares B, Paul Dominic .....	180
Oliva, Michele .....	523
Olsen E, Jorunn .....	161
O'Malley, Ronan .....	487
Omidbakhshfard, Mohammad Amin ..	571
Omranian, Nooshin .....	208
Ondzighi, Christine .....	349
Ono, Hirofumi .....	303
Oppenheimer G, David .....	280
Oppermann, Yasmin .....	192
Orellana, Ariel .....	219, 316
Orlowski, Sara .....	309
Osborne, Edward .....	22, 375
Otegui S, Marisa .....	13, 326, 332
Otegui, Marisa .....	349
Ouyang, Xinhao .....	513
Owen, Heather .....	414

**P**

Pacifci, Elena .....	38, 445
Padmanabhan S, Meenu .....	144
Pahari, Shankar .....	399
Pak, Jung-Hun .....	145
Palatnik F, Javier .....	490
Pallakies, Helge .....	449
Palme, Klaus .....	386, 387, 613
Panda, Satchidananda .....	50
Panoli, Aneesh .....	450
Panstruga, Ralph .....	239, 244
Paoli DE, Emanuele .....	499
Paponov, Ivan .....	386
Parcy, Francois .....	88
Parent, Jean-Sébastien .....	488
Park J, Esther .....	383
Park, Jin .....	121
Park, Jong-Yoon .....	400
Park, Meheea .....	146
Park, Soon-ju .....	57
Park, Sungjin .....	401
Park, Sunhee .....	499
Park, Youngju .....	253
Parker E, Jane .....	77, 88, 220, 233, 254, 269
Parsons T, Harriet .....	48
Paszkowski, Jerzy .....	479
Patel V, Rohan .....	209
Patel, Dhaval .....	395, 521
Patel, Ketan .....	120, 135
Patterson E, Sara .....	575
Patterson, Sara .....	340, 354
Peacock J, William .....	290, 477
Peaucelle, Alexis .....	36
Pecenkova, Tamara .....	329, 337
Peer, Natasha .....	192
Peine, Nora .....	77
Pelizzola, Mattia .....	33, 556
Pellegrini, Matteo .....	475
Pelletier, Julie .....	419, 433
Pelletier, Sandra .....	476
Peña-Castro, Julián .....	58
Peng, Jinying .....	205
Perault, Jean-Michel .....	102
Perez-Amador A, Miguel .....	35
Perez-Perez, Jose Manuel .....	451
Perez-Ruiz M, Juan .....	81
Perilli, Serena .....	38, 445, 451
Perry, Sharyn .....	466, 470
Peterson M, Kylee .....	429
Petricka, Jalean .....	127, 444
Petros, Robby .....	225
Petzold J, Christopher .....	351
Phinney S, Brett .....	50
Phinney, Brett .....	230
Picot, Emma .....	504
Pieck, Michael .....	185
Pillitteri J, Lynn .....	429
Pilot, Guillaume .....	194
Pineiro, Manuel .....	508
Ping, L .....	73
Pino Del Carpio, Dunia .....	545
Pinosa, Francesco .....	387
Piques, Maria .....	539
Piquot, Yves .....	552
Pizon, Joanna .....	323
Platt, Alex .....	131
Podany, Wendy .....	389
Poethig, R. Scott .....	468, 469
Poirier, Yves .....	166
Pollack, Shaul .....	368
Ponnu, Jathish .....	465
Poo, Cherise .....	624
Pope M, Brittany .....	402
Popescu, George .....	317
Popescu, Sorina .....	32, 317
Porto, Matthew .....	216
Posey, Garrett .....	363
Possenti, Marco .....	69
Pouzet, Cécile .....	222
Pratelli, Réjane .....	194
Provart J, Nicholas .....	95, 105, 209
Prunedá-Paz, Jose .....	51, 500
Prusinkiewicz, Przemyslaw ..	17, 452
Puelma, Tomas .....	94

Pyl, Eva-Theresa ..... 539

**Q**

Qi, Dong ..... 255  
 Qiao, Hong ..... 318, 556  
 Qin, Feng ..... 137  
 Qin, Genji ..... 355  
 Qin, Zhixiang ..... 390  
 Qingrun, Zhang ..... 534  
 Qu, Li-Jia ..... 355, 456, 572  
 Qu, Xiao-Qing ..... 278  
 Queitsch, Christine ..... 540, 551  
 Quellet, Mario ..... 345  
 Queval, Guillaume ..... 560  
 Quint, Marcel ..... 547, 590, 616

**R**

Ragni, Laura ..... 67  
 Raikhel, Natasha ..... 322  
 Raimondi, Patricia ..... 389  
 Raina, Ramesh ..... 256  
 Raines M, Tracy ..... 606  
 Ramos-Parra A., Perla ..... 460  
 Rangani, Gulab ..... 489  
 Ranty, Benoit ..... 240  
 Rashotte M, Aaron ..... 415  
 Rasmussen G, Carolyn ..... 319  
 Rast I, Madlen ..... 406  
 Rathjen, John ..... 28  
 Rautengarten, Carsten ..... 345, 351  
 Ravi, Maruthachalam ..... 30  
 Rawat, Reetika ..... 50  
 Reback A, Maxwell ..... 88  
 Rebecca, Brown ..... 157  
 Redding, Kevin ..... 179  
 Redditt, Thomas ..... 215, 257  
 Reddy, Vignyan ..... 21  
 Reece-Hoyes S, John ..... 199  
 Reece-Hoyes, John ..... 75  
 Reed W, Jason ..... 383  
 Reese C, John ..... 245  
 Reeves H, Paul ..... 383  
 Refahi, Yassin ..... 86  
 Reinhart, Brenda ..... 68, 397  
 Reinstädler, Anja ..... 239  
 Remblières, Céline ..... 263  
 Ren, Dongtao ..... 258  
 Ren, Fei ..... 330  
 Renault, Hugues ..... 113  
 Renna, Luciana ..... 320  
 Renou, Jean-Pierre ..... 476  
 Resch, Tatiana ..... 386  
 Resenchuk, Sergey ..... 365  
 Revers, Frederic ..... 548  
 Reyes C, Francisca ..... 13  
 Reymond, Philippe ..... 221, 260  
 Ribot, Cecile ..... 166

Rice, Judd ..... 475  
 Richardson, Colby ..... 321  
 Richardson, Steven ..... 104  
 Rickert, Joshua ..... 224  
 Ries, Amber ..... 115  
 Riha, Karel ..... 331  
 Risinger R, Jan ..... 132, 546  
 Ristova, Daniela ..... 147  
 Rivas, Susana ..... 222  
 Rivera Serrano E, Efraín ..... 322  
 Rivero, Luz ..... 356, 358, 363  
 Rizo-Rey, Jose ..... 608  
 Rizza, Annalisa ..... 522  
 Robaglia, Christophe ..... 116, 169  
 Robatzek, Silke ..... 246  
 Robert, Nadia ..... 77  
 Roberts M, Daniel ..... 134, 182  
 Roberts R, Mike ..... 559  
 Roberts, April ..... 120, 135  
 Roberts, Ianto ..... 422  
 Robert-Seilaniantz, Alexandre ..... 217  
 Robinson, Sarah ..... 452  
 Robledo-Hernández L., Ana ..... 460  
 Robles M, Linda ..... 186  
 Roby, Dominique ..... 222, 535, 552  
 Rocha, Pedro SCF ..... 195  
 Rodermel, Steve ..... 378  
 Rodibaugh, Natalie ..... 215, 257  
 Rodrigo-Peiris, Thushani ..... 274  
 Rodriguez E, Ramiro ..... 490  
 Rodriguez R, Verenice ..... 180  
 Rodríguez-Franco, Marta ..... 522  
 Rodríguez-Welsh F, María ..... 293, 322  
 Roeder, Adrienne ..... 453  
 Roesel, John ..... 408  
 Rogers E, Elizabeth ..... 627  
 Roguev, Assen ..... 472  
 Rojas-Pierce, Marcela ..... 293, 322  
 Rokhsar, Daniel ..... 487  
 Ronneberger, Olaf ..... 387  
 Roodbarkelari, Farshad ..... 331  
 Roque, Ligaya ..... 188  
 Rosas, Ulises ..... 147  
 Roschzttardtz, Hannetz ..... 170, 349  
 Rose, Annkatrin ..... 296, 321  
 Rosen D, Benjamin ..... 499  
 Rothstein J, Steven ..... 125  
 Rott, Matthias ..... 7  
 Rousseau, Frederic ..... 555  
 Roux J, Stanley ..... 279  
 Roux, Fabrice ..... 534, 535, 537, 548,  
     552  
 Roux, Milena ..... 28  
 Roy, Bijoyita ..... 18, 376  
 Ruberti, Ida ..... 69  
 Rubin, MJ ..... 73  
 Rugnone L, Matias ..... 524  
 Runions, Adam ..... 17

Runkel, Jeff ..... 389  
 Ruperti, Benedetto ..... 386  
 Rus-Alvarez, Ana ..... 475  
 Russell, Andrew ..... 215, 257  
 Russinova, Eugenia ..... 323, 555  
 Ruzza, Valentino ..... 69  
 Ryu, Choong-Min ..... 265  
 Rätsch, Gunnar ..... 22, 375

**S**

Sabatini, Sabrina ..... 38, 432, 445  
 Sack D, Fred ..... 403  
 Saedler, Heinz ..... 366  
 Safavian, Darya ..... 324  
 Saito, Kazuki ..... 110  
 Sajja, Uday ..... 299  
 Sakai, Tatsuya ..... 12, 398  
 Sakuragi, Yumiko ..... 350  
 Salemi R, Michelle ..... 50  
 Salinas, Julio ..... 148  
 Salinas, Paula ..... 67, 214  
 Salomé A, Patrice ..... 523  
 Salomon, Susanne ..... 246  
 Salt E, David ..... 109  
 Salzman A, Ron ..... 149  
 Samuels A, Lacey ..... 339  
 Sanchez E, Sabrina ..... 524  
 Sanchez, Pablo ..... 427  
 Sandberg, Göran ..... 435  
 Sandhu, Sumeet ..... 389  
 Sang, Yi ..... 88  
 Santi, Simonetta ..... 454  
 Santoro, Nick ..... 354  
 Santrucek, Jiri ..... 126  
 Santuari, Luca ..... 67  
 Sarikaya, Mehmet ..... 96  
 Sarioglu, Hakan ..... 220  
 Sarkar, Purbasha ..... 352  
 Sasaki, Shu ..... 404  
 Sasaki, Takayuki ..... 136  
 Sasha E, Dennis ..... 201  
 Sassi, Massimiliano ..... 69  
 Sato, Atsuko ..... 404  
 Sato, Masanao ..... 61  
 Sauer, Markus ..... 99  
 Sauer, Norbert ..... 90, 462, 467  
 Sauter, Margret ..... 192  
 Scacchi, Emanuele ..... 67  
 Schaeffner R, Tony ..... 405  
 Schafermeyer R, Kim ..... 511  
 Schaller, G. Eric ..... 213, 597, 607, 620  
 Scheller V, Henrik ..... 345, 350, 351  
 Scherer F., Günther ..... 163, 593  
 Scheres, Ben ..... 423  
 Schiefelbein, John ..... 438  
 Schlappi, Michael ..... 150  
 Schlereth, Armin ..... 187, 465

Schläppi, Klaus .....	7	Shaw L, Sidney .....	310
Schmid, Marcus .....	63	Shaw, McKenzie .....	597
Schmid, Markus .....	465	Sheard B, Laura .....	608
Schmidt, Stefanie .....	151	Sheard, Laura .....	42
Schmidt, Wolfgang .....	454	Shearer, Heather .....	249
Schmit, Anne-Catherine .....	331	Sheela Jali, Sathya .....	299
Schmitt, Johanna .....	71	Shen, Hui .....	53
Schmitz J, Robert .....	33, 514, 556	Shen, Zhouxin .....	31, 267, 284
Schmitz, Robert .....	487	Sheres, Ben .....	39
Schnittger, Arp .....	331	Sherrier, Janine .....	499
Scholl, Randy .....	356, 358, 363	Shi, Chun-Lin .....	575
Schreiber, Andreas .....	347	Shi, Huazhong .....	493
Schreiber, Lukas .....	355	Shi, Jiangli .....	530
Schrück, Kathrin .....	188	Shi, Jiao .....	456
Schroeder F, Dana .....	118	Shi, Leilei .....	285
Schroeder I, Julian .....	77, 115	Shi, Zhenying .....	234
Schubert, Daniel .....	436	Shih, Han-Wei .....	325
Schuettelpelz, Mark .....	99	Shim, Donghwan .....	303
Schultheiss, Sebastian .....	375	Shimada, Hiroaki .....	165
Schultz A, Elizabeth .....	399, 425, 455	Shin, Jinwoo .....	496
Schultz D, Matthew .....	33	Shinoda, Shoko .....	110
Schultz F, Thomas .....	54	Shinozawa, Hidefumi .....	609
Schultz-Larsen, Torsten .....	217, 259	Shinozaki, Kazuo ..	78, 117, 137, 140, 143, 570
Schulze, Waltraud .....	539	Shishova, Maria .....	593
Schulze-Lefert, Paul .....	7	Shiu, Shin-Han .....	19, 138, 369, 370, 542
Schumacher, Karin .....	323	Shkolnik-Inbar, Doron .....	380
Schumaker S, Karen .....	113, 128	Siddiqi, Imran .....	30
Schumaker, Karen .....	139	Siddiqui, Hamad .....	513, 526
Schumann, Nadine .....	547	Sieberer, Tobias .....	427
Schurdi-Levraud, Valerie .....	548	Sijacic, Paja .....	457
Schwab, Wilfried .....	269	Silvestro, Daniele .....	350
Schwartz, Jacob .....	50	Simon, Rüdiger .....	98, 406, 449, 545
Schwarz, Martina .....	427	Simpson G, Craig .....	524
Schwechheimer, Claus .....	368	Simpson, Craig .....	99
Schweizer, Fabian .....	260	Singh, Vijay .....	245
Schwessinger, Benjamin .....	28	Sinha, Neelima .....	417
Schymkowitz, Joost .....	555	Siriwardana L, Chamindika ..	132, 153
Schäffner R, Anton .....	114	Siriwardana S, Nirodhini .....	458
Seabolt, Savanna .....	121	Sisa, Miroslav .....	323
Sebastian, Jose .....	66, 394	Skibbe, Henrik .....	387
Sedbrook, John .....	349	Skirycz, Aleksandra .....	110
Seddon E, Alexander .....	19	Sklenar, Jan .....	28
Seeliger, Ingo .....	420	Skylar, Anna .....	333, 407
Segonzac, Cecile .....	28	Slotkin, R. Keith .....	486
Seguel, Aldo .....	214	Smidler, Andrea .....	111
Séguéla, Mathilde .....	170	Smith C, Halie .....	536
Segura, Vincent .....	131	Smith G, Laurie .....	319
Sehr, Eva Maria .....	427	Smith-White, Brian .....	365
Seidel, Claus A. M. ....	98	Snyder, Michael .....	216
Seki, Motoaki .....	100	Sohrabi, Reza .....	189
Seltmann A, Martin .....	106	Solheim, Cory .....	563
Sena, Giovanni .....	374	Solórzano-Lowell, Kathryn .....	235
Serino, Giovanna .....	432	Somerville C, Shauna .....	278
Serrano, Irene .....	152	Song, Chun-Peng .....	154
Shah, Bhavank .....	142	Song, Junqi .....	261, 270
Shah, Jyoti .....	225, 245	Song, Sang-Kee .....	438
Sharma, Nidhi .....	525		
Sharrock A, Robert .....	507		
		Song, Zhihong .....	49
		Soppe J, Wim .....	463
		Sorenson, Reed .....	101
		Soto Burgos, Junmarie .....	15
		Soto, Alvaro .....	94
		Sozzani, Rosangela .....	444, 459
		Spalding P, Edgar .....	198, 332, 543, 615, 619
		Spalding, Edgar .....	373
		Sparks, J. Alan .....	273
		Spartz K, Angela .....	395
		Spector L., David .....	285
		Spensley, Mark .....	504
		Spitzer, Christoph .....	326
		Spivey, Natalie .....	271
		Spooner, William .....	91
		Sreedharan T, Vipin .....	22
		Sreedharan, Vipin .....	375
		Sreekanta, Suma .....	264
		Srivastava C, Avinash .....	460
		Srivastava, Renu .....	125
		Srivastava, Vibha .....	489
		Stacey, Gary .....	499
		Stadler, Ruth .....	90, 462
		Stafstrom, Joel .....	155
		Stahelin V, Robert .....	339
		Stahl, Yvonne .....	98
		Staiger J, Christopher .....	301, 327
		Staiger, Dorothee .....	99
		Staudinger, Christana .....	162
		Steber M, Camille .....	604
		Stefano, Giovanni .....	328
		Steffen, Joshua .....	22, 375
		Stegle, Oliver .....	22, 375
		Stein C, Joshua .....	91
		Steinwand J, Blaire .....	353
		Steinwand, Blaire .....	574
		Stekelenburg, Tom .....	426
		Stekhoven, Daniel .....	93
		Stepanova N, Anna ..	27, 35, 186, 241
		Stewart L, Jodi .....	52
		Stingl E, Nadja .....	106
		Stiti, Naim .....	156
		Stitt, Mark .....	29, 187, 465, 539, 617
		Stokes E, Michael .....	610
		Stoller H, Jerry .....	149
		Stone L, Sophia .....	443
		Streitner, Corinna .....	99
		Strnad, Miroslav .....	323
		Strohm K, Allison .....	611
		Stuttmann, Johannes .....	254
		Styranko, Danielle .....	455
		Su, Shih-Heng .....	210
		Su, Tianying .....	319
		Su, Wei .....	308
		Sudkamp, Mitchell .....	34
		Suer, Stefanie .....	427
		Sugimoto, Hiroki .....	612

- Sugimoto, Keiko ..... 275, 431  
 Sulpice, Ronan ..... 539  
 Sumner W., Lloyd ..... 460  
 Sun H, Brian ..... 319  
 Sun, Bo ..... 492, 495  
 Sun, Jiaqiang ..... 613, 621  
 Sun, Kelian ..... 19, 138, 542  
 Sun, Tai-ping ..... 622  
 Sun, Tian-Hu ..... 190  
 Sun, Yuefeng ..... 569  
 Sundaresan, Venkatesan ..... 439  
 Sundaresan, Venkatesan ..... 450  
 Sundberg, Eva ..... 381  
 Sunde R, Gavin ..... 527  
 Sung, Frances ..... 333  
 Sussman R, Michael ..... 175  
 Suttangkakul, Anongpat ..... 307  
 Svedin, Elisabeth ..... 549  
 Swanson J, Robert ..... 550  
 Swarbreck, David ..... 371  
 Swarup, Ranjan ..... 286  
 Sweeney, Colleen ..... 276  
 Syed H, Naeem ..... 55  
 Synek, Lukas ..... 329, 337  
 Szatmári, Anna-Mária ..... 323  
 Szecowka, Marek ..... 29  
 Szumlanski, Amy ..... 401  
 Szymanski, Dan ..... 408  
 Szymanski1 B, Daniel ..... 339
- 
- T**
- Takada, Shinobu ..... 570  
 Takahashi, Nozomu ..... 50  
 Takahashi, Taku ..... 12, 392, 398  
 Takahashi, Yoshihiro ..... 262  
 Takahashi, Yuichiro ..... 12, 398  
 Takeda, Atsushi ..... 484  
 Takemoto J, Larry ..... 225  
 Takiguchi, Yuko ..... 570  
 Talbot, Mark ..... 290  
 Talloji, Prabhavathi ..... 191  
 Tamada, Yosuke ..... 497  
 Tamerler, Candan ..... 96  
 Tameshige, Toshiaki ..... 409  
 Tan J, Li Hui ..... 482  
 Tan, Xu ..... 42, 608  
 Tanaka, Tomoko ..... 565, 612  
 Tang, Yuhong ..... 460  
 Tarr T, Paul ..... 447  
 Tasaka, Masao ..... 413, 566  
 Tateda, Chika ..... 262  
 Tatemashu, Kiyoshi ..... 409  
 Tatusova, Tatiana ..... 365  
 Tavernini C, LeeAnna ..... 455  
 Taylor, Jennifer ..... 477  
 Taylor-Teebles, Mallorie ..... 75, 199, 211  
 Teaster D, Neal ..... 614  
 Tegowski R, Matthew ..... 458  
 Teng, Yibo ..... 513  
 Terpstra, Inez ..... 38, 445  
 Tershakovec, Tamara ..... 201  
 Tharmapalan, Pirashaanthy ..... 591  
 Thatcher R, Shawn ..... 157  
 Tholl, Dorothea ..... 189  
 Thomashow F, Michael ..... 19, 80  
 Thomason, Jim ..... 91  
 Tietz, Olaf ..... 613  
 To PC, Jennifer ..... 213, 620  
 Tobin, Elaine ..... 500  
 Tolstoy, Igor ..... 365  
 Tong, Wurina ..... 392  
 Toomajian, Chris ..... 22  
 Toomajian, Chris ..... 375  
 Torii U, Keiko ..... 96, 429  
 Tornqvist, Carl-Erik ..... 354  
 Torre, Sissel ..... 161  
 Toupalova, Hana ..... 329  
 Touraev, Alisher ..... 386  
 Toyokura, Koichi ..... 409  
 Toyota, Masatsugu ..... 566  
 Trémousaygue, Dominique ..... 263  
 Truman, William ..... 264  
 Traas, Jan ..... 86  
 Tsai YL, Allen ..... 410  
 Tsao, Tiffany ..... 250  
 Tsay, Yi-Fang ..... 177  
 Tschoep, Hendrik ..... 187  
 Tsiantis, Miltos ..... 17  
 Tsuda, Kenichi ..... 61, 264  
 Tsugeki, Ryuji ..... 409  
 Tsukagoshi, Hironaka ..... 127  
 Turchi, Luana ..... 69  
 Tyerman D, Stephen ..... 347  
 Tyler M, Brett ..... 212  
 Tyler, Brett ..... 248  
 Tör, Mahmut ..... 158
- 
- U**
- Ubeda-Tomás, Susana ..... 576  
 Uehlken, Christine ..... 462  
 Uhlířová, Radka ..... 382  
 Ullrich, Kristian ..... 547  
 Umulis, David ..... 408  
 Underwood, William ..... 278  
 Undurraga F, Soledad ..... 551  
 Uppalapati R, Srinivasa ..... 265  
 Urich, Mark ..... 33, 556  
 Urquhart, William ..... 107  
 Usadel, Bjoern ..... 351
- 
- V**
- Vaillant, Isabelle ..... 479  
 Valentova, Olga ..... 126
- 
- Van Breusegem, Frank ..... 111, 112, 573  
 Van Buskirk K, Elise ..... 528  
 Van Damme, Daniël ..... 323  
 Van de Peer, Yves ..... 112  
 Van den Ackerveken, Guido ..... 159, 247, 541  
 Van den Begin, Jos ..... 323  
 Van den Burg A, Harrold ..... 266  
 Van Houtte, Isabelle ..... 555  
 Van Leene, Jelle ..... 60  
 Van Norman M, Jaimie ..... 459  
 Van Parys, Thomas ..... 112  
 van Schie C, Chris ..... 267  
 van Schie, Chris ..... 31  
 van Wijk J, Klaas ..... 180  
 van Zanten, Martijn ..... 463  
 Vanderhaeghen, Rudy ..... 560  
 Varala, Kranthi ..... 372  
 Vatsan R, Anjana ..... 365  
 Vaughn N, Justin ..... 18, 376  
 Vazquez, Adeline ..... 534  
 Veerappan, Vijaykumar ..... 493  
 Venables J, Barney ..... 225  
 ver Loren Van Themaat, Emiel ..... 7  
 Vercammen, Dominique ..... 111  
 Verdonk, Julian ..... 349  
 Vermeersch, Leen ..... 560  
 Vermeirssen, Vanessa ..... 112  
 Vernoux, Teva ..... 86  
 Verslues E, Paul ..... 160  
 Vicentini, Renato ..... 476, 583  
 Vidal, Marc ..... 20  
 Vielhoefer, Prisca ..... 366  
 Vierstra D, Richard ..... 307, 369, 370, 502, 527, 531  
 Vilarrasa Blasi, Josep ..... 323  
 Vilhjalmsson J, Bjarni ..... 131  
 Villar, Corina ..... 163  
 Villiers, Florent ..... 300  
 Villoutreix, Romain ..... 537, 552  
 Vincent, Coral ..... 553  
 Vincenz, Michel ..... 476, 583  
 Vincill D, Eric ..... 615  
 Vlot, A. Corina ..... 220, 269  
 Vogel, John ..... 59  
 Voiniciuc, Catalin ..... 46  
 Vollmers, Christopher ..... 50  
 Volny, Matthew ..... 464  
 von Arnim G, Albrecht ..... 18, 376, 510  
 Vora, Hiral ..... 120, 207  
 Vosloh, Daniel ..... 29
- 
- W**
- Waduwara-Jayabahu, Ishari ..... 192  
 Wagner, Doris ..... 88  
 Wahl, Vanessa ..... 187, 465  
 Walhout JM, Albertha ..... 199

Walhout, A. J. Marian .....	75
Walker C, John .....	605
Wallace S, Ian .....	182
Walsh, Sean .....	92, 93
Walton, Jonathan .....	354
Wan, Angus .....	178
Wan, Jianmin .....	513
Wan, Xiang-yuan .....	513
Wang, Chieh-Ting .....	411
Wang, Fangfang .....	466
Wang, Guan-Feng .....	121
Wang, Guoying .....	530
Wang, Haiyang .....	513, 526
Wang, He .....	528
Wang, Jing .....	285, 493
Wang, Li .....	628
Wang, Lin .....	264
Wang, Pengtao .....	154
Wang, Renhou .....	553
Wang, Rui-Sheng .....	586
Wang, Shui .....	261
Wang, Wei .....	270
Wang, Xia .....	301
Wang, Xue-Chen .....	330
Wang, Xuemin .....	225
Wang, Yao .....	142, 414
Wang, Ying .....	529
Wang, Yingxiang .....	442
Wang, YiZhong .....	495
Wang, Yonghong .....	569
Wangdi, Tamding .....	265
Ware, Doreen .....	75, 91, 199
Wasaki, Jun .....	136
Washington, Isiah .....	389
Waßmann, Friedrich .....	355
Watahiki, Masaaki .....	404
Watanabe, Kanako .....	262
Watanabe, Keiro .....	409
Watanabe, Yuichiro .....	165, 484
Webb, Candace .....	500
Weckwerth, Wolfram .....	162
Weemen, Mieke .....	430
Wei, Hairong .....	377
Wei, Lirong .....	621
Wei, Peng-Cheng .....	330
Wei, Sharon .....	91
Weigel, Detlef .....	523, 528
Weijers, Dolf .....	85
Weimer, Annika .....	331
Weingartner, Magdalena .....	467
Weisshaar, Bernd .....	366
Weller, Benjamin .....	467
Wendell, Micael .....	161
Wenger P, Jonathan .....	616
Wenig, Ulrich .....	90
Werr, Wolfgang .....	420
Western L, Tamara .....	344, 346, 348
Whelan, James .....	563

**X**

Xia, Xinjie .....	195
Xia, Yang .....	308, 334
Xiang, Qu .....	447
Xiangchao, Gan .....	375
Xie, Zhixin .....	414
Xing, Denghui .....	412
Xu, Dan .....	150
Xu, Jian .....	621
Xu, Jie .....	555
Xu, Qing .....	522
Xu, Yifeng .....	495
Xu, Yingxiu .....	613
Xu, Zi-Qin .....	150
Xue, Hongwei .....	193
<b>Y</b>	
Yadav PR, Umesh .....	617
Yadav, Gitanjali .....	188
Yadegari, Ramin .....	139
Yakir, Esther .....	506
Yamaguchi, Ayako .....	88
Yamaguchi, Nobutoshi .....	88
Yamaguchi, Shinjiro .....	604
Yamaguchi-Shinozaki, Kazuko .....	78, 117, 137, 140, 143
Yamamoto, Kotaro .....	404
Yamaoka, Yasuyo .....	303, 335
Yan, Jingzhou .....	390
Yan, Shunping .....	261, 270
Yan, Xiufeng .....	197
Yan, Zhe .....	499
Yang Chum, Pak .....	562
Yang, Xiao-Yuan .....	171
Yang, Donglei .....	234
Yang, Hongxing .....	442
Yang, Li .....	469
Yang, Wannian .....	481
Yang, Xiaohui .....	336
Yanovsky J, Marcelo .....	524
Yasuda, Naomi .....	137
Ye, Huaxun .....	378
Ye, Songqing .....	395, 613
Yeung CT, Edward .....	419
Yin, Linlin .....	193
Yin, Yanhai .....	378
Ying, Emily .....	477
Yonehara, Ryo .....	413
Yonekura, Madoka .....	565
Yoo, Cheolmin .....	273
Yoo, Heejin .....	184
Yoo, Sang-Dong .....	110
Yoshida, Takumi .....	143
Yoshihara, Takeshi .....	619
Yoshimoto, Kaori .....	12, 392, 398
Yoshioka, Keiko .....	107
Yoshizumi, Takeshi .....	165, 570
Youens-Clark, Ken .....	91
Yu, Caihong .....	355
Yu, Jihyeon .....	496
Yu, Peng .....	395
Yu, Shi .....	194
Yu, Yingjie .....	290
Yuan, Li .....	336
Yuan, Youxi .....	185
Yue, Kun .....	422
Yumul, Rae .....	3
Yun, Jae-Young .....	480, 497
Yun, Jeonga .....	27
Yuzuak, Seyit .....	498

<b>Z</b>	
Zamir, Dani .....	57
Zampogna, Giulio .....	32
Zarsky, Viktor.....	329, 337
Zayed A, Yara.....	338
Zeilmaker, Tieme .....	159, 247
Zeng, Weiqing .....	271
Zhai, Jixian.....	157, 499
Zhai, Qingzhe.....	621
Zhang, Chong.....	530
Zhang, Chunhua.....	339, 408
Zhang, Hongtao.....	423
Zhang, Jon.....	91
Zhang, Junrui .....	531
Zhang, Lifang.....	75, 199
Zhang, Lihui.....	258
Zhang, Qingrun.....	537
Zhang, Rujia.....	195
Zhang, Ting .....	258
Zhang, Tingting.....	532
Zhang, Wenjing.....	620
Zhang, Xiaoran .....	390
Zhang, Xiaoyu.....	628
Zhang, Xu .....	524
Zhang, Yuelin .....	6, 237
Zhao, Dazhong.....	414
Zhao, Lifang.....	43
Zhao, Rongmin .....	142
Zhao, Yunde .....	450
Zheng, Bo.....	435
Zheng, Jiameng.....	293
Zheng, Ning .....	42, 608
Zheng, Qi .....	494
Zheng, Qiaolin .....	470
Zheng, Xiaobin .....	49
Zheng, Xiao-yu .....	271
Zheng, Yumei .....	470
Zheng, zuyu.....	533
Zhou, Binbin .....	263
Zhou, Jing .....	66, 394, 471
Zhou, Rongrong .....	35
Zhou, Wenkun .....	613, 621
Zhou, Xin .....	622
Zhou, Xuefeng .....	34
Zhou, Yun.....	520
Zhu, Lihuang.....	621
Zhu, Ling.....	53
Zhu, Shan .....	231
Zhu, Zhaohai .....	237
Zimmerli, Celine .....	166
Zipfel, Cyril.....	28
Zola, Jaroslaw .....	378
Zöll, Christian .....	463
Zou, Cheng....	19, 138, 369, 370, 542
Zwack J, Paul .....	415

# ICAR 2011

## 22nd International Conference on Arabidopsis Research

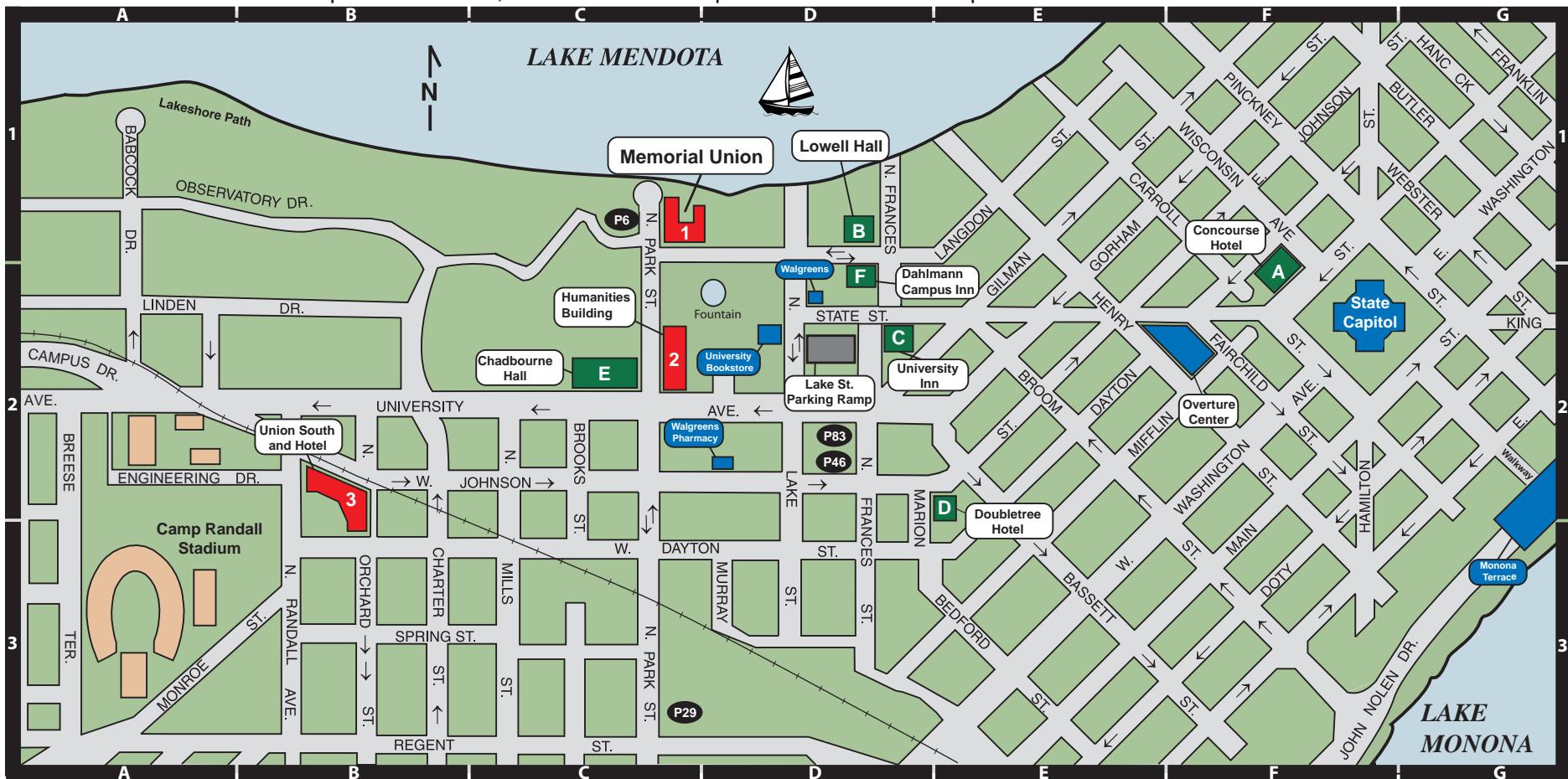
June 22– June 25, 2011



THE UNIVERSITY  
of  
**WISCONSIN**  
MADISON

### UNIVERSITY BUILDINGS, ACCOMMODATIONS, PARKING and POINTS OF INTEREST

Conference Site	Location	Accommodation	Location	Parking Lot	Location	Points of Interest	Location
1. Memorial Union	C1	A. Concourse Hotel	F2	Lot 6	C1	University Bookstore	D2
2 Humanities	C2	B. Lowell Hall	D2	Lot 29	C3	Walgreens	D2
3 Union South	B2	C. University Inn	D2	Lot 46	D2	Walgreens Pharmacy	D2
		D. Doubletree Hotel	D1	Lot 83	D2	Monona Terrace	G2
		E Chadbourne Hall	C2			Overture Center	F2
		F Dahlman Campus Inn	D1				





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# MEMORIAL UNION

MU Floor Legend		
	Floor	Room in Use
Fourth		
Third		
Second		
First		

## Arabidopsis Room Locations

## **Keynotes and Plenary Sessions**

- ① Union Theater (1st floor West)

## Concurrent Sessions

- ① Union Theater (1st floor West)  
→ 3650 Humanities (see campus map)

## **Poster Sessions**

- ② Tripp Commons (2nd floor East)
  - ③ Great Hall (4th floor)
  - ⑤ Main Lounge (2nd floor center)
  - ⑥ Capital View (4th floor)
  - ⑧ Langdon Room (4th floor)
  - ⑨ Reception Room (4th floor)

## Workshops

- ① Union Theater (1st floor West)
  - ⑦ Old Madison Room (3rd floor East)

## Exhibitors

- ③ Great Hall (4th floor)
  - ⑤ Main Lounge (2nd floor East)

## Meals

- ④ Inn Wisconsin (2nd floor East)**

Conference HQ

- ## A Annex Room (2nd floor center)

# ICAR

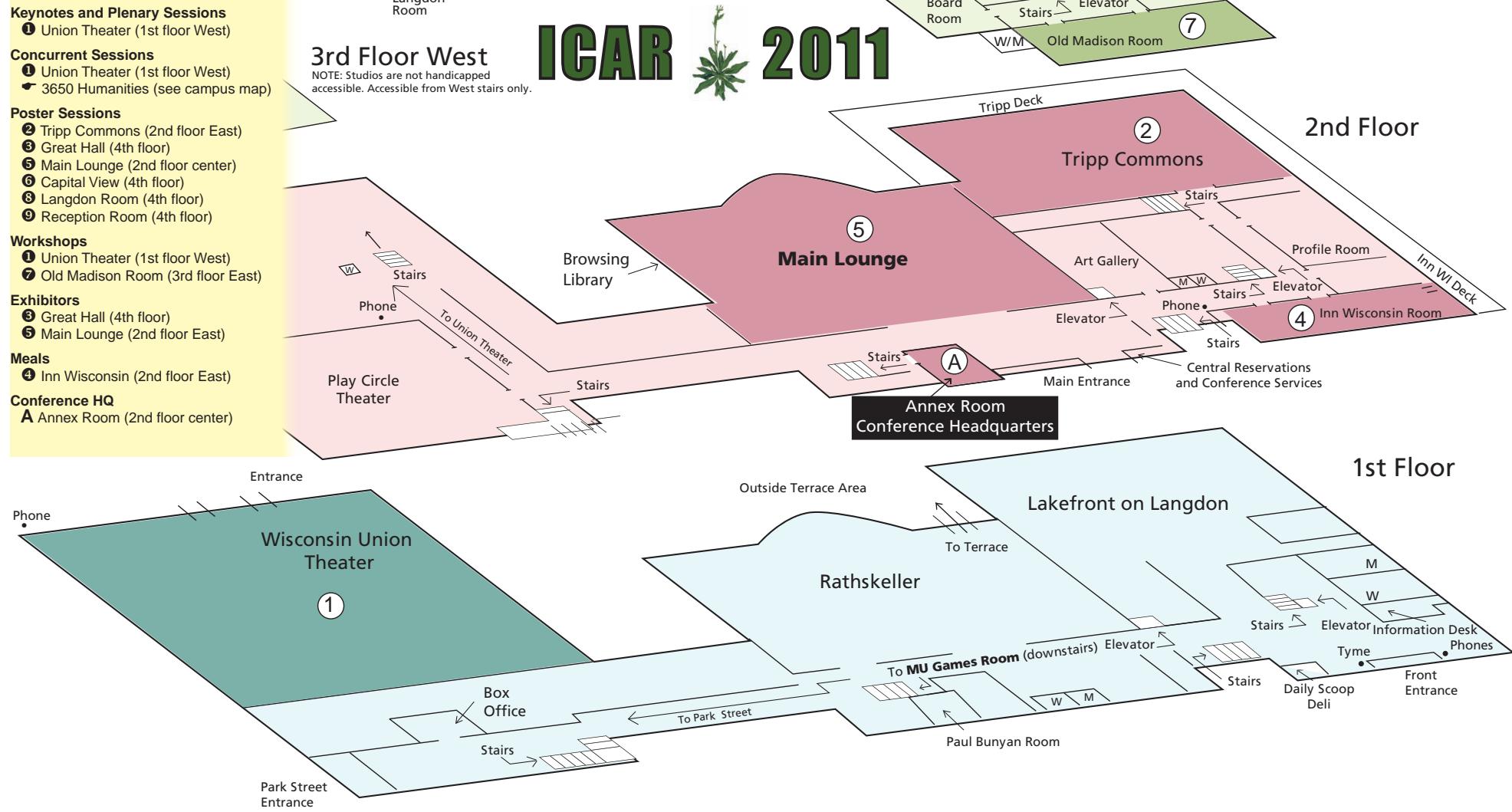
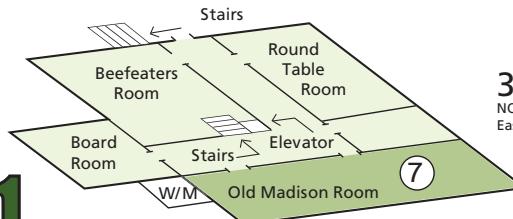
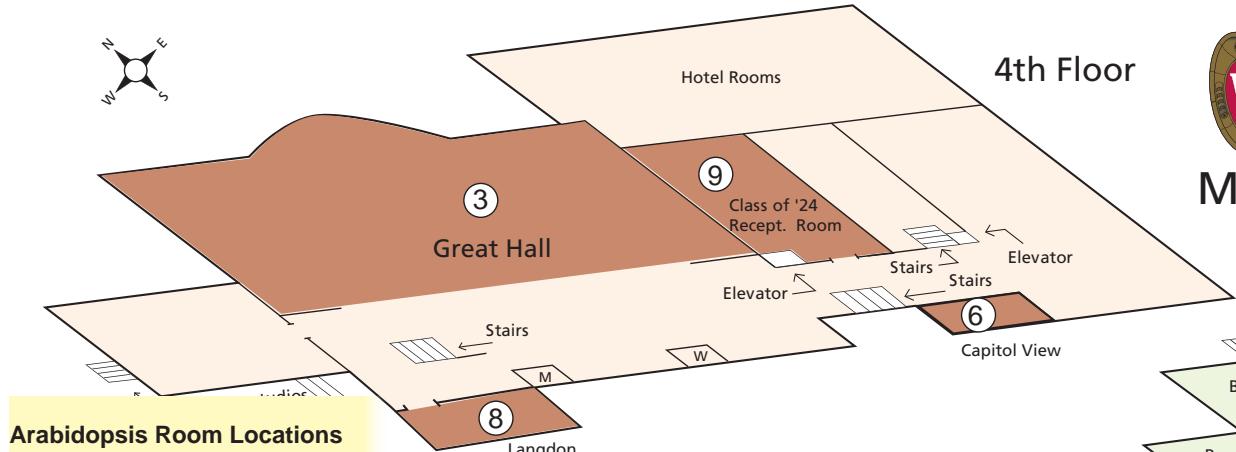


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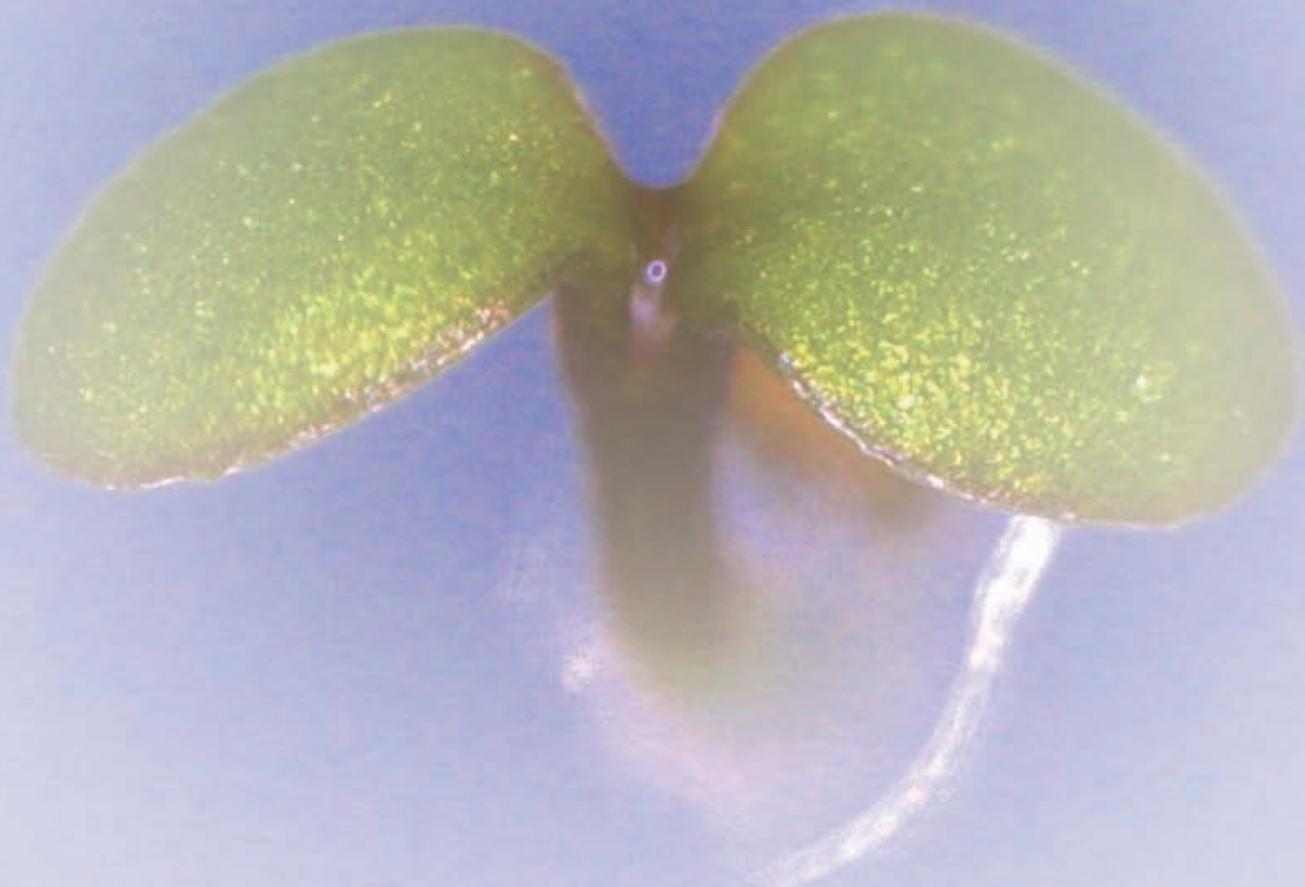


3rd Floor West

**NOTE:** Studios are not handicapped accessible. Accessible from West stairs only.



# Plant Resource for Our Future



**Arabidopsis mutants, full-length cDNA, T87 cell line**



**RIKEN BioResource Center**  
<http://www.brc.riken.jp/lab/epd/Eng/>

# 23<sup>rd</sup> International Conference on *Arabidopsis Research*

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3-7 July 2012: Hofburg Palace, Vienna, Austria



[www.icar2012.org](http://www.icar2012.org)

