

# 19th International Conference on Arabidopsis Research



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## Montreal, Canada

Hyatt Regency

July 23-27, 2008

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## **PROGRAM & ABSTRACTS**

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# **19<sup>th</sup> International Conference on ARABIDOPSIS RESEARCH**

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July 23 – July 27, 2008  
Hyatt Regency Hotel  
Montreal, Canada



## **Scientific Organizing Committee for the 19<sup>th</sup> International Conference on Arabidopsis Research**

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Scott Poethig ((University of Pennsylvania, Philadelphia, USA)<sup>2</sup>

Julian Schroeder (University of California, San Diego, USA)<sup>2</sup>

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<sup>1</sup> Coordinator of the Multinational Arabidopsis Steering Committee

<sup>2</sup> Member of the North American Arabidopsis Steering Committee

<sup>3</sup> Co-chair of the Multinational Arabidopsis Steering Committee

<sup>4</sup> Member of the local organizing committee

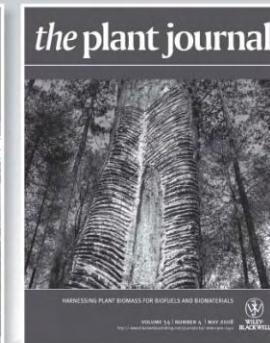
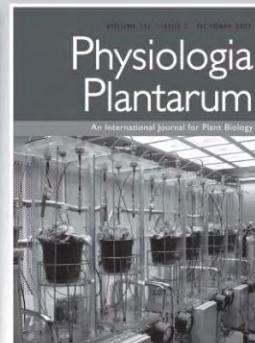
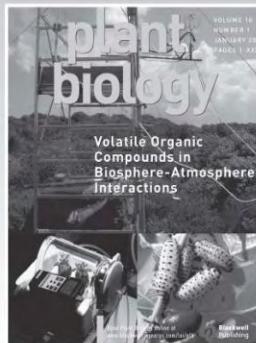
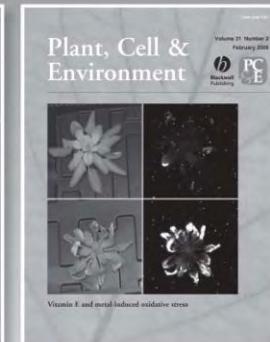
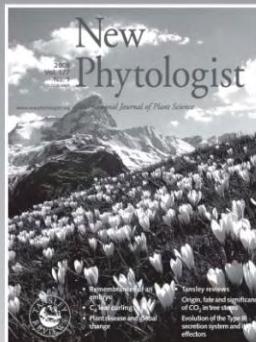
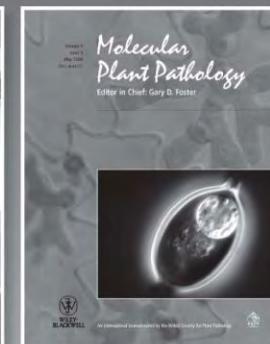
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Throughout this **program**, the numbers next to abstracts refer to abstract numbers, not the page number in the **abstract** part of this book.

## SESSION OVERVIEW

Wednesday, July 23, 2008 4:30 – 6:00 pm 6:30 – 7:30 pm 7:30 – 9:30 pm	Workshops 1 Keynote Lecture Opening Reception
Thursday, July 24, 2008 8:30 – 10:00 am 10:30 – 12:00 pm 1:30 – 3:00 pm 3:30 – 5:00 pm  7:00 – 8:30 pm 8:30 – 10:30 pm	Plenary I: Developmental Mechanisms Plenary II: Cell Biology Concurrent I: Developmental Mechanisms or Cell Walls Concurrent II: Evolution & Natural Variation or Signal Transduction Workshops 2 Poster Session I (Odd Numbered Abstracts Presented)
Friday, July 25, 2008 8:30 – 10:00 am 10:30 – 12:00 pm 1:30 – 7:00 pm 7:00 – 8:30 pm 8:30 – 10:30 pm	Plenary III: Metabolism Plenary IV: Interactions with the Environment Free Afternoon Workshops 3 Poster Session 2: (Even Numbered Abstracts Presented)
Saturday, July 26, 2008 8:30 – 10:00 am 10:30 – 12:00 pm 12:00 – 2:00 pm 2:00 – 3:30 pm  4:00 – 5:30 pm  7:00 pm	Plenary V: Genomics and Systems Biology Plenary VI: Genetic and Epigenetic Mechanisms Lunch with Exhibitors & Poster Session 3 (open session) Concurrent III: Biotic Interactions or Genetic & Epigenetic Mechanisms Concurrent IV: Abiotic Interactions or Evolution & Development Conference Banquet (please see page 246 for details on ordering tickets and transportation)
Sunday, July 27, 2008 8:30 – 10:00 am 10:30 am – 12:00 pm	Plenary VII: Signal Transduction Plenary VIII: Novel Tools and Techniques

# **CONFERENCE FUNDING & SUPPORT**

## **General Meeting Sponsors**



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# MEETING EXHIBITORS

## Platinum Exhibitor



Manufacturing & Developing of Growing Systems  
Yuval Importing – Booth 6 & 7

## Silver Exhibitor



Bio S&T – Booth 10

## Bronze Exhibitors



Araponics – Booth 11



Li-Cor – Booth 12



Quorum Technologies – Booth 4

## Other Exhibitors

American Society of Plant Biologists – Booth 14

Qubit Systems – Booth 9

BioChambers Inc – Booth 8

TAIR – Booth 1

Conviron – Booth 2

The iPLant Collaborative – Booth 15

MASC/Arabidopsis Meeting 2009 – Booth 5

Union Biometrika – Booth 3

NRC Research Press- Booth 16

Whatman – Booth 13



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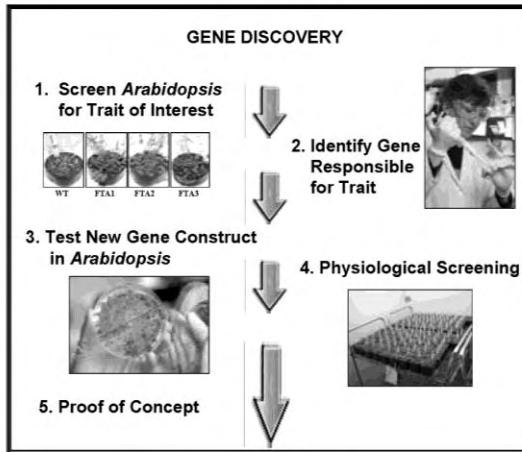
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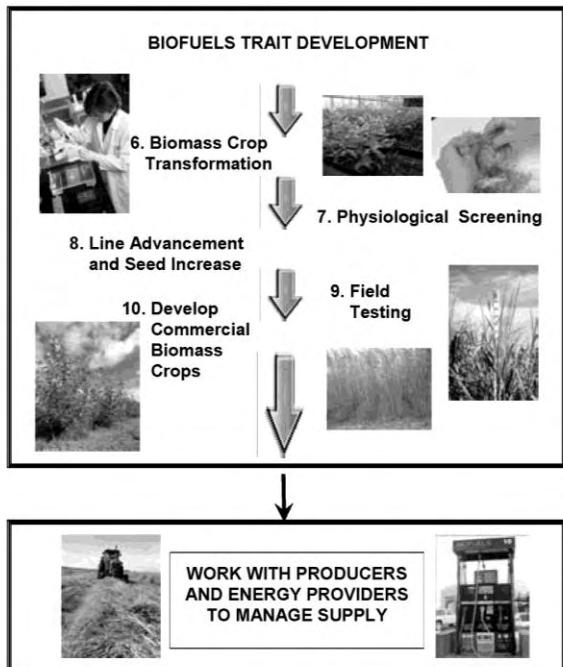
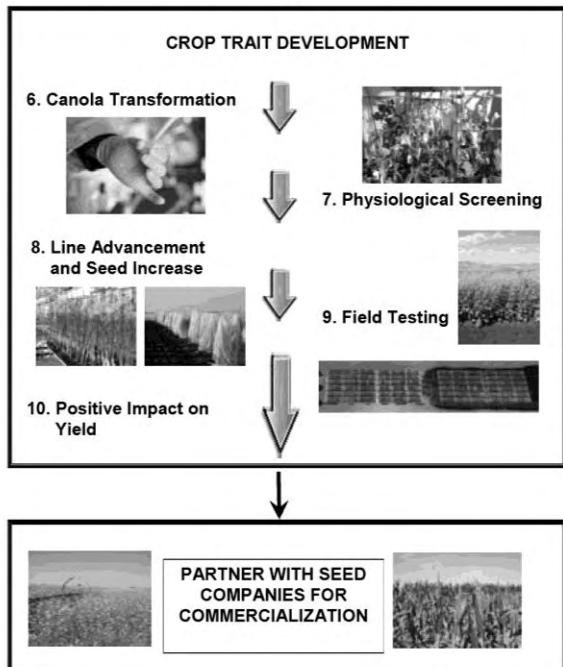
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# **PROGRAM OVERVIEW**

## ***Poster schedule***

All posters will remain up for the entire meeting and can be set up Wednesday evening beginning at 5:00 pm. 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 on page 57 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.

The Saturday lunch time **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.

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## **Wednesday, July 23, 2008**

12:00 – 7:00 pm	Registration Opens	Grand Salon Foyer
5:00 – 9:00 pm	Posters can be set up	Complexe Desjardins & Alfred Rouleau C
4:30 – 6:00 pm	Workshop Session 1A: Lead Organizer: Rodrigo A. Gutiérrez - <i>Frontiers in Plant Systems Biology</i>	Grand Salon
	Workshop Session 1B: Lead Organizer: Eva Huala - <i>Sources and strategies for gene structure, gene function, and metabolic pathway annotation at TAIR and AraCyc</i>	Alfred Rouleau A/B
6:30 – 7:30 pm	Keynote Address: Abstract K01: Chris Somerville, Energy Biosciences Institute and University of California, Berkeley, USA- <i>The Development of Cellulosic Biofuels</i> <i>This keynote address is sponsored by ASPB journals: Plant Physiology &amp; Plant Cell</i>	Grand Salon
7:30 – 9:30 pm	Welcome Reception	Jeanne-Mance & Terrasse

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## **Thursday, July 24, 2008**

7:30 am – 8:00 pm	Posters may be posted	Complexe Desjardins & Rouleau C
7:30 am – 8:00 pm	Registration Open	Grand Salon Foyer
8:30 – 10:00 am	Plenary Session I: <i>Developmental Mechanisms</i> 8:30 Abstract P101: Scott Poethig, <i>Session Chair, University of Pennsylvania – Philadelphia, USA – A Mirna-Regulated pathway for Vegetative Phase Change in Arabidopsis</i>	Grand Salon
	9:10 Abstract P102: Vivian Irish, Yale University, USA- <i>Regulation of Petal Organogenesis</i>	
	9:35 Abstract P103: Gerd Jürgens, University of Tübingen, Germany - <i>Axis Formation in Early Embryogenesis</i> <i>This session is sponsored by DBRI at McGill University and The Company of Biologists/Development Journal</i>	
10:00 – 10:30 am	Refreshment Break	Grand Salon Foyer
10:30 am – Noon	Plenary Session II: Cell Biology 10:30 Abstract P201: Natasha Raikhel, Session Chair, University of California-Riverside, USA- <i>Plant Endomembrane System and Chemical Genomics</i>	Grand Salon
	11:10 Abstract P202: Malcolm Bennett, University of Nottingham, UK – <i>Lateral Root Development: An Emerging Story</i>	
	11:35 Abstract P203: Jianping Hu, Michigan State University-East Lansing, USA – <i>Molecular Basis of Plant Peroxisomal Proliferation and Distribution</i> <i>This session is being sponsored by the Faculty of Science at McGill University</i>	
12:00 – 1:30 pm	Lunch	Jeanne-Mance Pavilion & Terrasse
1:30 – 3:00 pm	Concurrent Session 1A: <i>Developmental Mechanisms</i> 1:30 Abstract C1A01: Doris Wagner, Session Chair, University of Pennsylvania-Philadelphia, USA- <i>Regulation of the Onset of Reproduction in Arabidopsis</i>	Grand Salon
	1:55 Abstract C1A02: Yuval Eshed, Weizmann Institute, Israel - <i>Shaping the Arabidopsis Shoot By Short Range YABBY-Derived Signals</i>	
	2:20 Abstract C1A03: Fabio Fornara, Max Planck Institute for Plant Research, Cologne, Germany– <i>A High-Throughput Misexpression Screen Identifies Novel Genes Regulating Flowering From the Leaf</i>	
	2:33 Abstract C1A04: Giovanni Sena, New York University, USA - <i>A Functional Stem Cell Niche Is Not Required in Arabidopsis Root Tip</i>	

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*Regeneration*

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2:46 Abstract C1A05: Beth Krizek, University of South Carolina – Columbia, USA – *AIL/PLT Proteins Regulate Floral Patterning and Shoot Development in Arabidopsis Thaliana*

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Concurrent Session 1B: *Cell Walls* Alfred Rouleau A/B  
1:30 Abstract C1B01: Tobias Baskin, Session Chair, University of Massachusetts-Amherst, USA – *Twisted Roots: Growth Patterns and Cell Wall Structure*

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1:55 Abstract C1B02: Zheng-hua Ye, University of Georgia-Atlanta, USA – *Transcriptional Regulation of Secondary Wall Biosynthesis*

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2:20 Abstract C1B03: Gerasimos Daras, Agricultural University of Athens, Greece – *Semidominant-Negative CES A3 Mutant in Arabidopsis Inhibits Primary Cell Wall Formation*

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2:40 Abstract C1B04: Sebastian Wolf, Institute for Plant Science in Neuenheimer, Germany – *Processing of Type I Pectin Methyl esterase in the Golgi Apparatus: Prerequisite for Extracellular Targeting*  
This session is being sponsored by Dow AgroSciences

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3:00 – 3:30 pm Refreshment Break Grand Salon Foyer

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3:30 – 5:00 pm Concurrent Session 2A: *Evolution and Natural Variation* Alfred Rouleau A/B  
3:30 Abstract C2A01: Kirsten Bomblies, Session Chair, Max Planck Institute-Tübingen, Germany – *Opportunity of Misfortune – Outcrossing in Arabidopsis Thaliana*

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3:55 Abstract C2A02: Miltos Tsiantis, University of Oxford, UK – *EMBO sponsored Young Investigator Lecture – Cardamine Hirsuta as a Model System for Studies in Evolutionion of Plant Development*

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4:20 Abstract C2A03: Jesse Hollister, University of California-Irving, USA – *Population Genomics and Epigenomics of Arabidopsis Thaliana Transposable Elements*

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4:33 Abstract C2A04: Amity Wilczek, Brown University, Providence, Rhode Island, USA – *Seasonal and Geographic Variation in Sensitivity and Balance of Flowering Pathways*

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4:46 Abstract C2A05: Keith Adams, University of British Columbia, Vancouver, Canada – *Rapid Evolutionary Divergence in Alternative Splicing Patterns Following Gene and Genome Duplication*

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Concurrent Session 2B: *Signal Transduction* Grand Salon  
3:30 Abstract C2B01: Peter McCourt, Session Chair, University of Toronto, Canada – *A Role for Stigolactones in Arabidopsis: A New Plant Hormone*

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3:55 Abstract C2B02: Jose Alonso, North Carolina State University-

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Raleigh, USA – Ethylene – Auxin Interactions in *Arabidopsis*

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4:20 Abstract C2B03: Gregory Lampard, Stanford University, California, USA – *More Than a Simple Switch: Addressing Specificity in Arabidopsis MAPK Signalling Networks Using Stomatal Development as a Model*

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4:33 Abstract C2B04: Pierre Hilson, Ghent University, Belgium – *Golden Secretory Peptides Control Plant Gravitropism*

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4:46 Abstract C2B05: Sean Cutler, University of California, USA – *Chemical Genetic Identification of a New Family of ABA Response Factors*

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5:00 – 7:00 pm	Dinner	On your own
7:00 – 8:30 pm	Workshop Session 2A Lead Organizer: Nicholas Provart – <i>Advanced Bioinformatic Resources for Arabidopsis</i>	Grand Salon
	Workshop Session 2B Lead Organizer: Mary Wildermuth – <i>Laser Microtechniques and Applications with Arabidopsis</i>	Alfred Rouleau A/B
7:00 – 8:00 pm	Special Seminar Jim Collins, NSF Biology Director – <i>Priorities of the NSF Directorate</i>	Picardie
7:00 – 10:30 pm	Exhibits Open	Complexe Desjardins
8:30 – 11:00 pm	Posters Session 1: Odd numbered abstracts presented	Complexe Desjardins & Alfred Rouleau C

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## **Friday, July 25, 2008**

8:00 am – 1:30 pm	Registration Open	Grand Salon Foyer
8:00 am – 8:00 pm	Posters Open	Complexe Desjardins & Alfred Rouleau C
8:30 – 10:00 am	Plenary Session III: <i>Metabolism</i> 8:30 Abstract P301: Mary Schuler, Session Chair, University of Illinois-Urbana-Champaign, USA – <i>Cytochrome P450 Monooxygenases: Critical Components in Many Stress-Regulated Pathways</i>	Grand Salon
	9:10 Abstract P302: Kazuki Saito, Chiba University, Japan – <i>Integration of Metabolomics and Transcriptomics Towards Plant System Biology</i>	
	9:35 Abstract P303: Erich Grotewold, Ohio State University-Columbus, USA – <i>Anthocyanins: From Regulation to Sequestration</i> <i>This session is sponsored by Performance Plants</i>	
10:00 – 10:30 am	Refreshment Break	Grand Salon Foyer
10:30 am – Noon	Plenary Session IV: Interactions with the Environment 10:30 Abstract P401: Jeff Dangl, Session Chair, University of North Carolina-Chapel Hill, USA – <i>The Plant Immune System</i>	Grand Salon
	11:10 No Abstract Submitted: Olivier Voinnet, Institute of Plant Molecular Biology – CNRS, France -	
	11:35 Abstract P403: Alex Webb, University of Cambridge, UK – <i>Integrating Circadian and Environmental Signals</i>	
	<i>This session is sponsored by ISPMB</i>	
12:00 – 1:30 pm	Lunch	Jeanne-Mance Pavilion & Terrasse
1:30 – 7:00 pm	Free Afternoon – for networking, site-seeing, side meetings, etc.	On your own
7:00 – 8:00 pm	Registration Open	Grand Salon Foyer
7:00 – 8:30 pm	Workshop Session 3A Lead Organizer: Harvey Millar – <i>Plant Proteomics – Tools, Approaches, Standards and Breakthroughs in Studying the Proteome</i>	Grand Salon
	Workshop Session 3B Lead Organizer: Alexander Heyl – <i>Phytohormone Biosynthesis and Signal Transduction</i>	Alfred Rouleau A/B
7:00 – 10:30 pm	Exhibits open	Complexe Desjardins

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The diagram is a 3D pyramid divided into three horizontal sections. The top section is white with black text. The middle section is light gray with black text. The bottom section is dark gray with white text. The pyramid is tilted slightly to the right.

## **Saturday, July 26, 2008**

8:00 am – 7:00 pm	Registration Open	Grand Salon Foyer
8:00 am – 7:00 pm	Posters Open	Complexe Desjardins & Alfred Rouleau C
8:30 – 10:00 am	Plenary Session V: <i>Genomics and Systems Biology</i> 8:30 Abstract P501: Gloria Coruzzi, Session Chair, New York University, USA – <i>A Systems Approach to Nitrogen Regulatory Networks and the "Virtualplant"</i>	Grand Salon
	9:10 Abstract P502: Xing Wang Deng, Yale University, USA – <i>Global Experimental Annotation of Small (70~300NT) Non-Coding RNA Genes in Arabidopsis Thaliana</i>	
	9:35 Abstract P503: Ken Birnbaum, New York University, USA- <i>A Genomic View of Cell Identity</i> <i>This session is sponsored by Genome Canada.</i>	
10:00 – 10:30 am	Refreshment Break	Grand Salon Foyer
10:30 am – Noon	Plenary Session VI: <i>Genetic and Epigenetic Mechanisms</i> 10:30 Abstract P601: Jerzy Paszkowski, Session Chair, University of Geneva, Switzerland – <i>Backups of Epigenetic Regulation</i>	Grand Salon
	11:10 Abstract P602: Jian Kang Zhu, University of California-Riverside, USA – <i>Mechanism and Function of Active DNA Demethylation</i>	
	11:35 Abstract P603: Scott Michaels, Indiana University, USA – <i>A Novel Class of Histone Methyltransferases Is Requires for Heterochromatin Formation in Arabidopsis</i>	
12:00 – 2:00 pm	Exhibits open, lunch with exhibitors, open Poster Session	Complexe Desjardins & Alfred Rouleau C
2:00 – 3:30 pm	Concurrent Session 3A: <i>Biotic Interactions</i> 2:00 Abstract C3A01: Sheng Yeng He, Session Chair, Michigan State University-East Lansing, USA – <i>Bacterial Virulence Factoras as Molecular Probes of Basic Plant Cellular Functions</i>	Grand Salon
	2:25 Abstract C3A02: Tesfaye Mengiste, Purdue University, USA – <i>Dissecting Molecular and Cellular Processes Regulating Plant Responses to Necrotrophic Fungal Pathogens</i>	
	2:50 Abstract C3A03: Susanne Salomon, Max Planck Institute for Plant Research, Germany – <i>Genetic Analysis of Arbidopsis Defense Signaling in Response to PAMPS</i>	
	3:03 Abstract C3A04: Shahid Mukhtar, University of North Carolina at	

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Chapel Hill, USA – *Towards a Bacterial Effector-Arabidopsis Target Protein-Protein Interaction Network*

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3:16 Abstract C3A05: Mary Wildermuth, University of California-Berkely, USA – *Laser Microdissection as a Tool for Probing the Arabidopsis Response to the Powdery Mildew Golovinomyces Orontii at the Infection Site*

*This session is sponsored by Dupont Pioneer*

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Concurrent Session 3B: *Genetic and Epigenetic Mechanisms*

2:00 Abstract C3B01: Judith Bender, Session Chair, Brown University, USA – *Locus-Specific Control of DNA Methylation by the SUVH5 and SUVH6 Histone Methyltransferases*

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Alfred Rouleau

A&B

2:25 Abstract C3B02: Ilha Lee, Seoul National University, Korea – *The Function of SWR1 Complex for Floral Repression and Evolution of ARP6 Protein*

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2:50 Abstract C3B03: Marianne Hopkins, University of Waterloo, Canada – *Evidence for Non-Mendelian Inheritance of Ancestral Sequences in Arabidopsis*

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3:03 Abstract C3B04: Rebecca Schwab, Cold Spring Harbor Laboratory, USA – *Functional Consequences of Local Secondary Structures on MiRNA-Target RNA Interactions*

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3:16 Abstract C3B05: Matthew Endres, University of South Carolina-Columbia, USA – *A Plant Transcription Factor Plays a Required Role in Viral Suppression of Silencing*

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3:30 – 4:00 pm Refreshment Break Grand Salon Foyer

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4:00 – 5:30 pm Concurrent Session 4A: *Abiotic Interactions* Grand Salon  
4:00 Abstract C4A01: Stacey Harmer, Session Chair, University of California-Davis, USA – *The Plant Clock and its Output*

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4:25 Abstract C4A02: Eduardo Blumwald, University of California-Davis, USA – *Delayed Leaf Senescence Induces Extreme Drought Tolerance in Flowering Plants*

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4:50 Abstract C4A03: Chan Yul Yoo, Purdue University, USA – *ATGTL1 Transcription Factor Regulates Water Use Efficiency and Drought Adaption Through CA2+/Calmodulin Signaling*

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5:03 Abstract C4A04: Anjali Iyer-Pascuzzi, Duke University, USA – *Cell Type Specific Responses to Acid Stress in Arabidopsis*

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5:16 Abstract C4A05: Tessa Durham, University of Wisconsin-Madison, USA – *Variation in the Root Gravitropic Response Characterized With High-Throughput Computer Vision Analysis*

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Concurrent Session 4B: *Evolution and Development* Alfred Rouleau  
4:00 Abstract C4B01: Liam Dolan, Session Chair, John Innes Centre, UK

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– *An Ancient Mechanism Controls the Development of Cells With Rooting Functions* A&B

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4:25 Abstract C4B02: Faye Rosin, Harvard University, USA – *ABC Program and the Evolution of Novel Floral Organ Identity*

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4:50 Abstract C4B03: Hongchang Cui, Duke University, USA - *Shortroot and Scarecrow in Root Development and Land Plant Evolution: Old Protiens, New Functions*

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5:03 Abstract C4B04: Tie Liu, Stanford University, USA – *Functional Diversification of Stomatal Patterning Genes in Monocotyledons*

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5:16 Abstract C4B05: Eva Huala, TAIR/Carnegie Institute for Science, USA – *Arabidopsis as a Model for Plant Development*

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7:00 – 10:00 pm Conference Banquet

McGill Residence  
Ballroom

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**Please visit us at booth #4**

## **Sunday, July 27, 2008**

8:00 – 11:00 am	Registration Open	Grand Salon Foyer
8:00 – Noon	Posters Open	Complexe Desjardins & Alfred Rouleau C
8:30 – 10:00 am	Plenary Session VII: <i>Signal Transduction</i> 8:30 Abstract P701: Simon Gilroy, Session Chair, University of Madison-Wisconsin, USA – <i>Putting Plants in Touch with Their Feelings: Mechanotransduction in Arabidopsis</i>	Grand Salon
	9:10 Abstract P702: Sarah Liljegren, University of North Carolina-Chapel Hill, USA – <i>Membrane Trafficking and Receptor Kinase Signaling Control Organ Separation in Arabidopsis Flowers</i>	
	9:35 Abstract P703: Julin Maloof, University of California-Davis, USA – <i>Light Signaling: Insights From Time-Lapse Imaging and Quantitative Genetics</i>	
10:00 – 10:30 am	Refreshment Break	Grand Salon Foyer
10:30 am - Noon	Plenary Session VIII: <i>Novel Tools and Techniques</i> 10:30 Abstract P801: Scott Peck, Session Chair, University of Missouri-Columbia, USA – <i>Quantitative Phosphoproteomics reveal New Components of Host-Pathogen Interactions</i>	Grand Salon
	11:10 Abstract P802: Thomas Eulgem, University of California-Riverside, USA – <i>Dissecting Plant Defence Signaling By Chemical and Molecular Genetics</i>	
	11:35 Abstract P803: Blake Myers, Delaware Biotechnology Institute, USA – <i>Deep Transcriptional Profiling of Plant Small RNAs</i>	
12:00 pm	Remove Posters – any not removed will be discarded	Complexe Desjardins & Alfred Rouleau C

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## **END OF CONFERENCE**

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# **WORKSHOPS**

## **Overview Schedule of Workshops**

### **Wednesday, July 23: 4:30-6 pm**

- (1) *Frontiers in Plant Systems Biology* - Lead Organizer: Rodrigo A. Gutiérrez  
(2) *Sources and Strategies for Gene Structure, Gene Function, and Metabolic Pathway Annotation at TAIR and AraCyc* - Lead Organizer: Eva Huala

### **Thursday, July 24: 7:00-8:30 pm**

- (1) *Advanced Bioinformatic Resources for Arabidopsis* - Lead Organizer: Nicholas Provart  
(2) *Laser Microtechniques and Applications with Arabidopsis* - Lead Organizer: Mary Wildermuth

### **Friday, July 25: 7:00-8:30 pm**

- (1) *Plant Proteomics – Tools, Approaches, Standards and Breakthroughs in Studying the Proteome* - Lead Organizer: Harvey Millar  
(2) *Phytohormone Biosynthesis and Signal Transduction* - Lead Organizer: Alexander Heyl

## **Descriptions and Programs for Workshops**

### **Wednesday, July 23: 4:30 – 6:00 pm**

#### **1. Frontiers in Plant Systems Biology**

**Room location: Grand Salon**

Workshop Organizer: Rodrigo A. Gutiérrez (P. Universidad Católica de Chile)

The goal of the workshop organized by the MASC Systems Biology and Bioinformatics Subcommittees is to bring together groups that produce, integrate and model data from a systems perspective. We will have talks that address the new frontiers in genomic data collection for systems biology and the challenges in data storage, analysis and integration. We will have contributions from biologists performing cutting-edge systems research. We are requesting contributors to discuss their state-of-the-art research as well as providing a vision for systems research in plants. We hope this workshop will stimulate discussion on the role of systems biology research in addressing the grand challenges in plant biology. The workshop will also provide a venue for discussing a possible contribution to the iPlant Collaborative initiative. A representative from the iPlant initiative will communicate the goals of the initiative and lead a discussion on how best to use iPlant to advance systems biology research in Arabidopsis.

#### **Program**

4:30 pm      **Rodrigo A. Gutiérrez**, (P. Universidad Católica de Chile) Introduction

4:40 pm      **Xing-Wang Deng** (Yale University) "Data Generation for Systems Biology"

4:50 pm      **Chris Town** (J. Craig Venter Institute) "Data Integration"

5:10 pm	<b>Gloria Coruzzi</b> (New York University) "Data Modeling"
5:30 pm	<b>Steve Rounsley</b> (The University of Arizona) "The iPlant Collaborative"
5:50 pm	Open discussion.

## **2. Sources and strategies for Gene Structure, Gene Function, and Metabolic Pathway annotation at TAIR and AraCyc**

**Room location: Alfred Rouleau A/B**

Workshop Organizer: Eva Huala, Director, TAIR (Carnegie Institution for Science)

During this workshop, curators will discuss how they use both manual and computational methods to annotate different data types including gene structures, gene functions, and metabolic pathways. Highlights will include explanations of (a) the TAIR8 genome release, (b) on-going efforts to improve gene structure annotations, (c) the new TAIR-Plant Physiology collaboration, and (d) future updates to AraCyc generated with the help of PlantCyc, a new plant metabolic pathways database. Participants will gain a better sense of the different levels and types of support for the information they find at TAIR and they will develop an improved understanding of the decisions curators must make when assessing raw and published data. Curators will also provide advice on how members of the community can efficiently incorporate their data into TAIR and AraCyc. Participants will be encouraged to ask questions and to suggest additional datasets and evaluation protocols that could be introduced into the curation pipeline.

### **Program**

4:30 pm	<b>Philippe Lamesch</b> , curator (TAIR / Carnegie Institution for Science): "Gene Structure Annotation"
5:00 pm	<b>Debbie Alexander</b> , curator (TAIR / Carnegie Institution for Science): "Gene Function Annotation"
5:30 pm	<b>Kate Dreher</b> , curator (TAIR / PMN/ Carnegie Institution for Science) "Metabolic Pathway Annotation"

**Thursday, July 24: 7:00 – 8:30 pm**

## **3. Advanced Bioinformatic Resources for Arabidopsis**

**Room location: Grand Salon**

Workshop Organizer: Nicholas Provart, BAR (the Bio-Array Resource)

This workshop will cover the use of Arabidopsis bioinformatic resources for hypothesis generation and knowledge discovery.

### **Program**

7 pm	<b>TAIR</b> (Kate Dreher and Philippe Lamesch): GBrowse and AraCyc. GBrowse permits the exploration of Arabidopsis genomic information at many levels, by providing access to alternative gene models, methylation, promoter, and polymorphism data, VISTA plots of
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orthologous plant sequences, and numerous other data sets. Aracyc provides a powerful framework for exploring metabolic pathways in *Arabidopsis*. Data from "omics" experiments can be painted onto these pathways to allow further insight into these data sets.

- 7:20 pm      **ATTED-II** (Takeshi Obayashi): Gene coexpression networks can provide novel insight into biological processes. The use of ATTED-II to explore these networks will be discussed.
- 7:40 pm      **NASCArrays** (Sean May): Tools from NASCarrays, the Genespring Workgroup, and xspecies and other probe selection tools will be discussed along with the biological example of how xspecies can permit a neutral transcriptome phylogenetic analysis across the *Brassicaceae*.
- 8:00 pm      **Genevestigator** (Nicholas Provart): The powerful new Biomarker and Pathway analysis tools will be discussed.
- 8:15 pm      **The Bio-Array Resource, BAR** (Nicholas Provart): Aspects of Expression Angler including the Custom Bait feature, the eFP Browser, MarkerTracker, Arabidopsis Interactions Viewer and Promomer for cis-element prediction will be presented.

#### **4. Laser Microtechniques and Applications with *Arabidopsis***

**Room location: Alfred Rouleau A/B**

Workshop Organizer: Mary Wildermuth (University of California, Berkeley)

This workshop aims to bring together researchers currently using or interested in using laser microtechniques to discuss specific protocols, methodologies, and applications of laser microtechniques for use with *Arabidopsis*. This forum is important as the tissue preparation, cell isolation, and nucleic acid, protein, and/or metabolite extraction and analysis methods are non-trivial and can be tissue- and application-specific. This workshop will have talks given by groups pioneering the use and application of laser microtechniques with *Arabidopsis*. As many of the first applications of laser-isolated cells have focused on gene expression profiling, the panel discussion will also include researchers with expertise in complementary areas such as proteomic analyses.

#### **Program**

- 7 pm      **Mary Wildermuth** (UC Berkeley, USA) Welcoming/Introductory Remarks
- 7:10 pm      **Keith Lindsey** (Durham University, UK) "Laser-capture microdissection of the developing *Arabidopsis* embryo"
- 7:25 pm      **Mark Belmonte** (University of California, Davis, USA) "Compartment-specific gene expression during *Arabidopsis* seed development" Abstract ICAR1013
- 7:40 pm      **Richard MacKnight** (University of Otago, New Zealand) "Capturing the message: Insights into imprinting and endosperm biology using laser capture microdissection"

7:55 pm	<b>Neeru Gondotra</b> (Yale University, USA) "Adapting laser microdissection to difficult cell types and workflows"
8:10 pm	<b>Panel discussion</b> Facilitator: Mary Wildermuth  Panel members include: Mark Belmonte, University of California at Davis, USA; Divya Chandran, University of California at Berkeley, USA; Neeru Gondotra, Yale University, USA; Keith Lindsey, Durham University, UK; Richard MacKnight, University of Otago, New Zealand; Colin Turnbull, Imperial College London, UK; Klaas van Wijk, Cornell University, USA.

### **Friday, July 25: 7:00 – 8:30 pm**

#### **5. Plant Proteomics- Tools, Approaches, Standards and Breakthroughs in Studying the Proteome**

**Room location: Grand Salon**

Workshop Organizers: A. Harvey Millar (Perth, Australia), Klaas J. van Wijk (Cornell, USA), and Joshua L. Heazlewood (JBEI, Berkeley, USA)

Proteomics is a rapidly growing field and significant developments are being made in and led from Arabidopsis research. The MASC Proteomics Subcommittee has endeavored to establish a workshop presence at each ICAR since the Berlin meeting in 2004. This multi-faceted workshop provides some presentations from the MASC Subcommittee on issues of interest, direct research presentations selected from ICAR abstracts that relate to proteomic analysis, and some open discussion with workshop participants.

#### **Program**

7 pm	<b>Harvey Millar</b> (Perth, Australia) "Introduction to MASC Proteomics, Standards and the Workshop"
7.05 pm	<b>Joshua Heazlewood</b> (JBEI, LBL, USA) "New Arabidopsis Proteomic Resources"
7.15 pm	<b>Scott Peck</b> (Missouri-Columbia, USA) "How The Plant Cell Reviews Functional Genomics Papers"
7.20 pm	<b>Klaas van Wijk</b> (Cornell, USA) "Quantitative proteomics of chloroplast protein biogenesis and homeostasis mutants"
7.30 pm	<b>Berit Ebert</b> (MPI Molecular Plant Physiology, Germany) <i>Abstract ICAR1040</i> "Transcript, Protein and Metabolite Analysis of <i>Arabidopsis thaliana</i> Trichomes"
7.45 pm	<b>Mingjie Chen</b> (Missouri-Columbia, USA) <i>Abstract ICAR604</i> "Parallel microarray and proteomic analysis of a seed-specific acetyl-CoA carboxylase mutant reveals assorted changes in metabolism"
8 pm	<b>Sorina Popescu</b> (Yale, USA) <i>Abstract ICAR4056</i> "Analysis of MAPK signaling networks using <i>Arabidopsis thaliana</i> protein microarrays"
8.15 pm	Discussion and Questions

## **6. Phytohormone Biosynthesis and Signal Transduction**

**Room location: Alfred Rouleau A/B**

Workshop Organizer: Alexander Heyl (Free University, Berlin, Germany)

Plant hormones are key regulators in developmental programs, as well as in biotic and abiotic stress responses. As such this is a topic relevant for all researchers working in the field of plant biology. This is also reflected by the fact that phytohormones will appear in many talks at many different sessions of the meeting program.

This workshop is dedicated to the topic of plant hormone biology to highlight the recent progress made in this area and will include presentation of new findings and discussion of new ideas in this area of research.

### **Program**

- 7:00 pm     **Mary Wildermuth:** Signal Transduction- Temporal separation of free SA synthesis from its modification and activation of gene expression allows for dissection of SA metabolism and response; *Abstract ICAR4077*
- 7:20 pm     **Kavitha Kuppusamy:** Developmental Mechanisms- Brassinosteroid signaling in root epidermal patterning in *Arabidopsis thaliana*; *Abstract ICAR1069*
- 7:40 pm     **Stephen Howell:** Signal Transduction- Proteolytic processing of a plant peptide hormone by a subtilase in *Arabidopsis*; *Abstract ICAR4033*
- 8:00 pm     **Geraint Parry:** Signal Transduction- Regulating the Activity and Expression of Auxin Receptors in *Arabidopsis*; *Abstract ICAR4053*
- 8:20 pm     **Alexander Heyl:** Signal Transduction- Analysis of the function of B-type ARR in the cytokinin signal transduction using a dominant repressor; *Abstract ICAR4030*

# **KEYNOTE LECTURE ABSTRACT**

K01

THE DEVELOPMENT OF CELLULOSIC BIOFUELS

\*Somerville, Chris, Energy Biosciences Institute and University of California, Berkeley, USA

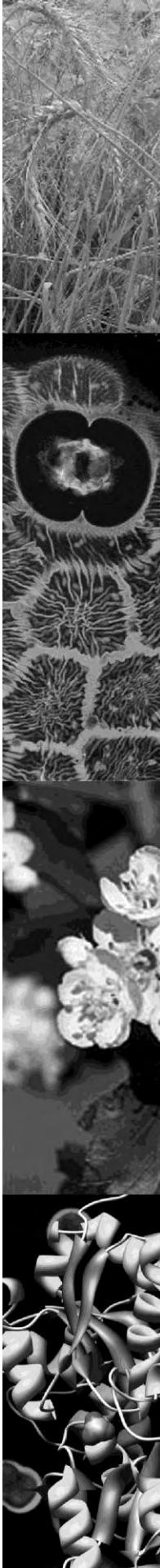
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The earth receives approximately 4000 times as much energy from the sun each year as the total projected human uses in 2050. Thus, because plants can be deployed on a large scale to capture and store solar energy, one way of moving toward the development of carbon neutral energy sources is to use plant biomass for production of fuels. In considering this possibility, the Secretary of Energy of the US has called for the replacement of 30% of the liquid fuels used in the US with biofuels by 2030.

Because of the large volume of fuel used by developed countries, the development of a large-scale biofuels industry may create competition with the use of arable land for food production. Thus, even though it is currently feasible to convert biomass to fuels by a variety of methods, there are many inefficiencies in the overall process that must be eliminated in order to make the most efficient use of land and capital. Many of these issues have been described in a workshop that was sponsored by the US Department of Energy to evaluate the scientific and technical issues associated with biofuel production in the US. The proceedings of that workshop are available online at  
<http://www.doegeonomestolife.org/biofuels/>

In brief, the efficient production of biofuels by biologically-based routes will require innovation in three main areas: production of feedstocks, conversion of feedstocks to sugars, and conversion of sugars to fuels. At present, the main feedstocks being used for fuel production are corn starch and sugar from sugarcane. However, the demand for fuel vastly exceeds the amount that can be produced from these feedstocks so it is expected that gasoline and diesel replacements will ultimately be derived from cellulosic biomass. In this respect there is renewed interest in identifying plants that have optimal biomass accumulation and understanding the production issues associated with large-scale cultivation and sustainable harvesting of such species. There has not been a major effort to improve herbaceous plants for enhanced biomass production and there are many outstanding questions. Additionally, the importance of enhancing soil carbon and nutrient retention while minimizing inputs will require an integrated approach to the development of cellulosic energy crops. Parallel technical developments on the biomass-to-fuels processing side also have important implications for how the industry is likely to develop. For instance, enzyme-based conversion technologies for biomass hydrolysis may be more sensitive to overall biomass composition than thermal decomposition methods. Thus, there are many opportunities to direct basic research on model organisms such as *Arabidopsis* directly toward outstanding problems related to bioenergy production. However, most importantly, because agricultural productivity is the key to making arable land available for any other purpose, research that is directed toward improving our understanding of basic biological processes in higher plants should be viewed as fundamental to the development of biofuels and all other uses of higher plants. Current trends in federal funding for research that lead away from basic research on *Arabidopsis* are ill-conceived.

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## **PLENARY SESSION ABSTRACTS**

P101

A MIRNA-REGULATED PATHWAY FOR VEGETATIVE PHASE CHANGE IN ARABIDOPSIS

\*Poethig, Scott, Wu, G, Conway, S, Smith, M, Wilmann, M and Yang, L University of Pennsylvania, Philadelphia, PA, USA spoethig@sas.upenn.edu

Arabidopsis undergoes several major transitions during its development. The most obvious of these is the transition from vegetative to reproductive growth, when the shoot stops making leaves and vegetative buds and begins to produce flowers. This event is preceded by the transition from a juvenile to an adult phase of vegetative development (vegetative phase change), which is marked by changes in leaf morphology and by an increase in reproductive competence. Microarray analysis of gene expression in 1 mm leaf primordia and fully expanded leaves from 6 different positions on the shoot demonstrates that many genes are differentially expressed during vegetative development, although only some are expressed in a phase-specific fashion. Phase-specific genes/traits are regulated by two temporally expressed miRNAs: miR156 and miR172. miR156 controls most of the morphological changes that occur during vegetative phase change through its effect on the expression of 10 members of the SPL family of transcription factors. miR172 acts downstream of miR156, and regulates a subset of phase-specific vegetative traits as well as flowering time via the AP2-like genes TOE1 and TOE2. The structure of the pathway(s) that control vegetative phase change, and the factors that specify the timing of this switch, will be discussed.

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P102

REGULATION OF PETAL ORGANOGENESIS

\*Irish, Vivian Yale University, New Haven, CT, USA vivian.irish@yale.edu

Organogenesis entails the regulation of cell division, cell expansion, cell and tissue type differentiation, and patterning of the organ as a whole. Arabidopsis petals have a simple laminar structure with a small number of cell types, facilitating the analysis of organogenesis. We have undertaken a number of studies aimed at defining the genetic pathways controlling petal organogenesis. The development of petals relies on at least two interdependent processes: specifying petal identity and regulation of second whorl organ growth. APETALA3 (AP3) encodes a MADS box transcription factor that is essential for petal identity specification. We have examined both how AP3 expression itself is regulated, as well as how AP3 regulates downstream target genes necessary for petal identity specification. We have shown that the F-box protein UFO acts as a transcriptional cofactor in the activation of AP3 expression. UFO appears to function via modulating the activity of the LEAFY transcription factor at the AP3 promoter. We have also characterized some of the genes regulated by AP3 in an effort to understand how petal identity is specified at the molecular level. In addition to transcriptionally upregulating a variety of genes required for petal development, AP3 also appears to transcriptionally downregulate photomorphogenesis pathways in petals. Thus AP3 is pivotal in acting as an integrator of light and developmental signals operating during petal organogenesis.

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P103

#### AXIS FORMATION IN EARLY EMBRYOGENESIS

\*Juergens, Gerd ZMBP - Developmental Genetics, Tuebingen University, Germany gerd.juergens@uni-tuebingen.de

Embryogenesis transforms the fertilised egg cell into a multicellular organism. Plant embryos establish a main body axis of polarity that harbours the stem-cell systems called primary shoot and root meristem at its top and bottom end, respectively. In *Arabidopsis*, axis formation originates from the asymmetric division of the zygote. The apical daughter cell generates a cluster of proembryo cells whereas the basal daughter cell makes a short file of extra-embryonic cells of which only the uppermost cell ("hypophysis") switches fate, initiating root meristem development. Molecular and cell-biological analyses suggest a model that links axis formation to cell polarity, membrane trafficking, polar auxin transport and auxin response. Although auxin might afford long-range coordination of plant development, cell specification in early embryogenesis appears to be a local affair involving small groups of neighbouring cells.

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P201

#### PLANT ENDOMEMBRANE SYSTEM AND CHEMICAL GENOMICS

\*Natasha Raikhel University of California, Riverside, CA, USA  
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Chemical genomics is an exciting new technology for studying gene functions in the context of living organisms or cell systems. The approach complements existing molecular and genetics tools (e.g. mutagenesis, RNAi) by allowing fine-tunable *in vivo* modulations of protein functions and cellular processes. For example, lethality and redundancy are common and challenging descriptors in genetic studies of the endomembrane system. Chemical genomics can be used to overcome these challenges and study protein trafficking mechanisms. We have performed a few chemical genomics screens and identified several useful compounds. Effect of these compounds on various markers of the endomembrane system was assessed. Analogs of these chemicals were tested to identify the chemical structures that are responsible for bioactivity of these molecules. Screens for resistant and hypersensitive mutants were carried out with the goal of identifying putative targets or to identify components of the pathway. An information about these targets and pathways will be discussed.

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P202

#### LATERAL ROOT DEVELOPMENT: AN EMERGING STORY...

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Lateral roots originate deep within the parental root from a small number of founder cells at the periphery of the vascular tissues that must emerge through intervening layers of tissues. Despite its importance to the integrity of the root system, little is known about the regulation of lateral root emergence. Our studies have recently revealed that lateral root emergence is a highly regulated process involving the active participation of cells in both new lateral root primordia and the parental root. The

hormone auxin originating from the developing lateral root appears to act as a local inductive signal which reprograms adjacent cells. Auxin induces the expression of a previously uncharacterized auxin influx carrier LAX3 in cortical and epidermal cells directly overlaying new primordia. Increased LAX3 activity reinforces the auxin-dependent induction of a selection of cell wall remodelling enzymes, promoting cell separation in advance of developing lateral root primordia. Auxin therefore appears to act as a common signal that synchronizes lateral root primordium initiation<sup>2</sup>, patterning<sup>3</sup> and emergence<sup>1</sup> processes.

1. Swarup et al (2008) The auxin influx carrier LAX3 promotes lateral root emergence. *Nature Cell Biology*, in press.
  2. de Smet et al (2007) Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* 134, 681-90
  3. Benkova et al (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115, 591-602
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P203

#### MOLECULAR BASIS OF PLANT PEROXISOMAL PROLIFERATION AND DISTRIBUTION

\*Jianping Hu MSU-DOE Plant Research Laboratory and Plant Biology Department, Michigan State University, East Lansing, MI 48824 [huji@msu.edu](mailto:huji@msu.edu)

Peroxisomes are multifunctional organelles that differ in size, abundance, and pattern of distribution depending on the species, cell type, developmental stage, and metabolic and environmental conditions. Plant peroxisomes play key roles in growth and development by orchestrating various metabolic pathways. To answer the question of how peroxisomal dynamics affects plant physiology and development, we employed forward and reverse genetics to establish a mechanistic model of peroxisome proliferation and movement in *Arabidopsis*. Our studies revealed common components of the machineries that control peroxisomal multiplication and distribution in diverse eukaryotic species, as well as features unique to plants. Three small protein families, namely, PEROXIN11 (a to e), DYNAMIN-RELATED PROTEIN3 (3A and 3B), and FISSION1 (A and B), mediate the proliferation of peroxisomes at various steps of the process. Among them, DRP3 and FIS1 also control the division of mitochondria. A phytochrome A-dependent light signaling pathway, which is composed of the bZIP transcription factor HYH and the peroxisomal protein PEX11b, induces peroxisome proliferation during seedling photomorphogenesis. Following proliferation, newly formed plant peroxisomes are separated from each other and travel on the actin-based cytoskeleton with the help of myosin molecular motors. We identified a peroxisomal protein highly likely to be the receptor for the myosins. Lastly, we analyzed the proteome of three major subtypes of peroxisomes from *Arabidopsis* and validated the proteomics data using subcellular localization study of YFP fusion proteins. Novel proteins that have not been associated previously with peroxisomes were discovered, which may uncover new functions for plant peroxisomes.

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P301

CYTOCHROME P450 MONOOXYGENASES: CRITICAL COMPONENTS IN MANY STRESS-REGULATED PATHWAYS

\*Schuler, Mary A. University of Illinois, Urbana, IL, USA maryschu@uiuc.edu

Of the many full-length P450 sequences characterized in the genomes of Arabidopsis, rice, papaya, grape and moss, a large number are involved in stress responses either for synthesis of signaling molecules or production of defense toxins. Understanding the importance of these catalytically versatile enzymes in various physiological functions and their functionally relevant evolutionary differences depends on developing accurate molecular models for their interactions with substrates, defining critical catalytic site residues and assessing their stress-responsive expression patterns. Our improvements in modeling procedures gained from the discrimination of bacterial and mammalian P450 template structures have enhanced our structural predictions of catalytic site residues impacting the reactivities of Arabidopsis P450s and provided a three-dimensional perspective on evolution within individual P450 subfamilies involved in fatty acid, flavonoid and lignin hydroxylations. High-throughput virtual screenings coupled with heterologous expression assays have identified substrates for several previously uncharacterized Arabidopsis P450s. Microarray analyses have identified several P450 loci important in metabolic responses to jasmonic acid signaling and many that are circadian-regulated under normal growth conditions. Physiological profilings of T-DNA knockout lines have identified several mediating fatty acid modifications that are specifically involved in stress responses to insect attack.

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P302

INTEGRATION OF METABOLOMICS AND TRANSCRIPTOMICS TOWARDS PLANT SYSTEMS BIOLOGY

\*Saito, Kazuki RIKEN Plant Science Center, Yokohama, Japan; Chiba University, Chiba, Japan  
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The completion of the whole genome sequence of several plants has made it possible to perform a systems analysis of transcriptome and metabolome involved in cellular process by determining gene-to-metabolite correlation. In silico co-expression analysis of genes involved in flavonoid metabolism in Arabidopsis was performed using a publicly available transcriptome database (Saito et al., TIPS, 13, 36 (2008)). A co-expression framework model of the genes involved in the pathways of flavonoid synthesis was inferred, leading to the suggestion of specific functions and co-regulation of the genes of pathway enzymes and transcription factors (Yonekura-Sakakibara et al., JBC, 282, 14932 (2007)). This strategy was also applied to the global responses under nutrient starvation and led to the identification of MYB transcription factors crucial for aliphatic glucosinolate production (Hirai et al., PNAS, 104, 6478 (2007)). Furthermore, the metabolite co-accumulation networks in a mutant of flavonoid biosynthesis revealed a particular enhancement of defense network primed in this particular mutant (Kusano et al., BMC Systems Biol, 1, 53 (2007)). Thus the functional genomics approach by integrated network analysis of metabolome with transcriptome provides an efficient way of identifying novel gene functions involved in plant metabolism. These studies lead to the systems understanding of plant cellular process for the basis for biotechnology application of crop improvements.

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P303

ANTHOCYANINS: FROM REGULATION TO SEQUESTRATION

\*Grotewold, Erich The Ohio State University, Columbus, OH 43210 grotewold.1@osu.edu

Anthocyanin pigments, derived from the flavonoid pathway, are regulated in most species by the cooperation of R2R3-MYB, basic helix-loop-helix (bHLH) and WD-repeat (WDR) proteins, providing one of the best studied examples of combinatorial control of plant gene expression. In contrast to the extensive knowledge acquired over the years on the regulation of the pathway, little is known on how anthocyanin pigments move within cells, from their site of synthesis, likely the cytoplasmic surface of the endoplasmic reticulum, to the site of accumulation, the large central vacuole. It is also unclear whether, and to what extent, pathway regulators participate in controlling the anthocyanin trafficking process. Taking advantage of unique auto-fluorescent properties of anthocyanins, which permit their visualization outside the vacuole, combined with the chemical complementation of mutants in the biosynthetic pathway, novel insights on the trafficking and vacuolar sequestration of these compounds are starting to emerge.

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P401

THE PLANT IMMUNE SYSTEM

\*Dangl, Jeff University of North Carolina – Chapel Hill, dangl@email.unc.edu

The major class of plant disease resistance proteins contains nucleotide binding sites and leucine rich repeat domains (the NB-LRR class). NB-LRR proteins condition resistance to pathogens with various extracellular, intracellular, haustorial, and insect feeding lifestyles, suggesting commonality in their mode of action. NB-LRR proteins make intra-molecular contacts in a signal competent multi-protein complex that includes a variety of co-chaperones required to achieve a final, folded and signal competent state. NB-LRR activation may be achieved by a release of intra-molecular inhibition via a “jack-knife opening” model, leading to activation of the NB.

It is now reasonably well established that bacterial type III effector proteins can function as virulence factors that act on one or more host target proteins following their delivery into host cells. The modifications of these host factors can be monitored by NB-LRR proteins. Thus, the intracellular branch of the plant immune system can recognize “modified self” inside the cell. Further, it is also known that some NB-LRR proteins interact directly with fungal virulence factors. How either the direct or indirect mode of pathogen recognition leads to rapid activation of defense response and HR is not yet clear.

I will summarize our group’s efforts to understand (1) how NB-LRR proteins are poised for activation; (2) how NB-LRR proteins monitor host target homeostasis; (3) how one pathogen, *Pseudomonas syringae*, has collected a large diversity of type III effectors that are evolving differentially across the population and (4) how superoxide and salicylic acid gradients might be sensed to bound hypersensitive cell death.

Supported by the NIH, NSF, DOE, and HFSP.

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P403

#### INTEGRATING CIRCADIAN AND ENVIRONMENTAL SIGNALS

\*Alex A.R. Webb Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK  
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The circadian clock is an internal time keeper essential for the coordination of cellular activities and photoperiodic sensing. We are investigating the interactions between environmental signals and the rhythmic regulation of signalling networks by the circadian clock. We have shown that correct adjustment interaction between the rhythms of the circadian network and the daily rhythms of the environment has large benefits for growth and competitive advantage of Arabidopsis.

We are particularly interested in the role of oscillations of cytosolic free Ca<sup>2+</sup> in the circadian signalling network. I will describe how systems approaches combined with transcriptomic, physiological, imaging and genetic tools have identified a new arm of the circadian clock in the cytosol containing circadian oscillations of cytosolic free Ca<sup>2+</sup> and cyclic ADP ribose. I will describe our new models of the network by which the circadian clock and environmental signals combine to regulate signalling and our data that demonstrate the presence of multiple independent circadian clocks in plants. The physiological consequences of the circadian regulation of signalling in ‘gating’ environmental inputs into plant cells and optimizing plant productivity and metabolism will be presented.

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P501

#### A SYSTEMS APPROACH TO NITROGEN REGULATORY NETWORKS AND THE “VIRTUALPLANT”

\*Coruzzi, Gloria, New York University, USA gloria.coruzzi@nyu.edu

Our goal is to understand how internal and external perturbations affect gene networks linking plant metabolism with development. Succeeding in this endeavor will allow us to (1) explain mechanistically how molecular network changes evoke responses and (2) predict molecular network states under untested conditions or in response to gene modifications. In the long term this approach should enable researchers to test biotechnological strategies for gene modification in silico, prior to testing in transgenic plants. Our approach starts with the integration of Arabidopsis genomic data into a “multinetwork” where the “edges” connecting gene “nodes” are supported by multiple data/evidence including: metabolic pathway connections, protein:protein and protein:DNA interactions, microarray data, microRNA:target datasets, and literature-based interactions predicted using a text-mining tool. At present, this Arabidopsis multinetwork contains approximately 7,000 nodes and 230,000 interactions between them. As proof-of-principle, we used this Arabidopsis multinetwork to identify the gene networks controlled transcriptionally by light, carbon and nitrogen signals based on the analysis of microarray data from specific organs and cell-types. In selected cases, the networks identified in wild-type plants have been validated using network analysis of microarray data from Arabidopsis signaling mutants. To support this type of plant systems biology analysis in the plant community, we have implemented a set of data integration, analysis and visualization tools into a system called the “VirtualPlant” ([www.virtualplant.org](http://www.virtualplant.org)). This system encompasses visualization techniques that render the multivariate genomic information in visual formats that facilitate the extraction of biological concepts and enable a “Systems Biology” view of the genomic data. While VirtualPlant relates specifically to Arabidopsis, the data structures, algorithms, and visualization tools developed are designed in a species-independent fashion. Thus, with the proper data uploads, the system can be used to visualize and model the molecular basis and underlying genomic

responses in any organism for which genomic data is available. This research is supported by NIH, NSF and DOE.

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P502

#### GLOBAL EXPERIMENTAL ANNOTATION OF SMALL (70~300NT) NON-CODING RNA GENES IN ARABIDOPSIS THALIANA

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Recent large-scale analytical efforts have revealed various aspects of the functional sequence elements in several model organisms, and thereby contributed to the understanding of the non-protein coding parts of genomes. However, small non-coding RNAs (ncRNA), that is, the cellular RNA species with size range of 70~300nt, have seldom been systematically annotated or extensively studied. Here, we report a genome wide analysis of small ncRNA genes in the flowering plant *Arabidopsis thaliana*. By 454 deep sequencing of the *A. thaliana* small non-coding transcriptome, we identified 1298 small RNA genes. These loci corresponded to 441 tRNAs and 857 other small ncRNAs, of which 566 of the non-tRNA genes have not been previously annotated or predicted. Furthermore, we compared our data with the recently *Arabidopsis* annotation TAIR8, and found various extend of discrepancy in defining the same ncRNA loci. We identified 363 (~100 novel) ncRNAs belonging to currently known ncRNA functional classes, snoRNA accounting for most (243, 80 novel) of these. The remaining 494 ncRNAs likely constitute novel or *Arabidopsis* specific ncRNA classes. We found ~200 (23%) *Arabidopsis* ncRNA genes are conserved (with more than 50% similarity) in rice genome, while only 108 of the homolog were detected in a similar rice ncRNA deep sequencing project, suggesting that the ncRNA genes evolved fast in sequence and function. Northern analysis of randomly selected ncRNAs suggested that more than 70% of the candidates have detectable expression in a selected tissue panel, some of which are tissue specific or developmentally regulated. Interestingly, a T-DNA insertion line showed down-regulated expressions of a cluster of five ncRNA genes, and a plant phenotype characterized by retarded vegetative growth and indented leaf shape, suggesting a possible role of ncRNAs in specific plant development peocess. Our results provides an extensive survey of the *Arabidopsis* small sized ncRNA inventory, and offers an opportunity to learn novel facets of an organism's genome coding capacity and plant biology.

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P503

#### A GENOMIC VIEW OF CELL IDENTITY

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One of the remarkable features of development is the ability to self organize tissues into specific cell types arranged in specific patterns. We use a combination of genomics, live imaging, and genetic techniques to ask how cells know what to become within a complex tissue and then once the decision is

made, how a specific cell type is constructed on the molecular level. We have developed several systems that enable us to incorporate global views of cell types as they acquire or lose their fate, including a root tip regeneration system, an *in vivo* cell identity transformation system, and a cell culture knockdown system. Using these systems, we have gained some new insights into the broad signals that organize patterning in the root tip. For example, we have compared a time-series transcriptional profile of regenerating root tips against our library of transcriptional cell states to show that differentiated cell identities can be re-specified within hours of their complete excision. Mutant analysis shows that stem cells are not needed for this respecification. Followup work shows that auxin flux itself may be one of the cues that can directly pattern root tissue independently of the stem cell niche. To utilize global data on cell identity, we have developed new bioinformatic tools which can be used to sensitively trace a shift in cell character. Using these tools to examine the effect of auxin on cell fate, we have treated roots with auxin and isolated specific cell types. We find that auxin induces different responses in different tissues pushing cells toward specific fates depending on the development history of the tissue. We also find that auxin can induce QC character in protoplasts. Overall, this leads to a model in which auxin has a direct role in specifying a number of cell types, depending on the underlying competence of the starting tissue. This implies auxin operates in a hierachal chain and we are using cell-based knockdown systems to explore these potential upstream factors as well as downstream cell-specific networks.

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P601

#### BACKUPS OF EPIGENETIC REGULATION

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In plants, heritable states of epialleles involve a self-reinforcing loop consisting of various epigenetic mechanisms in which CpG methylation plays the role of a central scaffold. Therefore, alternations of epigenetic states are usually associated with changes in DNA and chromatin modifications. I will focus on rather unusual epigenetic regulator, MOM1, which although is required for heritable maintenance of transcriptional gene silencing in *Arabidopsis thaliana*, its depletion evokes transcription without major changes in DNA methylation or histone modifications. Protein domains of MOM1 suggested an integral nuclear membrane protein with chromatin-remodeling and actin-binding activities. Unexpected recent results challenged these presumed MOM1 activities and demonstrated that less than 13% of MOM1 is necessary and sufficient for TGS maintenance. This active sequence encompasses novel Conserved MOM1 Motif 2 (CMM2). Its possible evolutionary origin and biological function will be discussed.

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P602

#### MECHANISM AND FUNCTION OF ACTIVE DNA DEMETHYLATION

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Active DNA demethylation is involved in many vital developmental and physiological processes in plants and animals. Recent genetic and biochemical studies in plants have demonstrated that a subfamily of DNA glycosylases function as DNA demethylases through a base excision-repair pathway. I will discuss the function of the ROS1 family of DNA demethylases in the model plant *Arabidopsis* and a possible mechanism of targeting the demethylases to specific loci.

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P603

A NOVEL CLASS OF HISTONE METHYLTRANSFERASES IS REQUIRED FOR HETEROCHROMATIN FORMATION IN ARABIDOPSIS

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In eukaryotes, chromatin modifications such as DNA and histone methylation are associated with epigenetic gene regulation and heterochromatin formation. The constitutive heterochromatin in *Arabidopsis thaliana* is marked by repressive chromatin modifications including DNA methylation, histone 3 dimethylation at lysine 9 (H3K9me2), and monomethylation at lysine 27 (H3K27me1). In contrast to DNA methylation and H3K9me2, the enzymes responsible for H3K27me1 at the constitutive heterochromatin remain unknown. The eukaryotic enzymes that have been demonstrated to methylate H3K27 in vivo are all homologs of the *Drosophila* SET-domain protein Enhancer of zeste (E(Z)). Despite the fact that CURLY LEAF (CLF), and SWINGER (SWN) are the only known H3K27 methyltransferases to be expressed in adult plants, H3K27 methylation at chromocenters is unaffected in *clf swn* double mutants. This has led to speculation that an unknown H3K27 methyltransferase is responsible for H3K27 methylation at the chromocenters. Our work shows that ATXR5 and ATXR6 are a divergent class of SET-domain proteins with H3K27 monomethyltransferase activity in vitro and in vivo. Furthermore, mutants show reduced H3K27me1 at chromocenters, partial heterochromatin decondensation, and transcriptional activation of repressed heterochromatic elements. Together, these results suggest that ATXR5 and ATXR6 comprise a novel class of H3K27 methyltransferases that play a critical role in the maintenance of heterochromatin.

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P701

PUTTING PLANTS IN TOUCH WITH THEIR FEELINGS: MECHANOTRANSDUCTION IN ARABIDOPSIS

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The sessile nature of the plant lifestyle requires exquisite sensitivity to environmental signals. These stimuli provide the information that controls much of plant behavior, ranging from decisions about when to grow or reproduce to whether to mount a defense response against a pathogen. Mechanical stimuli ranging from the buffeting by wind and rain, contact by herbivores and even the weight of an organ itself provide key information that governs plant morphogenesis. We are interested in defining the initial signaling events that link touch perception to subsequent growth responses. Mechanical stimulation of plants is well characterized as triggering a transient  $\text{Ca}^{2+}$  increase. However, the signal transduction

pathways elicited by such a Ca<sup>2+</sup>-dependent signaling system remains poorly defined. We have used fluorescent probes of intra and extracellular pH and ROS production to visualize the changes in these parameters in response to this Ca<sup>2+</sup> signal in the Arabidopsis root. Our research is revealing pH- and ROS-dependent regulation of root growth that is entrained to the mechanical environment of the plant through Ca<sup>2+</sup>-dependent regulation of plasma membrane proton transporters and ROS-producing enzymes, such as the NADPH oxidase C. Imaging of the changes in these signaling elements reveals they can act locally and also provide long range coordination of growth dynamics over the entire organ.

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P702

MEMBRANE TRAFFICKING AND RECEPTOR KINASE SIGNALING CONTROL ORGAN SEPARATION IN ARABIDOPSIS FLOWERS

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A distinctive feature of plants is their ability to release organs such as leaves, flowers, fruit, and seeds. Through our studies of organ separation in Arabidopsis, we have identified an ADP-ribosylation factor GTPase activating protein, NEVERSHED (NEV), which is required for shedding of the outer floral organs after pollination. Interestingly, mutations in NEV severely disrupt the structure of the golgi apparatus and cause vesicle hyperaccumulation in abscission zone cells at the time of shedding. To identify additional factors that may interact with or downstream of NEV, we conducted a screen for mutants that rescue organ shedding in nev flowers. Four dominant suppressor mutants were found to contain mutations predicted to affect the extracellular domain of the leucine-rich repeat receptor-like kinase (LRR RLK) SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 (SERK1). As golgi organization is restored in nev serk1 flowers, disruption of this organelle is likely primarily responsible for blocking abscission in nev flowers. Two additional recessive mutants that rescue shedding in nev flowers contain mutations affecting the extracellular and kinase domains of another predicted LRR RLK, EVERSHED (EVR). We have found that the abscission zones of nev serk1 and nev evr flowers show significantly increased cell expansion compared to wild type. A similar phenotype has been reported for plants constitutively expressing a putative ligand required for floral organ abscission, INFLORESCENCES DEFICIENT IN ABSISSION (IDA) (Stenvik et al 2006 Plant Cell 18:1567-1576). Thus, it is possible that higher levels of IDA or its activated receptor(s) may be present in nev serk1 and nev evr abscission zone cells. Currently, we are using the serk1 and evr mutants as tools to test whether the pathways regulating abscission controlled by NEV and IDA might intersect. These studies have revealed some unexpected findings and suggest the possibility that NEV may control organ separation by regulating the localization of members of particular receptor-like kinase complexes such as SERK1 and EVR.

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P703

LIGHT SIGNALING: INSIGHTS FROM TIME-LAPSE IMAGING AND QUANTITATIVE GENETICS

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Because plants depend on light for energy their development is highly modified in response to the light environment. One example is de-etiolation: seedling perception of light inhibits hypocotyl elongation, the appropriate response after emergence from the soil. Our time-lapse imaging of seedling growth revealed that in diurnal conditions cell-elongation peaks at dawn. Subsequent analysis revealed that this pattern results from the coordinated action of both the internal circadian oscillator and light signaling pathways. Specifically the clock regulates transcription of PIF4 and PIF5, growth-promoting transcription factors, whereas light regulates their protein stability. This work provides a detailed mechanism for clock/environment interactions.

A second example of light-regulated growth occurs during shade avoidance, a suite of traits that includes increased stem and petiole elongation, early flowering, and changes in resource allocation. Others have established that auxin is involved in shade-induced increases in cell elongation. To examine shade/auxin interactions in real-time, we used the synthetic auxin responsive promoter DR5 to drive expression of luciferase (LUC). We observed a strong increase in DR5:LUC bioluminescence after inducing the shade-avoidance response with end-of-day far red (EOD-FR) treatment. The pattern of induction suggests an increase in auxin biosynthesis and indeed we found that three putative auxin biosynthesis genes, YUCCA5, 8, and 9, are all induced by shade treatment. Mutant analysis shows that these genes are required for shade-induced increases in auxin signaling and full-elongation in response to EOD-FR, indicating a new connection between the light and auxin signaling pathways.

Finally, not all plants respond to the light environment in the same way; for example, *Arabidopsis* accessions show abundant natural variation in their light response. We are using quantitative genetics and association mapping to understand the genetic and genomic changes underlying variation in light signaling.

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P801

QUANTITATIVE PHOSPHOPROTEOMICS REVEAL NEW COMPONENTS OF HOST-PATHOGEN  
INTERACTIONS

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We recently developed a method for enriching phosphopeptides from complex mixtures to sequence in vivo protein phosphorylation sites from *Arabidopsis* by LC-MS/MS [1]. We have further refined the methodology to allow quantitative comparisons to examine changes in specific phosphorylation sites of plasma membrane proteins in response to treatment with a bacterial elicitor of defence responses, flagellin [2]. Strongly elicitor-responsive phosphorylation sites may reflect direct regulation of protein activity. We confirm this prediction for RbohD, an NADPH oxidase that mediates the rapid production of reactive oxygen species (ROS) in response to elicitors and pathogens. By complementing an RbohD mutant plant with different unphosphorylatable forms of RbohD, we show that only those sites that undergo differential regulation are required for activation of the protein. These experiments demonstrate the potential for quantitative phosphoproteomics to determine regulatory mechanisms on the molecular

level and provide new insights into innate immune responses. By pursuing reverse genetic investigations of differentially phosphorylated protein candidates, we have discovered that a specific syntaxin, AtSYP132, is an essential component for all forms of resistance against bacterial pathogens [3]. The protein appears to be the cognate t-SNARE required for secretion of antimicrobial proteins and/or compounds, implicating protein secretion as a major determinant of resistance. These results also provide examples of how proteomics can reveal important new aspects of biology that would otherwise escape detection by genetic or microarray experiments.

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P802

#### DISSECTING PLANT DEFENSE SIGNALING BY CHEMICAL AND MOLECULAR GENETICS

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Plants have an intricate immune system that responds to pathogen infections via a complex regulatory network. This network consists of at least three interconnected branches dependent on the phytohormones salicylic acid, jasmonic acid, and ethylene. Chemical genetics utilizes small molecules to specifically perturb defined regulatory mechanisms *in vivo* allowing for real-time control of homologous biological processes across species barriers. We use this approach to discover synthetic elicitors targeting components of the plant defense web. Combined with methods of molecular genetics, chemical genetics provides us with novel tools to study the behavior and architecture of the defense network and to discover new components of this regulatory system. We are performing high-throughput screens of diverse chemical libraries for synthetic elicitors that activate in *Arabidopsis* reporter genes containing promoters from the defense-associated gene clusters LURP (late up-regulated in response to *Peronospora*) and JEDI (Jasmonic Acid/Ethylene dependently induced). We also identified multiple pathogen-responsive *Arabidopsis* enhancer trap lines that will be used in screens for additional synthetic elicitors. Our goal is to establish a suite of synthetic elicitors with a variety of distinct target specificities. A comprehensive set of synthetic and natural defense elicitors combined with inhibitors and genetic mutants will allow for a detailed dissection of the plant defense network and is likely to serve as a powerful tool for the emerging field of systems biology. Blends of synthetic elicitors identified by our study may also facilitate the design of new pesticides providing protection to plants by efficiently stimulating their inherent defense mechanisms.

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P803

#### DEEP TRANSCRIPTIONAL PROFILING OF PLANT SMALL RNAs

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Small RNAs (21-24 nt) such as miRNAs and siRNAs are a powerful regulatory force in most eukaryotes because they can function to shut off genes at multiple levels. Deep sequencing of the small RNA component of the transcriptome is an important step toward elucidating the impact of small RNAs on individual genes and the genome as a whole. We have developed and applied small RNA profiling methods based on novel parallel sequencing technologies. Using these approaches in wildtype and mutant Arabidopsis lines, we have identified numerous new miRNAs and siRNA-generating loci from Arabidopsis (<http://mpss.udel.edu/>). We have been analyzing the small RNA component of grasses and other plant species, using comparative approaches to examine the small RNA profile and identify conserved small RNAs. In this context, our work in rice led to the identification of a novel class of natural-**antisense transcript miRNAs**. In maize, we've examined the small RNA complement of wildtype and a mutant of the *mop1* (mediator of paramutation1) gene. More recently, we have also developed a high-throughput and global approach to simultaneously identify miRNA-target RNA pairs; this represents an advance over predictive methods, as our approach simultaneously provides experimental data for both the miRNA and its targets. Applied in Arabidopsis, this approach has shown that most miRNA targets show a single abundant signature at the miRNA cleavage site, particularly in libraries from a mutant **deficient in the 5' to 3' exonuclease AtXRN4**. Taken together, these data are providing insights into the small RNA populations present in complex plant genomes.



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## **CONCURRENT SESSION ABSTRACTS**

C1A01

REGULATION OF THE ONSET OF REPRODUCTION IN ARABIDOPSIS

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One of the most dramatic developmental transitions in higher plants is that from vegetative to reproductive development. This transition requires reprogramming of cells at the flanks of the shoot apical meristem during the meristem identity (MI) switch to form the first flower primordia. This is followed by induction of the floral homeotic genes and flower patterning. Successful completion of these events is vital for species survival. The central regulator of both processes is the plant specific transcription factor LEAFY (LFY). Accumulating evidence suggests that LFY controls a complex regulatory network. Despite its importance in reproductive development, our knowledge of the components of this network and of its regulatory logic is still very limited. We are addressing this question using a combination of genomic and genetic approaches.

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C1A02

SHAPING THE ARABIDOPSIS SHOOT BY SHORT RANGE YABBY-DERIVED SIGNALS

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Shoot apical meristems (SAMs) are self-sustaining groups of cells responsible for the ordered initiation of all aerial plant tissues such as stems and lateral organs. The precise coordination of these processes argues for crosstalk between the different SAM domains. Products of YABBY genes are limited to the organ primordia domain situated at the periphery of all SAMs and are separated by a margin of 3-7 cells from the central meristem zone marked by WUSCHEL (WUS) and CLAVATA3 (CLV3). None-the-less, mutations in two YAB1 genes, FILAMENTOUS FLOWER (FIL) and YABBY3 (YAB3), display an array of defects including aberrant phyllotaxis that is coordinated at the central SAM zone. Thus, YAB1 activity affects sequentially and non-autonomously the phyllotaxis and growth of organ primordia. These effects support a role for short-range signaling. However, no evidence was found that YAB1 gene products are themselves mobile. A screen for suppression of YAB1 overexpression revealed that the YAB1-born signals are mediated in part by the activity of LATERAL SUPPRESSOR. This GRAS protein is expressed at the boundary of organ primordia and the SAM central zone (CZ), distinct from the YAB1 expression domain. Together, these results suggest that YAB1 activity stimulates signals from the organs to the meristem via secondary message or signal cascade, a process essential for organized growth of the SAM. That similar effects were found within organs too, hint for a general mode of action, whereby organ polarity is translated into short range growth regulating signals.

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C1A03

A HIGH-THROUGHPUT MISEXPRESSION SCREEN IDENTIFIES NOVEL GENES REGULATING FLOWERING FROM THE LEAF

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Arabidopsis is a facultative long-day plant in which flowering is promoted during inductive long days and delayed under short days. The genetic network monitoring the response to environmental and endogenous signals is complex and involves several factors that genetic and molecular studies have placed in different regulatory pathways, including the photoperiod, vernalization, autonomous and

gibberellin pathways. Inductive signals ultimately converge on a limited number of floral pathway integrators active in the shoot apical meristem (SAM).

The photoperiod pathway is responsible for sensing day length and comprises at its core the GIGANTEA (GI), CONSTANS (CO) and FLOWERING LOCUS T (FT) genes. GI acts as a transcriptional activator of CO, which in turn induces transcription of FT in the leaf in long days but not in short days. Both CO and FT are transcribed in the companion cells of the phloem. However, the FT protein can move through the vascular tissue to reach the SAM, where it eventually triggers flower formation.

Several proteins are known to act in the leaf vascular tissue to regulate flowering. To identify additional components required for flowering-time control and acting in the phloem companion cells, we performed a high-throughput misexpression screen, using the phloem-specific SUC2 promoter. Around 900 transcription factors belonging to different families were cloned under the control of SUC2 and transformed in *Arabidopsis*. Primary transformants were screened under inductive long days to identify both activators and repressors of flowering. About 30 transcription factors not previously implicated in flowering-time regulation were isolated on the basis of their altered response to LDs. By means of genetic analyses we placed some of these factors in the photoperiod pathway and characterized them in detail. Results will be discussed in the context of our current understanding of the molecular network that regulates flowering from the phloem.

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#### C1A04

#### A FUNCTIONAL STEM CELL NICHE IS NOT REQUIRED IN ARABIDOPSIS ROOT TIP REGENERATION

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Regeneration is the process of complete re-establishment of cellular identities and patterns of lost structures in an adult body. It is often assumed that organ regeneration in plants depends precisely on the same stem cells supporting continuous (indeterminate) growth during post-embryonic development, so that early re-appearance of a functional stem cell niche is usually expected for regeneration. One alternative hypothesis is that regeneration differs in this respect from indeterminate growth, and that re-patterning can emerge as a niche-independent, self-organizing process.

We use the *Arabidopsis* root as a model to investigate the stem cell niche role during plant organ regeneration. We use a novel approach of integrating over time confocal imaging of individual live roots with global transcriptional profiling of regenerating stumps after complete whole-tip excision, in various genetic and chemical backgrounds.

Our results suggest a rapid restoration of missing cell fate and function before the recovery of stem cell activity. Surprisingly, mutants deficient in stem cell niche maintenance were still able to re-establish the lost pattern and cell fates after root tip excision. Moreover, young leaves, lacking a stem cell niche and indeterminate growth, regenerated after partial excision, demonstrating the wide capacity to re-pattern organs without a central organizer.

These results separate the function of the stem cell niche in indeterminate growth from the regeneration processes. It appears then that fundamental aspects of organ regeneration in plants depends on a combination of cell fate plasticity and patterning mechanisms, independently of stem cell niche activity.

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#### C1A05

#### AIL/PLT PROTEINS REGULATE FLORAL PATTERNING AND SHOOT DEVELOPMENT IN ARABIDOPSIS THALIANA

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A mature *Arabidopsis thaliana* flower consists of four types of organs (sepals, petals, stamens, and carpels) that are arranged in a stereotypical pattern. This complex floral structure is elaborated from just a small number of floral meristem cells partitioned from the shoot apical meristem during reproductive development. The molecular mechanisms responsible for the initiation of floral organ primordia in defined positions within a floral meristem remain a mystery. An important regulator of growth within both floral meristems and developing floral organs is *AINTEGUMENTA* (ANT). ANT is a member of a small family of transcription factors called AIL/PLT proteins. Recent work in my lab has revealed that ANT and AIL6 act redundantly in early stages of flower development to regulate the expression of floral organ identity genes and the pattern of floral organ initiation. These two genes have additional functions in regulating plant height and architecture, vascular development, and organ growth. Furthermore, we have found that three AIL genes act redundantly in the shoot apical meristem to promote growth and organ initiation. In plants lacking these three gene products, the shoot apical meristem terminates after producing just a few abnormal leaves. Several pieces of evidence suggest connections between AIL/PLT function and auxin-regulated plant growth and development. AIL/PLT proteins may be components of an auxin "read-out" system that translates auxin gradients within plant tissues into developmental patterns.

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#### C1B01

#### TWISTED ROOTS: GROWTH PATTERNS AND CELL WALL STRUCTURE

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Roots of *arabidopsis* are well known to twist while they grow. When grown on vertical agar-filled plates, this tendency manifests itself as a concerted "skewing" towards the side of the plate. Twisting differs among *arabidopsis* accessions and is known to be enhanced by interference with cortical microtubules. That cortical microtubules influence twistiness suggests that cellulose orientation is involved; however, cell wall structure has been little studied in twisting roots. We are characterizing the spatial profiles of elemental elongation rate and twist and are comparing them to the alignment of cellulose microfibrils. For these experiments, we have developed a hydroponic system so that the root twists without mechanical interference from an agar substrate, and we are analyzing microfibril orientation specifically in the outer epidermal cell wall. We will relate the observed pattern of growth to cell wall structure and we will test the deduced relation with an engineering model of an *arabidopsis* root. In this way, we hope to straighten out twisting and thus to enlarge understanding of how cell wall structure in a multicellular tissue conditions morphogenesis.

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#### C1B02

#### TRANSCRIPTIONAL REGULATION OF SECONDARY WALL BIOSYNTHESIS

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Secondary walls are the major constituent of wood, which is the most abundant biomass produced by plants. Secondary walls are composed mainly of cellulose, lignin and hemicellulose. To make secondary walls, genes involved in the biosynthetic pathways of cellulose, lignin and hemicellulose need to be coordinately switched on. Understanding the molecular switches controlling secondary wall biosynthesis in wood is of importance in basic plant biology as well as for potential genetic engineering of wood quality and quantity in tree species. We have been using *Arabidopsis* as a model to characterize genes involved in transcriptional regulation of secondary wall biosynthesis. We uncovered key roles of several transcription factors in regulating secondary wall biosynthesis. One of these transcription factors, SND1, together with its homolog were found to be master switches activating the biosynthetic pathways of the secondary wall components (*Plant Cell*, 18, 3158-3170, 2006; *Planta*, 225, 1603-1611, 2007; *Plant Cell*,

19, 2776-2792). In addition, we discovered a number of additional players in the SND1-mediated transcriptional regulation of secondary wall biosynthesis. We hypothesize that a transcriptional network is involved in the activation of secondary wall biosynthetic genes during wood formation. Further studies of the transcriptional network regulating secondary wall biosynthesis will likely enable us to genetically alter the biosynthetic pathways of individual secondary wall components, and knowledge gained from such studies promises to lead to better strategies for genetic manipulation of wood quality and quantity.

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#### C1B03

#### SEMITDOMINANT-NEGATIVE CESA3 MUTANT IN ARABIDOPSIS INHIBITS PRIMARY CELL WALL FORMATION

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Cell walls represent key determinants of overall plant form, growth and development, including plant responses to environmental and pathogen-induced stress. Interactions between cellulose synthases (CesAs) are required for normal cellulose synthesis, suggesting that incorporation of defective CesA subunits into the cellulose synthase complex could potentially cause a dominant effect on cellulose biosynthesis. However, cesA mutations characterized up to date have shown to be recessive. Performing a genetic screen of an EMS mutagenized Arabidopsis population, we isolated thanatos (than) a semidominant-negative mutation with impaired cellular architecture. than homozygous seedlings become lethal, while the growth of heterozygous plants is dramatically decreased. Map-based cloning revealed an amino acid substitution (P578S) at the central cytoplasmic catalytic domain located between the TMD2 and TMD3 of AtCesA3 gene (At5g05170). The AtCesA3 mutant gene was introduced into wild type Arabidopsis plants causing acquisition of than negative dominant phenotype. Abnormal cellular organization and gapped cell walls were observed in than heterozygous sections. Cell wall components analysis by FTIR and direct cellulose content measurements confirmed a dramatic reduction in cellulose levels between homozygous and heterozygous than plants compared to the wild type. Multiple sequence alignment of the protein region flanking Proline 578 revealed a conserved consensus between plant and non-plant CesA genes. Ab initio analysis of AtCesA3 subdomain flanking the conserved Pro residue predicted that the amino acid substitution to Ser alters protein tertiary structure. These data suggest that the incorporation of a single than defective CesA3 subunit into the rosette complex prevents the production of  $\beta$ -(1,4)-glucose chain, which could in a way stall cellulose microfibril synthesis.

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#### C1B04

#### PROCESSING OF TYPE I PECTIN METHYLESTERASE IN THE GOLGI APPARATUS: PREREQUISITE FOR EXTRACELLULAR TARGETING

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The pectin matrix of the cell wall, a complex and dynamic network, has emerged as one of the key components of the cell wall affecting growth, cell shape and signalling processes. A hallmark of pectin structure is the methylesterification status of its major component homogalacturonan (HGA), which affects its biophysical properties and enzymatic turnover. Responsible for deesterification are pectin methylesterases (PMEs), encompassing a protein family of more than 60 isoforms in the Arabidopsis genome. The pivotal role of PME for pectin structure requires tight control, including post-translational regulation. Type 1 PMEs are characterized by an N-terminal pro region, which exhibits homology to pectin methylesterase inhibitors (PMEI). To elucidate its role, we explored the mechanism of its proteolytic

removal from the catalytic PME domain. Here, we demonstrate that the removal of the N-terminal pro region depends on conserved basic tetrad motifs, occurs in the Golgi, and is required for subsequent export of the PME core domain to the cell wall. Our results indicate that the pro region operates as an effective retention mechanism, keeping unprocessed PME in the Golgi. In addition, we provide evidence for the involvement of a subtilisin-like protease in PME processing and transport, enabling the active enzyme to act on its substrate in the cell wall.

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#### C2A01

#### OPPORTUNITY OR MISFORTUNE – OUTCROSSING IN ARABIDOPSIS THALIANA

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*Arabidopsis thaliana* is primarily selfing, but outcrossing – however rare – may nevertheless play an important role in generating phenotypic novelty and averting genetic meltdown due to accumulation of deleterious alleles. To begin gaining insight into how outcrossing affects progeny of lineages that are largely independently diverging, we intercrossed 293 wild *A. thaliana* accessions in 1430 combinations. 21 combinations showed strongly deleterious phenotypes attributable to hyperactivation of the plant immune system, and in at least two cases caused by known or suspected resistance (R) genes. Because R genes are especially diverse, it is logical that they might be among the earliest causes of genetic incompatibility among diverging lineages. Since we observe both strong positive and negative effects of hybridization in the laboratory, we are currently investigating the role that outcrossing may play in generating phenotypic novelty and adaptive opportunity in natural populations. We collected 1000 *A. thaliana* individuals from 95 ecologically diverse sites near Tübingen, Germany, and are genotyping them with 540 SNP markers across all 5 chromosomes. Among 950 plants genotyped to date at 149 SNP markers, we have identified 275 distinct whole-genome genotypes. 15 candidate heterozygotes had parental genotypes clearly identifiable in the same locale; the marker composition allowed identification of F1 as well as later generation offspring. The picture emerging from these results is that crossing can increase genetic diversity in *A. thaliana*; we are currently investigating how this impacts phenotypic diversity. Thus, though there may be some negative effects, spurts of heterozygosity and genetic novelty could also serve to reinvigorate *A. thaliana* populations and mitigate the disadvantages of selfing.

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#### C2A02

#### CARDAMINE HIRSUTA AS A MODEL SYSTEM FOR STUDIES IN EVOLUTION OF PLANT DEVELOPMENT

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A key problem in biology is to understand how diversity in organismal form is generated. Diversification in form of reproductively isolated species has been difficult to study because of the paucity of experimental systems where the developmental genetic changes underlying morphological variation can be accurately identified. To investigate this problem we study the genetic mechanisms underlying variation in form of the predominant photosynthetic organ of plants, the leaf. Leaf form can be classified as simple, where the leaf blade is entire as in the model organism *Arabidopsis thaliana*, or dissected where the blade is divided into distinct units called leaflets. Mechanisms that determine specification of dissected versus entire leaf shape and regulate the number, position and timing of leaflet production are poorly understood. To obtain an in-depth and unbiased understanding of these mechanisms we established *Cardamine hirsuta* - dissected leaf relative of *A. thaliana* - as a versatile experimental system where both forward and reverse genetics, and stable genetic transformation can be deployed for studying diversification of form. Here we discuss how comparisons between *A. thaliana* and *C. hirsuta* have illuminated our understanding of processes underlying the evolution of form.

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C2A03

POPULATION GENOMICS AND EPIGENOMICS OF ARABIDOPSIS THALIANA TRANSPOSABLE ELEMENTS

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Arabidopsis thaliana shared a common ancestor with its closest known relative, *Arabidopsis lyrata*, approximately 5 mya. Thus, these two species provide an ideal system for the study of the evolutionary processes leading to diversity within, and divergence between species. In this study, we measure the frequency of a genome-wide sample of 720 transposable element (TE) insertions in *A. thaliana*, and estimate the proportion of these insertions shared with *A. lyrata*. We compare both the TE site-frequency-spectrum and the ratio of polymorphic to fixed insertions with genome-wide nucleotide polymorphism data in order to estimate the strength and mode of natural selection on insertions. Using available epigenomic data, we show how variation in small RNA targeting of TEs relates to proliferation, copy-number stabilization, and quiescence of TE families. We also illustrate how TE-gene interactions affect, and are affected by, epigenetic TE-host interactions.

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C2A04

SEASONAL AND GEOGRAPHIC VARIATION IN SENSITIVITY AND BALANCE OF FLOWERING PATHWAYS

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Flowering is a critical life history event, and the seasonal timing of flowering is often under strong natural selection. To flower during favorable seasonal conditions, plants must integrate and respond appropriately to multiple environmental signals, such as day length, ambient temperature, and vernalization. These external signals mediate several converging developmental signaling pathways, which have been well characterized under laboratory conditions. However, very little is known about the balance and sensitivity of different pathways to complex, combinatorial environmental cues found under natural conditions. To measure the sensitivity of flowering time to perturbations in environmental signal perception in natural seasonal environments, we grew *Arabidopsis thaliana* mutants impaired in different **pathways in replicated field experiments across the species' native European climatic range, in Finland, England, western and eastern Germany, and Spain**. The relative contribution of signaling pathways varied widely across seasons and geographic locations; the photoperiod pathway predominated in determining the flowering time of summer annuals, and the vernalization pathway played a larger role in later autumn germinating cohorts. In spring and autumn cohorts in milder climates, mutational perturbation of every pathway had significant effects on flowering time. Moreover, most of the variation in flowering time across genotypes, sites, and seasons could be explained by a genetically informed photothermal model of progression toward flowering, in which mutants impaired in the ability to sense day length or vernalization cues differ in developmental responses to hourly temperature and photoperiod environments. Thus, by combining environmental data with information about pathway function, it is possible to predict the flowering behavior of *A. thaliana* across a broad range of climatic conditions.

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C2A05

RAPID EVOLUTIONARY DIVERGENCE IN ALTERNATIVE SPLICING PATTERNS FOLLOWING GENE AND GENOME DUPLICATION

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Polyplody and alternative splicing are two important processes that increase proteome diversity in plants. There are about 2,600 gene pairs in *Arabidopsis thaliana* that remain from the most recent paleopolyploidy event that occurred approximately 35 million years ago after divergence of the Brassicaceae from the Cleomaceae. We have studied the evolution of alternative splicing after genome duplication by comparing alternative splicing patterns between the genes in duplicated pairs. We used RT-PCR to examine alternative splicing in 50 gene pairs among cDNAs from eight organ types of *Arabidopsis thaliana*. For most genes we found differences in alternative splicing patterns between the gene pairs duplicated by polyplody. These differences include presence or absence of particular splicing forms and distinct organ specificity. Using three different abiotic stress experiments, we found that alternative splicing patterns in many gene pairs respond differently to stress. In addition we examined alternative splicing in several sets of tandemly duplicated genes, many of which were duplicated after the paleopolyploidy event. Alternative splicing was conserved in only about 20% of the gene pairs. Changes in alternative splicing patterns in duplicated genes may contribute to functional or regulatory divergence. Our results indicate that alternative splicing patterns in duplicated genes evolve rapidly on an evolutionary time scale.

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C2B01

A ROLE FOR STIGOLACTONES IN ARABIDOPSIS: A NEW PLANT HORMONE

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Parasitic weeds of the genera *Striga* and *Orobanche* are considered the most damaging agricultural agents in the developing world. In Africa alone *Striga* species have infested up to two-thirds of the arable land and are thought to cause tens of billions of dollars in lost crop yields. To ensure coordination with a host, parasitic plant seeds only germinate when they sense a group of related compounds, called strigolactones, which are released by the host root. Although this makes strigolactone synthesis and action a major target of biotechnology the parasitic lifestyle and the lack of molecular and genetic tools makes studies on these weeds problematic.

Although parasitic plants use strigolactones produced by the host as a germination stimulant, the first active strigolactone, Strigol was actually purified from a non-host cotton plant, which is not normally infected by *Striga* species. The ability of non-host plants to germinate parasitic plant seeds suggests strigolactones may exist ubiquitously in higher plants and have roles that are independent of host-parasite interactions.

Here we show using a combination of chemical and classical genetics that, as observed in parasitic plants, strigolactones can play an analogous role in seed germination in the model organism *Arabidopsis*. We identified mutants deficient in phytochromobilin synthesis that require strigolactones for good germination and show these mutants are inefficient at stimulating parasitic seed germination. A rice mutant deficient in phytochromobilin synthesis is also inefficient at stimulating *Striga* germination demonstrating that *Arabidopsis* may be useful in identifying genes for crop breeding programs against parasitic weeds. We are also able to show that many aspects of the phytochromobilin deficiency are rescued by strigolactone addition suggesting these compounds may play an important role in light and

retrograde signaling in plants. It therefore appears that strigolactones not only function as signaling molecules between parasitic and host plants but also act endogenously like a hormone in germination and early seedling growth.

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C2B02

#### ETHYLENE-AUXIN INTERACTIONS IN ARABIDOPSIS

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Survival of plants largely depends on their ability to coordinate internal programs, such as growth and development, with the external conditions of the ever-changing environment. Central to this integration process are plant hormones that act as executors of both internally and externally generated signals. It is remarkable that a limited set of plant hormones is capable of triggering a large number of tissue-specific developmental stage-dependent changes in response to particular environmental conditions. Evidently, interactions between different hormones (or hormones and other signaling pathways) are critical for achieving this wide diversity of plant responses. To start to understand the molecular mechanisms behind these interactions, we are focusing on two hormones, ethylene and auxin, and on their role in controlling growth of different plant tissues under different environmental conditions. Using a combination of genetic, cellular, and molecular approaches some of the genes and molecular mechanisms involved in ethylene-auxin crosstalk are being uncovered. Among the identified genes that participate in these interactions are WEI8/TAA1 and TARs, a new family of auxin biosynthetic genes. A detailed characterization of these genes not only confirms their role in the interaction between ethylene and auxin, but also supports the idea that local auxin production may play a key role during development as well as in response to environmental cues. Further characterization of these and other genes recently identified in the lab would provide a better understanding of the molecular network of ethylene-auxin interactions and their role in the integration of endogenous and exogenous signals.

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C2B03

#### MORE THAN A SIMPLE SWITCH: ADDRESSING SPECIFICITY IN ARABIDOPSIS MAPK SIGNALING NETWORKS USING STOMATAL DEVELOPMENT AS A MODEL

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Mitogen activated protein kinase (MAPK) signaling networks are common to all eukaryotes where they serve to translate vast arrays of input stimuli into discrete cellular outcomes. The scope of these networks is broad-ranging; MAPKs are involved in, amongst others, processes such as cell division and responses to environmental stimuli, hormones and pathogens. The *Arabidopsis* genome encodes upwards of 60 MAPKKKs, 10 MAPKKs and 20 MAPKs. Progress has been made identifying particular aspects of these networks that are essential to plant survival. However, the overwhelming involvement of a specific few MAPK components (namely MKK4/5 and MPK3/6) in virtually every biological context tested highlights the importance of addressing how and why a select few, ubiquitously expressed, kinases can direct specific responses.

Recently the YDA-MKK4/5-MPK3/6 signaling module was demonstrated to play a significant negative regulatory role in stomatal development (Wang et al., 2007). Stomatal development involves a multi-step process in which cells undergo asymmetric and symmetric divisions and display discrete morphological characteristics at each stage. The extent to which this module (and other MAPK modules) influences any particular stage was not resolved by these initial experiments. By expressing a collection of active/inactive MAPK component variants with promoters derived from the bHLH transcription factors that are specifically expressed in each discrete stage in stomatal development, we have created an *in vivo* assay

system for MAPK signaling that has allowed us to identify new players in stomatal development and provide novel insights into MAPK signaling specificity during each stage in the process.

Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. 2007. Plant Cell. 19(1):63-73.

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C2B04

GOLVEN SECRETORY PEPTIDES CONTROL PLANT GRAVITROPISM

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In a systematic reverse genetic screen for potential signaling peptides, we have identified a family of genes whose overexpression results in root agravitropic and wavy phenotypes. The family was called *GOLVEN* (*GLV*), meaning waves in Dutch. *GOLVEN* genes are specific to plants and code for small peptides that carry an N-terminal signal peptide and a C-terminal conserved motif, dubbed the GLV motif. When applied to *Arabidopsis* plantlets, short peptides derived from the GLV motif also induce dose-dependent agravitropic phenotypes. *GLV* gain- and loss-of-function mutants display root as well as hypocotyl gravitropic defects. Interestingly, *GLV1* and *GLV2* are transcribed asymmetrically in bending gravistimulated hypocotyls. This and further experiments investigating the potential mode of action of the *GOLVEN* proteins suggest that phytohormone activity can be modulated by secretory peptides and vice-versa. Such cross-talks might form regulatory feedback loops essential in complex signaling networks.

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C2B05

CHEMICAL GENETIC IDENTIFICATION OF A NEW FAMILY OF ABA RESPONSE FACTORS

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A new and complementary approach to genetic pathway dissection is to systematically identify small molecule “perturbagens” of the pathway in phenotype-based screens. These molecules can be then used as reagents to identify protein components of the pathway (i.e. the forward chemical genetic approach). The Cutler lab has an ongoing chemical genetic research program focused on identifying and characterizing new small molecule modulators of plant cell growth. This program has uncovered a new class of naphthalene sulfonamide growth regulators, called pyrabactins (for pyridyl containing ABA activators), which act as agonists (i.e. activators) of the seed ABA signal transduction pathway. A genetic screen for pyrabactin resistant mutants identified recessive alleles in a locus called Pyr1 (pyrabactin resistance 1) that encodes a Bet V protein family member. Structurally, this family is characterized by the presence of a hydrophobic ligand-binding pocket, otherwise their in vivo functions are poorly defined. An emerging theme in plant signal transduction is that plant hormones can induce downstream effects by modulating protein-protein interactions between receptors and effectors. In this context, the predicted ligand binding properties of Bet V proteins coupled to the requirement of PYR1 for pyrabactin activity, suggested that the binding of pyrabactin to PYR1 might trigger an ABA response by promoting a protein-protein interaction. Indeed, a yeast two-hybrid (Y2H) screen using PYR1 as bait identified a PP2C as an interactor. The PP2C-PYR1 interaction depends on the inclusion of pyrabactin in the yeast growth media, and thus behaves as a ligand induced protein-protein interaction. This interaction has been reconstituted in vitro, and is not triggered by an inactive analog of pyrabactin. Moreover, the interaction between PYR1 and the PP2C is triggered by ABA in both the Y2H and in vitro assays. Thus, we propose that PYR1 is a new ABA receptor. The *Arabidopsis* genome encodes ~65 Bet V family members, and 4 of the 12 closest relatives of PYR1 (named PYL1 - 4) also possess ABA receptor activity in the Y2H assay. Thus, our combined chemical and genetic approach has identified a new family of putative ABA receptors, which we call the PYR/PYL family. Genetic and biochemical characterization of the PYR/PYL family is ongoing.

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### C3A01

#### BACTERIAL VIRULENCE FACTORS AS MOLECULAR PROBES OF BASIC PLANT CELLULAR FUNCTIONS

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To successfully colonize plants, pathogens have evolved a myriad of virulence factors that allows them to manipulate host cellular pathways in order to gain entry into, multiply and move within, and eventually exit the host for a new infection cycle. In the past few years, substantial progress has been made in characterizing the host targets of pathogen virulence factors, providing unique insights into a variety of basic plant cellular processes. We are studying the compatible interaction between *Arabidopsis thaliana* and the bacterial pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (Pst DC3000), focusing on two virulence systems: the type III protein secretion system (TTSS) and the phytotoxin coronatine (COR). The TTSS delivers virulence effector proteins into plant cells to modulate host physiology. COR functions as a molecular mimic of the plant hormone jasmonic acid-isoleucine (JA-Ile). Study of the molecular action of effector proteins and coronatine is revealing several fundamental aspects of plant physiology, including vesicle traffic, stomatal function, jasmonate signaling, and innate immunity. Identification of the host targets of additional pathogen virulence factors promises to continue shedding light on fundamental cellular mechanisms in plants, thus enhancing our understanding of plant signaling, metabolism, and cell biology.

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### C3A02

#### DISSECTING MOLECULAR AND CELLULAR PROCESSES REGULATING PLANT RESPONSES TO NECROTROPHIC FUNGAL PATHOGENS

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Necrotrophic pathogens are diverse economically important fungal and bacterial species with destructive pathogenesis strategies. Despite their enormous economic significance as plant pathogens, host resistance mechanisms against necrotrophic pathogens are poorly understood. The molecular events associated with infection processes, infection related morphogenesis and plant resistance strategies to necrotrophic pathogens are different from that of obligate biotrophs. Recent studies have started to illuminate differences and similarities in host responses to pathogens of different life styles. Regulatory mechanisms in plant defenses to necrotrophic pathogens and how these relate to other defense pathways have been studied. Genome scale approaches identified a network of proteins mediating responses to *Botrytis cinerea* and *Alternaria brassicicola*, two typical necrotrophic fungi. Functional analyses of components of this network reveal the significance of different cellular processes in regulating plant resistance to fungal necrotrophs. Genetic analysis also reveals a complex network of interacting genetic factors. I will discuss the role of histone modification in plant defense to *B.cinerea* and *A.brassicicola*. This histone modification is closely linked to the regulation of transcription through interaction with a component of the *Arabidopsis* mediator, an evolutionarily conserved multi-subunit complex that regulates the function of RNA polymerase II in transcription. Mediator is required for diverse transcriptional activation processes including the expression of antimicrobial peptides in *Drosophila*. Thus, mediator may relay environmental cues including pathogen derived signals to RNA polymerase II, modulating expression of genes required for defense and other functions.

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### C3A03

#### GENETIC ANALYSIS OF ARABIDOPSIS DEFENSE SIGNALING IN RESPONSE TO PAMPS

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One of the first layers of active defense in plant-microbe interactions is based upon the recognition of pathogen associated molecular patterns (PAMPs). PAMPs are perceived by cell surface receptors that typically stimulate the generation of an oxidative burst, activation of a MAP kinase cascade, callose deposition and reduced seedling growth. Known ligand-receptor pairs in PAMP perception include flagellin (flg22) and EF-Tu (elf18) from bacteria, which are recognized by the Arabidopsis receptor kinases FLS2 and EFR, respectively. Additionally, the receptor kinase BAK1 is required for responses towards both PAMPs, and was described to form a complex together with activated FLS2. Importantly, fls2 mutants exhibit an enhanced susceptibility when phytopathogenic *Pseudomonas syringae* pv tomato DC3000 bacteria (PtoDC3000) were inoculated onto the leaf surface.

Although PAMP perception and signaling became a focus within the past years, many components contributing to PAMP-triggered immunity remain to be identified. Therefore, we applied forward genetic approaches to identify mutants impaired in flg22 responses. Screening an Arabidopsis gamma-irradiation population resulted in the identification of about 70 fli mutants (for flagellin-insensitive). We will present the further characterization of fli1 to fli8 mutants. Some fli mutants exhibited weak or no flg22-triggered callose deposition, while most were normal in the oxidative burst. In addition, most fli mutants showed normal elf18 responses, while some were also impaired in elf18-triggered callose deposition. Notably, fli1 to fli8 mutants are not allelic to FLS2 or BAK1, indicating that novel components in flg22 signaling could be isolated. First results also showed that fli mutants were more susceptible to infection with PtoDC3000. Currently, mapping of two fli mutants is in progress.

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#### C3A04

#### TOWARDS A BACTERIAL EFFECTOR-ARABIDOPSIS TARGET PROTEIN-PROTEIN INTERACTION NETWORK

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Diverse gram-negative bacteria deliver effector molecules via the type III secretion system (TTSS) directly into the host cell cytoplasm. These bacterial effector proteins target an increasing number of host proteins, often by mimicking eukaryotic proteins to interfere with disease resistance signaling pathways. We performed a whole-genome high-throughput screen for identifying *Pseudomonas syringae* type III effector genes from 15 different strains, pathogenic on an evolutionarily widely spread set of host genera. We identified more than 300 putative effectors that fall into over 80 families. In order to find host targets of all the type III effector families, we performed a large scale yeast two-hybrid (Y2H) screen. We generated 115 baits representing nearly all type III effector families from 15 *P. syringae* strains and screened them against a pathogen-induced cDNA library from Arabidopsis. We found that 30 type III effector proteins, representing only 20 type III effector families, interacted with 12 novel Arabidopsis proteins. This relatively compact interactome included examples where multiple effectors interacted with the same host protein, consistent with our previous findings. Currently, we are testing the functional relevance of the key host target proteins found in our Y2H screen.

We are expanding this interactome map by including two receptor protein families involved in plant defense. Cell-surface localized Pattern recognition receptors (PRRs) and cytosolic receptor NB-LRR proteins (nucleotide-binding, leucine-rich repeat) play crucial roles in disease resistance. We generated clones of coiled-coil (CC)/Drosophila Toll or mammalian Interleukin 1 receptor (TIR) domains from 140 NB-LRR proteins, and 180 kinase domains of Receptor like Kinase (RLKs, a sub-class of PRR). Using a stringent, high-throughput and semi-automated Y2H system, we are currently testing pairwise interactions among

bacterial effector proteins, Arabidopsis target proteins identified in our cDNA library screen, the N-termini of NB-LRR proteins and the PRR kinase domains. An initial draft of the Arabidopsis immunity interactome, consolidating all of our findings, will be presented.

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#### C3A05

#### LASER MICRODISSECTION AS A TOOL FOR PROBING THE ARABIDOPSIS RESPONSE TO THE POWDERY MILDEW GOLOVINOMYCES ORONTII AT THE INFECTION SITE

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Plant-pathogen interactions are marvelously complex and intricate. In order to dissect this complexity, we sought to develop a system for which we could evaluate, with spatial and temporal resolution, the progression of a virulent interaction. For this purpose, we chose the biotrophic fungus *Golovinomyces orontii* (formerly known as *Erysiphe orontii*), the causative agent of powdery mildew on leaves of *Arabidopsis* and other plants. We selected *G. orontii* for this study as it exclusively infects epidermal cells of *Arabidopsis* and it exhibits clearly defined stages of infection (i.e. germination, penetration, haustorial complex formation, and growth and reproduction) that are visible under the microscope. We evaluated a variety of methods for the isolation of powdery mildew-infected cells from leaf tissue and found UV laser microdissection (LMD) to be superior for our purposes. In order to prepare fragile *Arabidopsis* leaf tissue for LMD, we developed a novel tissue preparation method that resulted in excellent preservation of leaf internal structure including epidermal cells, as well as mRNA integrity [Inada and Wildermuth (2005) *Planta* 221: 9-16]. Optimization of mRNA isolation and amplification protocols for use in global expression profiling using Affymetrix *Arabidopsis* ATH1 GeneChips was then performed, with highly reproducible results obtained from ~2500 pooled cells (~2 ng RNA). Detailed and novel analysis of the impact of tissue preparation, LMD, and amplification protocols on mRNA degradation found that degradation was not an issue for ATH1 analyses. Furthermore, biological replicates were highly correlated ( $r = 0.98$ ). For our first dataset, we focused on the growth and reproduction stage of the powdery mildew infection (5 days post infection) in which we could visualize hyphal growth, the number of conidiophores per colony and conidia per conidiophore as quantitative indicators. Expression profiling was performed on LMD-isolated wild type *A. thaliana* Col-0 cells at individual sites of infection that were pooled for RNA isolation. Similar cells were also isolated and pooled from leaves of parallel uninfected plants. To examine genes impacted by the phytohormone salicylic acid (SA), we also included analysis of the SA biosynthetic mutant *ics1*. By examining cells localized to the site of infection, we identified both known and novel *Arabidopsis* genes, pathways, and processes associated with this infection phase. Putative novel regulators of the interaction were found and mutants in these regulators are being evaluated to determine their impact on powdery mildew growth and reproduction.

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#### C3B01

#### LOCUS-SPECIFIC CONTROL OF DNA METHYLATION BY THE SUVH5 AND SUVH6 HISTONE METHYLTRANSFERASES

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Transcriptional gene silencing mediated by DNA methylation plays important roles in genome stability and gene regulation. In *Arabidopsis*, DNA methylation is maintained by the combined action of three cytosine methyltransferase pathways. The DRM2 pathway initiates new DNA methylation imprints in response to small RNA signals and maintains methylation in non-CG contexts, the MET1 pathway

maintains methylation in CG contexts, and the CMT3 pathway maintains methylation in non-CG contexts. Our research focuses on the CMT3 pathway. In previous work we found that three histone H3 lysine 9 methyltransferases—SUVH4, SUVH5, and SUVH6—are required for CMT3-mediated DNA methylation. SUVH4 controls the majority of CMT3-dependent DNA methylation genome-wide whereas SUVH5 and SUVH6 make locus-specific contributions. For example, SUVH5 is required for non-CG methylation of AtMu1 transposons and SUVH6 is required for non-CG methylation of a transcribed inverted repeat gene duplication PAI1-PAI4. We are dissecting the sequence requirements for SUVH5 versus SUVH6 locus-specificity using SUVH transgene constructs transformed into a suvh4 suvh5 suvh6 mutant background, followed by analysis of DNA methylation patterning at the preferred SUVH5 target AtMu1 versus the preferred SUVH6 target PAI1- PAI4. The transgene approach has allowed us to map the determinants for locus-specificity to the SUVH coding sequences rather than the expression sequences. Transgenes with swaps between SUVH5 and SUVH6 coding sequence domains will further elucidate the mechanism of locus-specific epigenetic targeting.

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#### C3B02

THE FUNCTION OF SWR1 COMPLEX FOR FLORAL REPRESSION AND EVOLUTION OF ARP6 PROTEIN  
Kyuga Choi, Chulmin Park, and \*Ilha Lee National Research Laboratory of Plant Developmental Genetics, Department of Biological Sciences, Seoul National University, Seoul, 151-742, Korea  
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SWR1 complex (SWR1C) in yeast catalyzes the replacement of nucleosomal H2A with the H2AZ variant, which ensures full activation of underlying genes. We compared the phenotype of mutants in the homologs of SWR1C components in Arabidopsis. The mutations in SUF3 (=ARP6), PIE1 (=SWR1 homolog), and SWC6 caused similar developmental defects such as leaf serration, weak apical dominance, and early flowering by the reduction of FLOWERING LOCUS C (FLC), a strong floral repressor. SWC6 protein is colocalized with SUF3 in protoplast transfection assay. In yeast two hybrid analysis, PIE1, SUF3, SWC6, and SWC2 are directly interconnected and H2AZ interacts with both PIE1 and SWC2. Finally, knockdown of the H2AZs by RNA interference or artificial microRNA caused similar phenotype with swc6 or suf3. These results strongly suggest that SWR1 complex is conserved among eukaryotic organisms. Thus, we further analyzed how ARP6 protein is evolved and what constrains the evolution of ARP6 proteins. The recent results will be discussed.

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#### C3B03

EVIDENCE FOR NON-MENDELIAN INHERITANCE OF ANCESTRAL SEQUENCES IN ARABIDOPSIS  
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There exist in plant systems several known exceptions to the classical Mendelian laws of inheritance. Recently we have described an unprecedented mechanism of non-Mendelian inheritance in which the progeny inherit DNA sequences not found in the genomes of the parents. This process has been coined **"restoration"** since our findings indicate that sequences return to a previously existing ancestral state. In this study, we have demonstrated that restoration occurs in populations of hothead plants grown in isolation. Furthermore, in the presence of pollen donors restoration events are distinct from outcrossing events that may occur within the population. These findings demonstrate that the source of template that drives restoration is intrinsic to the plant itself.

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#### C3B04

FUNCTIONAL CONSEQUENCES OF LOCAL SECONDARY STRUCTURES ON MIRNA-TARGET RNA INTERACTION

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Post-transcriptional gene regulation is a wide-spread phenomenon that facilitates the spatial and temporal fine-tuning of gene expression in both plants and animals. Major players are small RNAs, which base-pair to target RNAs and trigger their cleavage or inhibit their translation. MicroRNAs (miRNAs), a mainly trans-acting class of small RNAs, has been shown to bind and regulate mRNA targets of high sequence complementarity in plants, and detailed determinants of target selection have been described. The transgenic expression of modified silencing triggers can exploit the endogenous silencing machinery to selectively inhibit one or several genes of interest, and artificial miRNAs (amiRNAs) do not exceed the targeting determinants of their endogenous counterparts. However, a significant fraction of amiRNAs does not confer detectable gene silencing. While unfavorable intrinsic properties of target genes, such as negative feedback regulation, might explain some of those instances, others require alternative explanations, since additional small RNAs targeting the same genes function very effectively. A likely explanation for decreased miRNA effectiveness is reduced accessibility of target sites, similar to recent observations in animals.

We have taken two experimental approaches to investigate the impact of local secondary structures in target sites on miRNA functionality. First, we generated numerous amiRNAs targeting only a few endogenous genes, and indeed found variable silencing efficiencies depending on the structural context of the target site. Furthermore, we directly modify the structural context of target sites by introducing silent mutations in the target site-surrounding region and analyze individual parameters that influence miRNA efficiency.

We will present our current model of miRNA-target RNA interaction, and discuss its impact on the evolution of miRNA-mediated gene regulation.

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#### C3B05

A PLANT TRANSCRIPTION FACTOR PLAYS A REQUIRED ROLE IN VIRAL SUPPRESSION OF SILENCING

\*Matthew Endres, Zhihuan Gao, Amy Wahba, Sizolowenksi Mlotshwa, Xin Ge, Lewis Bowman and Vicki Vance Department of Biological Sciences University of South Carolina Columbia, SC 29208  
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RNA silencing is a sequence-specific RNA degradation pathway that serves as an antiviral defense in plants. The double-stranded RNA (dsRNA) trigger of silencing is processed into primary siRNAs which direct destruction of complementary RNAs. The process can be amplified by transitive silencing, which produces secondary siRNAs from dsRNA generated via cellular RNA-dependent RNA polymerase(s). Here we report that two unrelated plant viral suppressors of silencing require an *Arabidopsis thaliana* transcription factor, RAV2/EDF2, to block silencing induced by a hairpin transgene. Neither potyviral P1/HCPo nor TCV p38 can block target RNA degradation directed by primary siRNAs in *rav2/edf2* mutant plants, although both still block secondary siRNA accumulation. These results suggest that RAV2/EDF2 is a key regulator of silencing pathways and a focal point of viral counter-defensive strategies.

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#### C4A01

THE PLANT CLOCK AND ITS OUTPUTS

\*Harmer, Stacey; Covington, Michael; Ellison, Cory; Martin-Tryon, Ellen; Rawat, Reetika; Schwartz, Koby Department of Plant Biology, University of California, Davis, USA  
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The circadian clock plays a pervasive role in the temporal regulation of plant physiology, environmental responsiveness, and development. Plants that have a clock whose periodicity matches rhythmic changes in the environment have an adaptive advantage over those that do not. We are interested in identifying the molecular components that comprise the plant circadian clock and understanding how the clock affects plant growth and development.

Although circadian rhythms are seen in most eukaryotes and even some prokaryotes, the molecular components underlying clock function are not conserved across higher taxa. We have carried out genetic

screens in *Arabidopsis* to identify clock-associated genes. One recently-cloned gene, XCT, is involved both in clock function and in plant responses to light. XCT is highly conserved across eukaryotes but its biochemical function is not known.

In our study of clock output pathways, we have found that the circadian clock modulates plant responses to the essential hormone auxin. Auxin-dependent transcriptional responses to both endogenous and exogenous hormone are regulated by the clock. We have recently identified a gene that helps link the plant clock with auxin response pathways.

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C4A02

DELAYED LEAF SENESCENCE INDUCES EXTREME DROUGHT TOLERANCE IN FLOWERING PLANTS.

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Drought, the most prominent threat to agricultural production worldwide, accelerates leaf senescence, leading to a decrease in canopy size, loss in photosynthesis and reduced yields. On the basis of the assumption that senescence is a type of cell death program that could be inappropriately activated during drought, we hypothesized that it may be possible to enhance drought tolerance by delaying drought-induced leaf senescence through the stress-induced synthesis of cytokinins. We generated transgenic plants expressing IPT, an isopentenyltransferase gene, driven by pSARK, a stress- and maturation-induced promoter. Remarkably, the suppression of drought-induced leaf senescence resulted in outstanding drought tolerance as shown by, among other responses, vigorous growth following a long drought period that killed the control plants. The transgenic plants expressing pSARK-IPT maintained high water contents and retained photosynthetic activity (albeit at a reduced level) during the drought. A comparison of the CO<sub>2</sub>-dependent rate of photosynthesis (A/Ci curve) from wild-type and transgenic plants showed that drought severely affected the RuBP regeneration and triose phosphate use of wild type plants but not of transgenic plants. Ultrastructural analysis of leaf parenchyma cells and metabolite analysis of leaf contents indicated the occurrence of enhanced photorespiration in the pSARK-IPT transgenic plants leading to CO<sub>2</sub> fixation under drought conditions. The transgenic plants displayed minimal yield loss when watered under reduced watering regimes of only 30% of the amount of water used under control conditions. Transgenic plants grown under reduced watering also displayed the induction of photorespiration observed under severe drought conditions. The production of drought-tolerant crops able to grow under restricted water regimes without diminution of yield would minimize drought-related losses and ensure food production in water-limited lands.

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C4A03

ATGTL1 TRANSCRIPTION FACTOR REGULATES WATER USE EFFICIENCY AND DROUGHT ADAPTATION THROUGH CA<sup>2+</sup>/CALMODULIN SIGNALING

\*Yoo, Chan Yul Jin, Jing Bo Miura, Kenji Gosney, Mike Jin, Yinhua Mickelbart, Michael V. Hasegawa, Paul M. Purdue University, West Lafayette, IN, USA  
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Drought is a major limitation to plant growth and productivity. Water use efficiency (WUE) is an important trait of drought adaptation in plants, but the genetic basis for how plants regulate WUE is not known. Plants have complex adaptation mechanisms that include sensing and signaling, and adaptation processes. Calcium (Ca<sup>2+</sup>) is a focal secondary messenger that is implicated in drought stress signaling of plants, and calmodulin (CaM) is presumed to be one of the primary Ca<sup>2+</sup> signature-decoding molecules. Genome-wide screening of expression libraries using labeled recombinant CaM has revealed that AtGT-2 (GT elements-binding proteins) family are potential CaM binding transcription factors. AtGTL1, one of the AtGT-2 family, encodes a putative Ca<sup>2+</sup>/CaM binding transcriptional activator. gtl1 T-DNA insertional mutations (gtl1-1, gtl1-2 and gtl1-3) substantially enhance the capacity of plants to

survive in response to severe water deficit stress by which maintain leaf relative water content during dehydration through reduced transpiration. Furthermore, gtl1 mutations increase WUE, indicating that AtGTL1 is a transcriptional regulator of drought adaptation and WUE. AtGTL1 transcript abundance decreased with dehydration stress and ABA treatment, which is consistent with the notion that the transcription factor is a negative regulator of drought adaptation response, which is important to maintain homeostasis for adaptation processes. We hypothesize that Ca<sup>2+</sup>/CaM-mediated GTL1 regulates drought stress signaling, adaptation and WUE through mechanism by which is linked to efficient carbon usage process. This research will provide functional understanding about how plants decode Ca<sup>2+</sup>/CaM signals to initiate stress adaptation processes and WUE that could enhance crop yield stability under water deficit conditions, and regulate central transcription factors by phytohormone signaling to change rapid gene expression, which is one of key-adaptation processes for plants.

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#### C4A04

##### CELL TYPE SPECIFIC RESPONSES TO ACID STRESS IN ARABIDOPSIS

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Plants have continuous development from a set of stem cells that gives them the plasticity to alter organ growth almost immediately upon response to abiotic stress. These developmental changes are the result of complex regulatory networks within specific cell types that compose whole organs. Understanding how these stresses are perceived and translated into developmental changes is an important goal in plant biology, as it will facilitate both an understanding of cell identity and the identification of novel alleles for crop improvement.

**The Arabidopsis root's simple radial structure, well-defined cell types, and developmental plasticity make it a tractable system to study environmental stresses.** Acid soils are found in approximately 50% of global arable lands. Though they are a limiting factor for food production both in the United States and developing nations, little is known about how plant roots respond to low pH. Here we present the first in-depth analysis of the **Arabidopsis root's** response to low pH at both the whole organ and cell type specific levels. We show that under acid stress, growth of the primary root is severely inhibited, and we identify new low pH responses, including agravitropic effects, and the alteration of lateral root development. Using 3 different microarray datasets comprising a whole root time course, 6 radial cell types, and 4 longitudinal sections covering developmental time, we examine the transcriptional program of the root under acid stress. We find that biological functions are differentially affected in specific cell types, and that these functions can be correlated to our observed phenotypes. Of the 6 cell types examined, the columella is the most responsive, and alterations in biological functions associated with gravity sensing are observed. We also examine T-DNA insertion lines in genes differentially affected by acid stress and identify a mutant hypersensitive to low pH. Finally, we compare the low pH stress response to those observed under high salt and iron deficiency to begin to understand universal stress responses.

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#### C4A05

##### VARIATION IN THE ROOT GRAVITROPIC RESPONSE CHARACTERIZED WITH HIGH-THROUGHPUT COMPUTER VISION ANALYSIS

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The way in which a plant responds to its environment is partially mediated by its genetic makeup. Variables external to genetic composition can also significantly influence how an organism responds to environmental stimuli. To better understand how non-genetic factors affect the root gravitropic response, computer-vision tools have been developed that enable the rapid extraction of morphometric data such

as tip angle and growth rate from high-resolution image series. We have compiled a dataset of over 1300 wild-type roots responding to gravi-reorientation over a ten-hour period. In creating this data set it is possible to establish and describe the range and types of root gravitropic responses displayed in *Arabidopsis* across any number of experimental axes; in this study we have characterized the responses of wild-type *Arabidopsis* (Columbia) across three axes: developmental age, media condition, and seed size. Global analysis of this multidimensional data set resulted in robust extraction of features such as pausing near 45 degrees, overshooting 90 degrees, and backbending to realign with the vertical. Overshooting and backbending behaviors were more frequent in younger seedlings coming from larger seeds grown on a complex medium. Of the entire dataset of 1339 individual roots reorienting to align with the gravity vector, the vast majority pause in their tip angle development near 45 degrees, independent of the experimental conditions. This characterization of root gravitropic behavior with respect to several non-genetic variables casts the response into a behavioral space rather than into a single, fixed behavior in a narrowly-defined environment. This dataset can serve as a foundation for many different types of experimental approaches including large-scale reverse genetic studies, QTL analysis, model building, and plasticity studies.

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#### C4B01

#### AN ANCIENT MECHANISM CONTROLS THE DEVELOPMENT OF CELLS WITH ROOTING FUNCTIONS

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Root hairs are required for the uptake of minerals and water from soil and for anchorage during growth and development of the root system in many plant species. Hairs elongate by a mechanism known as tip growth, where cell expansion is focussed to a restricted region of the cell surface. Similar cell types with rooting functions are found throughout the land plants. For example caulinema and rhizoids are tip-growing cells found in the mosses. In the ferns tip growing rhizoid cells are found in both gametophyte and sporophyte generations. This indicates that the production of tip-growing cells that interact with the substrate are found throughout the land plants. A cascade of transcription factors regulates the development of root hairs in the model angiosperm *Arabidopsis*. Early acting transcription factors control the fate of cells in the root epidermis – epidermal cells may be hair-bearing or hairless. We have evidence that the early acting genes also control the expression of a suite of late acting transcription factors that are required for late stages of root hair differentiation when hair outgrowth occurs. One of these genes, RHD6, is only expressed in the root hair cell where it promotes the transcription of three other related genes which are also required for root hair cell differentiation. Evidence will be presented that defines the regulatory interactions between these genes in *Arabidopsis* and I will demonstrate that RHD6 (At1g66470) and RHD6-LIKE (At5g37800) genes are part of an ancient mechanism that controls the differentiation of cells with rooting function in land plants.

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#### C4B02

#### ABC PROGRAM AND THE EVOLUTION OF NOVEL FLORAL ORGAN IDENTITY

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The enormous diversity of floral morphology exhibited by angiosperms is due to variation in many different aspects of floral development, including merosity, phyllotaxy, organ fusion, floral symmetry, floral organ identity and floral organ elaboration. The developmental genetic basis for this variability is only beginning to be explored. One question that has not been addressed is how new organ identity programs evolve. In this context, we have undertaken a study of the basal eudicot model *Aquilegia* (columbine) with a particular focus on modifications of floral organ identity. *Aquilegia* is exceptionally well suited for investigations of the evolution of floral developmental genetic pathways due to its evolutionary history, intriguing floral morphology and position as an emerging genetic model. The flowers of *Aquilegia*

exhibit three main innovative features relative to those of the core eudicot models: morphologically distinct petaloid sepals, the petal spur with its associated nectary, and the presence of staminodia between the fertile stamens and carpels. Our previous studies suggest that the *Aquilegia* homologs of the core eudicot ABC class genes have undergone complex patterns of subfunctionalization and possibly neofunctionalization following Ranunculid-specific gene duplication events. We are now extending these studies to include targeted functional knock-downs of all of the floral organ identity gene homologs. In addition, large scale EST sequencing in *Aquilegia* has made it possible to apply genome-level techniques to the question of how this genus has evolved both novel floral organ types and novel organ elaborations. This continued research has the potential to advance our understanding of both the conservation of the ABC program and the mechanisms by which it has been modified over evolutionary time.

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#### C4B03

#### SHORTROOT AND SCARECROW IN ROOT DEVELOPMENT AND LAND PLANT EVOLUTION: OLD PROTEINS, NEW FUNCTIONS

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SHORTROOT (SHR) and SCARECROW (SCR) are key regulators of root growth and development in *Arabidopsis thaliana*. Made in the stele, the SHR protein moves into an adjacent cell layer where it activates SCR transcription. SHR and SCR together in turn define a single layer of endodermis, and maintain the stem cell niche. To dissect the SHR/SCR developmental pathway, we have determined the genomewide locations of SHR direct targets using a ChIP-chip method that we developed. All known SHR targets were identified, thus validating our approach. Intriguingly, among the top-ranked SHR targets, whose functions are largely unknown, a number of genes appear to respond to stresses. Consistent with this observation, we found altered stress physiology in the shr and scr mutants, and mutants in some of the SHR targets. Interestingly, sequence analysis indicated that SHR and SCR homologs are present in *Physcomitrella patens*, a close relative to the first land plant, but not in the water-grown algae *Chlamydomonas reinhardtii*. Since *Physcomitrella* has neither endodermis nor a real root system, these results suggest that SHR and SCR may play an important role in the adaptation of early land plants to a harsh soil environment. To test this hypothesis, we will delete the SHR and SCR homologs in *Physcomitrella* by homologous recombination and test the knockout mutants for stress responses.

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#### C4B04

#### FUNCTIONAL DIVERSIFICATION OF STOMATAL PATTERNING GENES IN MONOCOTYLEDONS

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Although the vast majority of plants have stomata, the distribution of stomata as well as the shape of stomatal guard cells varies among species. The dumbbell-shaped stomata of grasses are generally believed to represent a more evolutionary advanced form than their kidney-shaped counterpart in dicots like *Arabidopsis*. Three bHLH-type transcription factors, SPCH, MUTE and FAMA control stomatal development in *Arabidopsis*. We are studying expression patterns and function during stomata development of the orthologues of these genes in monocots. We are interested in whether these genes play similar roles in stomatal development despite the different forms and patterns of stomata, or whether major changes have occurred in these genes' functions between different species.

In *Arabidopsis*, FAMA controls differentiation of guard cells. A knockout mutant of the rice orthologue (OsFAMA) showed a box-shaped stomata instead of the dumbbell shape and seedling lethality (suggesting that the mutant lacks stomatal functions). Overexpression of full length OsFAMA in

Arabidopsis lead to a phenotype similar to the one observed with the weak overexpression of AtFAMA. In addition, we detected OsFAMA expression in the epidermis of rice seedlings. In the homozygous plants, no FAMA transcript could be detected. Taken together, these data suggest that FAMA function in controlling guard cell differentiation is conserved between representatives of the monocots and the dicots despite the morphological divergence of these cell types.

SPCH and MUTE control stages of stomatal development that do not occur the same way in monocots. We were therefore interested in whether these genes have similar functions. OsMUTE is expressed in epidermal and primordia tissue in rice and ZmMUTE in a similar pattern in maize. Overexpression of OsmUTE in Arabidopsis resulted in an increase in small cell and stomata number both in hypocotyls and leaves, a phenotype distinct from the phenotype obtained by expressing AtMUTE. Interestingly, when overexpressed MUTE maize homologue ZmMUTE in Arabidopsis (35S::ZmMUTE), the maize version of MUTE produced a strong phenotype. Transgenic (T1) seedlings produced only a single ball-shaped or hammer-shaped cotyledon; their hypocotyls were severely reduced in length. 35S::ZmMUTE was exceedingly effective at promoting stomatal formation. Epidermal cells from all parts of the seedlings were converted into what appears to be guard cells. The SPCH gene is duplicated in the grasses, but we have not yet been able to detect expression or function of either version. Further detailed study of stomatal development in cereals may lead to improvement of food and biofuels crops, to better understanding of greenhouse effects or other aspects of climate change.

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C4B05

#### ARABIDOPSIS AS A MODEL FOR PLANT DEVELOPMENT

Category: Evolution and Development

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Despite the rapidly expanding set of fully sequenced plant genomes, Arabidopsis continues to play a central role in plant research as the most completely annotated plant species, with numbers of genes with an experimentally verified function far ahead of other plants and comparable to other major model organisms including fruitfly, nematode and mouse. As the key reference organism of the plant kingdom, Arabidopsis serves as the main source of gold-standard experimentally verified gene function information. To assist with this essential function, TAIR has recently added new tools and data that facilitate cross-genome comparisons. New GBrowse VISTA tracks integrate nucleotide alignment data hosted at JGI via a plugin module and include alignments for plants at a wide range of evolutionary distances. Other new GBrowse tracks at TAIR include promoter elements, Brassica ESTs and orthologs from a broad range of complete genomes. TAIR is also working to provide an integrated phenotype dataset by bringing in large datasets from other resources as well as individual contributions. Association of plant genes to controlled vocabulary terms describing developmental processes and anatomical structures applicable across all plant species is in progress, enabling cross-species analysis of important developmental genes. These tools can be used in concert to explore evolution and function of plant genes involved in developmental processes.

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# **POSTER ABSTRACTS**

## **DEVELOPMENTAL METABOLISM**

### **ICAR101**

PHOTOTROPIN SIGNALING IN THE REGULATION OF LEAF SHAPE AND STRUCTURE

Category: Developmental Mechanisms

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As the major photosynthetic organ, leaf changes its shape and structure in response to the light environment, which is recognized by means of photoreceptors. However, little progress has been made in understanding the mechanisms by which the leaf shape and structure are regulated by photoreceptor signals.

In *Arabidopsis thaliana*, the *PHOT1* and *PHOT2* genes are known to encode phototropins, a class of blue light photoreceptors. It is well established that phototropins mediate the hypocotyl phototropism, chloroplast relocation and stomatal opening. In this study, we investigated the effects of the phototropin on mesophyll cell elongation and leaf flattening. When plants are grown under intense blue light, palisade-tissue cells elongate in the leaf-thickness direction. In the *phot1phot2* null mutant, isotropic expansion in palisade-tissue cells were observed rather than the polar elongation, suggesting that phototropin signal regulates polar elongation in these cells. In addition, epinastic growth of rosette leaves has been reported in the *phot1phot2* mutant under white light. We confirmed that blue but not red light effectively flattened leaves. When grown under blue light, wild type seedlings had flat leaves, while the epinastic growth was observed under red light. The leaves of the *phot1phot2* mutant showed the epinastic growth under blue as well as red light. These results suggest that phototropins inhibit the epinastic growth in leaves in response to blue light. We further examined where in the leaves PHOT2 perceived blue light to mediated those responses. Towards this end, we established transgenic *Arabidopsis* lines in which PHOT2 was expressed in tissue-specific manners. On the basis of these analyses, spatial relationships between the phot2 signals and their physiological consequences will be discussed.

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### **ICAR102**

MIKC\* MADS-DOMAIN PROTEINS ARE IMPORTANT REGULATORS IN ARABIDOPSIS GAMETOPHYTE

Category: Developmental Mechanisms

\*Adamczyk, Benjamin J., Fernandez, Donna E. Department of Botany, University of Wisconsin-Madison  
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Members of a divergent clade of MADS-domain transcription factors, called MIKC\* proteins, have recently been shown to contribute to pollen function in *Arabidopsis*. MIKC\* proteins AGL66 and AGL104 have also been shown to heterodimerize with other clade members AGL30 and AGL65. We used double, triple, and quadruple mutants to disrupt each of the predicted heterodimers in pollen and found that each plays a role in the male gametophyte. Pollen carrying strong *agl66* and *agl104* alleles are especially compromised, suggesting that the disruption of all predicted heterodimers has the strongest effect on pollen. While this could suggest that functional redundancy exists between MIKC\* proteins and heterodimers, pollen competition assays have suggested that not all proteins or heterodimers may be completely interchangeable. Reciprocal crosses have also shown that a subset of MIKC\* proteins may have an additional role in the female gametophyte. We are currently using higher-order mutants to investigate these and other factors that could be involved in a MADS-based regulatory network during gametophyte development. Supported by the UW-Madison Graduate School.

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### **ICAR103**

IDENTIFICATION AND CHARACTERISATION OF CIS- AND TRANS-ACTING FACTORS REGULATING THE EXPRESSION OF THE ARABIDOPSIS FLOWERING-TIME GENE FT

Category: Developmental Mechanisms

\*Adrian, Jessika Max Planck Institute for Plant Breeding Research, Cologne, Germany Farrona, Sara Max Planck Institute for Plant Breeding Research, Cologne, Germany Albani, Maria Max Planck Institute for Plant Breeding Research, Cologne, Germany Coupland, George Max Planck Institute for Plant Breeding Research, Cologne, Germany Turck, Franziska Max Planck Institute for Plant Breeding Research, Cologne, Germany  
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The transition from vegetative to reproductive growth is tightly regulated. In *Arabidopsis*, cues from several floral transition pathways, such as the photoperiod and the vernalization dependent pathways, are integrated at the transcriptional regulation of *FLOWERING LOCUS T (FT)*.

Flowering in response to day length is mediated by CONSTANS (CO). Under inductive long-day conditions CO accumulates in the vasculature of cotyledons and leaves and stimulates *FT* transcription. CO contains two zinc-finger B-boxes and a CCT domain, features characteristic of some transcription factors. Nevertheless, physical association with regulatory sequences of the *FT* promoter could not be demonstrated. *GUS* reporter gene analysis in plants with increased CO expression (*35S::CO*) showed that *FT* levels are higher, but still restricted to the vascular tissue. It is possible that CO needs a so far unknown co-expressed activator to stimulate *FT*.

Through complementation and expression analyses of promoter deletion constructs in transgenic plants we aim to identify regulatory regions of *FT*. We applied transient bombardment assays to identify proximal *FT* promoter regions that are regulated by CO. Using sequence alignment of *FT* orthologous genes from other *Brassicaceae* we identified phylogenetic shadows in the proximal *FT* promoter. Mutational analyses of these putative *cis* elements in the transient bombardment assay suggest a role in the CO-mediated response. In addition analyses of the mutated elements in transgenic plants using *GUS* reporter genes confirmed their importance in *FT* regulation and responsiveness to CO. However proximal *FT* promoter sequences are required but not sufficient to drive gene expression.

The *FT* locus is targeted by the epigenetic repressor TERMINAL FLOWER 2 (TFL2) which is part of the Polycomb repressive pathway in plants. Lack of TFL2 causes high expression of *FT* in the vasculature of leaves. Expression analyses of plants carrying *FT* promoter *GUS* constructs showed that *FT* promoter fragments which are not sufficient to drive expression in wild type become active in *tfl2* plants.

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**ICAR104**

IDENTIFICATION OF A NOVEL GENE PUTATIVELY INVOLVED IN SEED MATURATION/GERMINATION

Category: Developmental Mechanisms

\*Amorim-Silva, Vitor Laranjeira, Sara Azevedo, Herlander Tavares, Rui Minho University, Portugal

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Functional screenings in the *Arabidopsis* biological model make use of phenotype-centred forward and reverse genetic approaches, namely with the use of loss-of-function mutant analysis. Based on a reverse genetics approach, the expression analysis of osmotic- and salt-stressed *Arabidopsis* plants, made available through the Affymetrix microarray database (NASC), led to the identification of one gene (At2g47770) that is putatively involved in conferring tolerance to these abiotic stresses. At2g47770 codes for a benzodiazepine receptor-related protein of unknown molecular function, similar to the peripheral-type benzodiazepine receptor of *Solanum tuberosum*. We have isolated a T-DNA insertion mutant line that is homozygous for the mutation and are currently screening for phenotype. Preliminary results seem to suggest the strong involvement of At2g47770 on seed maturation/germination.

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**ICAR105**

A CELLULOSE SYNTHASE-LIKE D PROTEIN REQUIRED FOR POLLEN FUNCTION

Category: Developmental Mechanisms

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The life cycle of plants comprises two alternating generations, the diploid sporophyte and the haploid gametophyte. Mechanisms underlying development and function of higher-plant gametophytes are still poorly understood, which is mainly due to the technical difficulties of identifying relevant gene functions by mutant phenotypes. Screening a collection of 1,300 Ds gene-trap insertion lines, we isolated gametophytic mutants detectable by their reduced transmission of the Ds transposon-borne kanamycin resistance marker. One of the genes identified in this screen is a member of the transmembrane cellulose synthase-like D family of proteins (CSLD). In reciprocal crosses, the *cslA* mutant line showed a complete block of resistance marker transmission through the pollen. The transmission through the embryo sac was not affected. Morphological characterization of the *cslD* mutant revealed a defect in pollen maturation or function. Two additional alleles showed the same phenotype and pollen specific transmission failure of the mutated allele. Expression analyses by means of RT-PCR showed that the *CSLD* gene is exclusively transcribed in anthers. We are currently investigating the expression at different stages of pollen development by real-time PCR. A *pCSLD::GUS* reporter line showed strong expression in pollen and germinating pollen tubes. The cellulose synthase superfamily consists of the cellulose synthase family (CESA) and the cellulose synthase-like family (CSL). Members of the CESA family have been shown to be involved in the synthesis of cellulose. Not much is known about the function of the CSL proteins, but they are thought to be processive polysaccharide  $\beta$ -glycosyltransferases. There are six CSL protein families in *Arabidopsis*, with altogether about 30 genes that are structurally similar. These genes encode polypeptides that differ from the CESA polypeptides so that they may not participate in the synthesis of cellulose, but rather catalyze the synthesis of non-cellulose polysaccharides. In order to determine the polysaccharide specificity of CSLD and/or function of the CSLD protein in pollen maturation or function, we are currently generating recombinant proteins to perform *in vitro* functional assays as well as sub-cellular localization studies.

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**ICAR106**

ARABIDOPSIS MUTANTS RESISTANT TO ANTIPROLIFERATIVE AGENTS

Category: Developmental Mechanisms

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Cancer and other diseases involving abnormal cell-proliferation are often treated with antiproliferative agents. Many of these agents selectively target rapidly dividing cells by inhibiting basic cellular mechanisms necessary for cell division. The cell division machinery is similar in plants and animals, and plant meristems harbor groups of rapidly dividing cells that are sensitive to antiproliferative agents. We believe that these features make plants interesting as model systems for studying the effects of antiproliferative agents. Specifically, resistant plant mutants could provide information about the molecular mechanisms behind drug cytotoxicity. We have established a screening system and isolated a number of *Arabidopsis* mutants, which we are currently characterizing.

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**ICAR107**

TRAUCO, A TRITHORAX GROUP GENE HOMOLOGUE THAT CONTROL EARLY EMBRYOGENESIS IN ARABIDOPSIS THALIANA

Category: Developmental Mechanisms

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Embryogenesis is a critical stage during plant life cycle, in which an unicellular zygote develops into a multicellular organism through cell divisions, cell polarity and cell differentiation. Coordinated gene expression is thus necessary for proper embryo development. Polycomb and trithorax group genes are members of an evolutionarily conserved machinery that maintains the correct expression patterns of key developmental regulators, respectively by repressing and activating gene transcription. We have identified *TRAUCO* (*TRO*) in *Arabidopsis thaliana*, a gene homologous to trithorax group of genes. *TRO* is a nuclear gene product, expressed during embryo development. We identified a zygotically lethal mutant of *TRO*, which impaired early embryogenesis events. Expression of Polycomb-group regulated genes was derepressed in mutant embryos. The *TRO* mutant

embryos arrested at the globular stage were fully rescued by a *TRO* expression clone, demonstrating that *TRO* mutation is a true loss-of-function. Our data have established that *TRO* is the first trithorax homologue in plants that control early embryogenesis.

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**ICAR108**

TWO-DIMENSIONAL PATTERNING BY A TRAPPING/DEPLETION MECHANISM: THE ROLE OF TTG1 AND GL3 IN ARABIDOPSIS TRICHOME FORMATION  
Category: Developmental Mechanisms

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Trichome patterning in *Arabidopsis* serves as a model system to study, how single cells are selected within a field of initially equivalent cells. Current models explain this pattern by an activator-inhibitor feed back loop. Here, we report that also a novel mechanism is involved by which patterning is governed by the removal of the trichome promoting factor TRANSPARENT TESTA GLABRA1 (TTG1) from non-trichome cells. We demonstrate by clonal analysis and misexpression studies that *Arabidopsis* TTG1 can act non-cell autonomously and by microinjection experiments that TTG1 protein moves between cells. While TTG1 is expressed ubiquitously, TTG1-YFP protein accumulates in trichomes and is depleted in the surrounding cells. TTG1-YFP depletion depends on GLABRA3 (GL3), suggesting that the depletion is governed by a trapping mechanism. To study the potential of the observed trapping/depletion mechanism, we formulated a mathematical model enabling us to evaluate the relevance of each parameter and to identify parameters explaining the paradox genetic finding that strong ttg1 alleles are glabrous, while weak alleles exhibit trichome clusters.

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**ICAR109**

CELL-TYPE-SPECIFIC AUXIN RESPONSES IN THE ARABIDOPSIS ROOT  
Category: Developmental Mechanisms

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Auxin is a phytohormone crucial to many different aspects of growth and development. One of the key questions in the field of auxin research is how this single molecule can elicit its diverse effects during development, in various tissues and in particular in specific cell types. Auxin distribution is highly regulated through directed transport mediated by polar localization of efflux carriers. This distribution can be altered in response to environmental stimuli such as light, gravity or wounding, inducing changes in growth patterns of the plant. Transcriptional responses to auxin are known to be regulated by ARFs and Aux/IAAs in a proteasome-dependent manner. Previous cell-type-specific gene expression profiling of the *Arabidopsis* root has demonstrated that these regulators of auxin-induced transcription have distinct cellular expression patterns. To assess how individual cell types respond differentially to high auxin concentrations, we have generated cell-type-specific gene expression profiles utilizing protoplasting of fluorescent marker lines expressing GFP in particular cell types and consequent FACS (Fluorescence Activated Cell Sorting) of the GFP-positive protoplasts. The roots of seedlings treated with IAA or mock treated were harvested after two hours, protoplasted and sorted. Microarray analysis has shown that the various assayed cell types have both overlapping and distinct responses to treatment. ANOVA of three replicates for all tested cell types and treatments yielded clusters of genes whose expression is broadly or exclusively induced or repressed by auxin treatment, certain gene clusters even show opposite effects on their expression in different cell types. Analysis of the four marker lines assayed so far has already greatly expanded the list of auxin responsive genes. We have found a total of 7,434 genes to be significantly responsive to auxin. Surprisingly, 6,469 of these were similarly responsive across all tested cell types, 4,027 showed universal induction and 2,422 were repressed in all cell types. 965 genes were differentially regulated by auxin in particular cells. Interestingly, a large part of these cell-type-specific auxin responses took place in the vasculature. A detailed functional analysis of the acquired data set will be presented.

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**ICAR1010**

ASCORBIC ACID INFLUENCES FLOWERING TIME THROUGH THE PHOTOPERIODIC AND THE AUTONOMOUS PATHWAY

Category: Developmental Mechanisms  
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Ascorbic acid protects plants against abiotic stress. The antioxidant is also involved in the control of flowering. In order to understand how ascorbate mechanistically affects flowering time, we studied the four ascorbic acid-deficient *Arabidopsis* mutants *vtc1-1*, *vtc2-1*, *vtc3-1* and *vtc4-1* when grown under short and long days, respectively. The *vtc* mutants flowered and senesced before the wild type irrespective of the photoperiod. Expression analyses of genes controlling flowering in *Arabidopsis* revealed that transcript levels of the circadian clock genes *LHY* and *TOC1* as well as genes of the connected photoperiodic flowering pathway, including *G1*, *CO*, and *FT*, are significantly higher in the *vtc* mutants than in the wild type under both short and long days. Early flowering of the *vtc* mutants was associated with a stronger expression of the floral meristem identity gene *LFY*. Furthermore, gene expression analyses suggest a regulatory role of *PHYA*, *PHYB*, *CRY1*, and *CRY2* photoreceptors in promoting flowering in the *vtc* mutants. When the early flowering *vtc1-1* mutant was crossed to late flowering mutants, including *gi-1*, *co-2*, and *ft-1*, the *vtc1-1 gi-1*, *vtc1-1 co-2*, and *vtc1-1 ft-1* double mutants all exhibited a delayed flowering phenotype, suggesting that functional *G1*, *CO*, and *FT* are required for early flowering when ascorbic acid levels are low. Further support for a role of ascorbate in regulating flowering time comes from the fact that wild-type plants sprayed with a precursor of ascorbic acid are late flowering compared to wild-type plants sprayed with water. The late flowering phenotype in the ascorbate precursor-sprayed plants correlated with lower mRNA levels of circadian clock and photoperiodic pathway genes. Collectively, this study provides first insights as to how ascorbate regulates flowering, which we suggest occurs predominantly through the photoperiodic pathway. The contribution of other pathways in regulating flowering time in *vtc* mutants is discussed.

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**ICAR1011**

ELUCIDATING THE MECHANISMS OF NITROGEN HYPERSENSITIVITY OF THE *ARABIDOPSIS THALIANA VTC1-1* MUTANT

Category: Developmental Mechanisms

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This study aims to elucidate the mechanism by which root elongation and the formation of lateral roots is inhibited in the ascorbate-deficient *Arabidopsis* mutant *vtc1-1*. Three other ascorbate-deficient mutant alleles, *vtc2-1*, *vtc3-1*, and *vtc4-1*, were also characterized in respect to root development. However, these three mutant alleles are not altered in root development compared to the wild type, suggesting that the short root phenotype in *vtc1-1* is not caused by ascorbate deficiency. In order to investigate the cause of the altered root development in *vtc1-1*, we tested the hypothesis that *vtc1-1* may be differentially sensitive to nutrients. We found that root elongation in *vtc1-1* is inhibited by high levels of ammonium, whereas wild-type root development is normal when plants were grown on increasing concentrations of ammonium chloride. Furthermore, the inhibition in root elongation correlated with heightened levels of nitric oxide in *vtc1-1*. Transcript levels of nitric oxide synthase were significantly higher in the mutant than in the wild type. Furthermore, when seedlings were germinated in the presence of the nitric oxide donor SNP, root elongation was strongly inhibited in the wild type, whereas the already short-root phenotype of *vtc1-1* was hardly affected. In contrast, the nitric oxide inhibitor cPTIO had a promoting effect on root elongation in *vtc1-1*. Taken together, our data suggest that *vtc1-1* is hypersensitive to ammonium. We hypothesize that ammonium is converted into nitric oxide that serves as ligand for gyanylate cyclase, which converts GTP into cGMP, which has been shown to inhibit root elongation. Alternatively, the genetic defect in *vtc1-1*, which has down-regulated GDP-mannose pyrophosphorylase, may be responsible for the inhibition of root development through altered N-glycosylation of proteins. This in turn may result in altered cell division activity of the root apical meristem. Additional results in support of our hypotheses are discussed.

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**ICAR1012**

PATERNAL CONTROL OF EMBRYONIC PATTERNING IN ARABIDOPSIS THALIANA

Category: Developmental Mechanisms

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Embryonic development relies on robust spatial and temporal coordinates and multi-cellular organisms have evolved strategies to derive such coordinates from external and intrinsic cues.

Little is known about the spatio-temporal cues coordinating plant embryogenesis.

The first division of the *Arabidopsis* zygote is asymmetric and generates two daughter cells with fundamentally different developmental fates: while the apical cell produces the spherical pro-embryo, the basal cell forms the mostly extra-embryonic suspensor. We have previously shown that the MAPKK kinase gene *YODA* (*YDA*) acts as a molecular switch promoting extra-embryonic fate in the cells of the basal cell lineage.

Here, we report that activation of the *YDA* MAP kinase cascade is linked to fertilization through an unusual parent-of-origin effect and offer a mechanistic basis for our finding. We provide evidence that the receptor-like cytoplasmic kinase gene *SHORT SUSPENSOR* (*SSP*) acts upstream of *YDA* in this signaling event. Our data suggests that *SSP* mRNA accumulates in sperm cells of mature pollen and is delivered to the zygote during fertilization where *SSP* triggers the activation of the *YDA* MAP kinase cascade. Thus, *SSP* constitutes a pollen-derived temporal cue that links the onset of *YDA*-dependent signaling to fertilization.

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**ICAR1013**

IDENTIFICATION OF NOVEL CELL, TISSUE AND REGION-SPECIFIC GENE EXPRESSION PATTERNS WITHIN THE DEVELOPING ARABIDOPSIS SEED USING LASER-CAPTURE MICRODISSECTION TECHNOLOGY

Category: Developmental Mechanisms

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Differentiation of the *Arabidopsis* seed is programmed by a complex network of regulatory genes and pathways partitioned in a compartment and cell type-specific manner. However, due to the relatively small size of the *Arabidopsis* seed, global expression patterns within various cell and tissue types as well as various seed regions have been next to impossible to capture. We have identified all of the genes expressed in each compartment of developing seeds using laser-capture microdissection to isolate specific regions, tissues and cell types from the embryo, endosperm and seed coat. These compartments include the embryo proper and suspensor, the micropylar, peripheral and chalazal endosperm as well as the chalazal and general seed coats. Affymetrix ATH1 GeneChip hybridization experiments and qRT-PCR validations have been carried out to profile the diverse mRNA populations responsible for transforming a seed from early to late stages of *Arabidopsis* seed development. GeneChip analysis has provided a profile of active gene sets and their corresponding expression levels responsible for various processes including endosperm development, thereby providing potential insights into biological function. We captured all major endosperm compartments of the developing seed including the micropylar, peripheral and chalazal regions during the earliest stages of development and the cellularized endosperm of differentiating seeds. Gene expression patterns responsible for seed filling and hormone metabolism have been examined and are shown to be partitioned both spatially and temporally within this non-persistent yet fundamental region. Novel roles for the endosperm and its relationship to other compartments within the *Arabidopsis* seed will be discussed.

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**ICAR1014**

MOLECULAR DISSECTION OF CLV3 PEPTIDE SIGNALLING.

Category: Developmental Mechanisms

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Recent identification of CLAVATA3 (CLV3), a CLV3/ ESR-related (CLE) protein, functioning as a dodecapeptide with two hydroxyproline residues in plants, has enlightened the importance of peptide hormones and corresponding receptor-like kinases (RLKs) for inter- and intracellular communication in plant development. By genetic means, the CLV3 dodecapeptide is considered as a ligand for receptor complex(es) consisting of CLAVATA1 and CLAVATA2 to control shoot apical meristem size in *Arabidopsis* and, indeed, has been shown to interact with the CLV1 ectodomain recently. However, the precise molecular details of CLV3 peptide maturation, transport, reception and signalling in a target cell, still remain to be solved. To answer these questions, we focused on identifying further loci involved in the CLV signalling. Our screening of EMS-mutagenized Col-0 population for the CLV3 synthetic peptide insensitivity identified a number of potential candidates. We also examined a collection of insertional mutants in the selected 313 transcription factors and found several mutants with weakened sensitivity to the CLV3 synthetic peptide. Current progress in this study will be presented.

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### ICAR1015

THE WDR PROTEIN EMLA IS REQUIRED FOR GAMETOGENESIS AND SEED DEVELOPMENT

Category: Developmental Mechanisms

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Unique to the plant lifecycle is the existence of a multicellular haploid gametophyte generation. The gametophytes produce the gametes and interact to create the double fertilization product, the embryo and the endosperm. Although major progress has been achieved in this field in the past few years, our current knowledge on the genetic and molecular basis of gametophyte development and function is still limited. In an Ac/Ds transposon mutagenesis screen we have identified several lines that display segregation distortion of a selectable marker, thus indicating that a gene vital to the gametophyte generation has been disrupted. In one line a WD-40 repeat protein (WDR) encoding gene was disrupted, and we termed this gene *EMLA* (*EML*). Both Real-time PCR and reporter gene constructs showed that *EML* is expressed during embryo sac development, at early stages of pollen development and also in early embryo development. Phenotype examination of *eml-1* lines revealed that pollen development arrested at the binucleate to trinucleate stage. *EML* has two splice variants and *eml-1* only affect one of these. A second mutant allele, *eml-2*, affects both splice variants but showed no defects in pollen development. In both alleles, however, embryo and endosperm development arrested at various stages from the zygote stage to the early globular stage. The two alleles could not complement, thus indicating also a recessive zygotic requirement for *EML*. In 35S::EML-GFP overexpression experiments an extracellular and/or membrane localization of EML is indicated and overexpression result in arrested ovules and pollen defects. We are currently investigating the complementation ability of the two splice variants using cDNA driven by the endogenous promoter in addition to complementation by the genomic sequence of *EML*. Preliminary results suggest that the genomic sequence of *EML* complements the *eml-1* seed phenotype. EML is conserved across species and is therefore likely to be part of a basic cellular mechanism. In order to explore the function of *EML* and its role in gametophyte and seed development, we currently perform yeast-2-hybrid screens to identify EML interaction partners.

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### ICAR1016

JACKDAW CONTROLS EPIDERMAL PATTERNING IN THE ARABIDOPSIS ROOT MERISTEM

Category: Developmental Mechanisms

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We have previously shown that, in the *Arabidopsis* root meristem, the transcription factor JACKDAW (JKD) regulates the movement of the cell fate determinant SHORTROOT and restricts its action to one single layer: the endodermis. Here we provide evidence that JKD regulates epidermal cell fate and thus root hair patterning. JKD is required for normal expression of root hair patterning genes early in the embryo. Furthermore, root hair regulatory network acts downstream of JKD. Domain specific mis-expression of JKD indicates a non cell autonomous action to establish position dependant epidermal cell specification in the *Arabidopsis* root meristem. Our data suggest that JKD acts by triggering an inductive signal from the ground tissue that would enable cells in the epidermis to interpret their appropriate fate relative to their position "vis a vis" the cortex.

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### ICAR1017

UNRAVELLING THE BASIS OF APOMIXIS IN THE GENUS BOECHERA

Category: Developmental Mechanisms

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The genus *Boechera* (Brassicaceae) comprises a large group of North American plants that are phylogenetically very close to the model plant *arabidopsis*. The genus is highly polymorphic, with plants exhibiting different chromosome numbers, genetic backgrounds, morphologies and reproductive modes. It is the only taxon in the Brassicaceae that can reproduce clonally through seed (apomixis). Cytological studies have shown that apomorphic reproduction in *Boechera* spp. involves an incomplete female meiosis leading to unreduced female gametes, and embryo development

through parthenogenesis of an unreduced egg cell. Similar variants of meiosis were also observed during microsporogenesis, leading to the formation of unreduced pollen. The large degree of natural (reproductive) variation within the genus combined with its relatedness to *arabidopsis* make *Boechera* a potentially powerful model system for molecular-genetic studies on apomixis. However, very little is known about the life histories and reproductive biology of the genus. We are taking a multidisciplinary approach to establish a framework and develop new tools for understanding the molecular-genetic and evolutionary basis of reproductive development in this genus. In this context cytogenetic analyses have been used to describe the genomic organization, meiotic chromosome behaviour and reproductive mode of different sexual and apomorphic *Boechera* germplasm, while microarray-based transcriptomics have been used to describe the gene expression changes that distinguish sexual reproduction from apomorphic reproduction.

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### **ICAR1018**

#### IDENTIFICATION AND ANALYSIS OF A SUPPRESSOR OF ABSCISIC ACID INSENSITIVE 3

Category: Developmental Mechanisms

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Seed maturation represents the last stage of seed development and prepares the seed to survive the unfavorable conditions that it will encounter after dispersal. During seed maturation the embryo accumulates storage products, becomes metabolically quiescent and tolerant to desiccation, and acquires dormancy.

Seed maturation in *Arabidopsis* is controlled by four major regulators: FUSCA 3 (FUS3), LEAFY COTYLEDON 1 (LEC1), LEC2, and ABSCISIC ACID INSENSITIVE 3 (ABI3). Although the relationship between these genes has been investigated, very little is known about the factors that contribute to their regulation. The abi3 mutant produces non-dormant seeds with a reduced sensitivity to ABA, absence of chlorophyll degradation and intolerance to desiccation. A genetic screen to identify suppressors of the strong mutant allele abi3-5 led to the isolation of the suppressor of abi3 (sua) mutant. Mutations in sua rescue the abi3-5 phenotype by splicing a small cryptic intron and producing a slightly shorter but functional ABI3 splicing variant. SUA is a ubiquitously expressed nuclear protein, it localizes in different subnuclear compartments at different developmental stages and it contains two RNA-binding domains. These SUA features and its effects on ABI3 mRNA splicing suggest its involvement in pre-messenger RNA metabolism. The mutant allele of sua in a wild-type background shows increased dormancy and ABA sensitivity, which implies an independent role of SUA in the control of seed dormancy possibly through the regulation of other target genes.

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### **ICAR1019**

#### MOLECULAR CHARACTERISATION OF DOMINANT REPRESSORS OF THE CYTOKININ DEFICIENCY SYNDROME

Category: Developmental Mechanisms

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Cytokinins are a class of plant hormones controlling numerous aspects of plant development throughout the life cycle. The catabolic inactivation of cytokinins is catalyzed by cytokinin oxidases/dehydrogenases (CKX) and in many plant species they are responsible for the majority of metabolic cytokinin inactivation. *35S:CKX* transgenic *Arabidopsis* plants show a cytokinin-deficiency phenotype, which mainly consists of a dwarfed shoot with smaller apical meristems and an enhanced root system.

We carried out mutagenesis of *35S:CKX1*-overexpressing transgenic *Arabidopsis* plants which display a strong cytokinin-deficiency phenotype and screened for mutants that showed a partial reversion of the cytokinin deficiency syndrome.

We isolated several recessive and dominant second site suppressor mutations termed *rock* (*repressor of cytokinin deficiency*), which are being characterised. Using map-based cloning we identified the dominant *rock2* and *rock3* mutant alleles as missense mutations in the *AHK2* and *AHK3* genes, which encode histidine kinase cytokinin receptors. These putative gain-of-function receptor alleles caused an almost complete reversion of the cytokinin-deficiency syndromes of *35S:CKX1* plants. Detailed phenotypic analysis of *rock2* and *rock3* single as well as *rock2 rock3* double mutants in wild type and *35S:CKX1* background revealed partially specific changes in cotyledon expansion, root growth, flower size, flower induction and leaf senescence.

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### **ICAR1020**

#### EXAMINING TOTIPOTENCY VIA LEAFY COTYLEDON2-MEDIATED SOMATIC EMBRYOGENESIS

Category: Developmental Mechanisms

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Totipotency is a unique aspect of plant development. The molecular mechanisms that underlie totipotency are largely unknown. We are interested in dissecting the molecular networks leading to totipotency by defining the role of the transcription factor LEAFY COTYLEDON2 (LEC2) in inducing somatic embryogenesis. Somatic embryogenesis is the de-differentiation and re-differentiation of somatic cells into embryo-like cells, an elegant example of totipotency. Over-expression of the *LEC2* gene in vegetative tissues induces somatic embryo development and can also lead to a 'seed-maturation' type environment in vegetative tissues. Here we explore how these two major over-expression phenotypes provide clues about the switch to an embryogenic cell fate induced by LEC2. We divide somatic embryogenesis into two components: an initiation step which often requires auxin,

and a competence of tissue to respond to said initiation step. Utilizing ATH1GeneChips® profiling RNA changes in transgenic plants carrying an inducible form of the LEC2 protein and chromatin-immunoprecipitation (ChIP) experiments we demonstrate that LEC2 directly regulates auxin biosynthesis genes and indirectly alters auxin responses. This provides biological information about how LEC2 initiates somatic embryogenesis. We also propose roles for LEC2 in altering gibberellin (GA) and abscisic acid (ABA) responses and hypothesize how these changes may contribute to a tissue's competence to respond to auxin and develop somatic embryos.

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**ICAR1021**

TWO DISTINCT, INTERACTING CLASSES OF NUCLEAR ENVELOPE-ASSOCIATED COILED-COIL PROTEINS ARE REQUIRED FOR THE TISSUE-SPECIFIC NUCLEAR ENVELOPE TARGETING OF ARABIDOPSIS RANGAP

Category: Developmental Mechanisms

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The Ran GTPase plays essential roles in multiple cellular processes, including nucleocytoplasmic transport, spindle formation and post-mitotic nuclear envelope (NE) re-assembly. The cytoplasmic Ran GTPase activating protein RanGAP is critical to establish a functional RanGTP/RanGDP gradient across the NE, and is associated with the outer surface of the NE in metazoan and higher plant cells. Arabidopsis RanGAP association with the root tip NE requires a family of likely plant-specific nucleoporins combining coiled-coil and transmembrane domains (CC-TMD), WPP-domain interacting proteins (WIP). We have now identified by tandem affinity purification coupled with mass spectrometry a second family of CC-TMD proteins, structurally similar, yet clearly distinct from the WIP family, that is required for RanGAP NE-association in root-tip cells. A combination of loss-of-function mutant analysis and protein-interaction data indicates that at least one member of each NE-associated CC-TMD protein family is required for RanGAP targeting in root tip cells, while both families are dispensable in other plant tissues. This suggests an unanticipated complexity of RanGAP NE targeting in higher plant cells, contrasting both the single-nucleoporin anchor in metazoans and the lack of targeting in fungi and proposes an early evolutionary divergence of the underlying plant and animal mechanisms.

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**ICAR1022**

EXPLORING THE TRANSCRIPTIONAL REGULATORY NETWORK OF ROOT EPIDERMAL CELL SPECIFICATION USING A SYSTEM BIOLOGY APPROACH

Category: Developmental Mechanisms

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The cell differentiation process in the *Arabidopsis* root epidermis provides a simple and powerful model to study regulatory gene networks. So far more than 10 genes have been defined by molecular genetics approaches that play a role in the position-dependent specification of the two root epidermal cell types – trichoblast and atrichoblast. The regulatory relationship between many of these genes has been established and mutants are available for analysis. Thus, the root epidermis system provides a unique opportunity to assemble a relatively simple gene network that can serve as a paradigm for the construction and analysis of more complex networks in *Arabidopsis*. Different mutants defective in root epidermal cell differentiation and containing the *WER::GFP* transgene are used to develop this network. The *WER::GFP* transgene is a specific root epidermis marker, which allows us to select epidermal cells by fluorescence activated cell sorting. The expression profiles of the mutants are then analyzed by microarrays and a epidermal gene network is built by a Bayesian network approach. This approach enables us to expand our knowledge about root cell differentiation and its regulatory network, to find new players of the network, to test predictions and establish an exemplary model network.

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**ICAR1023**

MODELING VASCULAR PATTERNING IN THE SHOOT OF ARABIDOPSIS THALIANA

Category: Developmental Mechanisms

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Long distance transport in plants is accomplished by a vascular system composed of two different tissues: xylem, that transports water and solutes from roots to aerial parts, and phloem, that distributes photo-assimilates after photosynthesis. Despite the importance of the vascular system in plant development, the molecular mechanism that controls the radial periodic arrangement of vascular bundles within the shoot apical meristem is not yet known.

Plant hormones have been shown to be important regulators of vascular-cell differentiation. The characterization of BRI1-like (BRASSINOSTEROID INSENSITIVE 1) family members BRL1 and BRL3 have pointed that brassinosteroids (BRs) are important regulators of vascular cell differentiation. In this study, we carried a quantitative characterization of the vascular pattern in the inflorescence stem of a number of BR-signaling and synthesis mutants available. The vascular parameters quantified have been computerized and used to build up a mathematical model able to simulate BRs contribution to provascular division and differentiation during vascular development in the stem. The current model points towards a mechanism for vascular patterning based on cell-to-cell interactions as opposed to long-range signaling.

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**ICAR1024**

AUXIN CONTROLS LATE STAMEN DEVELOPMENT IN ARABIDOPSIS FLOWERS

Category: Developmental Mechanisms

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In autogamous plants, during late stamen development microspores develop into pollen grains, filaments elongate and anther dehiscence causes pollen release on the stigma. Based on the phenotypes observed upon increasing auxin sensitivity in tobacco stamens, we had previously suggested an involvement of auxin in anther dehiscence and filament elongation (Cecchetti et al. 2004). To better assess the role of auxin in *Arabidopsis* late stamen development, we analysed auxin synthesis and accumulation by means of *in situ* hybridizations of the auxin biosynthetic genes *YUC2* and *YUC6*, and via the expression of the auxin responsive construct *DR5:GUS*. The role of auxin transport was also investigated. The data obtained strongly point to auxin response in anthers at the end of meiosis, mostly due to local synthesis. The phenotype of *tir1* *afb* mutants defective in auxin perception was analysed with respect to anther dehiscence, filament elongation and pollen maturation, showing a precocious release of mature pollen grains in shortened filaments (Cecchetti et al. 2008). A model of the role of auxin in controlling the different processes in late stamen development will be presented.

Cecchetti et al. (2004) Plant J., 38, 512-525

Cecchetti et al (2008), Under revision.

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**ICAR1025**

CVP2 AND CVL1-MEDIATED LIPID SIGNALING AS A REGULATOR OF THE ARF GAP, SFC/VAN3 IN ESTABLISHMENT OF FOLIAR VEIN PATTERNS

Category: Developmental Mechanisms

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To gain insight into the molecular mechanisms governing the spatial regularity of vein patterns in plant foliar organs, we previously used a genetic approach to identify several cotyledon vascular pattern (*cvp*) mutants in *Arabidopsis*. In contrast to the closed vein pattern in wild type foliar organs, *cvp2* mutants have an increase in free vein endings and a resulting open vein network. CVP2 encodes an inositol polyphosphate 5' phosphatase (5PTase). 5PTases hydrolyze various inositol substrates to terminate signal transduction events and/or provide lipid ligands for appropriate targeting of lipid binding proteins. CVP2LIKE1 (CVL1) is the closest relative to CVP2 in the 15 member *Arabidopsis* 5PTase gene family. Although *cvl1* mutants appear wildtype, when combined with *cvp2*, the double mutants have a highly discontinuous vein pattern and closely resemble the vascular patterning mutant *scarface/vascular network defective3 (sfc/van3)*. *cvp2* enhances the *sfc/van3* phenotype and CVP2, CVL1 and SFC are similarly expressed in developing vascular cells in embryos, roots, and leaves, further suggesting that the genes reside in the same pathway. SFC/VAN3 encodes a PH domain-containing ADP-ribosylation factor-guanosine activating protein (ARF-GAP) that likely functions as a regulator of vesicle transport. ARF GAPs are known downstream targets of 5 PTases in other systems. CVP2 and CVL1 5PTase activity, as assayed in yeast, generate the SFC binding lipid, presumably for SFC subcellular localization. We propose that CVP2 and CVL1-mediated lipid signaling regulate SFC ARF-GAP activity in the maintenance of vascular continuity.

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**ICAR1026**

ANALYSIS OF *HUA2 LIKE (HULK)* GENE FAMILY IN *ARABIDOPSIS THALIANA*

Category: Developmental Mechanisms

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*HUA2* gene is a putative pre-mRNA processing factor involved in the regulation of flowering time and floral development. There are three other *HUA2 LIKE (HULK)* genes in *Arabidopsis* genome, named *HUA2 LIKE1 (HULK1)*, *HUA2 LIKE2 (HULK2)*, and *HUA2 LIKE3 (HULK3)*. The *HULK* gene family of proteins range from 1347 to 1445 amino acids, and they share 19 to 53% amino acid identities over their entire length. In addition, these *HULK* proteins contain the same conserved domains identified in *HUA2*: PWW domain, RPR (pre-mRNA processing domain), proline-rich region containing PPLP repeats (no PPLP repeat in *HULK3*), and nuclear localization signals. *HULK* proteins have not been identified in animal species, indicating that this family of proteins may play roles unique to the plant development. Developmental and tissue specific expression patterns of members of *HULK* gene family are largely overlapping, suggesting possible functional redundancy. T-DNA insertional lines of *HULK1*, *HULK2* and *HULK3* did not show any obvious phenotypes. However, double mutant combinations showed distinct phenotypes: early and late flowering (depending on the combination), stem fasciation, early sterile flowers, reduced fertility and embryo defects. Some of the triple mutant combinations are not viable. Constitutive expression of individual members resulted in meristem-related phenotypes. These data suggest that *HULK* gene family members may have redundant roles in flowering time and other aspects of plant development.

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**ICAR1027**

THE DRN/DRNL AND CUC GENES CONVERT RADIAL TO BILATERAL SYMMETRY IN THE GLOBULAR EMBRYO VIA AUXIN-DEPENDENT PATHWAYS.

Category: Developmental Mechanisms

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A fundamental phase of *Arabidopsis* embryogenesis comprises the transition of a globular embryo with radial symmetry to the heart stage with two planes of bilateral symmetry, a change wrought by cotyledon initiation and outgrowth, with a requirement for cotyledon boundary separation.

The AP2 domain-encoding paralogues *DORNROESCHEN* (*DRN*) and *DORNROESCHEN-LIKE* (*DRNL*) redundantly control embryonic patterning and cotyledon development, with single *drn* or *drnl* mutants showing monocotyl or polycotyl and *drn drnl* double mutants completely lacking cotyledons. Mutant embryos uncover an asymmetry in *CUP-SHAPED COTYLEDON* (*CUC*) or *SHOOTMERISTEMLESS* (*STM*) gene expression, demonstrating that *DRN* and *DRNL* provide apical positional information essential for SAM positioning and boundary specification. Double mutants between *drn* or *drnl* and any of the *cuc* mutants show a significant increase in penetrance of cotyledon defects and a triple *drn drnl cuc2* mutant shows almost complete penetrance of cotyledon defects, suggesting that *DRN/DRNL* and *CUC* are the only pathways required for normal cotyledon specification.

*DRN* functions both upstream of auxin signalling/perception/response as shown by DR5::GFP and PIN1 expression, and downstream of auxin, as demonstrated by functional auxin response elements in the *DRN* promoter. Expression of DR5::GFP is also affected in the *cuc* mutants, suggesting the *CUC* genes also function in an auxin-dependent manner. The integration therefore, of *CUC* and *DRN* and *DRNL* pathways involving auxin are necessary for cotyledon specification.

We have also shown that *DRN* is downstream of *MONOPTEROS* (*MP*) and different epistatic genetic interactions suggest that *DRN* and *DRNL* can be differentially positioned with respect to *MP* function.

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### **ICAR1028**

DECAPITATION INDUCED ADVENTITIOUS REGENERATION (DIAR): A NEW RAPID SHOOT REGENERATION SYSTEM IN ARABIDOPSIS IN WHICH PRESUMPTIVE LATERAL ROOT PRIMORDIA ARE CONVERTED INTO NEW SHOOT APICAL MERISTEMS

Category: Developmental Mechanisms

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Regeneration via adventitious shoot apical meristems (SAMs) is a widespread ability in plants and this developmental flexibility has been successfully exploited during decades of in vitro culture. However, traditional tissue culture techniques typically rely upon high exogenous concentrations of auxin and cytokinin, and may take weeks to regenerate shoots from undifferentiated callus. Here we report, for the first time, adventitious shoot regeneration in the model plant *Arabidopsis thaliana* without the need for exogenous hormones and in the absence of callus. New SAMs are induced in the hypocotyl and uppermost root rapidly (4-10 days) following decapitation. The process involves the conversion of presumptive lateral root primordia (LRP) into new SAMs. Reporters for the SAM specific genes *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*) are first expressed in organs with shared anatomical features of root and shoot meristems (48 hours post-decapitation). There is a functional requirement for the *WUS* gene in decapitation-induced shoot regeneration, but not *CLV3*. We have investigated the role of various hormones in the process and have been able to induce similar patterns of shoot regeneration and SAM reporter gene expression using sequential applications of physiological concentrations of auxin and cytokinin. Using this inducible system and confocal microscopy we have followed the loss of fluorescent root meristem (RAM) reporters and the up-regulation of SAM specific genes during the conversion of LRP to shoot meristems. We have found that expression of root meristem/primordia specific markers and SAM specific reporters are mutually exclusive.

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### **ICAR1029**

ROOT HAIR-SPECIFIC GENES IN THE ARABIDOPSIS GENOME

Category: Developmental Mechanisms

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Cell differentiation of multicellular organisms ultimately relies on a portion of genes whose expression is modulated in a cell type-specific manner. Characterization of cell type-specific genes is the primary task to understand cell differentiation processes. *Arabidopsis* root hair cells have been a model to study cell differentiation. Here, we report novel root hair cell-specific genes which were screened by a series of both *in silico* and experimental filtration procedures: (1) genome-wide screening of the genes with well-defined root hair-specific cis-element (RHE) in the promoter, (2) filtering root-specific genes from the RHE-containing genes, (3) further filtration of the genes that are suppressed in root hair-defective mutants, and (4) experimental confirmation by promoter analysis. These procedures gave rise to 19 new root hair-specific genes that have not been studied yet. These new root hair genes include many protein kinases and cell wall-related genes which could provide further insight into the processes for root hair morphogenesis and tip growth. Functional analysis of some of these root hair genes revealed that they play a role for hair growth. This study demonstrated that a defined cis-element can serve a useful starting key for genome-wide screening of cell type-specific genes, and identified novel root hair-specific genes that are implicated in root hair development.

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### **ICAR1030**

ABC TRANSPORTERS IMPORTANT FOR POLLEN WALL DEVELOPMENT.

Category: Developmental Mechanisms

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Pollen grains are coated with lipophilic materials synthesized at the tapetum layer cells and pollen cytosol. We searched for transporters that might

be involved in transporting the lipid materials onto the pollen surface. ABC proteins are known to transport lipid materials in diverse organisms. We identified two ABC transporters that are specifically expressed in anther using RT-PCR assay, promoter-GUS assay and in silico search using microarray database.

Mutant plants that are deficient in expression of the two genes were tested for pollen viability. The pollen grains of double knockout mutant were less viable under normal condition, and their coats were often shrunken and distorted. Based on these results, we propose that the two ABC transporters are involved in pollen wall formation. These and other data supporting the possibility will be presented.

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### **ICAR1031**

#### IDENTIFICATION OF CIS-REGULATORY ELEMENTS AND EXPRESSION ANALYSIS OF VOZ GENES FROM ARABIDOPSIS THALIANA

Category: Developmental Mechanisms

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Two Plant specific transcription factors AtVOZ1 and AtVOZ2 (*A. thaliana* Vascular plant One Zinc finger protein) identified earlier as novel proteins interacting with cis-acting of Arabidopsis V-PPase gene AVP1.

These proteins showed significant sequence similarity, and homologs of the proteins have been found in mosses to higher plants. All VOZ proteins form various species share the conserved domain-A, this domain has property to bind with DNA in sequence specific manner. (Mitsuda et al;2003 & Choudhary et al ;unpublished data).

AtVOZ1 expressed specifically in phloem tissues where as AtVOZ2 gene expressed in both xylem and phloem tissues. Further detailed expression analysis of AtVOZ revealed that these genes are vascular specific genes expressed in all most all organ vasculature.

The expression pattern is quite ubiquitous it starts from very early stages of the embryogenesis to mature plants even in senescence leaves.

In this study to dissect the regulatory region of VOZ genes we have used GUS/GFP –reporter assay system. We have cloned different length of 5'- regulatory region in GUS/GFP- reporter constructs, the cloned 3.77 kb, 2.5 kb, 2 kb 5'-regulatory region in the GUS/GFP reporter transgenic plants shown the phloem specific AtVOZ1 expression.

**But 1.6 kb 5'-regulatory region was sufficient to show phloem specific AtVOZ1 expression. The 1.7 kb 5'-regulatory region of AtVOZ2 has shown xylem and phloem specific expression.**

The T-DNA mutant line with in the 1.6 kb 5' regulatory region of AtVOZ1 gene has shown the thick stem phenotype and increased xylem tissues. This might be the indication of de-repression of some regulatory elements lie with in the 1.6 kb region to control phloem specific expression .Further detailed analyses are in progress.

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### **ICAR1032**

#### THE CHARACTERIZATION OF MUTANT TO AMBIENT ENVIRONMENTAL CONDITION.

Category: Developmental Mechanisms

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Plants adapt fluently the environmental condition. We isolated the mutant which shows multiple responses to growth condition. Closed plastic ware grown plants have a normal aerial part and floral transition. But, opened grown plants have abnormal leaves, several shoot, and delayed flowering. Closed air transition to opened air promptly caused the mutant phenotypes. This mutant increased the number and length of lateral root compared to wt. above all, this mutant showed the resistance of exaggerated apical hook formation on 10uM ACC. Because plant hormones regulated the development to be adapted their ambient environment. I tested the expression of the several hormone specific marker genes. We have found that at least the expression of *PBP1* and *ERF1* were up-regulated and *ABI2* was down-regulated in the mutant.

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### **ICAR1033**

#### IDENTIFICATION OF TARGET GENES OF THE MADS DOMAIN PROTEIN AGAMOUS BY CHIP-ON-CHIP

Category: Developmental Mechanisms

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The regulation of transcription is the main mechanism controlling gene activity in complex biological systems. A key step in regulating gene expression, among others, is the sequence-specific binding of transcription factors (the trans-elements) to their DNA recognition sites (the cis-elements). A well-studied family of transcription factors in plants is the MADS domain family, for which a wealth of genetic, molecular and evolutionary data is available. The generally accepted model is that MADS domain proteins form higher-order complexes and bind to CArG-boxes present in the vicinity of their target genes. However, our knowledge about target genes is still very limited. To gain more knowledge about target genes and their

specific CArG-boxes, we are using chromatin immunoprecipitation (ChIP) in combination with a microarray hybridization procedure (ChIP-on-chip). The latest results will be presented in elucidating targets of the MADS domain protein AGAMOUS by using *Arabidopsis* full genome promoter microarrays.

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**ICAR1034****A BONA FIDE LA PROTEIN IS REQUIRED FOR EMBRYOGENESIS IN ARABIDOPSIS THALIANA**

Category: Developmental Mechanisms

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Searches in the *A. thaliana* genome using the La motif as query revealed the presence of eight La or La-like proteins. Using structural and phylogenetic criteria, we identified two putative genuine La proteins (At32 and At79) and showed that they are both expressed throughout plant development but at different levels and under different regulatory conditions. At32, but not At79, restores *S. cerevisiae* La nuclear functions in non-coding RNAs biogenesis and is able to bind to plant 3'-UUU-OH RNAs. We conclude that these La nuclear functions are conserved in *Arabidopsis* and supported by At32 we renamed AtLa1. Consistently, AtLa1 is predominantly localized to the plant nucleoplasm and was also detected in the nucleolar cavity. The inactivation of AtLa1 in *Arabidopsis* leads to an embryonic lethal phenotype with deficient embryos arrested at early globular stage of development. In addition, mutant embryonic cells display a nucleolar hypertrophy suggesting that AtLa1 is required for normal ribosome biogenesis. The identification of two distantly related proteins with all structural characteristics of genuine La proteins suggests that these factors evolved to a certain level of specialization in plants. This unprecedented situation provides a unique opportunity to dissect the very different aspects of this crucial cellular activity.

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**ICAR1035****PHENOTYPIC ANALYSIS OF ASK GENE LOSS OF FUNCTION ALLELES IN ARABIDOPSIS**

Category: Developmental Mechanisms

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Genetic studies have indicated the importance of targeted post-translational protein degradation as a key mechanism for the regulation of gene expression in plants. One of the main pathways involved in targeted protein degradation is the Ubiquitin/26S proteasome pathway. One highly studied class of E3 ligase are the SCF class whose quaternary structure typically includes 4 canonical polypeptides - Cullin, Rbx, Skp and an F-box subunit. In *Arabidopsis*, the Skp family of SCF subunits is encoded by 21 Skp1-like ASK genes compared to 1 orthologous gene in humans or yeast.

Phylogenetic analyses based on deduced amino acid sequence and expression profiles result in the clustering of the ASK gene family to distinct clades. The phylogeny suggests the possibility of functional redundancy between related Skp proteins. This suggestion is supported by genetic observation that ask1/ask2 double mutant exhibited a marked severe phenotype involving developmental delay and seedling lethality, whereas these defects were not detected in mutant lines carrying either ask1 or ask2 mutants alone<sup>(1)</sup>.

To elucidate the function of select members of the ASK gene family in *Arabidopsis*, we have undertaken a program of functional studies of a subset of the predicted ASK family of genes using two reverse-genetic resources; T-DNA insertions derived from the SIGNAL resource<sup>(2)</sup> targeting selected members of the gene family, and transgenic plants expressing artificial microRNA constructs designed to suppress the abundance of one or more ASK transcripts<sup>(3)</sup>. Example data will be presented describing aberrant plant growth and development phenotypes among select loss-of-function alleles of ASK3 or ASK10. The genetic resources developed by this study are the basis for further studies into the specific targeting functions and molecular mechanisms by which ASK genes contribute to plant patterning and development.

**1.F. Liu et al., Plant Cell 16, 5 (2004).**

**2.J. M. Alonso et al., Science 301, 653 (2003).**

**3.R. Schwab, S. Ossowski, M. Riester, N. Warthmann, D. Weigel, Plant Cell 18, 1121 (2006).**

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**ICAR1036****THEORETICAL MODELLING REVEALS COMPETITIVE COMPLEX FORMATION AS THE CORE OF TRICHOME PATTERNING ON ARABIDOPSIS LEAVES**

Category: Developmental Mechanisms

\*Simona Digiuni (1), Swen Schellmann (1), Florian Geier (3), Bettina Greese (3), \*Martina Pesch (1), Katja Wester (1), Burcu Dartan (1), Valerie Mach (1), Bhylahalli Purushottam Srinivas (1), Jens Timmer (2), Christian Fleck (2), Martin Hulskamp (1) 1 University of Cologne, Botany III, Gyrhofstr. 15, 50931 Cologne 2 University of Freiburg, Department of Mathematics and Physics, Hermann-Herder-Str. 3a, 79104 Freiburg, Germany 3 University of Freiburg, Department of Biology, Schaenzlestr. 1, 79104 Freiburg, Germany

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Trichome patterning in *Arabidopsis* serves as a model system for de novo pattern formation in plants. It is thought to typify the theoretical activator-inhibitor mechanism although this hypothesis has never been challenged by a combined experimental and theoretical approach. By integrating the key genetic and molecular data of the trichome patterning system we develop a new theoretical model which allows to directly test the effect of experimental interventions and to predict patterning phenotypes. We show experimentally that the trichome inhibitor TRIPTYCHON is transcriptionally activated by the known positive regulators GLABRA1 and GLABRA3. Further, by particle bombardment of protein fusions with GFP we show that TRIPTYCHON and CAPRICE but not GLABRA1 and GLABRA3 can move between cells. Finally, theoretical considerations suggest promoter

swapping and basal over-expression experiments by means of which we are able to discriminate three biologically meaningful variants of the trichome patterning model. Our work demonstrates that the mutual interplay between theory and experiment can reveal a new level of understanding of how biochemical mechanisms can drive biological patterning processes.

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**ICAR1037**

## MECHANICAL INDUCTION OF DE NOVO LATERAL ROOT INITIATION IN ARABIDOPSIS

Category: Developmental Mechanisms

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Lateral roots are essential if a plant is to explore efficiently its underground environment. Though more than seventy genes of Arabidopsis have been shown to affect lateral root development, the plant hormone auxin has emerged as a central regulator. However, auxin does not control lateral root development alone: other regulators such as ABA, cytokinin and brassinosteroids also regulate lateral root development, suggesting that the hormonal regulation of root development is complex. Lateral root initiation is also closely integrated with a range of environmental stimuli such as water availability, nutrients, pathogens, and symbionts. All of these factors influence the shape of the root system, making the control of root architecture an ideal model system to study the environmental control of plant development. Auxin accumulation at the site of lateral root initiation happens very early, even before the first cell division. Without any morphological changes to signify that the initiation process has begun, it has been very difficult to investigate events upstream of this accumulation. In this study we demonstrate that lateral root initiation is a consequence of root bending, either in response to gravity or by direct manipulation. We report on the series of molecular events that follow lateral root induction, with respect to auxin biosynthesis. This work for the first time enables the manual positioning of incipient lateral roots, allowing us now to investigate the "black box" of initiation signals prior to the accumulation of auxin at the initiation site, moving us closer to the link between the environment and organogenesis.

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**ICAR1038**

## CONTROL OF ASYMMETRIC CELL DIVISIONS IN THE PLANT STOMATAL LINEAGE BY A NOVAL POLARIZED PROTEIN

Category: Developmental Mechanisms

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Asymmetric cell division (ACD), in which daughter cells that differ in cell shape, size, and cellular components are created with different cell fate potentials, is a common mechanism for generating cell diversity. Studies from worms, flies, and vertebrate revealed the importance of intrinsic factors for determining the cell polarity in the process of ACD. Many of these factors are asymmetrically localized inside the cell, such as the cortically localized PAR proteins and the cell fate determinant Numb. ACDs are seen in embryonic, root and stomatal development in plants, but how these divisions are carried out is much less well understood. None of the proteins that are asymmetrically localized for cell fate in animals have obvious homologues in plants. ACD of a meristemoid mother cell (MMC) is the first event during stomatal differentiation in Arabidopsis, and it is important in regulating the overall patterned distribution of stomata. To better understand how this ACD was regulated, we screened an EMS population for mutants defective in stomatal asymmetric division and patterning. We recently mapped and cloned a mutant that disrupted ACD in the stomata lineage and exhibited increased numbers of stomata and stomatal lineage ground cells (SLGCs), many of which are found in clusters. The corresponding gene encodes a unique protein with no recognizable function domains, no paralogues in Aroidopsis, and no homologues outside of the Brassicas. We refer to this locus as BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL). BASL protein exhibits a remarkable expression pattern. A BASLpro::GFP-BASL reporter that rescues the basl null mutant localizes to nuclei of MMCs, divided meristemoids (the smaller daughter cell of a MMC, which may asymmetrically divide again or differentiate to guard mother cell) and their sister cells at the early stages of stomatal development. Later, BASL moves to a polarized plasma membrane region of MMCs and the meristemoid sister cells, away from the meristemoids. By creating reporters with different regions of the BASL protein, we identified specific domains responsible for specific sub-cellular localization. Most interestingly, the BASL protein region that is localized only to a highly asymmetric region of the plasma membrane is sufficient to rescue the stomatal development defects in the basl mutant. We believe that BASL is a key regulator of asymmetric division in plants and provides a foothold to identify proteins involved in this process. We have already carried out a yeast-two-hybrid screen for BASL interacting proteins. Greater than 80% of the interactors contained the same specific protein domain, and this domain suggests potential functions for BASL during development.

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**ICAR1039**

## DUAL ROLES FOR THE RECEPTOR KINASE CLV1 IN REGULATION OF FRUIT ORGAN NUMBER

Category: Developmental Mechanisms

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In species such as tomatoes and peppers, increases in fruit size are directly correlated with increases in organ number. Previous studies of floral meristems of Arabidopsis mutants with increased numbers of floral organs indicated that the extra organs are produced due to the presence of extra cells. For example, mutation of individual components of the CLAVATA meristem maintenance pathway increases the number of cells in the floral meristem and also produces extra fruit organs<sup>1</sup>. Through the characterization of a new allelic series of mutants in the receptor kinase *CLAVATA1* (*CLV1*), we have identified a second signaling mechanism that regulates fruit organ number by restricting cell proliferation in developing fruit. This developmental process is temporally distinct from the pathway functioning in floral meristems, acts specifically in developing fruit and also requires CLV1. Finally, we speculate that loss of this fruit-specific mechanism leads to the ectopic activation of the transcription factor network known to

regulate fruit organ specification, resulting in the production of fruit with extra organs. Supported by NSF IBN-0347675.

1. Clark, S., Running, M., and E. Meyerowitz (1993) *Development* 119: 397-418.

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**ICAR1040****TRANSCRIPT, PROTEIN AND METABOLITE ANALYSIS OF ARABIDOPSIS THALIANA TRICHOMES**

Category: Developmental Mechanisms

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Plants evolved diverse epidermal cell types with particular functions as adaptations to life on land such as gas-exchange, water evaporation and protection of the plant against mechanical and irradiation damage as well as herbivore and pathogen attacks. Due to their location on the leaf surface, epidermal cell-types provide a feasible and physiologically interesting model system for spatial analysis. Here we present a comprehensive spatial analysis of Arabidopsis leaf hairs in comparison to epidermal pavement and basal cells. Microsampling was applied to provide the basis for cell-specific analysis. To ensure a thorough coverage of all aspects transcripts, proteins and metabolites were studied. Microarray analyses revealed the cell type-specific transcriptome of trichomes and pavement cells. Numerous genes higher or exclusively expressed in trichomes were detected and confirmed by promoter:GUS analysis. In most cases these genes were associated with responses to pathogens, or biotic and abiotic stimuli. Nonetheless, spatial dissection of the transcriptome has also allowed insights into the localized mediators of hormone inputs as well as it provided information on cell-specific effects of so far uncharacterized genes.

In a proteomic approach SELDI disclosed molecular weights of cell type-specific marker proteins. However, identification of proteins highly abundant in trichomes was achieved using 2DE and nano-LC-MS/MS. Most of the identified proteins are implicated in metabolic processes and proteins involved in responses to biotic and abiotic stimuli and pathogens were detected.

GC-TOF-MS analysis of trichomes, pavement, and basal cells clearly demonstrated the metabolite partitioning among specialized epidermal cell types and enhanced the understanding of spatial aspects of physiology.

Analysis of cell wall extracts revealed a unique polysaccharide composition of trichome cells as compared to whole leaf extracts.

Altogether, this study led to the identification of known as well as so far uncharacterized transcripts, proteins and metabolites specifically abundant or enriched in trichomes. Evidence emphasizing a role of trichomes in defense-related processes was obtained. Thus, it can be concluded that trichomes are defense organs that serve as physical and molecular barrier.

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**ICAR1041****SUMO PROTEIN SUBSTRATES IN ARABIDOPSIS**

Category: Developmental Mechanisms

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Posttranslational modification of proteins by the covalent attachment of Small Ubiquitin-like Modifier (SUMO) has recently been implicated in the regulation of function of many proteins. Although having topological similarity with Ubiquitin, SUMO modification is not directly involved in protein degradation. In Arabidopsis, a mutation that impairs the function of a major SUMO-specific protease (ESD4) causes extreme early flowering as well as other phenotypes including alteration to phyllotaxy, leaf and siliques morphology, decreased fertility and determination of the shoot apical meristem. In order to investigate how protein SUMOylation is involved in such diverse developmental processes, we sought to identify SUMO substrates in Arabidopsis. We used both Yeast-Two hybrid assays and a bioinformatic approach and identified a large number of potential SUMO substrates. To test the SUMOylation of these substrates, we developed *E. coli* strains that express the basal SUMO conjugation pathway components as well as either SUMO1 or SUMO3. Substrates were then introduced into this strain and their SUMOylation was tested by immunoblot analysis using anti-substrate and anti-SUMO antibodies. To test the SUMOylation of these proteins in planta, we are developing a system whereby an Agrobacterium tumefaciens strain carrying a test substrate and another carrying a tagged SUMO are co-infiltrated into *Nicotiana benthamiana* leaves and SUMOylation is tested by immunoblot analysis. Using these approaches, we are preparing an inventory of Arabidopsis SUMO substrates.

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**ICAR1042****EFFECT OF SUCROSE ON COMPENSATED CELL ENLARGEMENT IN *FUGU5* MUTANT COTYLEDONS**

Category: Developmental Mechanisms

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In leaves of *Arabidopsis thaliana*, decreased cell proliferation activity often triggers excessive cell enlargement, a phenomenon that we named compensation. We have isolated and characterized five mutants (*fugu1-fugu5*) that exhibit compensation (Ferjani et al., 2007). The cotyledons of *fugu5* mutant are oblong when germinated on rockwool and exhibit strong compensation. Under these growth conditions, cell division is almost completely lost in mesophyll cells of *fugu5* cotyledons. However, we found that morphological phenotype of *fugu5* is recovered *in vitro* cultures. Histological analyses showed that in *fugu5* cotyledons grown *in vitro*, the number and size of cells recovered to wild-type levels, indicating the

presence of factors that can completely cancel compensated cell enlargement in *fugu5*. In order to identify those factors, we analyzed *fugu5* phenotype on MS medium with different supplements. Our results revealed that sucrose was necessary and sufficient for *fugu5* phenotype recovery. Exogenously supplemented glucose had similar effect to sucrose, but fructose was ineffective. Equimolar concentration of sorbitol did not affect *fugu5* phenotype. 3-O-methyl glucose, a non-metabolizable analog of glucose, also showed no positive effect. Taken together, these results indicate that sucrose should act either as a metabolite or as a signaling molecule, rather than as an osmolite. Furthermore, we showed that exogenous addition of sucrose induced the expression of *CYCB1;2::GUS* reporter gene 3 days after the start of imbibition. In the absence of sucrose no signals of the reporter GUS activity were identifiable at all despite the accumulation of *CYCB1;2* mRNA in *fugu5* to similar level as the WT. These findings suggest a translational or a post-translational regulation of *CYCB1;2* in *fugu5* background. Cloning of *FUGU5* gene revealed that it is *AVP1*, which encodes for a vacuolar type H<sup>+</sup>-pumping pyrophosphatase (H<sup>+</sup>-PPase). Although none of the presented phenotypes have been analyzable due to the very strong phenotype of *avp1-1* null mutant (Li et al., 2005), analyses of our own mutant alleles revealed for the first time that *FUGU5/AVP1* plays a crucial role in balancing cell number and cell size during development.

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**ICAR1043**

ARCHITECTURAL DIGEST: THE ROLE OF LIGHT AND PHYB IN THE GENERATION OF PLANT FORM.

Category: Developmental Mechanisms

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Branching is an obvious process contributing to plant architecture, and plays an important role in determining a plant's ecological/evolutionary fitness and suitability for agricultural use. Our long term goal is to understand how environmental signals coordinate the generation of plant form, especially branching. Phytochrome B (phyB) is understood to transduce light signals (R:FR) that control axillary bud outgrowth. Arabidopsis branching studies are challenging due to the quantitative nature of the process and the constellation of variables that can logically be considered. A comprehensive quantitative analysis of the role of phyB demonstrates that many aspects of branching are altered in *phyB* mutant Arabidopsis, with phyB acting negatively in some branching processes and positively in others. Some of the effects attributed to loss of phyB function may be partially or wholly abrogated by mutations in hormonal pathways that give rise to a hyperbranching phenotype. Therefore, phyB likely acts at least partially upstream of hormonal branching regulators. Loss of function of the branching integrators TBL1 (BRC1) or TBL2 (BRC2) results in hyperbranching, parameters of which may be partially suppressed by the *phyB* mutation. Loss of phyB function in the *tb1/tb2* double mutant affects various aspects of branching differentially, but overall suggests downstream and overlapping roles of the integrators. Growth under high R:FR (unshaded) and low R:FR (simulated shade) regimens further revealed unique roles for TBL1 and TBL2 in determining several architectural characteristics. Analyses of gene expression in axillary buds prior to elongation provide evidence elaborating the functions of phyB, hormones and the branching integrators TBL1 and TBL2 in branching responses to light signals.

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**ICAR1044**

THE ROLE OF THE SIGNALING MOLECULE CLE8 IN ARABIDOPSIS SEED DEVELOPMENT

Category: Developmental Mechanisms

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In diploid plants, seed development is initiated by the process of double fertilization which gives rise to a diploid embryo and a triploid endosperm. The two fertilization products grow in a concerted fashion, implying that communication takes place between these two tissues, but up until now little is known about the nature of this interaction at the molecular level. We have identified a putative signaling molecule, *CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR)-related (CLE) 8*, as an important player in *Arabidopsis* seed development. Members of the *CLE* gene family encode small polypeptides that work as signaling molecules in cell-to-cell communication. Although no functional information is available for the vast majority of the *CLE* genes, a few are known to function in different aspects of plant development. CLV3 acts in one of the best characterized ligand-receptor signaling pathways in *Arabidopsis* to regulate shoot and floral meristem size. CLV3 binds to the extracellular domain of the receptor like kinase CLAVATA1 (CLV1) leading to a signal cascade whose ultimate result is to restrict the domain of expression of the *WUSCHEL (WUS)* transcription factor gene. We have determined that *CLE8* is expressed in the endosperm and young developing embryos and that *CLE8* protein is secreted to the apoplast. A *CLE8* mutant allele has been identified that shows transcriptional mis-regulation of genes known to regulate endosperm proliferation, embryo development and seed size, suggesting that *CLE8* is involved in these processes. *Arabidopsis* plants over-expressing *CLE8* produce bigger and heavier seeds and show up-regulation of some members of the *WUSCHEL-LIKE HOMEBOX (WOX)* gene family. These findings, together with the known regulation of meristem size by *CLV3* and *WUS*, suggests that different members of the *CLE* and *WOX* gene families may be recruited in various *Arabidopsis* tissues to regulate a variety of developmental processes.

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**ICAR1045**

FUNCTIONAL ANALYSES OF *ARABIDOPSIS MAB4/ENP* INVOLVED IN POLAR AUXIN TRANSPORT

Category: Developmental Mechanisms

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The phytohormone auxin is transported by polar auxin transport system from cell to cell, leading to the asymmetric auxin distribution. Polar auxin transport is dependent on the activity of auxin efflux carriers, PIN-FORMED (PIN) proteins, localized in the plasma membrane with polarity. Recently, NONPHOTOTROPIC HYPOCOTYL 3 (NPH3)-like protein MACCHI-BOU 4/ENHANCER OF PINOID (MAB4/ENP) has been reported to regulate polar auxin transport through the control of subcellular localization of PIN proteins.

Although the MAB4/ENP protein has NPH3 and BTB/POZ domains, the function remains to be clarified. To isolate genes encoding proteins that interact with MAB4/ENP, we carried out a yeast two-hybrid screen. As a result, we identified several candidate genes. This time, we will present the results of our research on these genes, such as expression and subcellular localization analyses. In addition, there are at least five *MAB4/ENP*-like genes in the *Arabidopsis* genome. Although T-DNA insertion lines disrupting these loci did not result in any distinct phenotypes, multiple mutants between *mab4/enp* and some loci displayed severe defects than *mab4/enp* single mutants in organogenesis. These results suggest that these genes function redundantly with *MAB4/ENP* in organogenesis. Furthermore, analysis results on expression analysis of these genes will be also presented.

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**ICAR1046**

SIL1 IS A SANT DOMAIN-CONTAINING TRIHELIX TRANSCRIPTIONAL REPRESSOR OF SEED MATURATION GENES IN ARABIDOPSIS

Category: Developmental Mechanisms

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Progression through embryogenesis to seed maturity is regulated by the concerted interaction of major stage-specific developmental regulators. Such regulatory network is repressed prior to germination and the repression state is maintained during seedling development so that embryonic genes are not expressed in vegetative tissues. A new member of the plant-specific trihelix family of DNA-binding transcription factors, SIL1, was isolated from *Arabidopsis* based on its interaction with the 49-bp promoter region of 2S seed storage protein (SSP) gene *At2S3*, which has dual positive and negative roles for the regulation of seed storage protein expression. SIL1 possesses domains conserved in the trihelix family of proteins, which is similar to the SANT domain, and belongs to a subfamily of 6b-interacting protein 1-like proteins (SILs). Gene expression analysis reveals that SIL1 responds to ABA and functions as a negative regulator of seed maturation program in seedlings as disruption of *SIL1* markedly derepresses expression of the 2S SSP genes *At2S1-4* and the major oilbody protein oleosin gene *Oleo2* in an ABA-dependant manner along with the gene expression profile bearing a resemblance to late embryogenesis. In developing siliques, *SIL1* mutation leads to the earlier expression of both master regulatory genes *LEC2*, *FUS3* and *ABI3* and seed storage product genes *At2S1-4*, cruciferin *CRC* and *Oleo2*, indicating the temporal regulation of seed maturation genes by SIL1. SIL1 specifically recognizes GT-element that is overlapped the ACCT-box and closely associated with RY repeats in the *At2S* promoters. Our results suggest that SIL1 plays an important role in maintaining a repression state for the *Arabidopsis* napin and oleosin genes in seedlings and contributing to the correct developmental regulation of most seed maturation genes in developing siliques through a mechanism by which SIL1 actively interferes with or inhibits directly major transcriptional activators.

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**ICAR1047**

ACTIVATION TAGGING IDENTIFIES A GARP FAMILY TRANSCRIPTION FACTOR PAT1/KAN4 INVOLVED IN SEED PROANTHOCYANIDIN DEPOSITION, LEAF DEVELOPMENT AND CYTOKININ SIGNALING IN ARABIDOPSIS

Category: Developmental Mechanisms

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A novel patchy seed coat line *pat1* was observed in a histochemical screen for proanthocyanidin-deficient transparent testa mutants from a new activation-tagged population of *Arabidopsis*. This mutant also showed serrated leaves, uneven leaf shape and curvature, short crinkled fruits and longer time to maturity compared with Columbia. Genetic analysis indicated that the leaf shape phenotype was dominant and linked to the seed coat phenotype. Analysis of the single T-DNA insertion site and nearby genes revealed that these phenotypes were caused by activation of a GARP family MYB-like transcription factor *ATS* (*aberrant testa shape*), previously referenced as *KAN4* and involved in ovule integument development. Over-expression of the *KAN4* cDNA recreated the leaf shape and seed coat phenotype of *pat1*. A PAT1-GFP fusion protein was located mostly in the nucleus, consistent with a regulatory function for the native PAT1 protein. *PAT1* is expressed mainly in the vasculature of young seedlings and in the shoot and root apex and flowers of mature plants. Hormone treatment shows that *pat1* is less sensitive to the cytokinin benzyl adenine than Columbia. Microarray analysis of mature leaf transcripts between *pat1* and Columbia suggested that PAT1 functions as a central transcriptional regulator of lateral organ development genes and cytokinin response genes. Together, our results show that the *PAT1* is important for ovule integuments, leaf development, cytokinin signal transduction and consequently proanthocyanidin deposition.

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**ICAR1048**

THE C-TERMINAL DOMAIN OF THE TRANSCRIPTION FACTOR FUSCA3 CONTROLS PROTEIN STABILITY AND LOCALIZATION IN A HORMONE-DEPENDENT MANNER

Category: Developmental Mechanisms

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*FUSCA3* (*FUS3*) is a key transcriptional regulator controlling embryonic-to-vegetative phase transition by regulating abscisic acid (ABA) and gibberellin acid (GA) levels in *Arabidopsis*. The *FUS3* protein is destabilized by GA while stabilized by ABA. Here we report that destabilization of *FUS3* and subsequent degradation is mediated by its c-terminus. A truncated *FUS3* lacking this domain is expressed at high levels and is insensitive to the destabilizing and stabilizing effects of GA and ABA, respectively. Moreover, *FUS3* c-terminus alone is also sufficient to cause protein degradation when attached to heterologous proteins. Finally, we also show that *FUS3* c-terminus is required for correct protein localization and normal *FUS3* function. These results suggest that *FUS3* turnover, sensitivity to hormones and localization are mediated by its c-terminus.

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**ICAR1049**

AIR12: A PUTATIVE COMPONENT OF ABA SIGNALING AND PLANT ARCHITECTURE IN *ARABIDOPSIS THALIANA*

Category: Developmental Mechanisms

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Our group is interested in improving the performance of *Brassica* crops. *B. carinata* is generally more resistant to abiotic and biotic stress than other *Brassica* species and as such we chose to identify genes whose expression was induced by copper chloride in this plant. Since a highly efficient transformation system was available for *B. carinata* we initially investigated the function of some of these genes by antisense suppression. Antisense transgenics for a gene designated *CIL1* (Copper Induced in Leaves) had reduced apical dominance and lateral root production in addition to altered response to abiotic stress and abscisic acid (ABA). BLAST queries indicated high similarity between *CIL1* and *AIR12* (Auxin Induced in Roots) from *Arabidopsis thaliana*. Since the resources for molecular analyses in *A. thaliana* are greater than are available for *Brassicas* we are proceeding with our functional analyses in *Arabidopsis*. The available data suggests that *AIR12* may either be an extracellular matrix constituent anchored to the outer leaflet of the plasma membrane via a glucosyphosphatidylinositol (GPI) moiety or be secreted into the apoplast. Similar to antisense *CIL1* *B. carinata* transgenics, *AIR12* T-DNA knockout (ko) plants show reductions in lateral root number and root H<sub>2</sub>O<sub>2</sub> concentration as well as increased tolerance to salt stress compared to wild type. The knockout plants are also less sensitive to ABA and germinate twice as quickly as wild type. *AIR12* T-DNA ko plants were transformed with a *35S:CIL1-GFP* translational fusion to investigate gene complementarity and localization of *CIL1*. Microscopic examination of *AIR12*ko-CILGFP plants indicates that *CIL1* localizes to the plasma membrane and apoplast. Given the changes in plant architecture, osmotic stress response, ABA sensitivity and reactive oxygen species content that we observe in *AIR12* ko plants we believe that the protein is a component of an ABA signal transduction pathway.

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**ICAR1050**

THE MOLECULAR BASIS OF PLANT YIELD

Category: Developmental Mechanisms

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The demand for more plant-derived products is increasing spectacularly to feed a rapidly growing world population, produce more plant-derived feed and supply our ever-growing energy needs. To cope with this demand while using less arable land, a profound increase in crop yield will have to be achieved.

Biomass production is a multi-factorial system in which processes, such as photosynthesis, water and mineral uptake, mobilization of starch and lipid reserves, and response to environment, ultimately determine the activity of meristems that give rise to new cells, tissues, and organs. Whereas a considerable amount of physiological research has been done on yield performance of crops, little is known about the molecular networks determining growth rates. Many genes have been described in *Arabidopsis* that, when mutated or ectopically expressed, lead to faster growth, often due to the formation of larger structures. These "intrinsic yield genes" (IYGs) are involved in various processes whose interrelationships are mostly unknown. However, published experiments to measure the effects of IYGs on growth under optimal conditions were carried out under often very different growth conditions and with different ecotypes, making comparisons virtually impossible.

To this end, we recently initiated a large-scale project (yield booster) to compare the effects of "yield genes" under standardized conditions in the same genetic background and to analyze the cellular and molecular bases underpinning the increased leaf growth under optimal conditions. The cellular basis of the enhanced growth is being studied by kinematic analysis and various 'omics' technologies are used to decipher the molecular networks orchestrating the observed growth effects.

A literature search identified 37 IYG lines producing enlarged leaves. The growth behavior of 9 IYG lines was analyzed and, in our conditions, we were able to confirm the enlarged leaf surface of 6 lines. We found that the increased leaf area is mainly due to an increase in cell number. These lines are now used for expression profiling, metabolite analysis in order to identify molecular pathways directly related to the improved growth. The long-term goal is to develop computational models describing the molecular basis of plant yield and to use these models to improve crop productivity.

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**ICAR1051**

ARABIDOPSIS *RACK1* GENES REGULATE PLANT DEVELOPMENT WITH UNEQUAL GENETIC REDUNDANCE

Category: Developmental Mechanisms

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Receptor for activated C kinase 1 (RACK1) is a versatile scaffold protein that binds numerous signalling molecules from diverse signal transduction pathways in mammals. The protein sequence of mammalian RACK1 is highly conserved in plants. Moreover, unlike non-plant organisms which contain a single *RACK1* gene, some plants have more than one *RACK1* genes. In particular, the *Arabidopsis* genome contains three *RACK1* homologous genes, designated as *RACK1A*, *RACK1B* and *RACK1C*, respectively. Previous studies indicated that the loss-of-function alleles of *RACK1A* displayed multiple defects in plant development. However, the function of the other two members, *RACK1B* and *RACK1C*, and the relationship between the three *Arabidopsis* *RACK1* genes were unknown. Here we provide genetic evidence that unlike in *RACK1A*, loss-of-function mutations in *RACK1B* or *RACK1C* do not confer apparent defects in plant development. However, loss-of-function mutations in *RACK1B* or *RACK1C* strongly enhanced the *rack1a* mutant's developmental defects, including reduction in rosette leaf production and reduction in root development. Further, an extreme developmental defect was observed in *rack1a rack1b rack1c* triple mutant. These results suggested that *RACK1* genes are critical regulators of plant development, and that there is unequal genetic redundancy among three *Arabidopsis* *RACK1* homologous genes. We demonstrated that *RACK1B* and *RACK1C* are in principal functionally equivalent to *RACK1A*, and that both the difference in gene expression level and the cross-regulation are the molecular determinants of unequal genetic redundancy of *RACK1* homologous genes in regulating plant development.

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**ICAR1052**

IDENTIFICATION OF MICRORNAs REGULATED BY THE CHANGE OF AMBIENT TEMPERATURE

Category: Developmental Mechanisms

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MicroRNAs regulate many aspects of development in *Arabidopsis*. Especially recent studies have reported the roles of microRNAs in regulation of flowering time. But, it is not yet to be clearly established the relation between the microRNAs and flowering time control. We had expected that there are some microRNAs which is affected by change of ambient temperature. So we checked the wild-type microarray data at 23°C and 16°C. We could find out 22 microRNAs and checked the expression level of the selected microRNAs on the ambient temperature. As a result, some microRNAs appeared difference of the expression level. Also we checked the expression pattern of some of microRNAs in autonomous pathway mutants. We expect that these are one of the regulator related to ambient temperature.

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**ICAR1053**

CONTROL OF ARABIDOPSIS ROOT DEVELOPMENT THROUGH ACTION OF THE TYPE-B RESPONSE REGULATORS

Category: Developmental Mechanisms

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Cytokinins play essential roles in plant morphogenesis. Elevated levels of cytokinin release apical dominance, inhibit root elongation, delay senescence, and enhance shoot regeneration in cultured tissues. The cytokinin response is mediated by a two-component signaling pathway that culminates in regulation of the type-B response regulators (type-B ARR family). The type-B ARRs are transcription factors that contain a receiver domain with a conserved aspartate residue that allows for regulation through phosphorylation. Several members of the type-B ARR family are expressed in the root and here we have taken a mutant-based approach to elucidate their roles in regulation of root growth. Our results demonstrate roles for type-B ARR family members in root elongation, cell division, vascular development, and gene expression. To determine the mechanistic basis for type-B ARR action, we have examined the ability of various modified versions to rescue mutant phenotypes.

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**ICAR1054**

GDP1, A PUTATIVE RNA-BINDING PROTEIN, PARTICIPATES IN THE CONTROL OF LEAF SHAPE AND CELL PROLIFERATION

Category: Developmental Mechanisms

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One of unique features of leaf development is its determinate growth. We previously reported that *ANGUSTIFOLIA3* (*AN3*) encoding a putative transcription coactivator plays a role in the promotion of cell proliferation in leaf primordia. To understand how *AN3* controls cell proliferation, we carried out microarray analysis using *an3*- and wild-type leaf primordia. For a subset of affected genes, changes in the expression levels were confirmed by RT-PCR analysis. Among them we focused on a gene that encodes an unknown protein with an RNA-binding motif known as G-patch domain. We named this gene *GDP1* (*G-PATCH DOMAIN PROTEIN 1*) and further characterized. *GDP1* is expressed strongly in early leaf primordia and weakly in differentiating tissues and in the *an3* background *GDP1* expression was substantially reduced. We next analyzed its loss-of-function phenotype. Three *gdp1* alleles were obtained and all them developed narrow and pointed leaves, and contained about 30% fewer cells as compared with the wild-type leaves. These results suggest that *GDP1* plays a role in the promotion of cell proliferation at least partially through the direct or indirect transcriptional control by *AN3*.

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**ICAR1055**

INVOLVEMENT OF THE ATORK1 RECEPTOR KINASE IN GAMETOPHYTE DEVELOPMENT

Category: Developmental Mechanisms

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We report on a Ser/Thr receptor kinase from *Arabidopsis thaliana*, *AtORK1*, involved in gametophyte development (*Arabidopsis thaliana* ovule receptor kinase 1). It was first isolated with three other genes as a homologue of a previously characterized receptor kinase from *Solanum chacoense*. A phylogenetic analysis revealed a strong link between *AtORK1* (At3g17840) and three other receptor-like kinases (At1g48480, At3g02880, At5g16590). The expression analysis showed a restricted expression profile for *AtORK1*, being highly expressed in developing siliques and much weakly in the other tissues. The expression profile for the three other homologues was much broader. It was also shown that *AtORK1* is specifically up-regulated during female and male reproductive organ development. The analysis of insertion lines for all homologues resulted in no obvious phenotype. Therefore, to characterize the function of the receptor kinase, a dominant negative mutant in *Arabidopsis* for *AtORK1* was created and resulted in a fertility-associated phenotype. The lines analyzed showed reduced pollination rates due to pollen lethality, leading to short siliques and low seed yield. Pistil defects also accounted for this phenotype, as aborted ovules could be seen as early as the two-nuclear stage of the female gametophyte development. The bimolecular fluorescence complementation (BiFC) approach under transient transfection in onion cells was used to

study the action mode of the four homologues. It was found that each receptor can homodimerize in the overexpression system. The heterodimerization between the different receptors was also investigated to resolve the possible functional redundancy and gain insight on the interaction process.

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**ICAR1056**

SEED LONGEVITY RESEARCHES IN ARABIDOPSIS AND SACRED LOTUS

Category: Developmental Mechanisms

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Seed longevity is an important character of seed and grain from both ecological and agricultural perspectives, but the genetic basis and biochemical factors for seed aging is still not very clear. To date, it is reported that a great many factors, such as phospholipase D, oligosaccharides, sHSP, vitamin E, ABA and LEA Protein, are contributed to seed longevity in Arabidopsis and other species, while ascorbic acid, glutathion and catalase are not involved. Seeds of the sacred lotus seem to be the best materials of studying seed longevity, for lotus seeds hold the **world's record for seed** longevity and can survive under the treatment of extreme high temperature. To investigate the molecular mechanism of seed longevity, four genes from the seeds of sacred lotus, NnHSP21, NnHSP17.5, NnMT2-A, and NnMT3, were ectopic overexpression in Arabidopsis under the control of the CaMV35S and Arah3 promoter which is a seed-specific and highly efficient promoter from peanut, respectively. On the other hand, NnHSP21 and NnHSP17.5 were subcloned into pET-14b and expressed in E.coli as a fusion protein with a six histidine tag. Further researches should be continued.

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**ICAR1057**

LEAF POLARITY IS REGULATED BY ANTAGONISTICALLY DIRECT INTERACTIONS OF TRANSCRIPTION FACTORS AND GENES INVOLVED IN AUXIN RESPONSES

Category: Developmental Mechanisms

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Normal biological functions of leaves such as intercepting light and exchanging gases during photosynthesis rely on proper differentiation of adaxial (dorsal)-abaxial (ventral) identity. Although several families of transcriptional regulators have been identified that act antagonistically in establishing leaf polarity, their targets and the molecular basis for the regulatory circuitry are largely unknown. KANADI1, a transcription factor belonging to the *myb*-related GARP family, is an essential abaxial determinant. In this study, we characterized the DNA binding specificity of KAN1 both *in vitro* and *in vivo*. We identified the 6 base pair motif that the *Myb-like* domain in KAN1 binds *in vitro*. We also found that KAN1 acts primarily as a transcriptional repressor *in vivo* and directly regulates several genes implicated in auxin responses and one in gibberellin (GA) metabolism. In addition, we investigated in detail a specific target *ASYMMETRIC LEAVES2*, a key promoter of adaxial leaf fate. We found that KAN1 directly interacts with the *AS2* promoter and represses its transcription in abaxial cells. Mutation of a single nucleotide in a KAN1 binding motif in the *AS2* promoter abolishes KAN1 targeting leading to ectopic expression of *AS2* in abaxial cells and conferring a dominant, adaxialized phenotype. These results demonstrate the significant role of *KAN1* in the transcriptional regulation of leaf developmental patterning events and they also serve as a launch pad for researchers to explore additional regulators in the interaction network controlling leaf morphogenesis.

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**ICAR1058**

ANALYSIS OF THE *RLR50* MUTANT THAT SHOWS REDUCED LATERAL ROOT FORMATION IN *ARABIDOPSIS THALIANA*.

Category: Developmental Mechanisms

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Lateral root (LR) formation is important for the establishment of root architecture in higher plants. Previous studies have shown that Auxin Response Factor7 (ARF7) and ARF19 regulate LR formation via direct activation of *LOB-domain16* (*LBD16*) and *LBD29* genes in *Arabidopsis thaliana*. To understand the molecular mechanism of LR formation, we isolated the novel recessive mutant *rlr50-1* (*reduced lateral root formation 50*) that had the decreased number of LRs. The *rlr50* mutant showed reduction of not only emerged LRs from the primary root but also LR primordia. However, our analysis with the use of G2-M cell cycle marker cyclinB1;1::GUS indicated that the *rlr50-1* mutation increased the frequency of the spot which expressed cyclinB1;1::GUS without cell division in pericycle layer. These observations suggest that the *rlr50-1* mutation retards initial cell divisions in LR primordium formation. In the *rlr50-1* mutant, auxin-induced LR formation was reduced but auxin-inhibited primary root growth was not affected. Auxin-induced expression of *LBD16* and *LBD29* genes was not affected by *rlr50-1* mutation, suggesting that the *rlr50-1* mutation does not directly affect ARF-Aux/IAA signaling in roots. *RLR50* gene encodes an unknown protein with cytochrome b5-like heme/steroid binding domain and is expressed in almost all organs of the plant. In addition, functional GFP-tagged RLR50 proteins were localized in the cytosol of cultured cells and of root cells. Taken together, we conclude that RLR50 is a novel positive regulator of LR formation in *Arabidopsis thaliana*.

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**ICAR1059**

*ARABIDOPSIS* LATERAL ROOT FORMATION IN CULTURE: TWO APPROACHES, TWO MUTANT CATEGORIES, AND AT LEAST TWO MECHANISMS.

Category: Developmental Mechanisms

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The complex root system architecture of adult plants is derived almost exclusively from the post-embryonic production of lateral roots. This post-embryonic development allows plants to incorporate environmental information into decisions they make about when and where to produce lateral roots. We and others have utilized an agar plate assay to observe lateral root formation in the model plant *Arabidopsis thaliana*. We have found that when we lower the osmotic potential of the growth media, lateral root formation is repressed (repressive media) when compared to media that lacks osmotica (permissive media). I have undertaken two different approaches to identify genes that are involved in the repression of lateral root formation: a natural variation approach comparing *Arabidopsis* accessions Columbia (Col) and Landsberg erecta (Ler), and a forward genetics approach screening T-DNA mutants in the Col background. Both of these approaches were successful in identifying naturally-occurring or mutated alleles that result in abundant lateral root formation in plants grown on repressive media. Intriguingly, concurrent work in our laboratory demonstrated that uptake of sucrose from the culture media into plants' aerial tissues is necessary and sufficient for lateral root formation. Therefore, I have analyzed 10 recessive T-DNA mutants, Ler, and a near-isogenic line (NIL) that harbors a promotive Col allele in the Ler background to see if aerial tissue sucrose uptake could explain the observed increase in lateral root formation. Indeed, I have found evidence that increased lateral root formation in Ler, the NIL, and five of the mutants is most likely caused by increased aerial tissue sucrose uptake. In contrast, the remaining five mutants form lateral roots in the absence of aerial tissue contact with sucrose, suggesting a mechanism downstream or independent of sucrose uptake by aerial tissues. I am taking a closer look at the potential mechanism of action of two of these mutants, and will present current data on their further characterization.

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**ICAR1060**

## A GENETIC SCREEN FOR GENES INTERACTING WITH AS2 DURING LEAF DEVELOPMENT

Category: Developmental Mechanisms

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During plant development, leaves are initiated from the shoot apical meristem (SAM) and develop along adaxial-abaxial, proximal-distal and medial-lateral axes. The loss-of-function mutations in the *ASYMMETRIC LEAVES2* (*AS2*) genes of *Arabidopsis thaliana* cause pleiotropic phenotypes in leaves such as downward curling of leaves, reduced complexity of leaf venation pattern, generation of lobes, and slightly shorter petiole. *AS2* encodes a plant specific protein that contains *AS2*/LOB domain. *AS2* transcripts are accumulated in the adaxial domain of young leaves. *AS2* acts as a transcriptional repressor with *AS1* for class 1 *KNOX* genes, and abaxial factor genes, *ETT*, *KAN2*, *YAB5*. To identify new factors that function together with *AS2* in leaf development, we performed a genetic screen for enhancers and suppressors of the *as2-1* mutant. We found many mutants that enhanced the phenotype of *as2-1*. Some mutants had more lobed leaves, and some mutants had filament-like leaves that may defect the development of adaxial-abaxial leaf polarity. These phenotypes were similar to those of mutants that are previously reported as enhancers of *as2*. We also found a mutant that did not generate leaves but meristematic tissues. The mutant produced callus-like cells on MS medium without any plant hormone. We will present characteristics and genetic analysis of these mutants.

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**ICAR1061**A LOSS-OF-FUNCTION MUTANT IN *MIR319A* EXHIBITS DEFECTS IN PETAL AND STAMEN DEVELOPMENT

Category: Developmental Mechanisms

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The *Arabidopsis* flower is comprised of four types of organs: sepals, petals, stamens, and carpels. *dornröschen-2* mutants exhibit defects primarily in stamen development. A modifier screen in the *dornröschen-2* background led to the isolation of loss-of-function allele in the *miR319a* gene (better known as *miR-JAW*). The *miR319a<sup>129</sup>* loss-of-function mutant results in defects in both petal and stamen development; specifically, the petals are narrow and are of variable height while the stamen filaments are short. The *miR319a<sup>129</sup>* mutation results in a single base change in the mature miRNA. *miR319* targets a subset of TCP transcription factor genes (e.g. *TCP2*, *TCP3*, *TCP4*, *TCP10*, and *TCP24*); in *miR319a<sup>129</sup>* mutants, RNA levels for the TCP targets are increased. It is surprising that *miR319a<sup>129</sup>* mutants have a phenotype since *miR319* is redundantly encoded by a family of three genes: *miR319a*, *miR319b*, and *miR319c*. Expression analysis indicates that *miR319a*, but not *miR319c*, is expressed throughout developing petals and stamens.

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**ICAR1062**EARLY IN SHORT DAYS7 (*ESD7*) IS AFFECTING THE CATALYTIC SUBUNIT OF DNA POLYMERASE EPSILON, ACCELERATES FLOWERING TIME AND ALTERS LEAF AND ROOT DEVELOPMENT

Category: Developmental Mechanisms

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The suitable control of the floral transition is crucial for reproductive success in flowering plants. During the last years, several early-flowering mutants have been characterized although much of their interactions with the inductive pathways of flowering are not known. In a collection of Ds-containing T-DNA lines, we have isolated the *esd7-1* mutation, which cause early flowering independently of photoperiod conditions. This mutant is smaller in size and appears less vigorous than wild-type plant. The aerial part of the *esd7-1* mutant shows narrowed leaves and alterations in the pattern of vegetative growth. Flowers and siliques from the mutant are also smaller than wild type ones. Moreover, *esd7-1* primary root shows a significant decrease on elongation with higher production of adventitious roots.

*ESD7* has been identified through a map-based cloning approach and encodes the catalytic subunit of DNA polymerase epsilon (AtPOL2A), which is involved in other organisms in diverse processes such as DNA replication, DNA repair, chromatin remodelling and transcriptional silencing. *esd7-1* is a

hypomorphic allele whereas KO alleles display an embryo-lethal phenotype, suggesting that this gene is essential for the proper embryo development and viability.

*ESD7/AtPOL2A* is expressed ubiquitously at low levels in all the tissues analyzed and its expression is up-regulated by genotoxic stress. In fact, the mutant shows higher sensitivity to these agents than wild type plants and altered expression of genes involved in DNA repair mechanisms by homologous recombination such as *RAD51*.

Genetic analyses indicate that *ESD7* is involved in the repression of multiple flowering pathways, and that *esd7* early flowering phenotype is totally dependent on FT and SOC1 functional proteins. Genetic relationships between *ESD7* and several chromatin remodelers that participate in the control of flowering time as well as transcriptomic analysis involving *esd7-1* will be presented.

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### ICAR1063

RESTORATION OF *ACAU1/S1* PLANT SHAPE UNDER DIFFERENT TEMPERATURE AND DIFFERENT NUTRIENT CONDITIONS

Category: Developmental Mechanisms

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*acaulis1 (acl1)* mutants are first reported in 1993 by Tsukaya and his colleagues (Tsukaya et al., 1993, Development 118, 751-764). The severe *acl1-1* plants have tiny curly leaves, greatly reduced rosettes in size, and short inflorescences, which are only slightly longer than the height of the rosettes. A cluster of two or three flowers was produced at the top of its inflorescence stem. The weak allele *acl1-3* plants also have small leaves, small rosettes and short inflorescence stems. It was surprising, at that time, that even the severe *acl1-1* plants were able to grow as the same height and rosette size as wild type at 28°C.

We recently re-started the morphological observation of *acl1* and also *acl2* mutants under several different growth conditions. The temperature was indeed the important factor that controls the plant shape of *acl1* and *acl2* plants. Moreover, we newly found that the nutrient conditions are effective to change the growth of *acl1* plants. *ACL1* and *ACL2* may share the same pathway in part. However, there are differences between *acl1* and *acl2* mutants on the restoration of plant shapes under several growth conditions. The fact that the *acl1-3 acl2-1* double mutant shows stronger phenotype than the single mutants also suggests that *ACL1* and *ACL2* have other functions in independent pathways.

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### ICAR1064

FUNCTIONAL CONSERVATION OF LOTUS JAPONICUS ROOTHAIRLESS1/SЛИPPERY AND ARABIDOPSIS BASIC HELIX-LOOP-HELIX PROTEINS REVEALS NOVEL PLAYERS IN ROOT HAIR DEVELOPMENT.

Category: Developmental Mechanisms

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Root hairs increase root surface area, facilitating physical anchorage to a substrate while providing a large interface through which nutrients and water are absorbed. Root hairs are also important for plant-microbe interactions, highlighting the key role of these tip growing cells in biotic and abiotic interactions of the root. In the model legume *Lotus japonicus*, deleterious mutations in the *ROOTHAIRLESS1/SЛИPPERY (LjRHL1/SLP)* locus prevent root hair formation. Inoculation of this mutant with symbiotic bacteria leads to the formation of empty nodule structures, thus uncoupling bacterial colonization of the root from nodule organogenesis (1). We show here that mutations in the *LjRHL1/SLP* gene, encoding a presumed basic helix-loop-helix transcription factor, were responsible for lack of root hairs in the *L. japonicus Ljrh1* and *slp* allelic mutants. Among the 162 affiliates of the *Arabidopsis* bHLH protein family, *LjRHL1/SLP* showed the highest homology with subfamily XI, which comprises five predicted bHLH transcription factors (At4g30980, At2g24260, At5g58010, At1g03040 and At4g02590). We will discuss the results of cross species complementation experiments demonstrating the functional conservation of *L. japonicus LjRHL1/SLP* and At4g30980, At2g24260, At5g58010 but not At1g03040 and At4g02590 proteins. Partial redundancy of At4g30980, At2g24260, and At5g58010 may explain why mutations in these loci have not generated a root hair phenotype in *Arabidopsis*.

1. B. Karas *et al.*, Plant Physiol. 137, 1331 (2005).

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### ICAR1065

DEVELOPMENTAL ANALYSIS ON THE TOOTH IN ARABIDOPSIS

Category: Developmental Mechanisms

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Serration found along the leaf margin is an important key to identify plant species. Genetic analyses of arabidopsis have generated variety of serration mutants, but the mechanisms of tooth development are largely unknown. As a first step to unravel the mechanisms, we followed tooth development in the wild-type sixth leaves using microscopes.

We first looked for distinctive features of cell size and number along the leaf margin that could give rise to a tooth protrusion. We noticed that margin cells at the tooth tip and sinus were smaller than the other margin cells in a young leaf. Later, the cells at the sinus began to elongate while the cells at the tooth tip remained small. This indicates a locally regulated cell division and differentiation mechanism to form teeth. To understand how teeth are spaced out, margin cells were counted through development. Cell division at the margin ceased when the cell numbers between neighboring teeth reached around 16. Considering that the distance between teeth varies within a leaf, the distance is likely to depend on the extent of cell elongation.

The consistent cell number also suggests that teeth are formed by repetition of an unknown mechanism.

An auxin transport inhibitor results in smoother leaves and auxin accumulates at the tip of developing tooth (Aloni et al., 2003, *Planta*; Hay et al., 2006, *Development*). We examined if auxin is also involved in determining tooth initiation sites. Prior to tooth emergence, DR5::GFP, an indicator for auxin accumulation, was detected in the epidermis where a tooth was predicted to develop. This suggests that auxin marks future tooth initiation sites. The hydathode is found at the tooth tip in *Arabidopsis*. Characteristics of hydathodes were observed as soon as tracheary elements of xylem developed at the tooth tip. Hydathode formation might be related to xylem maturation and this is in agreement with its function to discharge excess water.

Additionally, cell-file-like patterns of epidermal and mesophyll cells were observed in young leaves. The patterns have a proximodistal axis at the base, and the axis curves toward the tooth. Unlike roots in which one growth direction and division plane result in a cell file, well-balanced two directions of growth and two orientations of cell division that are perpendicular to each other seem to be a key to these patterns.

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**ICAR1066**

INTER-CELL-LAYER SIGNALING DURING FLORAL DEVELOPMENT MEDIATED BY THE ATYPICAL RECEPTOR-LIKE KINASE STRUBBELIG

Category: Developmental Mechanisms

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The coordination of cellular behaviour within a tissue or organ requires intercellular communication that is poorly understood in plants. Signaling by the receptor-like kinase STRUBBELIG (SUB) is repeatedly required during *Arabidopsis* development in processes such as floral morphogenesis and ovule development<sup>1</sup>. At the cellular level *SUB* regulates cell shape and the cell division plane in a specific cell layer of floral meristems. In addition, *SUB*, also known as *SCRAMBLED (SCM)*, affects root hair patterning<sup>2</sup>. Interestingly, we have previously shown that phosphotransfer activity of SUB is not required in vivo indicating that SUB is a so-called atypical or dead receptor-like kinase<sup>1</sup>. As little is known about signaling by atypical receptor kinases in plants and in animals *SUB* signal transduction also serves as an excellent model to investigate the mechanistic basis of signal transduction by this class of unusual receptor kinases. Here we provide evidence that *SUB* acts in a non-cell-autonomous fashion and mediates cell morphogenesis and cell fate across clonally distinct cell layers in a radial inside-out signaling process in floral primordia, young ovules and root meristems. Using a combination of forward genetics and a systematic comparison of whole genome transcriptome profiles we have also identified three genes, *DETORSQUEO (DOO)*, *QUIRKY (QKY)*, and *ZERZAUST (ZET)*, that are involved in *SUB*-dependent processes. The data shed light on the mechanisms that depend on signaling through the atypical receptor-like kinase SUB.

<sup>1</sup>Chevalier et. al. 2005 PNAS 102: 9074-9079

<sup>2</sup>Kwak et. al. 2005 Science 307: 1111-1113.

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**ICAR1067**

THE GRF-INTERACTING FACTOR (*GIF*) GENE FAMILY IS REQUIRED FOR THE SHOOT APICAL MERISTEM FUNCTION IN *ARABIDOPSIS THALIANA*

Category: Developmental Mechanisms

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The *GRF-INTERACTING FACTOR (GIF)* gene family comprises three members and encodes transcription coactivators that interact with GRF proteins, transcription activators. We have previously demonstrated that *GIF7*, also known as *ANGUSTIFOLIA3*, is involved in regulating cell numbers of lateral organs such as leaves and flowers, determining their growth and shape. Similar expression pattern of *GIF* gene members prompted us to study in more detail the biological role of *GIF2* and *GIF3* genes as well as *GIF1*. Combinations of *gif1*, *gif2*, and *gif3* mutations showed synergistic enhancement of *gif1* phenotype, resulting in severely small and narrow lateral organs, indicating an overlapping function between *GIF* genes. We also found that numbers of cells comprising the shoot apical meristem (SAM) was remarkably reduced in the triple mutants and moderately in *gif1* and *gif2* double mutants, suggesting that small size of the mutant lateral organs may be ultimately due to reduction in the SAM cell numbers. In addition, the *gif* triple mutants displayed rapid germination and short plastochrony. Based on these phenotypical analyses, we propose that the *GIF* gene family may act as an important regulator of the SAM function.

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**ICAR1068**

FUNCTIONAL ANALYSES OF SHOOT AND ROOT MERISTEMS USING SYNTHESIZED CLV3 DODECAPEPTIDE

Category: Developmental Mechanisms

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Postembryonic development in plants is dependent on the activity of shoot and root meristems. CLAVATA3 (CLV3), a putative peptide ligand of *Arabidopsis thaliana*, regulates the stem cell population in the shoot apical meristem (SAM). Previously, we have been reported that functional CLV3 encodes dodecapeptides with two hydroxy proline residues, and chemically synthesized CLV3 peptide also function in our in vitro bioassay system, resulted in reduced SAM size (Kondo et al. 2006). In order to confirm synthetic peptide act on through endogenous CLV pathway, we determined whether CLV1 and CLV2 are involved in SAM defective phenotype by treating *clv* mutants with CLV3 peptide. As a result, *clv1* and *clv2* plants, but not *clv3* plants, showed resistance to SAM defect caused by CLV3 peptide. This suggests synthetic CLV3 peptide act on through endogenous CLV1/CLV2 receptor complex.

We also examined other 26 chemically synthetic CLE peptides, which correspond to the predicted products of 31 *Arabidopsis CLE* genes, and found

functional redundancy between these peptides. Here, we will show the effects of chemically synthetic CLE peptides on shoot and root meristems. Together with the analysis of CLV3 peptide-resistant-mutants, we will discuss about CLE function in higher plants.

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**ICAR1069****BRASSINOSTEROID SIGNALING IN ROOT EPIDERMAL PATTERNING IN ARABIDOPSIS THALIANA**

Category: Developmental Mechanisms

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Readily-accessible epidermal cells have played a central role in dissecting cell fate determination during plant development. Cell fate decisions in *Arabidopsis* root epidermis are made at early stages of development. Epidermal cells in contact with two underlying cortical cells differentiate into root hair cells (H cells/ trichoblasts), whereas cells that contact only a single cortical cell differentiate into mature hairless cells (N cells/ atrichoblasts) (1). This position-dependent patterning, in combination with constrained orientation of cell divisions, results in alternating hair and non-hair cell files throughout the root.

In this study, we provide evidence to indicate that **brassinosteroids (BRs), a family of growth regulatory phytohormones, are required to maintain position-dependent cell fate specification in *Arabidopsis* root epidermis**. Experiments documenting the number of hair or non-hair cells in N and H positions under different conditions strongly suggest that the **loss of BRs leads to an increase in non-hair cells in H positions**. Consistent with this, a GUS reporter for the *GLABRA 2 (GL2)* (1) gene, normally expressed only in the N cell position, is aberrantly expressed when either BR signaling or synthesis is blocked. Cross-sections of *GL2::GUS* roots in a *brassinosteroid Insensitive 1 ( bri1)* (2) background reveal many cases where cells in contact with two cortical cells aberrantly express the *GL2::GUS* reporter. In contrast, the loss of BR synthesis or signaling has little to no effect on the GUS expression pattern of *ENHANCER OF GLABRA 3 (EGL3)* (1), a gene that gets normally expressed in hair cells. Prior to this study, SCRAMBLED (SCM) (3), a leucine-rich repeat receptor-like kinase was the only known mediator of positional cues in the root epidermis. For the first time, this study points to a cell-type specific role for BRs in root hair formation and provides evidence for a novel mechanism for interpreting cell position.

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**ICAR1070****LIMITATION OF ARABIDOPSIS SHOOT ORGAN GROWTH REQUIRES OPTIMAL 26S PROTEASOME FUNCTION**

Category: Developmental Mechanisms

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Although the final size of plant organs is influenced by environmental cues, it is generally accepted that the primary size determinants are intrinsic factors. These internal signaling pathways exert positive and negative control on growth processes, such as cell proliferation and cell expansion. Here we show that one of the internal signaling pathways that repress *Arabidopsis* shoot organ growth requires the RPT2 subunit of the 26S proteasome.

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**ICAR1071****FUNCTIONAL CHARACTERIZATION OF THE CYTOKININ-ACTIVATING ENZYMES IN ARABIDOPSIS**

Category: Developmental Mechanisms

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Cytokinins, adenine derivatives, play a crucial role in various aspects of plant growth and development. The initial product of *de novo* cytokinin biosynthesis is cytokinin nucleotide. To become biologically active, the nucleotide has to be converted to a nucleobase. There are two pathways for producing active cytokinin species from the nucleotide: two-step pathway and direct pathway. Recently, LONELY GUY (LOG) has been identified from rice as cytokinin-activating enzyme catalyzing a reaction of the direct pathway. To elucidate the functions of cytokinin-activating enzyme in *Arabidopsis*, we characterized the *Arabidopsis LOG* family genes (*AtLOGs*). In *Arabidopsis* genome, nine genes (*AtLOG1 -AtLOG9*) are predicted as homologs of rice LOG. All *AtLOG* cDNAs with expected length were obtained by RT-PCR. The *AtLOG6* and *9* seem pseudogenes because of the existence of a premature stop codon in each cDNA. The cytokinin-specific phosphoribohydrolase activities were detected from *AtLOG1, 2, 3, 4, 5, 7, 8* proteins as well as rice LOG. Analysis of *AtLOGs::GUS* fusion genes revealed that each *AtLOGs* genes show the different expression patterns. To elucidate the functions of *Arabidopsis LOG* family genes *in vivo*, we produced and analyzed transgenic *Arabidopsis* lines overexpressing *AtLOGs* under the control of CaMV 35S promoter. These transgenic plants showed drastic change of cytokinin levels and cytokinin-related phenotypes. Possible role of the *AtLOGs* genes in plant growth and development will be discussed.

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**ICAR1072****MECHANISMS CONTROLLING SEED SIZE IN BRASSICACEAE**

Category: Developmental Mechanisms

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Seed size plays a key role in the yield, quality and profitability of oilseed rape (*Brassica napus*), with ancillary effects on the early stages of crop establishment through its contribution to seedling vigour. Seed growth in the Brassicaceae is controlled and co-ordinated by three main components: endosperm, seed integument and embryo. The embryo is the primary storage organ. However, the development and ultimate size of the embryo is determined by early development of endosperm and integuments. Currently we know of two mechanisms that increase seed size in the reference species Arabidopsis: increased endosperm proliferation during early seed development and increased growth of seed integuments. Although Arabidopsis seeds have ephemeral endosperms, increasing endosperm proliferation early in seed development increases the final size and weight of the seed. Crosses which generate 'paternal excess' in the seed (e.g. diploid 2x seed parent X tetraploid 4x pollen parent) increase endosperm size by increasing the rate and duration of mitosis. Extending the growing period of the endosperm produces a larger endosperm resulting in a larger embryo. Increased growth of seed integuments is evident from loss-of-function mutations in the transcription factor AUXIN RESPONSE FACTOR 2, syn. MEGAINTEGUMENTA (ARF2/MNT) which result in extra cell divisions within the integument prior to fertilization, a larger seed cavity, and ultimately large and heavy seeds containing large embryos. We expect much of the information gained from studying Arabidopsis to be relevant to the 400x larger *Brassica* crop seeds. We have recently verified that early endosperm proliferation due to paternal excess also occurs in *Brassica*. By carrying out reciprocal interploidy crosses (2x X 4x), we are characterising this endosperm-led modulation of seed size in *Brassica* spp. Using a set of nine monosomic addition lines that provide a full set of *B. rapa* (AA) chromosomes and one each of the haploid *B. oleracea* (C) chromosomes, we have recently identified a line that produces significantly larger seed. The use of addition lines allows us to target the changes in parental contribution to 'excess' by testing different sections of the genome. The data from these studies, together with a comparative genomics approach will help attribute QTL for seed size in *Brassica* to specific candidates, as well as highlight mechanisms that are being characterised in Arabidopsis.

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### **ICAR1073**

MOLECULAR AND GENETIC DISSECTION OF SCRAMBLED EXPRESSION IN ARABIDOPSIS ROOTS

Category: Developmental Mechanisms

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The epidermal cells of Arabidopsis roots determine their fate in a position-dependent manner. The epidermal cells developing on two cortical cells adopt the root hair cell fate; on the other hand, the epidermal cells on a single cortical cell develop as non-hair cells. SCRAMBLED (SCM), a leucine-rich repeat receptor-like kinase, mediates positional signaling and cell fate determination. We confirm that SCM-GFP protein is localized in the plasma membrane and distributed in epidermis, cortex, endodermis, stele, quiescent center and columella root cap initials. In epidermis, SCM-GFP protein is shown to accumulate evenly in younger (lower) meristem cells. Interestingly, in older (upper) part of the epidermal meristem, SCM-GFP protein accumulates preferentially in root hair cells. We demonstrate that the epidermal expression of SCM-GFP protein is necessary and sufficient for the epidermal patterning of roots by molecular dissection analysis of SCM expression. Furthermore, a root hair cell-specific promoter is more efficient for establishing the pattern than a non-hair cell-specific promoter. These results suggest that (1) SCM protein localized in the plasma membrane of epidermal cells is responsible for the epidermal patterning of roots and (2) although the primary determinant for the positional signaling is the distribution of a putative positional signal at an early stage, the root hair cell-preferential accumulation of SCM protein is necessary for the fine tuning of the cell fate pattern.

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### **ICAR1074**

FORMATION OF SECOND WHORL ORGANS IN THE ARABIDOPSIS FLOWER INVOLVES AUXIN INFUX AND IS SENSITIVE TO DISTORTION OF THE FLOWER MERISTEM SHAPE

Category: Developmental Mechanisms

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We are interested in how organ primordia are initiated within flowers, and in identifying the genetic factors and pathways involved in this process. Flowers of mutants of the *PETAL LOSS* (*PTL*) gene of *Arabidopsis* show a progressive loss in the ability to generate petals, and the sepals are wider, closer together and sometimes fused. *PTL* encodes a trihelix transcription factor that is expressed between sepal primordia (in the inter-sepal zone) during early stages of flower development. *PTL* may have a role in the suppression of growth between sepals, and a downstream role that localises a signal required for petals to arise in the adjacent petal initiation zone.

To identify enhancers, particularly redundant factors involved in floral organ initiation, an EMS mutagenesis screen was conducted in *ptl* mutant background. We identified a recessive enhancer mutant (*no petals, nop*) lacking petals, in a *ptl* mutant background. The *NOP* gene was positionally cloned and identified as *AUX7*, an auxin influx carrier.

Here we provide further evidence that auxin influx is necessary for petal development. This is only revealed when the flower meristem shape has been modified, either by the loss of *PTL* function or that of other genes including *CUC1*, *CUC2* and *EEP1* which maintain the flower meristem shape. We propose that auxin functions as a signal to promote the initiation of petals. Changes in flower meristem shape apparently cause disruptions to the signal, and that the initiation of second whorl primordia is especially sensitive to variation in its strength.

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### **ICAR1075**

ARF-GEFs REGULATE STOMATAL DISTRIBUTION AND MORPHOGENESIS

Category: Developmental Mechanisms

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Arabidopsis stomata consist of two kidney-shape guard cells around a pore and control gas exchange between shoots and the atmosphere. GNOM and GNOM-LIKE1 (GNL1) are ARF-GEFs meaning that they are guanine-nucleotide exchange factors (GEFs) that act upon ADP-ribosylation factor (ARF) small GTPases. GNOM and GNL1 control processes ranging from embryo development to the localization of PIN proteins. *gnom* mutants displayed a range of stomatal defects with phenotypic severity following an allelic series. By contrast, *gnl1* plants had fewer types of abnormalities. Some of the defects found were phenocopied by drug treatment of wild-type plants. Our data suggest that GNOM acts more broadly during stomatal development than GNL1.

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### **ICAR1076**

#### STAGE-SPECIFIC RESTRICTION OF CELL CYCLING BY THE R2R3 MYB FOUR LIPS

Category: Developmental Mechanisms

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Stomata are hydrostatic valves required for shoot gas exchange. Each stoma develops after a symmetric division that produces a pair of guard cells, a division that takes place in the guard mother cell (GMC). FOUR LIPS (FLP) is an R2R3MYB protein required to limit GMC symmetric divisions to one as shown by the presence of clusters of touching and laterally aligned stomata in *f/p* mutants. Mutations in *MYB88*, a *FLP* paralog, show no stomatal defects, but enhance the extent of stomatal clustering in a *f/p myb88* double mutant (Lai et al., TPC, 2005). To further probe FLP function we evaluated the effects of *FLP* overexpression, defined the expression window of a *FLP* translational fusion, and determined the relationship of *FLP* to the functions and expression patterns of several cell cycle genes. Our results suggest that FLP restricts the expression of cell cycle genes prior to guard cell differentiation.

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### **ICAR1077**

#### CELL-TO-CELL COMMUNICATION AND XYLEM PATTERNING IN ARABIDOPSIS ROOT

Category: Developmental Mechanisms

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A key question in developmental biology is how patterning of different cell types is organized to form organs and body plans. This normally involves extensive communication between cells and tissues. In plant roots, tissue organization is highly conserved over evolutionary time. The vascular cylinder, or stele, located in the middle of the root for conducting nutrients and water, is surrounded by endodermis, cortex, and epidermis. In the stele, two distinct water conducting xylem cell types, protoxylem and metaxylem differentiate in the centripetal direction. This coordinated tissue organization is very important for root function, yet the mechanism by which it is achieved is largely unknown. Here we show that xylem patterning in the root is controlled by a retrograde microRNA signal that emanates from the endodermis. Genes encoding these retrograde microRNAs are directly activated by the transcription factor SHORT ROOT (SHR), which is produced in the stele and moves into the endodermis. The activation by SHR is crucial for setting up the mRNA gradient of class III HD-ZIP transcription factors which is highest in the center of the stele and lowest in the stele periphery. The gradient is mediated via microRNA mediated mRNA degradation. We also show that this mRNA gradient is important for the dosage-dependent regulation of class III HD-ZIP transcription factors for xylem patterning. This intricate regulatory pathway involving cell-to-cell movement, transcriptional regulation, and posttranscriptional regulation provides new insight into how cells gain their identities via cross-talk during organ development.

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### **ICAR1078**

#### THE ROLE OF APL AS A TRANSCRIPTIONAL REGULATOR IN SPECIFYING VASCULAR IDENTITY

Category: Developmental Mechanisms

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The vascular system of higher plants confers efficient conduction and provides mechanical support. It consists of two kinds of conducting tissues, xylem and phloem. Phloem transports the products of photosynthesis and provides paths for translocation of proteins and mRNAs involved in plant growth and development. Although there are some reports of gene expression characteristic to phloem, the molecular basis of phloem development is still largely unknown. The APL transcription factor (Altered Phloem Development) was identified as the first gene specifying vascular tissue identity. Based on cell sorting coupled with genome-wide microarray analysis, we have been able to uncover phloem abundant regulatory genes dependent on APL. The results indicate that APL is a key node for transcriptional activation of gene expression characteristic to phloem development and for transcriptional repression of gene expression characteristic to xylem development. We are currently studying the possible functions of the identified genes in phloem development. Interestingly, some of the identified regulatory genes are related to each other, indicating subfunctionalisation of gene families related to phloem development.

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### **ICAR1079**

#### LARGE-SCALE ANALYSIS OF THE ARABIDOPSIS GRAS FAMILY

Category: Developmental Mechanisms

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GRAS proteins belong to a plant-specific transcription factor family. Currently, 33 GRAS members including a putative expressed pseudogene have been identified in the *Arabidopsis* genome. With a reverse genetic approach, we have constructed a "phenome-ready unimutant collection" of the GRAS genes in *Arabidopsis thaliana*. Of this collection, we focused on loss-of-function mutations in 23 uncharacterized GRAS members. Under standard conditions, homozygous mutants have no obvious morphological phenotypes compared with those of wild-type plants. Thus, we have begun to characterize the mutant phenotypes in specific tissues and/or under specific conditions, and our preliminary results will be discussed. Furthermore, expression analysis by qRT-PCR, microarray data mining, and promoter:GUS reporter fusions revealed their tissue-specific expression patterns. Our analysis of protein-protein interaction and subcellular localization of individual GRAS members indicated their roles as transcription regulators. In yeast two-hybrid (Y2H) assay, we confirmed the protein-protein interaction between SHORT-ROOT (SHR) and SCARECROW (SCR). Interestingly, we identified another SHR-interacting candidate, SCARECROW-LIKE23 (SCL23), which is the most closely related to SCR, suggesting that SCL23 plays a role in the SHR-involved developmental pathways. Our large-scale analysis of the *Arabidopsis* GRAS gene family provides an opportunity for the comprehensive evaluation on the roles of the members of this gene family. In addition, our phenome-ready unimutant collection will be a useful resource to better understand individual GRAS proteins that play diverse roles in plant growth and development.

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#### ICAR1082

#### ROOT SYSTEM ARCHITECTURE IN ARABIDOPSIS GROWN IN CULTURE IS CONTROLLED BY SUCROSE UPTAKE IN THE AERIAL TISSUES

Category: Developmental Mechanisms

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Culture systems have long been used to study root system development and the effects of environment on root system architecture. Our investigations into the mechanisms controlling these processes allow us to present a comprehensive model for the regulation of lateral root formation in *Arabidopsis* seedlings grown in culture. We demonstrate that direct contact between the aerial tissues and sucrose in the growth media is necessary and sufficient to promote emergence of lateral root primordia from the parent root. Sucrose enters the aerial tissues and acts as a metabolite to coordinately increase both shoot system development and lateral root formation. Mild osmotic stress, previously shown to switch root system development towards an unbranched architecture, is perceived by the root, which then sends an ABA signal to the aerial tissues. ABA causes a decrease in the permeability of aerial tissues and hence reduces uptake of sucrose from the culture media and therefore reduced lateral root formation. Consistent with these findings, osmotic repression of lateral root formation in culture can be overcome by mutations that cause the cuticle of a plant's aerial tissues to become more permeable. Indeed, we report here that the *lateral root development2* (*lrd2*) mutant, which overcomes osmotic repression of lateral root formation, carries a point mutation in *Long Chain Acyl-CoA Synthetase 2* (*LACS2*), a gene essential for cutin biosynthesis. Together, our findings: 1) Strongly impact the interpretation of experiments that utilize *Arabidopsis* grown in culture to study root system architecture; 2) Identify sucrose as an unexpected regulator of lateral root formation; 3) Demonstrate important mechanisms by which roots communicate information to aerial tissues and receive information in turn; and 4) Provide new insights into the regulatory pathways that allow plants to be developmentally plastic while preserving the essential balance between above-ground and underground organs.

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#### ICAR1083

#### ROLE OF PERIANTHIA IN STEM CELL CONTROL

Category: Developmental Mechanisms

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Plant stem cells continuously proliferate and are able to give rise to all cell types of the organism. To understand plant growth and development it is, therefore, of central importance to study the mechanisms of stem cell maintenance and differentiation. Recent findings indicate that the bZIP transcription factor **PERIANTHIA (PAN)** is one of the key players in regulating the fate choice between proliferation and differentiation in *Arabidopsis* shoots. Genetic and molecular studies indicate that PAN on the one hand affects the function of the stem cell niche in the shoot apical meristem, while on the other hand it is also necessary for the activation of the differentiation gene **AGAMOUS (AG)** in flowers. We have found that PAN directly binds to AG regulatory sequences and that this regulatory interaction is required for AG activation and proper termination of stem cell maintenance during flower development. In addition, PAN itself seems to be target of complex regulatory mechanisms, both at the transcriptional, as well as the posttranscriptional level, adding another layer of complexity. Taken together, PAN is embedded into a complex regulatory network and plays important roles in stem cell regulation that had previously gone unnoticed.

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#### ICAR1084

#### RHODANESE IS ESSENTIAL FOR EMBRYO DEVELOPMENT IN ARABIDOPSIS THALIANA

Category: Developmental Mechanisms

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Seed development is a pivotal process in the life cycle of angiosperms. We found that mutation in a rhodanese (*RHD*) gene results in a shrunken seed phenotype in *Arabidopsis thaliana*. The vegetative growth and development of *rhd* mutant are identical to those in the wild type. However, the embryo development of the *rhd* mutant is delayed and the majority of embryos arrest at the heart-stage, resulting in shrunken and wrinkled seeds. The germination of the mutant seeds is also affected. Only about 25% of the seeds from null *rhd* mutant plants can germinate and more than half of the seedlings show different degrees of cotyledon defects. The rhodanese activity in *rhd* mutant plants is much lower in comparison to wild-type plants. Expression of a wild-type *RHD* gene was able to rescue the shrunken seeds phenotype. These results indicate that the *RHD* gene play an important role in embryo and seed development.

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### **ICAR1085**

ROOTLESS: A PHD DOMAIN PROTEIN CONTROLLING ROOT SPECIFICATION IN THE ARABIDOPSIS EMBRYO

Category: Developmental Mechanisms

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In a search for the molecular function of proteins, called PVIPs, identified through their interaction with a plant virus protein, we have uncovered novel players role in root specification. Phenotypic characterisation of mutants, crosses with mutants in known auxin-related genes involved in embryonic root development, and localised expression patterns compared with reporters of auxin concentration have been used in PVIP functional analysis. The phenotypic features of the double mutant have led us to propose the name ROOTLESS1 and -2 (RTLS1 and RTLS2) for PVIP1 and PVIP2. RTLS1 and RTLS2 have PHD domains, which in other proteins have been shown to affect transcription through interactions with chromatin. Double homozygous null mutants produce defective seedlings completely lacking any root tissue. *rtls1 rtls2* phenotypes were very similar to *mp*, *bdl*, and other mutants in the auxin signalling pathway. Crosses to *mp*, *bdl*, and *pin1* mutants confirmed a positive relationship between RTLS1 and 2 and auxin signalling in defining the root during early embryogenesis. Mutant embryos showed altered basal cellular phenotypes. Reporters (*pDR5:GFP*) of auxin accumulation showed a delay in the development of the auxin maxima in basal embryonic tissues although later embryos became auxin overproducers. The *rtls1 rtls2* mutant showed widespread changes in the transcription of auxin-related genes. Promoter::reporter fusions showed that RLS expression was predominantly in the root tip and auxin-rich regions of the apical tissues. Our current hypothesis is that *rtls1 rtls2* mutants are defective in auxin perception or signal transduction at the transcriptional level possibly through an interaction with chromatin, which would represent a new level of control in auxin-directed functions.

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### **ICAR1086**

SOL2 MEDIATES CLE SIGNALING PATHWAY IN ARABIDOPSIS

Category: Developmental Mechanisms

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The plant meristem is maintained by intercellular signalling through dodeca-CLE peptides which regulate multicellular meristematic homeostasis in higher plants. The *Arabidopsis suppressor of LLP1 2 (sol2)* mutant, which suppress the short root phenotype caused by *CLAVATA3/ESR-related(CLE)19*-overexpression, showed a resistance to CLAVATA3 (CLV3) peptide and maintains the size of root meristematic zone in the presence of CLV3. The *sol2* mutant showed a defect in floral organ development and produced increased number of carpels, which is the typical phenotype of *c/lv* mutants. We investigated 26 synthetic CLE peptide function in the *clv1-4*, *clv1-6*, *clv1-13*, *clv2-1*, and *sol2* mutants, and suggest that at least three CLE pathways regulate root apical meristem homeostasis. Positional cloning of the *sol2* mutant identified a single amino acid substitution in a transmembrane domain of a receptor-like kinase protein. Transcriptional analysis by GeneChip revealed that many genes encoding cell signalling enzymes, including DC1-domain transcription factors, peptidase and receptor kinases were upregulated at the root tip in *sol2* mutant. Taken together, we suggest diverse SOL2 functions, including the regulation of shoot and root apical meristem homeostasis through the CLE signaling pathway.

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### **ICAR1087**

TRANSCRIPTION FACTORS INVOLVED IN CELL-FATE SPECIFICATION IN THE ARABIDOPSIS ROOT

Category: Developmental Mechanisms

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Plants are able to grow after embryogenesis generating new organs such as leaves, flowers and roots. Specialized proliferative tissues (meristems) located at the tips of roots and shoots continuously generate new cells for the plant. The root meristem comprises an organizing center (the quiescent center) surrounded by stem cells that divide asymmetrically to regenerate themselves and produce daughter cells, which are the precursor of each of the root lineages. Asymmetric cell divisions of the cortex/endodermis stem cell require the SHORT-ROOT (SHR) transcription factor (TF), which moves from the stele to the endodermis, as well as a related transcription factor, SCARECROW (SCR) that carries it into the nucleus. In addition, two zinc-finger proteins, MAGPIE (MGP) and JACKDAW (JKD) can differentially activate or restrain the SHR/SCR feedback loop. To further understand transcriptional regulation of cortex/endodermis specification, TFs differentially regulated in microarray analyses of whole roots and sorted endodermis/cortex cells were selected and specifically over-expressed in the ground tissue (the cortex and the endodermis). For some of them, we observed an altered radial root pattern indicating their involvement in cell-fate specification. Study of these TFs allowed us to further dissect the SHR network, leading to a better understanding of how certain genes act cooperatively with SHR while others would be activated to produce the asymmetric cell divisions of the cortex/endodermis stem cell daughter.

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**ICAR1088**

A NIMA-RELATED PROTEIN KINASE SUPPRESSES ECTOPIC OUTGROWTH OF EPIDERMAL CELLS THROUGH ITS KINASE ACTIVITY AND THE ASSOCIATION WITH MICROTUBULES.

Category: Developmental Mechanisms

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To study cellular morphogenesis genetically, we isolated loss-of-function mutants of *Arabidopsis thaliana*, designated *ibo1*. The *ibo1* mutations cause local outgrowth in the middle of epidermal cells of the hypocotyls and petioles, resulting in the formation of a protuberance. In *Arabidopsis*, the hypocotyl epidermis differentiates into two alternate cell files, the stoma cell file and the non-stoma cell file, by a mechanism involving TRANSPARENT TESTA GLABRA1 (TTG1) and GLABRA2 (GL2). The ectopic protuberances of the *ibo1* mutants were preferentially induced in the non-stoma cell files, which express GL2. TTG1-dependent epidermal patterning is required for protuberance formation in *ibo1*, suggesting that IBO1 functions downstream from epidermal cell specification. Pharmacological and genetic analyses demonstrated that ethylene promotes protuberance formation in *ibo1*, implying that IBO1 acts antagonistically to ethylene to suppress radial outgrowth. *IBO1* is identical to *NEK6*, which encodes a NIMA-related protein kinase (Nek) with sequence similarity to Neks involved in the microtubule organization in fungi, algae, and animals. The *ibo1-7* mutation, in which a conserved Glu residue in the activation loop is substituted by Arg, completely abolishes its kinase activity. The intracellular localization of GFP-tagged NEK6 showed that NEK6 mainly accumulates in cytoplasmic spots associated with cortical microtubules and with a putative component of the  $\gamma$ -tubulin complex. The localization of NEK6 is regulated by the C-terminal domain, which is truncated in the *ibo1-2* allele. These results suggest that the role of NEK6 in the control of cellular morphogenesis is dependent on its kinase action and association with the cortical microtubules.

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**ICAR1089**

STABLE ACCUMULATION OF DOG1 PROTEIN IN THE PRESENCE OF MULTIPLE ISOFORMS DERIVED FROM ALTERNATIVE SPLICING

Category: Developmental Mechanisms

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*DELAY OF GERMINATION 1 (DOG1)* was identified as a major determinant of natural variation for seed dormancy between the accessions Ler and Cvi of *Arabidopsis thaliana*. Expression of *DOG1* is seed-specific and transcript level correlates with dormancy. A loss-of-function mutation in *DOG1* results in a completely non-dormant phenotype.

*DOG1* is alternatively spliced into five different transcripts, which produces three isoforms. We have taken a transgenic complementation approach to check the functionality of these isoforms and have found that none of the single isoforms driven by the native promoter was able to complement the non-dormant phenotype of the *dog1* mutant. However, expression by CaMV 35S promoter yielded both non-dormant and dormant transformants. Quantitative RT-PCR of these transgenic lines has shown that the transcript level largely differed between non-dormant and dormant classes, and DOG1 protein was only detected in the plants with dormant phenotype. Accumulation of DOG1 protein was observed in wild-type seeds, although transcript level in wild type was much lower than that in non-dormant transgenic overexpressors. These results suggested that the single isoforms are unstable and are only able to accumulate and induce dormancy in the *dog1* mutant when transcript/protein level is above a certain threshold. Stable accumulation of DOG1 protein is likely to require multiple isoforms derived from alternative splicing.

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**ICAR1090**

WOX FAMILY GENES *PRS* AND *WOX1* ARE ESSENTIAL FOR LATERAL GROWTH AND DEVELOPMENT OF MARGINAL TISSUES OF LATERAL ORGANS

Category: Developmental Mechanisms

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*PRS*, encoding a WOX family transcription factor, is required for development of some lateral or marginal structures, including lateral sepals, marginal tissues of sepals and stipules. *PRS* is specifically expressed in marginal region of all lateral organ primordia except for carpels. Overexpression of *PRS* results in excessive proliferation of L1 cells. These imply that the marginal expression of *PRS* promotes the formation of marginal tissues and cell proliferation in lateral organs. However, although *PRS* is expressed also in the margin of the prospective blade region of leaf primordia, *prs* mutant does not display any morphological defects in leaf blades, suggesting that *PRS* redundantly act with other factors in leaf development.

*WOX1* is the *WOX* family gene sharing the highest sequence similarity to *PRS* in the homeodomain. Like *PRS*, *WOX1* expression was detected in lateral organ primordia. To investigate whether the function of *PRS* and *WOX1* overlap or not, we characterized phenotypes of *prs wox1* double mutant. In *prs wox1* double mutant, marginal epidermal cell files were completely missing and the number of hydathodes at the edge of leaf blades was dramatically decreased. Furthermore, leaves, sepals and petals in *prs wox1* double mutant were much narrower than those in wild type, *prs* or *wox1* single mutant. *prs wox1* double mutant had reduced cell number along the mediolateral axis in these organs. These results indicate that *PRS* and *WOX1* redundantly control the development of marginal tissues and promote lateral growth of lateral organs by regulating cell proliferation.

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**ICAR1091**

HANABA TARANU IS REQUIRED TO POSITION THE PROEMBRYO BOUNDARY IN *ARABIDOPSIS* EMBRYOGENESIS

Category: Developmental Mechanisms

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Cell fate decisions in the early embryo must be executed within tight space limitations, making the definition of boundaries between tissues at these early stages critical to pattern formation. As early as the first division of the zygote, a basal lineage is established which forms a predominantly extraembryonic support structure known as the suspensor, while the apical cell divides to give the proembryo. The juxtaposition of these two fates is associated with a weak auxin maximum in proembryo cells, presumably due to expression of apically-localized PIN-FORMED7 (PIN7) efflux receptor in the suspensor and apolar PIN1 in the proembryo. Later this serves as an inductive boundary across which the proembryo signals the uppermost suspensor cell (termed hypophysis) to initiate root meristem formation. Here we show that the GATA transcription factor *HANABA TARANU* (*HAN*) is required to position the proembryo boundary. Mutation of *HAN* results in an apical shift in the embryonic fate map such that the lower cells of the embryo take on characteristics of the suspensor. Both PIN1 and PIN7 localization are altered in *han* mutants, leading to a shifted auxin maximum and, remarkably, hypophysis-independent root formation at a central position in the embryo. *HAN* therefore represents one of the earliest known regulators of *PIN* gene expression – a boundary gene required for separating key lineages in the embryo and positioning root initiation correctly along the developing apical-basal axis.

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### ICAR1092

AGAMOUS CONTROLS GIANT KILLER, A MULTIFUNCTIONAL DETERMINANT OF REPRODUCTIVE ORGAN PATTERNING AND DIFFERENTIATION

Category: Developmental Mechanisms

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The *Arabidopsis* homeotic protein AGAMOUS (AG), a MADS domain transcription factor, specifies reproductive organ identity during flower development. We identified a direct target of AG, GIANT KILLER (GIK) by binding assay and expression analysis. GIK protein contains an AT-hook DNA binding motif, which is widely found in chromosomal proteins, and binds to nuclear matrix attachment regions (MAR) of DNA elements. Over-expression and loss-of-function of GIK exhibited wide-ranging defects in patterning and differentiation of reproductive organs. We show that GIK directly regulates the expression of several key transcriptional regulators, including ETTIN/AUXIN RESPONSE FACTOR 3 (ETT/ARF3) that patterns the gynoecium, through binding to the MARs of target promoters. Overexpression of GIK causes a swift and dynamic change of repressive histone modification in the ETT promoter. We propose that GIK acts as a molecular node downstream of the homeotic protein AG, regulating patterning and differentiation of reproductive organs through chromatin organization.

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### ICAR1093

CYTOKININ SIGNALING REGULATES CAMBIAL ACTIVITY

Category: Developmental Mechanisms

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Although a substantial amount of plant biomass originates from the activity of vascular cambium, molecular basis of radial plant growth is still largely unknown. Our work has previously indicated that enhanced cytokinin signaling can stimulate cambial activity during *Arabidopsis* root development (Mähönen *et al.* *Curr. Biol.* 2006 16:1116). To address whether cytokinins are required for normal cambial activity, we studied cambial cytokinin signaling in two hardwood tree species; poplar and birch. We observed a peak in the expression of putative cytokinin receptor genes in the cambial cells. For functional studies we engineered transgenic poplar trees expressing a cytokinin catabolic gene from *Arabidopsis*, *CYTOCKININ OXIDASE 2*, under the promoter of a birch cytokinin receptor. Reduced cytokinin signaling correlated with decreased radial growth and low cambial activity. Thus, our results indicate that cytokinins are major regulators required for cambial development. To identify components acting downstream of cytokinin signaling we are studying gene functions regulating the maintenance and proliferation of cambial stem cells in *Arabidopsis* roots. Through cell sorting and global gene expression profiling we have identified several cytokinin regulated genes whose expression is enriched in cambial cell files. We are currently studying the functions of identified candidate genes in the regulation of cambial development.

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### ICAR1094

MLO FUNCTION(S) IN PLANTS: TOWARDS THE GENETIC CONTROL OF THE ROOT CURLING PHENOTYPE ASSOCIATED WITH *ATMLO4* AND *ATMLO11* MUTANTS

Category: Developmental Mechanisms

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MLO proteins constitute a plant-specific family of seven-transmembrane domain proteins comprising 10 to 15 members per plant species. Barley MLO is the founder of this protein family, and based on amino acid similarity 15 MLO homologs have been identified in *Arabidopsis thaliana*. So far, barley MLO and its functional ortholog, AtMLO2, are the only members for which functional data are available: they are described as negative regulators of defence responses against appropriate powdery mildew fungi. However, each *AtMLO* gene has a unique expression pattern and is differentially regulated, and they can be grouped in at least four distinct phylogenetic clades. These data possibly reflect a putative functional diversification within the protein family.

In the effort of elucidating function(s) of MLO proteins in plants, it has been observed that *Atmlo4* and *Atmlo11* mutants exhibit a dramatic circling root growth pattern under *in vitro* culture conditions. In this project, we propose to explore the biological mechanisms associated with MLO function by identifying potential regulators implicated in the *root curling* phenotype observed in *Atmlo* mutants.

The cellular localisation of an MLO4-GFP fusion protein in the *Atmlo4* root tip has been determined by confocal microscopy. Then, to further characterise *Atmlo4* mutants, a chemical genetic approach has been employed. By screening of chemical libraries, it has been observed that compounds described as auxin transport- or vesicle trafficking-inhibitors and flavonoids are able to reverse the *root curling* phenotype of *Atmlo* mutants. The putative implication of MLO in auxin- and flavonoid-related mechanisms has been tested.

In addition to generate *Arabidopsis* suppressors of the *root curling* phenotype, a chemical re-mutagenesis approach has been performed from *Atmlo* plants. This allowed to identify nine suppressor mutants. A characterisation of these mutants has been carried out and map-based cloning of selected suppressing genes is in process.

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**ICAR1095**

AND YET IT TURNS – THE CELL CYCLE WITH AND WITHOUT THE CENTRAL CYCLIN-DEPENDENT KINASE CDKA;1

Category: Developmental Mechanisms

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The core of the eukaryotic cell cycle machinery is controlled by heterodimers of Cyclins and Cyclin-Dependent Kinases (CDKs). In multicellular organisms, families with partly redundant members of these regulators have evolved to take over specific functions. However, it appears to be a universal theme that at least one CDK containing the conserved Cyclin binding PSTAIRE motif is essential for basic cell cycle progression and thus survival of the organism. The *Arabidopsis* genome contains one single PSTAIRE-domain CDK, CDKA;1, which is able to complement the *cdc2/cdc28* mutants in yeast. We and others previously described *cdka;1* T-DNA insertion mutants without identifying homozygous individuals segregating from heterozygous parents. Yet, closer analyses revealed the existence of tiny, rootless plants, viable only in cell culture conditions. These homozygous *cdka;1* mutants stem from embryos of the same size as wild type embryos, but they contain only a fraction of the usual cell number. After germination, the shoot apical meristem becomes active and produces very small few-celled leaves and stout shoots, while the root meristem is not functional. How can plants, in contrast to all other eukaryotes investigated so far, survive without a PSTAIRE-domain CDK? Prime candidates for the backup system revealed in *cdka;1* mutants are the plant-specific CDKBs with a PPTALRE/PPTTLRE cyclin-binding domain. Current experiments show that a *proCDKA;1::CDKB* construct is able to partly rescue the *cdka;1* phenotype and that *cdka;1::cdkb* double mutants are not viable. These results underline the key position of CDKA;1 for cell cycle control, but also indicate that during their evolutionary adaptation, the CDKBs retained a function to drive both entry S and M phase.

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**ICAR1096**

PARTNERS OF THE PETAL LOSS PROTEIN IN THE ESTABLISHMENT OF PERIANTH DEVELOPMENT

Category: Developmental Mechanisms

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In the past decade, the intricate involvement of transcription factors in establishing the architecture and organogenesis of the flower is being revealed. We are characterizing the role of the PETAL LOSS (PTL) trihelix transcription factor. There is evidence that the PTL protein is involved in growth suppression between emerging sepals, leading to their separation and allowing sufficient space for petals to emerge internal to the inter-sepal zone. To identify proteins that interact with PTL, we conducted a yeast two-hybrid screen. As well as the PTL protein itself, we isolated independent clones of the Snf1-related protein kinase1 AKIN10. We were able to reproduce these interactions *in vitro* by pull-down assays. Through the use of bimolecular fluorescence complementation, we were also able to show interactions *in planta*. Further, we determined that PTL homodimerization involves the PTL central domain, and that the non-kinase domain of AKIN10 is sufficient to bind PTL. We are currently studying the phosphorylation status of PTL by AKIN10. A TILLING mutant of AKIN10 shows a pleiotropic disrupted phenotype, but in the flower the petal phenotype is reminiscent of a mild *ptl* mutant as they are smaller, sometimes filamentous and slightly misoriented. Promoter analysis of AKIN10 is currently being undertaken by fusions to GUS, and preliminary data indicate that PTL expression is included in the AKIN10 transcription domain. We are also over-expressing the kinase in the region defined by PTL expression to see if this will affect in any way the regulation of PTL. AKIN10 is known to sense energy deprivation and target a range of transcription factors involved in metabolism. Our findings suggest that AKIN10 signaling may extend to transcription factors involved in normal morphogenesis.

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**ICAR1097**

TRANSCRIPTIONAL ACTIVATION OF THE DICER-LIKE PROTEIN 2 AFTER AUXIN TREATMENT

Category: Developmental Mechanisms

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Transgenic *Arabidopsis* plants with the promoter of all four DCL proteins fused to GFP were screened with different phytohormones for activation or repression. For the developmentally important hormone auxin, activation of the promoter of DCL2 was detected. Also the level of mRNA of the

endogenous DCL2 was elevated. Interestingly PromoterDCL2:Gus fusion showed a very specific expression pattern in the youngest leaves of transgenic Arabidopsis seedlings and disappeared in older leaves. The influence of auxin on the small RNA pathway is discussed.

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**ICAR1098**

ARABIDOPSIS LIGHT-INDEPENDENT PROTOCHLOROPHYLLIDE OXIDOREDUCTASE A (PORA) RESTORES BULK CHLOROPHYLL SYNTHESIS TO A *PORB* *PORC* DOUBLE MUTANT

Category: Developmental Mechanisms

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In angiosperms the strictly light-dependent reduction of protochlorophyllide (Pchlde) to chlorophyllide is catalyzed by NADPH:Pchlde oxidoreductase (POR), chlorophyllide is subsequently modified to form chlorophyll. The *Arabidopsis thaliana* genome encodes three structurally related but differentially regulated *POR* genes *PORA*, *PORB* and *PORC*. *PORA* and *PORB* are coexpressed strongly early in development: during etiolation, germination and greening at the seedling cotyledon stage. *PORB* and *PORC* are co expressed both during seedling development and throughout the later life of the plant, and therefore are responsible for post-cotyledon bulk chlorophyll synthesis. While single *porB-1* or *porC-1* null mutants display no distinct light-grown phenotypes, the *porB-1 porC-1* double mutant displays a severe *xantha* (highly chlorophyll-deficient) phenotype and has reduced thylakoid membrane stacking in its plastids. In *porB-1* mutant etioplasts there are reduced amounts of prolamellar bodies (Frick et al. (2003) Plant J., 35, 141-153). Constitutive overexpression of *PORA* produces slightly larger prolamellar bodies in etioplasts in wild type (Sperling et al. (1997) Plant J., 12, 649-58). Constitutive overexpression of *PORA* restores prolamellar bodies in etioplasts of the *porB-1 porC-1* double mutant. Furthermore, bulk chlorophyll synthesis and thylakoid stacking are restored in the light-grown *PORA*-overexpressing *porB-1 porC-1* double mutant. Chlorophyll production is restored to normal levels in the *PORA*-rescued *porB-1 porC-1* double mutant plants. A *porB-1 porC-1* double mutant can therefore be functionally rescued by the addition of the overexpression of *PORA*, indicating that *PORA* alone is sufficient to support bulk chlorophyll synthesis and plant development throughout the life cycle in the absence of *PORB* and *PORC*.

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**ICAR1099**

PATTERNING OF ROOT EPIDERMAL CELLS IN ARABIDOPSIS: IDENTIFYING NEW MOLECULAR COMPONENTS IN POSITION-DEPENDENT CELL-FATE SPECIFICATION

Category: Developmental Mechanisms

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The *Arabidopsis* root epidermis is a simple and convenient model to study cell-fate specification and patterning in plants. Root epidermal cells adopt one of two possible fates, hair or non-hair, in a position-dependent manner. Epidermal cells located over two cortical cells will develop into a hair cell, while epidermal cells located over only one cortical cell will adopt a non-hair cell fate. Previous work has identified a transcriptional regulatory network within epidermal cells that generates and maintains cell fate. This network includes the patterning genes *WEREWOLF* (*WER*), *GLABRA3* (*GL3*), *ENHANCER OF GL3* (*EGL3*), *CAPRICE* (*CPC*) and *GLABRA2* (*GL2*). Recently, our lab identified *SCRAMBLED* (*SCM*), a leucine-rich repeat receptor-like kinase, which transmits positional information to the aforementioned downstream network. While this discovery has provided insight into the molecular mechanism controlling position-dependent fate specification, many questions remain unanswered. We are currently employing both forward genetic and genomic approaches to identify additional signaling components involved in conveying positional information to developing root epidermal cells. Two mutants with defects in epidermal cell-type patterning were isolated in a forward genetic screen. We are using map-based cloning to identify the disrupted genes, and we are investigating how these genes influence the gene-regulatory network within the epidermal cells by characterizing the mutant effect on spatial expression of *WER*, *GL2* and *EGL3*. Concurrently, we are investigating the role of genes identified in a microarray experiment as being differentially expressed in wild type versus *scm* root tissues. The identification and analysis of additional signaling components will provide insight into the molecular mechanism responsible for position-dependent cell-fate specification.

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**ICAR1100**

D- TYPE CYCLINS IN STOMATAL DEVELOPMENT

Category: Developmental Mechanisms

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A key developmental innovation of land plants was the evolution of specialized epidermal structures called stomata. Stomata consist of a pair of guard cells that mediate gas and water-vapour exchange between plants and the atmosphere. In *Arabidopsis*, the stomatal lineage arises from a protodermal cell that divides asymmetrically to give rise to a meristemoid that can undergo further asymmetric division before producing a guard mother cell (GMC). The GMC then divides symmetrically to create the two guard cells. This pathway requires stringent control by the machinery that regulates the cell cycle. Cell cycle progression is controlled by the activity and substrate specificity of cyclin-dependent protein kinases (CDKs), which is modulated by association with their regulatory cyclin subunit. D-type cyclins play an important role in cell cycle progression and re-entry in response to external signals, and expression studies in *Arabidopsis* of the 10 D-type cyclins indicate transient expression of three D-type cyclins at specific stages during stomatal development. CYCD function in stomatal development has been studied by the gain and loss of function, revealing control of distinct aspects of cell cycle progression during stomatal development by specific CYCDs. In addition, putative CDK and KRP partners for the stomatal D-type cyclins have been identified.

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**ICAR1101**

AN AUXIN SENSITIVITY MECHANISM IS INVOLVED IN LATERAL ROOT DEVELOPMENT UNDER PHOSPHATE DEPRIVATION CONDITIONS.

Category: Developmental Mechanisms

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Lateral root development is a major determinant of plant root architecture, contributing considerably to spatial configuration of the root system and substantially determining the ability of a plant to secure anchorage, water and nutrient uptake.

Root system architecture depends on both genetic determinants and postembryonic developmental processes that are under the influence of environmental factors. Among them, phosphate(Pi) limitation is a constraint for plant growth in many natural and agricultural ecosystems. As a result, plants possess Pi-sensing mechanisms that allow them to respond and adapt to conditions of limited Pi supply, including increases in formation and growth of lateral roots. In *Arabidopsis thaliana* these developmental modifications are mainly mediated by the plant hormone auxin, however, remains to clarify how auxin is directing those responses.

Here, we assessed if the alteration of root system architecture under Pi-starvation is mediated by modifications in auxin sensitivity in root cells. An increased expression of the auxin-responsive gene markers DR5:uidA and BA3:uidA, as well as an enhanced lateral root formation in Pi-deprived seedlings treated with auxin transport inhibitors suggest that Pi starvation increased auxin sensitivity in roots particularly in pericycle cells. In addition we demonstrate that auxin sensitivity and increased lateral root formation in response to Pi-deprivation are mediated by the auxin receptor TIR1 and the transcription factor ARF19. Our results demonstrate that Pi starvation modify auxin sensitivity by increasing the expression of TIR1, which in turn provoke an enhanced degradation of the AUX/IAA auxin response repressors and thus liberating ARF transcription factors to activate lateral root formation and emergence.

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**ICAR1102**

THE ROLE OF THE BAH-PHD-CONTAINING PROTEIN SHORT LIFE (SHL) IN THE CONTROL OF DEVELOPMENTAL TRANSITIONS IN ARABIDOPSIS

Category: Developmental Mechanisms

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Chromatin remodelling processes play crucial roles in the establishment and maintenance of gene expression patterns that control plant development. We are analysing the role of a small family of *Arabidopsis* nuclear proteins characterised by containing a BAH domain and a PHD finger; both motifs are found in transcriptional regulators involved in chromatin remodelling. We have previously isolated the locus *EARLY BOLTING IN SHORT DAYS* (*EBS*) that encodes a member of this family involved in the repression of flowering and the regulation of other developmental processes such as floral development and seed dormancy in *Arabidopsis*. *EBS* is required to repress the expression of the floral integrator *FT* under non-inductive photoperiodic conditions.

To further understand the role of this plant specific family of proteins involved in developmental regulation and related to chromatin remodelling factors, we have analysed loss-of-function alleles of another member of this family, *SHORT LIFE* (*SHL*), with the same modular architecture as *EBS*. Our results show that *SHL* also has a role in the control of flowering time. Moreover, double mutant analyses indicate that *SHL* has partially but not totally redundant functions with *EBS* in the control of the floral transition and other developmental processes. The analyses of genetic interactions between *SHL* and other genes that participate in the control of flowering time as well as the effect of *shl* loss-of-function mutations in the expression of these genes will be discussed. In addition, recent progress in understanding the biochemical role of these proteins and their histone binding properties, and natural variation data with implications in the evolution of the family will be also presented.

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**ICAR1103**

GENETIC INTERACTION BETWEEN *ULTRAPETALA1* AND *KANADI1* ESTABLISH CARPEL AND LEAF POLARITY AXES IN *ARABIDOPSIS THALIANA*.

Category: Developmental Mechanisms

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Carpels and leaves are evolutionarily related organs, as carpels are thought to be modified leaves. Both are polar structures that exhibit asymmetry in both their proximo-distal (basal-apical) and adaxial-abaxial axes. The components of the pathways responsible for establishing each axis of polarity are not exclusively restricted to one axis, and seem to have been recruited through evolution to provide multiple redundant specifications for organ development. There is some knowledge of the pathways that regulate polarity in *Arabidopsis*, but how they cross-talk and how combinatorial control between transcription factors contributes to establishing polarity axes is still unclear. We found that the putative transcription regulators *ULTRAPETALA1* (*ULT1*) and *KANADI1* (*KAN1*) interact genetically to regulate several organ polarity pathways. *ULT1* is an important negative regulator of stem cell accumulation in shoot and floral meristems and *KAN1* promotes abaxial cell fate during the establishment of the polarity axes in carpels and leaves. We have determined that *ult1* mutation suppresses the adaxial-abaxial polarity defect of *kan1* carpels and leaves. Conversely, *ult1* mutation enhances the apical-basal polarity defect of *kan1* carpels. We propose that in early stages of carpel and leaf development, the *ULT1* and *KAN1* regulatory pathways oppose one another to establish adaxial-abaxial polarity. Later during carpel development *ULT1* and *KAN1* are co-expressed

and may regulate common downstream target genes to establish the apical-basal polarity axis. We are testing our hypothesis by investigating which genes are responsible for the suppression of the *kan1* adaxial-abaxial carpel defect by *ult1* and which are possible target genes of *ULT1* and *KAN1* in regulating apical-basal carpel polarity.

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**ICAR1104****ATTZF-DEPENDENT mRNA TURNOVER IS CRITICAL FOR PLANT GROWTH AND DEVELOPMENT**

Category: Developmental Mechanisms

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mRNA turnover represents an important regulatory step of post-transcriptional gene silencing. In humans, AU-rich element (ARE) mediated degradation (AMD) pathway plays a critical role in controlling mRNA half-life of many important growth factors. In this pathway, RNA-binding tandem zinc finger proteins (TZFs) act as key recognition and recruiting factors for mRNA turnover taking place in cytosolic foci termed as P-bodies (PB) and stress granules (SG). In Arabidopsis and Rice our lab has identified a group of TZF proteins that localize to cytosolic foci similar to PB and SG. The Arabidopsis TZF proteins (AtTZFs) can bind ARE-containing 3'UTRs *in vitro*, and co-localize with several conserved SG and PB components. Due to gene redundancy, no apparent loss-of-function phenotypes could be found. However, overexpression of AtTFZ1 caused dramatic and pleiotropic growth and developmental changes from single cell to whole plant level, indicating possible effects on mRNA turnover of multiple growth regulators. To test this hypothesis, we conducted microarray analysis to identify genes with reduced expression in AtTFZ1 overexpression plants. By applying gene network analysis on these down-regulated genes, we reveal a novel pathway that contains membrane and cell wall modifying enzymes, a secreted peptidase, a peptide hormone precursor, and a putative peptide hormone receptor. Work is in progress to determine their relationship in signaling cascades and if these genes are subjected to AtTFZ1-mediated mRNA decay.

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**ICAR1105****FUNCTION AND REGULATION OF SMALL RAB-A1 GTPASES IN ARABIDOPSIS**

Category: Developmental Mechanisms

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Rab proteins are a family of small GTPases, which are involved in membrane trafficking in eukaryotes. In *Arabidopsis*, 57 Rab proteins have been identified. Genomic comparison shows that there is a large elaboration in the Rab-A subfamily whereby 26 members can be categorized into 6 structural subclasses from Rab-A1 to A6. Studies indicated that members in the Rab-A2/A3 and Rab-A4s subclasses might be required during cytokinesis and the formation of cell walls respectively. In this study, we focus on the functional analysis of the Rab-A1 subclass, which consists of 9 members. Microarray revealed that Rab-A1h and Rab-A1i are pollen specific, Rab-A1e is root specific and the others are generally expressed in the tissues tested. Semi-quantitative RT-PCR results confirmed most of the expression patterns whereas some discrepancies also existed. Two generally and highly expressed members Rab-A1b and Rab-A1c as well as pollen specific Rab-A1i were selected for detailed functional study. Promoter-GUS staining indicated that *RAB-A1b* is expressed in both meristematic and elongating zones of primary roots, while *RAB-A1c* is restricted in meristematic zone. Both are expressed in leaves and in some parts of flowers such as pollen grains. The GFP fused RAB-A1c protein is localized to many small mobile punctate structures in the cell, which are partially co-localized with FM4-64. We will study the relationship between the RAB-A1 labeled compartment and Golgi, as well as other post-Golgi compartments such as those labeled by RAB-A4b, RAB-A2 and RAB-F2b. Currently we are in the process to generate knock-out mutants for *RAB-A1c*, *RAB-A1b* and *RAB-A1i* in order to examine their function in membrane trafficking and cell development.

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**ICAR1106****LATERAL SUPPRESSOR FUNCTION IN AXILLARY MERISTEM DEVELOPMENT IS STRONGLY DEPENDANT ON ITS 3'REGULATORY SEQUENCES**

Category: Developmental Mechanisms

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The aerial architecture of plants is determined by the activities of the shoot apical meristem (SAM) and lateral meristems. These new meristems are formed in the axils of leaves and will develop into side shoots, leading to the large diversity of plant forms. Many genes regulating the initiation of axillary meristems are expressed in very specific domains at the adaxial side of young leaf primordia. One of these is the *Arabidopsis LATERAL SUPPRESSOR (LAS)* gene, which belongs to the GRAS gene family and encodes a putative transcription factor. The *lateral suppressor* mutant fails to initiate axillary meristems during the vegetative phase of development, indicating that *LAS* is a key regulator of meristem initiation. In order to elucidate the first steps of lateral meristem formation we are searching for upstream regulators of *LAS*. A promoter analysis using deletion constructs demonstrated that at least 820 bp upstream of the ATG are necessary for complementation, whereas a promoter fragment of 800 bp does not lead to a restoration of the wild-type phenotype. Furthermore, the 3'-regions of the *Arabidopsis LAS* and the tomato *Ls* genes are required for complementation of the *las/l*s mutants. In tomato, complementation of the *l*s phenotype was also obtained, when the 5'-promoter region was replaced by other promoters whose expression domains do not overlap with that of *l*s. This led to the hypothesis that the 3'-elements might account to a large extent for the very specific *LAS/Ls* mRNA accumulation profile. Phylogenetic footprinting revealed two highly conserved regions in the 3' regulatory sequence, one of which can also be found in monocots. Experiments have been initiated to validate putative regulatory elements in the *LAS/Ls* genes, with the aim to analyze them in detail. Some of the identified regulatory elements were subjected to a Yeast One-Hybrid screen, which led to the identification of candidate regulators of *LAS*.

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**ICAR1107****PHENOCRITICAL PERIODS IN LIGHT QUANTITY SENSITIVITY DURING THE INITIAL HOURS OF ADVENTITIOUS SHOOT ORGAN REGENERATION IN ARABIDOPSIS THALIANA**

Category: Developmental Mechanisms

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Previous plant tissue culture studies have shown that light affects adventitious organ regeneration on a time scale of days to weeks of dark/light exposure. This research demonstrates that these effects can actually occur within hours in *Arabidopsis thaliana*. By exposing *Arabidopsis* cotyledon explants to light-dark shifts, we found that light exposure during the first 24 hours after explant excision conditioned the frequency of shoot regeneration 3-5 weeks later. Whereas early exposure to high light decreased shoot regeneration in ecotypes Ler-0 and DijG, as little as two hours of darkness post-excision reduced this effect. Two critical light intervals were identified during the first 24 hours after excision: higher light during the first 12 hours promoted shoot regeneration when followed by darkness, whereas further light for an additional 12 hours or more, followed by darkness, inhibited shoot regeneration. These light responses were dependent on an auxin and cytokinin treatment proposed to be required for dedifferentiation. It is hypothesized that these 12-hour periods identify a light-sensitive signal(s) or cellular damage period(s) critical for regeneration. To test this, *Arabidopsis* mutants, chemical inhibitors and visual reporters are being used to identify the genetic pathway(s) and essential step(s) involved in regeneration as well as the environmental conditions that promote this process.

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### **ICAR1108**

PYRAMID SCREENING: A METHOD TO COMBINE MULTIPLE GENETIC SCREENS INTO A SINGLE SCREEN FOR ECONOMY AND MINING OF CROSS-TALK ALLELES – APPLICATION TO THE ISOLATION OF SHOOT REGENERATION MUTANTS

Category: Developmental Mechanisms

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Informative alleles can be identified by creating a non-permissive condition for a trait of interest and then screening for mutants that overcome the imposed condition. Separate genetic screens are conducted for each stress or condition, a potentially time-consuming effort. In severed *Arabidopsis thaliana* leaves, high light, suboptimal hormone exposure and old age, were each independently found to reduce the frequency of regeneration of a replacement shoot organ. Rather than conduct three separate enhancer screens to dissect these three pathways, a laborious process, we combined the three suboptimal “stress” conditions such that only when combined was our trait of interest (shoot regeneration) abolished. We chose a pyramided “stress” combination such that no one stress was primarily responsible for loss of our trait, thus ensuring that we could recover enhancer alleles in any of the three pathways of interest. Screening of 18,000 mutagenized plants resulted in 12 SHOOTING UP (stu) mutants. Secondary screening revealed that we had recovered alleles that were both specific for a pathway (light, hormones or age) or which acted through two or three of these pathways. Our approach, which we refer to as pyramid screening, represents an economical method for enhancer screening of multiple pathways in parallel (3 screens in 1) and has the potential to recover alleles that cross-talk between multiple pathways that underlie a complex trait such as organ regeneration. Pyramid screening should be widely applicable across species.

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### **ICAR1109**

TWO NATURAL VARIATION QTLS ACT COOPERATIVELY TO BYPASS THE CALLUS INDUCTION MEDIA COMPETENCY STEP NECESSARY FOR EFFICIENT IN VITRO SHOOT REGENERATION FROM EXCISED ARABIDOPSIS THALIANA COTYLEDONS

Category: Developmental Mechanisms

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Across plant species, a high auxin/low cytokinin (Callus Induction Media, CIM) pre-treatment is often required to make excised plant tissues competent to regenerate de novo roots or shoots in vitro. CIM has been speculated to promote dedifferentiation of somatic cells. In a natural variation screen of 60 ecotypes in *Arabidopsis thaliana*, we identified a subset of ecotypes, including Nossen-0 (No-0), which could regenerate efficiently in the absence of a CIM treatment whereas other ecotypes, such as Landsberg erecta (Ler-0), required CIM. Using two previously identified QTLs that promote shoot regeneration in a Ler-0 x Columbia cross, we found that the corresponding No-0 chromosome segments conferred onto Ler the ability to regenerate in the absence of a high auxin/low cytokinin treatment, thus bypassing the competency requirement. The two QTLs acted cooperatively as dosage-dependent genetic enhancers of one another. Furthermore, in a Ler near isogenic line (NIL) population, we found that the No-0 QTL region, linked to Chromosome 5 marker nga129, as well as the No-0 QTL region, linked to Chromosome 4 marker g4539, caused intact NIL-Ler hypocotyls to have altered growth responses to auxin and cytokinin in intact seedlings. This result suggests that an important gene regulating organ regeneration competency in vitro normally helps to regulate hormone responses in intact plants. The NIL populations with segregating QTLs were used for global gene expression analysis using Affymetrix ATH1 microarrays to examine their effects on hormone and other pathways.

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### **ICAR1110**

T-DNA INSERTIONS IN THE EUKARYOTIC ELONGATION FACTOR ONE ALPHA GENE FAMILY DISRUPTS ROOT MORPHOLOGY IN ARABIDOPSIS THALIANA.

Category: Developmental Mechanisms

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Eukaryotic Elongation Factor One Alpha (eEF1A) is a multifunctional protein involved in protein synthesis and degradation, binding actin and microtubules, and several different signal transduction pathways in the cell. The regulation of eEF1A's many functions is not understood. *Arabidopsis thaliana* has four eEF1A genes forming a small gene family. We have taken a loss of function approach to gain a better understanding of the importance and functions of each eEF1A family member. To accomplish this task we identified forty T-DNA knockout lines containing a T-DNA insertion in the 5' nontranslating, coding, or 3'UTR region of each eEF1A gene. Seeds were germinated and grown on vertical plates containing 1/2x MS media under normal growth conditions. The most dramatic phenotype observed was a change in root morphology. Several T-DNA lines displayed a root length of 2.4 mm compared to 5 mm root length for wild type seedlings at three days post germination. The difference in root length increased to 4.5 mm shorter for the T-DNA lines compared to wild type seedlings at six days post germination. The short root phenotype was observed in T-DNA lines with insertions in At1g07920, At1g07930, and At5g60390. At ten days post germination short roots were observed only in lines with insertions in At1g07920 and At5g60390. The short root seedlings with T-DNA insertions in At1g07930 had grown and were equal in length to the wild type

seedlings. Several of the T-DNA lines with short roots at six days post germination also showed a low germination rate and a short inflorescence stalk later as mature plants. The importance of this phenotype in respect to the function of each gene will be presented.

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**ICAR1111**

## CYTOKININ RESPONSE FACTORS: DOMAINS, INTERACTIONS, AND EXPRESSION

Category: Developmental Mechanisms

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Cytokinin is an essential plant hormone that affects numerous aspects of plant growth and development. Cytokinin Response Factors (CRFs) are newly described transcription factors linked to the cytokinin signaling pathway that have been shown to be involved in cotyledon, leaf, and embryo development. In order to better understand how CRFs are involved in regulating cytokinin response we have taken several different approaches to examine these genes and proteins. One common feature of CRF proteins is an N-terminal domain that appears to be unique to a small subset of AP2/ERF proteins. Extensive protein sequence alignment searches have allowed us to define this approximately 60 AA domain as plant specific and always coincident with an AP2/ERF domain. Using these criteria more than 80 different CRF domain containing proteins have been identified in nearly all land plants with available sequence. Further significance of these results will be discussed. Another approach that we are taking to study CRF proteins is to examine their interactions and initial results of Y2H and split YFP analyses on CRF protein-protein interactions will be presented. An entirely different approach is to examine when and where CRFs are expressed in the plant during development and how that expression is affected by cytokinin. CRF GUS and GFP expression patterns during development will be shown with special attention to leaf development and the different stages of lateral root initiation. Additionally, we will update previous work on changes in CRF expression in mutants and overexpression lines and how that relates to a better understanding of CRF function.

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**ICAR1112**

## CONTROL OF LEAF DEVELOPMENT BY MIR396

Category: Developmental Mechanisms

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Leaf development involves the concerted action of various hormone signalling pathways and transcription factor networks. Some of the identified key transcriptional regulators include *AINTEGUMENTA*, *JAGGED*, *BLADE ON PETIOLE*, *TCPs* and *GROWTH REGULATING FACTORS (GRFs)*. Here, we present insights in the role of miR396, a microRNA that regulates seven transcription factors of the plant specific *GRF* family.

Transgenic plants with high miR396 levels have smaller leaves with fewer cells. Microarray analysis of 35S: *miR396* plants revealed that this is likely a consequence of reduced mitotic activity. Nevertheless, leaf size was partially maintained by a compensating increase in cell size.

We also performed a detailed analysis of the regulation of *GRF2* by *miR396*. To do this, we prepared several reporters where we fused the  $\beta$ -glucuronidase reporter to the *GRF2* gene. The motif recognized by miR396 was mutagenized to make a *GRF2* reporter version either insensitive or hypersensitive to the miRNA. The results obtained from this analysis indicate that miR396 regulates the spatio-temporal pattern of *GRF* expression during leaf development. The participation of miR396 during *Arabidopsis* development will be discussed.

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**ICAR1113**

## PHOTOACTIVATION OF GFP REVEALS DYNAMIC INTERRELATIONSHIPS OF SECRETORY PATHWAY ENDOMEMBRANES DURING CELL DIVISION

Category: Developmental Mechanisms

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Endomembranes - once described as a set of discrete membranes - are now, in some cases, thought to be continuous and interconvertable. This is especially so of the nuclear envelope and endoplasmic reticulum. Although these have defined roles and spatial locations during interphase, proteins of the nuclear envelope recycle to the ER as the NE breaks down during prophase of mitosis. We have been using experimental techniques that allow us to examine the behaviour and interrelatedness of the set of membranes including NE, ER, Golgi bodies, plasma membrane and tonoplast during cell division. Observational studies are complemented by photobleaching and photoactivation experiments in which the dynamics of proteins are measured. In particular, photoactivation of GFP in discreet regions of the plasma membrane and ER allows us to observe any limitations to movement of proteins within these membranes as mitosis proceeds. How and when in mitosis do these membranes segregate into daughter cells? To study this, we observe *Arabidopsis* root tips growing *in situ* with markers of the different organelle membranes in different fluorescent colours. Co-localization analysis techniques are critical to assess the degree of separation between membranes as cell division proceeds through formation of the new cell plate. Golgi bodies have previously been shown to associate with the surface of the ER and we hope to be able to quantify Golgi / ER association during cell division. Ultimately, we hope to answer a temporal question about Golgi body reduplication - does it coincide with cell division or do Golgi body numbers per cell drop at cell division and re-establish later in the cell cycle?

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**ICAR1114**

## A MUTANT IN GERANYLGERANYL DIPHOSPHATE SYNTHASE 1 (GGPS1) OF ARABIDOPSIS THALIANA THAT AFFECTS CHLOROPLAST DEVELOPMENT

## IN ADULT LEAVES

Category: Developmental Mechanisms

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In plants, plastid development can be critical for survival throughout all phases of the life cycle. Research in our lab has focused on two classes of mutants in *Arabidopsis thaliana* that affect the proper development of chloroplasts in a stage specific manner. In the first, we study mutations that affect plastid development in embryo-derived cells, resulting in a seedling-specific albinism phenotype. A second stage of plastid development that we study is in the adult leaf, exemplified by a novel variegated leaf mutant in *Arabidopsis thaliana*. Positional cloning of this mutant revealed that it encodes a geranylgeranyl diphosphate synthase, previously identified as *GGPS1*. This gene is one member of a small gene family consisting of five isozymes plus a related protein (Okada et al. 2000) and appears to be the major plastid-localized member of this gene family. *GGPS1* catalyzes the formation of the twenty-carbon isoprenoid geranylgeranyl diphosphate (GGPP), which serves as a precursor to various biochemical pathways, including carotenoid biosynthesis and the formation of side-chains for several prenyllipids such as the chlorophylls. GGPP is also a precursor in the formation of diterpenes such as the plant hormone gibberellic acid (GA). The *ggps1* mutant has an amino acid substitution immediately downstream of an aspartate-rich domain critical for catalytic activity, thus the mutation in *ggps1* is likely to affect the production of downstream products in these pathways. We found that total chlorophyll and carotenoid levels are reduced in *ggps1*. Within a variegated leaf, chloroplast development in the cells of green sectors appears normal, whereas cells in white sectors contain abnormal plastids with numerous inclusion bodies and poorly developed thylakoid membranes. Interestingly, phenotypes typically associated with a reduction in GA are not seen in *ggps1*, suggesting that GA biosynthesis is not noticeably altered in the mutant.

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## ICAR1115

### FUNCTIONAL CHARACTERIZATION OF LATERAL ORGAN JUNCTION (*LOJ*) GENE AND ITS PROMOTER IN *ARABIDOPSIS THALIANA*

Category: Developmental Mechanisms

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Isolation and characterization of genes and promoters from plant origin is considered as one of the priority research for genetic improvement of crop plants through transgenic approach. The T-DNA insertion mutagenesis-based gene and promoter traps have gained wide popularity in isolation of genes and promoters from the model plant, *Arabidopsis thaliana*. A T-DNA based promoter trapping approach in *Arabidopsis* by our laboratory has led to the identification of a novel tissue-specific promoter regulating the gene expression in lateral organ junctions (LOJs). The promoter region was located in the upstream sequence of a pentatricopeptide repeat protein coding gene (*At2g39230*), which was designated as *Lateral Organ Junction (LOJ)* gene.

The present study involves functional characterization of the *LOJ* gene and its promoter in *Arabidopsis*. The microarray database-assisted *in silico* analysis revealed *LOJ* gene is functional in the shoot apex and nodes, especially in the meristematic tissues. The overexpression and RNAi silencing of the *LOJ* gene although exhibited alterations in the *LOJ* gene transcript quantity, no altered phenotypes were discernible at the plant level, indicating possible involvement of redundant gene(s) in restoring *LOJ*/gene functions.

The *in silico* analysis of the immediate upstream sequence of the *LOJ* gene revealed presence of relevant *cis*-regulatory elements. Deletion based functional analysis of the *LOJ* promoter helped us in identification of two different regulatory domains specifying different tissue- specificity. A distal part of the *LOJ* promoter identified with an enhancer element confers LOJ tissue-specificity in the transgenic *Arabidopsis* plants and is active independent of orientation and distance. The proximal part of the *LOJ* promoter is responsible for conferring gene expression in the developing anthers and seeds in a temporal fashion. The *LOJ* enhancer prevails over the anther- and seed- specific regulation when present together. The efficacy of the *LOJ* promoter in conferring LOJ-tissue-specific regulation in a heterologous plant system was tested in *Brassica juncea* var. *varuna*. The regulatory elements identified from the above study holds promise in genetic engineering of plants for targeted gene expression in lateral organ junctions.

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## ICAR1116

### HUA2 INTERACTS WITH FCA AND IS REQUIRED FOR ACCUMULATION OF *FCA-1* TRANSCRIPT AND *FLC* PRIMARY TRANSCRIPT

Category: Developmental Mechanisms

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Shoot development in plants progresses continuously during postembryonic development through initiation of primordia that can give rise to either vegetative or reproductive structures. Determination of primordial fate depends on both endogenous and environmental signals resulting in highly plastic shoot morphology adapted to specific environmental conditions. In *Arabidopsis thaliana* a complex network has evolved to integrate endogenous and environmental signals. *FLOWERING LOCUS C (FLC)*, a floral repressor, integrates inputs from vernalization and autonomous pathways, and various activators of *FLC* expression including the *HUA2*. Autonomous pathway genes *FCA* and *FY*, form a complex to suppress the activity of *FLC*. It was previously shown that *FCA* and *FY* interact through *FCA-WW* and *FY-PPLP* domains. *HUA2*, an activator of *FLC*, has C-terminal end (CT-*HUA2*) with five PPLP repeats, suggesting that it may interact with proteins containing the WW domain. Since *HUA2* and *FCA* contain compatible interaction domains, and both of them affect the same downstream gene *FLC*, we investigated whether *HUA2* and *FCA* interact. We show that CT-*HUA2* is required and sufficient for interaction with *FCA-WW* domain. We further identified that *HUA2* is required for the expression of *FCA-1* transcript and for the late flowering of *fca-1* mutant plants via the effect *HUA2* has on the synthesis of *FLC* primary transcript.

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## ICAR1117

#### ANALYSIS OF HIGH PLOIDY MUTANTS

Category: Developmental Mechanisms

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Endoreduplication, *i.e.* the replication of chromosomes without intervening mitoses, often occurs in cell types that undergo specialization or those that have high metabolic activities. The increased amount of the nuclear DNA often correlates with the ability of plant cells to undergo massive post-mitotic enlargement, sometimes up to hundreds of times their original size, but the molecular mechanisms that mediate this control remain largely unknown. Recently, we identified four new mutations *high ploidy 1-4* (*hip1-4*) from a screen for *Arabidopsis* plants that display altered cell size and ploidy phenotypes. Ploidy levels in *Arabidopsis* wild type seedlings range from 2C to 32C with C being the haploid DNA content. In contrast, the ploidy level of the *hip* mutants reaches up to 128C or 256C, suggesting HIP1-4 are involved in pathways that negatively regulate endoreduplication. This phenotype is also accompanied by severe dwarfism and defects in the maintenance of functional meristem. Our current work focuses on the in depth analyses of these phenotypes and the identification of the mutated genes. We will utilize these new mutant lines for the identification of novel modulators of cell size and ploidy.

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#### ICAR1118

##### MULTIPLE MONOPTEROS-DEPENDENT PATHWAYS ARE INVOLVED IN LEAF INITIATION.

Category: Developmental Mechanisms

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Initiation of leaves at the flanks of the shoot apical meristem occurs at sites of auxin accumulation and pronounced expression of auxin-inducible *PIN* genes, suggesting a feedback loop to progressively focus auxin in concrete spots. Since *PIN* expression is regulated by Auxin Response Factor (ARF) activity, including *MONOPTEROS* (*MP*), it appeared possible that *MP* affects leaf formation as a positive regulator of *PIN* genes and auxin transport. Here we analyze a novel, completely leafless phenotype arising from simultaneous interference with both auxin signaling and auxin transport. We show that *mp pin1* double mutants, as well as *mp* mutants treated with auxin-efflux inhibitors, display synergistic abnormalities, not seen in *wild type* regardless of how strongly auxin transport was reduced. The synergism of abnormalities indicates that the role of *MP* in shoot meristem organization is not limited to auxin transport regulation. In *mp* mutant background, auxin transport inhibition completely abolishes leaf formation. Instead of forming leaves, the abnormal shoot meristems dramatically increase in size harboring correspondingly enlarged expression domains of *CLAVATA3* and *SHOOTMERISTEMLESS*, molecular markers for the central stem cell zone and the complete meristem respectively. The observed synergism under conditions of auxin efflux inhibition was further supported by an unrestricted *PIN1* expression in *mp* meristems, as compared to a partial restriction in *wild type* meristems. Auxin transport-inhibited *mp* meristems also lacked detectable auxin maxima. We conclude that *MP* promotes the focusing of auxin and leaf initiation in part through pathways not affected by auxin efflux inhibitors.

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#### ICAR1119

##### FKD1 IS REQUIRED FOR PROPER PIN1 LOCALIZATION IN DEVELOPING LEAF VEINS.

Category: Developmental Mechanisms

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The formation of a reticulate vein pattern in *Arabidopsis* leaves is dependent upon the canalization of auxin into cell files by the action of localized PIN1, an auxin efflux protein. Canalization of PIN1 to particular cell files and localization of PIN1 to particular cellular membranes is dependent upon endosomal cycling that allows rapid and flexible changes to PIN localization. How the localization of PIN1 is controlled during the elaboration of vein pattern is largely unknown. We have previously described plants mutant for FORKED1 (FKD1), which have reduced leaf response to auxin and show an open vein pattern through lack of distal vein meeting in cotyledons and leaves. The reduced auxin response might be due to either defective auxin signaling or transport, leading to inefficient auxin canalization. FKD1 encodes a hypothetical protein with a Domain of Unknown Function 828 (DUF828) domain and a pleckstrin homology-like, plant (PH-like) region. Interestingly, another gene required for the reticulate vein pattern, SCARFACE (SFC) also contains a PH domain. SFC encodes an ARF-GAP that influences PIN localization in roots following brefeldinA treatment. FKD1::GUS fusions indicate that FKD1 is expressed throughout the developing plant vasculature. Consistent with a role for FKD1 is the canalization process, the narrowing of FKD1 expression to a particular cell file is dependent upon auxin transport. To test the idea that FKD1 is involved in PIN1 localization, we observed PIN1::GFP localization in *fkd1* leaves. Whereas in *wild type*, procambial cell files are predicted by continuous loops of cells localizing PIN1::GFP to one cell face, in *fkd1* leaves PIN1::GFP is never localized within cells of the distal vein region, resulting in discontinuous loops. Based on this data, we suggest that FKD1 represents a novel component of the PIN1 localization mechanism.

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#### ICAR1120

##### THE ROLE OF JABBER JAW IN STOMATAL DEVELOPMENT

Category: Developmental Mechanisms

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Stomata are microscopic pores on botanical epidermis that regulate gas and water exchange. Several genes in *Arabidopsis thaliana* have been identified as important to the development and correct spacing of stomata, which may be defined as stomata separated by at least one neighboring epidermal cell. Mutations in these genes result in overproduction, underproduction, or unusually clustered stomata. *JABBER JAW* (*JB*) has recently

been identified as playing a role in stomatal patterning. This gene was originally found in a microarray-based expression profiling experiment designed to identify genes important to stomatal precursor development. *jb/jb* mutant plants exhibit small clusters of stomata, indicating that JBJ is a negative regulator of stomatal formation and contributes to patterning. Two transgenic constructs were designed to further examine the role of JBJ in stomatal development. In the first, the 35S CaMV promoter was used to constitutively overexpress JBJ, and in the second an estrogen-inducible promoter was used to express JBJ in tissues on demand. Quantitative analysis of stomatal patterning defects and development in the epidermis of multiple plant organs will be presented. The aim of this work is to further our understanding of cell-signaling pathways that control tissue patterning and stem cell behaviors in plants.

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**ICAR1121****IDENTIFICATION OF THE NATURAL ROLES OF IDA-LIKE (IDL) PROTEINS AND CHARACTERISATION OF THE IDA SIGNALING PATHWAY IN ARABIDOPSIS**

Category: Developmental Mechanisms

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Small signaling peptides may play an important role in the coordination of plant growth and development. *INFLORESCENCE DEFICIENT IN ABCSSION (IDA)*, expressed in floral abscission zones (AZs), is required for floral organ abscission in *Arabidopsis*. Five IDA-like (IDL) proteins, with a conserved 20 aa motif (EPIP) at the C-terminus, are found in *Arabidopsis*. *35S:IDA* and *35S:IDLs* plants have early floral organ abscission and ectopic abscission of organs with preformed abscission zones, indicating functional redundancy and the possibility that the IDL proteins may function in other cell separation processes. Here we show distinct expression of *IDLs* in columella root cap, base of the pedicel, floral AZs, vascular tissue and guard cells of young seedlings. To identify the natural roles of IDL proteins in *Arabidopsis*, expression of *IDLs* will be modified using an inducible two components system for overexpression and amiRNA for RNA silencing. Additionally, we show that a double mutant between *HAESA (HAE)* and *HAESA-LIKE 2 (HSL2)* is epistatic to *35S:IDA*, suggesting that IDA may function as a ligand for the receptor-like kinases HAE and HSL2. Transcriptome profiling using RNA from AZs of *hae hsl2* and *ida* will be performed to disclose genes that may be regulated by the IDA HAE HSL2 pathway.

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**ICAR1122****EFFECT OF COLD TEMPERATURE STRESS ON AUXIN RESPONSE AND THE GROWTH OF ARABIDOPSIS THALIANA ROOT**

Category: Developmental Mechanisms

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During environmental stresses, plants adapt to the condition for survival and reproduction in multiple ways including changing the hormonal responses. Among the hormones, auxin, which has long been recognized as an important phytohormone, controls every aspect of growth and development. However, little is known about the effect of temperature stress on auxin response. To understand the mechanistic basis of cold temperature stress and auxin response, we characterized the root growth of *Arabidopsis thaliana* at 23°C after pre-incubating the seedlings at 4°C. The time course assay revealed that 8-12hr pre-incubation at 4°C inhibited the root growth and reduced the gravity response compared to that of untreated controls. This effect was reversible as the gravity response returned to normal with time after transferring to 23°C. The auxin-signaling mutant *axr1-3*, which shows a reduced gravity response, responded to cold treatment like wild-type indicating that auxin transport rather than auxin signaling mechanism is affected by cold stress. Consistently, the expression of the auxin responsive marker was found to be altered in cold treated plants accumulating GUS staining in the outer layer cells of the root meristem suggesting that auxin flow in this region is stopped by cold stress. This idea was supported by the fact that trafficking of PIN2 protein, which plays an important role in basipetal auxin transport, was inhibited by cold stress. Taken together these results, we hypothesize that cold stress affects the auxin transport machinery, reduces the basipetal transport of auxin and hence alters the gravity response.

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**ICAR1123****THE ESCRT RELATED CHMP1A AND B PROTEINS MEDIATE THE LOCALIZATION OF THE AUXIN EFLUX CARRIER PIN1 AT THE PLASMA MEMBRANE AND ARE REQUIRED FOR EMBRYO DEVELOPMENT.**

Category: Developmental Mechanisms

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ESCRT-mediated sorting has emerged as a versatile pathway that controls important cellular processes. The central concept is the sorting of biosynthetic and endocytosed proteins into internal vesicles of late endosomes that are then released into the lumen of the vacuole after endosome-vacuole fusion. This process is facilitated by ESCRT and ESCRT-related proteins. Many components of this pathway have been characterized in fungi, animals and plants but cargo-proteins have only been identified in the first two kingdoms.

The CHMP1A and B proteins are similar to mammalian CHMP1A and yeast Did2p which are involved in the dissociation of ESCRT III complex from endosomal membranes. Whereas yeast mutants are viable, *Arabidopsis chmp1a:b* double mutants are heavily retarded in embryo development and die shortly after germination. In this study we show that the *Arabidopsis* CHMP1 proteins are required for embryo development and that the auxin efflux

carrier PIN1 is a target of the *Arabidopsis* ESCRT pathway. The localization of PIN1 protein is crucial for axis formation of the *Arabidopsis* embryo and the shift from radial to bilateral symmetry. In accordance with this, *chmp1a;b* embryos develop into ball-like structures that either form no cotyledons at all or develop multiple cotyledons that in most cases fail to expand. In wild type embryos PIN1 is predominantly expressed in developing cotyledons and procambial strands but in *chmp1a;b* double mutants PIN1 protein is found throughout the whole embryo. Whereas in wild type PIN1 protein is localized asymmetrically to the plasma membrane of one side of the cell, it is directed to the vacuolar membrane in *chmp1a;b* double mutants. PIN1 loaded bodies are frequently found close to the central vacuole and resemble class E compartments found in yeast ESCRT mutants. Interestingly PIN1 seems to accumulate in *chmp1a;b* embryos compared to wild type, indicating a failure to efficiently degrade PIN1.

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#### **ICAR1124**

THE AUXIN RESPONSE FACTOR6 AND 8 REGULATE FLOWER ORGANOGENESIS BY REPRESSING THE EXPRESSION OF CLASS 1 *KNOX* GENES IN *ARABIDOPSIS THALIANA*.

Category: Developmental Mechanisms

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At the flower opening, petals and stamens elongate dramatically in a short period. The *Arabidopsis* mutants *auxin response factor6* (*arf6*) and *arf8* showed delayed elongation of floral organs at the flower opening. In the *arf6 arf8* double mutant, flowers never opened until senescence. Such a phenotype is also observed in the mutants that have a defect in jasmonic acid (JA) biosynthesis or JA signaling. We found that the level of *DEFECTIVE IN ANTER DEHISCENCE1* (*DAD1*) mRNA was markedly decreased in the *arf6 arf8* inflorescences, which resulted in the reduction of JA amount in the organs. The *arf6 arf8* double mutant carrying *ProPISTILLATA-DAD1* gene partly recovered the amount of JA, suggesting that the ARF6 and ARF8 are required for the activation of *DAD1* gene expression. Along with the deficiency of JA, *arf6 arf8* showed several developmental defects, such as aberrant vascular patterning and lack of epidermal cell differentiation in petals. We found that the class 1 *KNOX* genes were expressed ectopically in the developing floral organs of *arf6 arf8*. A mutation of *SHOOT MERISTEMLESS* (*STM*) partially rescued the defects of JA production. Moreover, *Pro35S-BREVIPEDICELLUS*, *Pro35S-KNAT2*, and *Pro35S-KNAT6* plants exhibited the defects in floral organ development, including the downregulation of *DAD1* expression and the aberrant vascular patterning. It is suggested that the most defects in *arf6 arf8* are attributed to the misexpression of class 1 *KNOX* genes. It has been reported that the expression of class 1 *KNOX* genes are repressed in developing organs by *ASYMMETRIC LEAVES1* (*AS1*) and *AS2*. The expression levels of class 1 *KNOX* in flower buds were additively increased in the *as1 arf6 arf8* triple mutant. Taken together, we concluded that the ARF6 and ARF8 repress the class 1 *KNOX* genes in developing floral organs in parallel with the AS1 and AS2 to progress the development of these organs.

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#### **ICAR1125**

ANALYSIS OF *GORGON*, A NOVEL MUTANT DEFECTIVE IN SHOOT APICAL MERISTEM AND ORGAN DEVELOPMENT.

Category: Developmental Mechanisms

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In higher plants, nearly all above ground organs are derived from the shoot apical meristem. A functional shoot apical meristem is maintained through tightly controlled balance between the proliferation of cells and the differentiation of organ primordia. It is thus important to understand the molecular mechanism underlying shoot apical meristem formation and its maintenance, and organ formation from it. We isolated a recessive *Arabidopsis* mutant named *gorgon*, (*gor*), which exhibited drastic expansion of the shoot apical meristem. The expansion of the *gor* meristem initially becomes apparent in young seedlings. As the plant starts bolting, the *gor* meristem continues to expand and produces numerous pin-shaped protrusions from its periphery but fails to form branches or flowers. Shortly after, the *gor* meristem terminates and produces numerous flowers or branches from its entire surface. Moreover, some of these flowers produce extra flowers or floral organs from an additional whorl inside the carpels. These observations show that *gor* is defective in the regulation of shoot apical meristem size and floral organ development. We next focused on five-day-old seedlings to examine the early influence of the *gor* mutation on shoot apical meristem development. Histological analysis showed that the number of small, densely cytoplasmic cells that comprised the meristem was increased in the expanded *gor* meristem. Consistently, expression domains of *SHOOT APICAL MERISTEMLESS* and *WUSCHEL*, which are positive regulators for meristem maintenance, were highly expanded. The expression domain of a reporter gene for *CLAVATA3*, which normally restricts the meristem size, was also expanded, suggesting that the enhanced growth of the *gor* meristem overcame the negative effect of *CLAVATA3* on meristem size.

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#### **ICAR1126**

A NOVEL GENE *ENLARGED FIL EXPRESSION DOMAIN2* (*ENF2*) REGULATES THE POSITION OF THE BOUNDARY BETWEEN THE ADAXIAL AND ABAXIAL DOMAINS IN THE LEAVES.

Category: Developmental Mechanisms

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In many land plants, proper adaxial/abaxial differentiation in their leaves is crucial for efficient photosynthesis. It is a known example of such differentiation that the palisade and spongy tissues are formed in the adaxial and abaxial mesophyll, respectively.

Among several genes regulating adaxial/abaxial differentiation, *PHABULOSA* (*PHB*) and *FILAMENTOUS FLOWER* (*FIL*) are known as each ones being expressed specifically in the adaxial or abaxial domains, respectively, during leaf primordial development. We have shown that the expression domains of *PHB* and *FIL* are mutually exclusive without overlap or gap, suggesting the existence of a sharp boundary bisecting the leaf primordia into the adaxial and abaxial halves. However, it remains unsolved how the position of this adaxial/abaxial boundary is determined.

We isolated an *Arabidopsis* mutant *enlarged fil expression domain2* (*enf2*) whose leaf primordia have larger expression domain of *FIL* than that in wild type plant. On the other hand, *enf2* mutant had smaller expression domain of *PHB* corresponding to the larger expression domain of *FIL* without overlap or gap, indicating that the position of the adaxial/abaxial boundary was shifted toward the adaxial direction in this mutant. The differentiated mesophyll in *enf2* leaves was composed of decreased amount of palisade-like tissue and inversely increased amount of spongy-like tissue, possibly reflecting the aberrant position of the adaxial/abaxial boundary.

*ENF2* gene was turned out to encode a novel plastid-targeted protein by map-based cloning. *ENF2* knock out plants showed more severe phenotype in *FIL* expression pattern than the *enf2* allele. Interestingly, *ENF2* knock out plants were albino, while *enf2* allele exhibited pale green phenotype. Our results suggested that a plastid function involving the *ENF2* gene determines the proper position of the adaxial/abaxial boundary, and is also required to the development of plastid itself.

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### **ICAR1127**

#### THE INDETERMINATE DOMAIN FAMILY HAS DIVERSE ROLES IN CONTROLLING *ARABIDOPSIS* DEVELOPMENT

Category: Developmental Mechanisms

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The *INDETERMINATE DOMAIN* (*IDD*) genes encode a plant-specific family of putative zinc finger transcription factors. The founding member of the *IDD* family, *INDETERMINATE1* (*ID1*) controls flowering time in maize. The *Arabidopsis* genome contains 16 *IDD* genes, which appear to have diverse roles in development. *AtIDD3*, *AtIDD8* and *AtIDD10* form a highly homologous subgroup, sharing overlapping functions in both root and shoot morphogenesis. In contrast, loss-of-function of *AtIDD15* causes a reduced gravitropic response in *Arabidopsis* inflorescence stems. Gravity-sensing amyloplasts in the shoot endodermis of *atidd15* mutants sediment more slowly than wild type suggesting a defect in gravity perception. This is correlated with lower amyloplast starch levels, which may account for the reduced sensitivity to gravity in *atidd15* mutants.

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### **ICAR1128**

#### THE PARALOGOUS PARPs RCD1 AND SRO1 SHARE DEVELOPMENTAL FUNCTIONS IN ARABIDOPSIS

Category: Developmental Mechanisms

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RADICAL-INDUCED CELL DEATH1 (RCD1) and SIMILAR TO RCD ONE1 (SRO1) are the only two proteins encoded in the *Arabidopsis* genome containing both a putative poly (ADP-ribose) polymerase (PARP) catalytic domain and a WWE protein-protein interaction domain. Proteins with a similar domain structure have been found in other eukaryotes. PARPs mediate attachment of ADP-ribose units from donor NAD<sup>+</sup> molecules to target proteins and have been implicated in a number of processes including DNA repair, apoptosis, transcription, and chromatin remodeling. Like *RCD1*, *SRO1* is expressed in all plant organs. We have isolated mutants in both *RCD1* and *SRO1*, *rcd1-3* and *sro1-1*, respectively. *rcd1-3* plants display similar phenotypic defects to those reported for previously isolated alleles, most notably reduced stature. In addition, *rcd1-3* mutants display abnormal phyllotaxy, abnormal floral organs, increased lateral root number and length, and shorter primary roots. *sro1-1* plants display some subtle developmental defects in the roots but otherwise develop normally. The lack of visible phenotypes in *sro1-1* mutants could be due to redundancy with *RCD1*. However, *rcd1-3* plants are early flowering under both long and short days, in contrast *sro1-1* plants flower late. This suggests that the genes do not always function equivalently. Loss of a single dose of *SRO1* in the *rcd1-3* background increases the severity of dwarfing, suggesting these two genes may share some functions. Consistent with this, *rcd1-3; sro1-1* double mutants display severe developmental problems like embryo and seed abnormalities, reduced germination, extreme dwarfism, abnormal flowers, and short siliques. The basis for these defects and the exact nature of the redundancy between these two paralogous genes is currently being examined.

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### **ICAR1129**

#### ARABIDOPSIS MBF1S PLAY A CRUCIAL ROLE FOR CONTROLLING LEAF CELL EXPANSION THROUGH REGULATING THE EXPRESSION OF ENDOREDUPLICATION-RELATED FACTORS.

Category: Developmental Mechanisms

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Multiprotein Bridging Factor 1 (MBF1) is known as a transcriptional co-activator that enhances transcription of its target genes by bridging between transcription factors and TATA-box binding protein in several organisms. *Arabidopsis thaliana* has three MBF1 genes (*AtMBF1a*, *1b*, *1c*), however, the detail of AtMBF1-related signal transduction pathway remains unclear. In this study, chimeric genes encode a chimeric transcriptional co-repressor, AtMBF1s-SRDX, were constructed to reveal the functions of three subtypes of AtMBF1s. We expected reduction of expression levels of target genes under control of transcription factors cooperating with AtMBF1s by dominant negative effect of over-expression of AtMBF1s-SRDX. Transgenic *Arabidopsis* overexpressing AtMBF1-SRDX (AtMBF1-SRDX<sup>OE</sup>) showed extremely dwarf phenotype under continuous white light condition, and its cells in leaf were much smaller than that from WT. We found that ploidy levels of leaves from AtMBF1s-SRDX<sup>OE</sup> were dramatically reduced compared to that from WT, and expression levels of several negative regulators of endoreduplication were also elevated in AtMBF1s-SRDX<sup>OE</sup> than those in WT. These observations suggest that AtMBF1s-SRDX may interact with factors which regulate negative regulators of endoreduplication, and induce the reduction of ploidy levels in leaf by elevating the expression of these negative factors. These observations strongly indicate that AtMBF1s play crucial role for leaf morphogenesis and expansion through cell cycle regulation in *Arabidopsis*.

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**ICAR1130**

KNOCKDOWN OF *ARABIDOPSIS* GLYCINE DECARBOXYLASE COMPLEX BY RNAI LEADS TO CHLOROSIS AND EARLY SENESCENCE

Category: Developmental Mechanisms

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The mitochondrial glycine decarboxylase complex (GDC) catalyzes the oxidative decarboxylation of glycine in the photorespiratory pathway. With the cooperation of serine hydroxymethyltransferase, GDC is responsible for the cycling of serine and glycine in the mitochondria. Here, we employed over-expression and RNA interference (RNAi) strategies to investigate the roles of H and P subunits of GDC in *Arabidopsis*. Plant expression vectors with full length cDNA or hairpin cDNA fragments were constructed and introduced into *Arabidopsis* (Columbia). Homozygous lines of the four transgenic varieties containing a single T-DNA insertion in the haploid chromosome were used for downstream analysis. The over-expression transgenic plants containing H or P subunit did not show any apparent phenotypes. In contrast, transgenic plants containing either RNAi- H or P showed strong chlorosis and early senescence after flowering. Real-time PCR revealed that the transcript level of the target gene was reduced to the levels ranging from 2% to 40% of those of the wild type. Western blot analysis showed that the corresponding protein was reduced to 30%. Chlorophyll analysis showed that both RNAi lines (H or P) had lower contents in various stages during plant growth. Profiling of free amino acids showed that glycine accumulation was increased by 30-50 folds in the RNAi-lines when compared to the wild type plants. In addition, total and individual free amino acids (e.g. serine, glutamine, histidine, tryptophan, and etc) were significantly increased in the RNAi lines. When GFP was fused to the promoter region of the H subunit, expression was observed in the hypocotyls, vascular bundles and leaf stomas. Strong fluorescence was detected in mitochondria in a subcellular localization experiment. Currently, the role of GDC in the regulation of glycine and serine is being examined at various stages of development in *Arabidopsis*.

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**ICAR1131**

ISOLATION AND DETERMINATION OF THE STRUCTURE OF NATIVE CLV3 PEPTIDE FROM CAULIFLOWER

Category: Developmental Mechanisms

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CLV3 is the peptide ligand that controls the number of non-differentiated cells in shoot apical meristem. Here, we first isolated and determined the structure of native form of the CLV3 peptide in the apoplast of cauliflower head tissue. We developed an efficient method to extract peptides from plant tissues and to select the biofunctional peptides. Meristem-rich tissues were collected by peeling the surface from cauliflower head. The apoplastic proteins were extracted and the peptides of 1 to 2 kDa were isolated by gel filtration column. Next, the peptides were fractionated with a cation exchange column, and each fraction was used for bioassay. Peptides in each fraction were added into MS liquid medium, and *Arabidopsis* seeds were germinated in the medium. Then, fractions, inducing abnormal structures in the shoot or root meristem, were selected. We further characterized a fraction and found it contained CLV3 peptides. LC-MS/MS analysis and protein sequencing of CLV3 showed that cauliflower CLV3 (BobCLV3) was a 12-aa peptide. The sequence was identical with *Arabidopsis* CLV3 (AtCLV3), however all three proline residues in BobCLV3 were not hydroxylated different from AtCLV3. This result suggested that CLV3 have variations on proline-hydroxylation among species or organs. Other than BobCLV3, we could also find several fractions that affected on the structure of SAM.

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**ICAR1132**

A ROLE FOR STRIGOLACTONES IN GERMINATION AND EARLY SEEDLING DEVELOPMENT IN ARABIDOPSIS

Category: Developmental Mechanisms

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Parasitic weeds of the genera *Striga* and *Orobanche* are considered the most damaging agricultural agents in the developing world 1,2. In Africa alone *Striga* species have infested up to two-thirds of the arable land and are thought to cause tens of billions of dollars in lost crop yields. To ensure coordination with a host, parasitic plant seeds only germinate when they sense a group of related compounds, called strigolactones, that are released by the host root. Although this makes strigolactone synthesis and action a major target of biotechnology the parasitic lifestyle and the lack of molecular and genetic tools makes studies on these weeds problematic. Here we show using a combination of chemical and classical genetics that, as observed in parasitic plants, strigolactones can play an analogous stimulatory role in seed germination in the model organism *Arabidopsis*. We identified mutants deficient in phytochromobilin synthesis that require strigolactones for good germination and also show these mutants are inefficient in stimulating parasitic seed germination. Rice mutants deficient in phytochromobilin synthesis are also inefficient at stimulating *Striga* germination demonstrating *Arabidopsis* may be useful in identifying genes for crop breeding programs against parasitic weeds. We are also able to show that many seedling phenotypes observed in phytochromobilin mutants are rescued by strigolactone addition suggesting these compounds may play an important role in light and retrograde signal transduction pathways in higher plants.

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**ICAR1133**

ECTOPIC EXPRESSION OF AN F-BOX PROTEIN ALTERS INFLORESCENCE ARCHITECTURE

Category: Developmental Mechanisms

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In *Arabidopsis*, the F-box gene family encodes a large number of proteins, most of which have not been characterized, but are postulated to act as substrate selectors for proteasome-mediated protein degradation. Many recent reports document the importance of various F-box proteins in

developmental and metabolic signaling. Our interest in these proteins stems from microarray analyses of inflorescences of wildtype and *brevipedicellus* (*bp*) mutants. In *bp*, several F-box proteins are upregulated, suggesting that BP represses these genes in wildtype plants to condition normal inflorescence development. We therefore undertook analyses to examine the function of these proteins and of how they might contribute to the pleiotropic phenotypes of the *bp* mutant. Yeast-2-hybrid screens revealed that one of the F-box proteins binds to phenylalanine ammonia lyase 1 (PAL1), the gateway enzyme of the phenylpropanoid pathway. These studies are currently being complemented by PAL enzyme assays on F-box mutants and overexpression lines in addition to wildtype and *pal1* mutants. Transgenic lines in which the BP promoter drives F-box expression exhibit defects in phyllotaxy, which is manifest as alterations in axillary branch angles and as the emergence of inflorescence meristems in addition to floral meristems in the axils of some cauline leaves.

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**ICAR1134**

## COMPLEXITIES OF SYSTEMIC REGULATION AND FUNCTION OF CYTOKININS

Category: Developmental Mechanisms

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Cytokinins are multifunctional systemic hormones with high mobility in both xylem and phloem. Similar to the original discoveries in *rms* shoot branching mutants of pea, we show that the homologous *max* mutants of *Arabidopsis* exhibit massively down-regulated xylem sap cytokinin content, and that this is governed by the shoot branching phenotype via an unknown long-distance feedback signal transmitted from shoot to root. We have now found opposite trends in phloem where cytokinin content is increased in *max* lines. However, the predominant cytokinin types in phloem are very different from those in xylem, suggesting independent pools, or potentially exchange from phloem to xylem but not vice versa.

We have initiated a systematic analysis of cytokinin biosynthesis (*IPT*) and degradation (*CKX*) in wild-type and *max* genotypes, aiming to reveal the molecular basis of xylem, phloem and tissue cytokinin dynamics. Studies elsewhere with promoter-reporter fusions indicate complex, highly specific sites of *IPT* and *CKX* expression. Contrary to our hypothesis that vascular-expressed *IPT* and *CKX* genes would be altered in *max* mutants, we instead discovered tissue-specific down-regulation of *CKX1* in *max* shoots, and both up- and down-regulation of *CKX5* in *max* roots. This indicates that at least part of the regulatory mechanism acts through cytokinin degradation. Interestingly, plants with *CKX* overexpression (*CKX-OE*) differ in phenotype from *max* mutants, and *CKX-OE max* lines have additive phenotypes. Reciprocal shoot-root grafts among *CKX-OE*, *max4* and WT lines revealed that branching of *CKX-OE* shoots was independent of root genotype, but *CKX-OE* roots slightly increased branching of both *max4* and WT shoots. This contrasts with *max4*-WT grafts where *max4* branching was strongly repressed by WT roots. The need for systems models to address the spatial and temporal complexity of systemic cytokinin regulation is discussed.

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**ICAR1135**

## IN PLANTA LOCALIZATION AND DYNAMICS OF FLUORESCENTLY TAGGED MADS BOX TRANSCRIPTION FACTORS

Category: Developmental Mechanisms

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Members of the MADS box transcription factor family are among others involved in the initiation and differentiation of the floral organs in plants. To investigate the dynamic behavior of selected MADS box transcription factors in living plants, C-terminal GFP fusions were made and introduced into *Arabidopsis thaliana* (Columbia). Genomic clones of *AGAMOUS* (*AG*), *SEPALLATA3* (*SEP3*), *FRUITFULL* (*FUL*) and *APETALA1* (*AP1*), including the upstream regulatory regions, were used to generate the GFP protein fusion constructs. Using confocal microscopy the temporal and spatial localization patterns of these GFP-tagged transcription factors were analyzed during flower development. This work revealed differences between localizations of mRNA and protein, and provided new insights into the subcellular localization of these proteins.

Also, GFP tagged cDNA clones of *AG*, *SEP3*, *AP1*, *PISTILLATA* (*P*) and *APETALA3* (*AP3*) under the control of the L1 specific *AtML1* promoter were made to see if MADS box proteins are able to move from the epidermal layer to the inner cell layers of plant tissues. In addition to this, the effect of the epidermal (over)expression of these MADS box transcription factors on plant morphology and flowering time is being studied in wild type plants and in their respective mutant backgrounds.

Future work with similar constructs but different fluorescent tags, like the photoswitchable mEosFP, will allow us to study more precisely the dynamics of intra/intercellular trafficking of the (complexes of) MADS box transcription factors in their native environment. The latest results of these studies will be presented.

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**ICAR1136**

## IDENTIFICATION AND CHARACTERIZATION OF A NOVEL TRICHOME SPECIFIC PROMOTER FROM A T-DNA TAGGED MUTANT POPULATION OF ARABIDOPSIS THALIANA

Category: Developmental Mechanisms

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An *Arabidopsis thaliana* mutant line, T200 exhibiting trichome specific GUS expression was identified from a promoter trapped mutant population. GUS expression analyzed at different developmental stages revealed prominent expression in the trichome of developing leaves. The GUS expression was more prominent in the trichomes of the rosette leaves present at the proximal end of leaves.

Southern analysis indicated the presence of two copies of T-DNA. TAIL-PCR and Inverse PCR were employed to clone the flanking sequences. T-DNA insertion was found to be at two distinct locations: (i) in the IVth intron of a putative GTP binding protein at loci At4g39890.1 and (ii) upstream region of a putative Ethylene response factor (patent pending). Orientation of transcription of both these genes is the same as that of GUS. Restriction profile for T-DNA insertion corroborates the results of Southern analysis.

Upstream sequences (~1.0kb) of both these genes were cloned in binary vector pBI101. Transgenics obtained from these constructs were analyzed in T2 generation for GUS expression. Trichome specific GUS expression was observed in the transgenics obtained from the constructs containing the upstream sequences of Ethylene Response Factor encoding gene.

Deletion analysis is being carried out to further characterize the promoter sequence. In silico analysis has revealed the presence of certain *cis-acting* elements.

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**ICAR1137**

THE PLASTID CLPPR PROTEASE FAMILY MEMBERS SHOW DIFFERENTIAL CONTRIBUTIONS TO EMBRYOGENESIS AND LEAF DEVELOPMENT

Category: Developmental Mechanisms

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Plastids play an essential role in embryo and seedling development. In this study we address the role of the CLPP/R family encoding for a tetradecameric protease complex consisting of five soluble catalytic ClpP (1,3-6) and four non-catalytic ClpR (1-4) proteins. We isolated a dozen null mutants and knock-down mutants for five CLPP/R genes and we characterized embryo and seedling development using a wide range of tools. Developing siliques of heterozygous plants for the various CLP genes showed Mendelian segregation of white and green seeds, indicative of absence of chlorophyll accumulation in homozygous plastids during embryogenesis. Light microscopy showed either strongly delayed or a complete block embryo development. Except for CLPR1, all null alleles were seedling lethal under autotrophic conditions, with development blocked prior or in the cotyledon stage. Under heterotrophic growth conditions, homozygous seeds for some of the genes either developed into extremely small plantlets with sterile flowers. Overexpression of ClpPR proteins in these various mutant lines, as well as double mutants, showed partial redundancies between specific sets of subunits within the complex. Large scale comparative proteome analysis of selected mutant lines pinpointed to specific functional defects within the plastid. RTPCR of the Clp gene family in various mutants showed a limited role of transcriptional regulation and together with the protein analysis, this indicates a dominating role of post-translational regulation. We conclude that the ClpPR gene family is critical for embryo and seedling development, plastid size, number and plastid function. Plastid do not have a proteasome and the Clp protease system, possible together with other plastid localized proteolytic systems, fulfill this important regulatory role.

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**ICAR1138**

CDH1/CCS52A TYPE ACTIVATORS OF THE ANAPHASE PROMOTING COMPLEX/CYCLOSOME LINK CELL CYCLE PROGRESSION TO CELL

DIFFERENTIATION IN ARABIDOPSIS

Category: Developmental Mechanisms

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During plant development, cell division and differentiation are tightly coordinated in order to maintain post-embryonic growth and control organ development. The cell cycle switch protein CCS52A, a plant homologue of the yeast and animal CDH1-type activator of the Anaphase Promoting-Complex, is involved in endoreduplication and cell cycle exit in *Medicago* species. Unlike other plants, there are two CCS52A isoforms in *Arabidopsis*, AtCCS52A1 and AtCCS52A2. We show that they stimulate differentiation by favoring endoreduplication and cell expansion in rosette leaves. In addition, AtCCS52A1 controlled endoreduplication in a number of other tissues. A specialized function was acquired by AtCCS52A2 in apical meristems. Mutations in AtCCS52A2 provoked a dwarf phenotype and the architecture of the root and shoot apical meristems was perturbed. The different functions of AtCCS52A1 and AtCCS52A2 were reflected by their distinct expression patterns during plant development. Taken together, the data suggest that multiple APC/CCS52A complexes exist in *Arabidopsis* with different localizations and functions during plant development.

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**ICAR1139**

ANALYSIS OF CELL-TO-CELL MOVEMENT OF REGULATORY FACTORS IN ARABIDOPSIS ROOT EPIDERMIS

Category: Developmental Mechanisms

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The MYB gene *CAPRICE* (*CPC*) is one of the regulators of epidermal cell differentiation, and represses the expression of the homeodomain-leucine zipper gene *GLABRA2* in root-hair cells. Analysis of *CPC::CPC:GFP* transgenic plants indicated that the CPC protein moves from hairless cells to root-hair cells. Using yeast two-hybrid screening, we identified a Factor Interacting with CPC (FIC), which might regulate the cell-to-cell movement of CPC. GFP fluorescence was observed in both root-hair and hairless cell files in transgenic plants expressing a *FIC::FIC* coding sequences:2XGFP construct. On the other hand, GFP fluorescence was seen only at the root tip in plants expressing *FIC::FIC*:2XGFP. Our results indicate that FIC also performs cell-to-cell movement. We are performing *in situ* hybridizations with a *FIC* probe to confirm this cell-to-cell movement. In addition, we are using tobacco leaf assays to investigate the possible *in vivo* interactions between CPC and FIC

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**ICAR1140**

#### DEVELOPMENT OF THE SECONDARY VASCULAR CAMBIIUM IN ARABIDOPSIS POLARITY MUTANTS

Category: Developmental Mechanisms

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The vascular cambium is a lateral meristem that gives rise to secondary vascular tissue, and in trees is responsible for the production of wood. In Arabidopsis stems, vascular bundles (VBs) are arranged around the stem periphery and produce xylem into the central pith and phloem outwards into the cortex. During secondary vascular development, cambium arises in the interfascicular regions between adjacent VBs, forming a continuous ring of fascicular (i.e. VB) and interfascicular cambium around the stem. Xylem and phloem production continues in a polar fashion and eventually, no distinction can be made between vascular tissue deriving from the original VBs or from the secondary interfascicular cambium. Mutations in members of the class III homeodomain leucine zipper (HD-ZipIII) and KANADI transcription factor families result in defects in lateral organ polarity as well as disruptions to the patterning of stem VBs. Gain-of-function HD-ZipIII mutants and loss-of-function KANADI mutants have VBs with xylem surrounding phloem. To determine whether the vascular patterning defects in HD-ZipIII and KANADI mutants affect the development of the cambium during secondary tissue formation, stems of HD-ZipIII and KANADI mutants were examined following secondary growth. In HD-ZipIII and KANADI mutants, radialized VBs were distributed randomly throughout the stem, and were embedded within or entirely excluded from the bulk of the secondary vascular tissue, which arose from the secondary cambium. Polarity establishment in the secondary cambium appeared normal, with xylem being produced into the pith and phloem into the cortex. The secondary cambium also formed an unbroken ring around the stem, despite being unable to connect with the fascicular cambium of individual VBs. This implies that patterning of the secondary cambium is not affected by aberrant patterning of the stem VBs. Furthermore, the polarity of the secondary cambium appears to be established independently of the HD-ZipIII and KANADI polarity pathways.

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#### ICAR1141

#### PATTERNING OF THE EARLY EMBRYO BY DISTINCT AUXIN RESPONSE MACHINERIES

Category: Developmental Mechanisms

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Auxin controls numerous developmental processes in plants by regulating gene expression. These transcriptional responses are mediated by ARF transcription factors and their Aux/IAA inhibitors. In the embryo, MP/ARF5 and BDL/IAA12 control root meristem initiation by specifying an extraembryonic suspensor cell as hypophysis, the root meristem precursor. However they do so in a non-cell autonomous way in part by promoting auxin transport to the adjacent cell. How auxin accumulation in this cell is translated into a gene expression response and cell fate change is completely unknown. We found that IAA10 expression is limited to suspensor cells, and that gain of function *iaa10* mutations specifically interfere with suspensor and hypophysis. Hence IAA10 represents the Aux/IAA component of the local auxin response machinery. To identify the ARF counterpart of this auxin response, we systematically analyzed the expression of all ARF genes in the embryo by means of promoter-GFP fusions. We identified a pair of closely related ARFs (ARF9 and 13) that are co-expressed in the suspensor. Double knock-out lines of these ARFs showed that both genes redundantly control suspensor development. Interestingly, the suspensor-specific IAA10/ARF13/ARF9 auxin response machinery is functionally distinct from the embryo-specific IAA12/ARF5 machinery, as evidenced from misexpression and promoter-swap experiments. However, both machineries are required for root formation.

We will present our latest results in understanding the mechanistic basis for auxin-dependent cell type specification in the embryo.

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#### ICAR1142

#### ANALYSIS OF THE LITTLE ZIPPER GENE FAMILY IN ARABIDOPSIS

Category: Developmental Mechanisms

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The *LITTLE ZIPPER* (*ZPR*) gene family is a small family of proteins that consists mostly of a leucine zipper domain. The leucine zipper domain is similar to the domain found in class III homeodomain leucine zipper (HD-ZipIII) transcription factors. The Arabidopsis genome contains five HD-ZipIII proteins [PHABULOSA (PHB) PHAVOLUTA (PHV), REVOLUTA (REV), AtHB-8 and AtHB-15] and four ZPR proteins [ZPR1-ZPR4]. We have shown that REV can induce the expression of all *ZPR* genes in Arabidopsis. ZPR proteins can heterodimerize with REV and this interaction results in the formation of heterodimeric complexes that can no longer bind to HD-ZipIII binding elements, thus forming a negative feedback loop. We show that the *ZPR* genes have overlapping expression patterns with HD-ZipIII genes indicating that their function is to modulate HD-ZipIII activity in the cells where they are expressed. Plants overexpressing *ZPR* genes show severely abaxialized phenotypes resembling plants having reduced expression of *PHB*, *PHV* and *REV*. However, plants overexpressing a ZPR protein carrying a mutation that disturbs the leucine zipper domain do not display any mutant phenotype. We further present evidence that the mutated protein no longer interacts with REV and does not interfere with the DNA-binding ability of REV, thus indicating that the leucine-zipper domain is required for ZPR function. Plants carrying T-DNA insertions in the *ZPR* genes were isolated and these plants do not show any obvious developmental defects indicating that they can act redundantly. However, *zpr3 zpr4* double mutants have an enlarged shoot apical meristem and severe developmental defects indicating that *ZPR* genes are involved in the maintenance of the stem cells in the shoot apex.

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#### ICAR1143

#### ISOLATION AND CHARACTERIZATION OF *MUM EHANCERS (MEN)* AFFECTING ARABIDOPSIS SEED COAT DIFFERENTIATION

Category: Developmental Mechanisms

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Pollination triggers not only embryo development, but also the differentiation of the ovule integuments to form a specialized seed coat. One specialization found in a number of species is the production of pectinaceous mucilage in the epidermal cells of the seed coat. This mucilage is released upon wetting and forms a gel capsule that is thought to aid seed hydration and germination. The mucilage secretory cells of the *Arabidopsis* seed coat undergo a complex differentiation process in which cell growth is followed by the synthesis and secretion of a large amount of mucilage. Mucilage secretion to the apoplast in the outer tangential portion of the cell is accompanied by constriction of the vacuole and formation of a cytoplasmic column in the centre of the cell. Secondary cell wall synthesis then leads to the production of the volcano-shaped columella beneath the mucilage pocket.

A number of genes have been identified affecting mucilage secretory cell differentiation, including *MUCILAGE-MODIFIED4* (*MUM4*). *mum4* mutants produce a reduced amount of mucilage and have a flattened columella. Cloning of *MUM4* revealed that it encodes a rhamnose synthase (also known as *RHM2*) that is developmentally upregulated to provide rhamnose for the backbone of the primary pectin found in *Arabidopsis* mucilage. In order to identify further genes acting in mucilage secretory cell differentiation, pectin synthesis and secretion, a screen for enhancers and suppressors of the *mum4* phenotype was performed. Eleven *mum* enhancers (*men*) have been identified, two of which result from defects in known mucilage secretory cell genes (*MUM2* and *MYB61*). Currently the remaining *mum4 men* lines are being characterized for their effects on seed coat differentiation, mucilage production, germination, resistance to pathogens and overall developmental phenotypes. Preliminary mapping of several *Men* has identified loci on chromosomes I and V.

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#### **ICAR1144**

A FRAMEWORK FOR THE GENETIC CONTROL OF SHOOT LATERAL ORGAN SIZE IN *ARABIDOPSIS*.

Category: Developmental Mechanisms

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While one of the main factors influencing final shoot lateral organ size is the duration of cell proliferation, changing the size of a flattened organ, such as the leaf, requires coordination of the patterns of cellular proliferation, differentiation and expansion that occur during development. During leaf development in *Arabidopsis* a front of general cell-division arrest moves progressively from the tip to the base, followed by a gradient of cellular differentiation. Although most cells distal to the primary arrest front differentiate, dispersed meristematic cells continue proliferation to produce specialized cell types, such as stomata guard cells. The *PEAPOD* genes *PPD1* and *PPD2* coordinate the arrest of this dispersed meristematic cell proliferation. White, D. (2006) PNAS 103, 13238-13234. I have proposed a revised model with two separate and independent cell-proliferation arrest fronts regulating leaf development: a primary front (shape and progression influenced by class II *TCP* genes- *CIN* in *Antirrhinum*, *JAW-D/MIR 139A* acting on *TCP's* in *Arabidopsis*), and a secondary front involving *PPD1/PPD2*. Furthermore, it appears that the extent of lamina growth maybe regulated by a balance between the activities of genes promoting and arresting cell proliferation. To test these concepts I have examined the influence of combinations of loss and gain-of-function alleles of *PPD* and *ANT/TEGUMENTA (ANT)* on shoot lateral organ size and shape. Both *ppd* and *ant* mutations and over expression transgenes were also combined with *JAW-D*. Results indicate that *ANT* influences the maintenance of cell proliferation in the dispersed meristematic phase of lamina development, as well as the earlier proliferative cell division phase. Increased size promoted by *ANT-OE* was counteracted by *PPD-OE*, and the positive curvature caused by extended dispersed meristematic cell proliferation in *ppd* is limited by *ant*. The jagged leaf margin of *JAW-D* was reduced in *ppd;JAW-D*, *PPD-OE;JAW-D* and *ANT-OE;JAW-D*, but exaggerated in *ant;JAW-D*. These results support a framework for the genetic control of shoot lateral organ size, composed of a balance between the action and timing of positive regulators promoting the maintenance and two arrest fronts the limitation of cell proliferation during development.

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#### **ICAR1145**

A MIRNA-MEDIATED VEGETATIVE PHASE CHANGE PATHWAY IN ARABIDOPSIS

Category: Developmental Mechanisms

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miRNAs are ca. 21-nt small RNAs involved many important developmental processes in both animals and plants. In *Arabidopsis*, miR156 and miR172 have been shown to affect flowering. Here we show that these two miRNAs and their targets also play important roles in vegetative phase change. miR156 regulates vegetative phase change through its effects on the transcription factors *SPL3*, *SPL4*, *SPL5*, *SPL9* and *SPL10*, whereas miR172 regulates this process via its effect on the *AP2*-like genes *TOE1* and *TOE2*. Molecular and genetic evidence indicates that miR172 acts downstream of miR156, and that *SPL9* and *SPL10* mediate the effect of miR156 on miR172 expression. *SPL3*, *SPL4* and *SPL5* have less significant effects on vegetative phase change than *SPL9* and *SPL10*, and appear to operate independently of miR172.

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#### **ICAR1146**

FUNCTIONAL GENOMICS OF TRANSCRIPTION FACTORS EXPRESSED DURING EMBRYO DEVELOPMENT IN ARABIDOPSIS AND BRASSICA

Category: Developmental Mechanisms

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Embryogenesis represents a critical phase in the life cycle of sexually reproducing plants. During this phase the embryos undergo a number of key developmental switches to establish the body plan as well as organizational template for postembryonic processes. To identify the key regulatory factors that are involved in these processes, we have used genome wide expression profiling for key phases of embryogenesis from zygote to maturity in *Arabidopsis* and *Brassica napus* (canola – crop species). From the global analysis, we have identified a number of transcription factors that are

**expressed differentially during embryogenesis. These include a group of "Zinc Finger" (ZF) transcription factors that display** unique expression patterns. To address the functions of these putative transcriptional regulatory factors, we have used both loss and gain of function approaches. A range of embryonic and post embryonic phenotypes were observed with mutant and ectopic expression of several of the "Zinc Finger" transcription factors. The embryonic phenotypes include altered body plan with restricted, expanded axis, large cotyledons, more cotyledons, seed number. With ZF6, ZF7 we have observed compact and dwarf architecture; and several of the other ZFs displayed leaf phenotypes. The functional implications of the transcriptional factor studies will be presented

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#### **ICAR1147**

ARABIDOPSIS POLYADENYLATION FACTOR CLPS3 FUNCTIONS IN GAMETOPHYTE, EMBRYO AND POST-EMBRYONIC DEVELOPMENT

Category: Developmental Mechanisms

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**Polyadenylation factor CLP1, a predicted ATP/GTP binding protein, is essential for mRNA 3'-end processing** in yeast and mammals. CLP1 also is a RNA kinase in human tRNA splicing process. Two Arabidopsis orthologs, designated as CLPS3 and CLPS5 (CLP1 Similar protein 3 and 5), of human CLP1 (or yeast Clp1p) has been identified based on sequence similarity and phylogenetic analyses. CLPS3, together with FY, was previously shown to be a subunit in the affinity purified PCFS4-TAP complex involved in the alternative polyadenylation of FCA pre-mRNA and therefore flowering time control (Xing et al 2008). In this study, we further explored the components in an affinity purified CLPS3-TAP complex and identified Arabidopsis CPSF100 in the complex. This result suggested that PCFS4 complex interacted with CPSF complex in vivo, probably through CLPS3. Analysis of transgenic plants containing CLPS3-GFP protein fusion or CLPS3 promoter-GUS fusion suggested that CLPS3 is a nuclear protein and was universally expressed throughout the life cycle. Characterization of the T-DNA insertion mutant of CLPS3 revealed that CLPS3 is essential for embryo development. Genetic analysis of hemizygote at CLPS3 locus also revealed the reduced transmission of female gametophyte, but not male gametophyte, suggesting that CLPS3 is important for megagametophyte development. Over-expression of CLPS3 caused a range of developmental abnormality including altered leaf shape, phyllotaxy, flowering time (early), and shape and number of flower organs. However, no apparent abnormality was observed in root development and seed setting. To explore the molecular basis of early flowering time and altered phyllotaxy of CLPS3 over-expression plants, the expression of FCA and a set of genes involved in SAM development are under investigation.

Reference:

Xing D, Zhao H, Xu R and Li QQ. 2008. Arabidopsis PCFS4, a homologue of yeast polyadenylation factor Pcf11p, regulates FCA alternative processing and promotes flowering time. Plant Journal, in press. (doi: 10.1111/j.1365-313X.2008.03455.x)

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#### **ICAR1148**

A ROLE FOR BLADE-ON-PETIOLE1 AND 2 IN CONTROL OF ARABIDOPSIS INFLORESCENCE AND FLORAL ARCHITECTURE

Category: Developmental Mechanisms

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The transition from vegetative to reproductive development in Arabidopsis is controlled by both endogenous and environmental signals. LEAFY (LFY) and APETALA1 (AP1) are key regulators of this transition and expression of these genes in organ primordia produced by the inflorescence meristem confers floral fate. We now show that two NPR1-like genes, BLADE-ON-PETIOLE 1 and 2, function together with LFY and AP1 to promote the transition to floral fate. Double mutants bop1 bop2 show only subtle defects in inflorescence and floral architecture but in combination with lfy or ap1 reveal synergistic enhancement of floral-meristem identity defects including changes in internode elongation, floral organ phyllotaxy, bract formation, and ectopic meristem activity. Mutation of bop1 bop2 also enhances floral-organ identity defects associated with weak alleles of lfy and ap1 and enhances floral bract formation in LATE-MERISTEM IDENTITY1, a downstream target of LFY. Molecular analysis shows that BOP1/2 function redundantly with LFY and AP1 in down-regulation of the inflorescence identity gene AGAMOUS-LIKE24, an important first step in the conversion of shoots to flowers during the transition to reproductive development.

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#### **ICAR1149**

VASCULAR-RELATED NAC-DOMAIN7 IS INVOLVED IN DIFFERENTIATION OF ALL TYPES OF XYLEM VESSELS IN ARABIDOPSIS ROOTS AND SHOOTS

Category: Developmental Mechanisms

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We have shown that an *Arabidopsis thaliana* NAC domain transcription factor, *VASCULAR-RELATED NAC-DOMAIN7* (*VND7*), plays a pivotal role in regulating root protoxylem vessel differentiation (Kubo et al., 2005, *Genes Dev.*). In order to understand the mechanisms underscoring *VND7* function in vessel differentiation in detail, we conducted extensive molecular analyses in yeast (*Saccharomyces cerevisiae*), Arabidopsis, and *Nicotiana tabacum* L. cv. Bright Yellow 2 (tobacco BY-2) cells. The C-terminal region of *VND7* was required for its transcriptional activation in yeast and Arabidopsis. Expression of the C-terminus-truncated *VND7* protein under the control of the native *VND7* promoter resulted in inhibition of normal development of metaxylem vessels in roots and vessels in aerial organs, as well as protoxylem vessels in roots. The expression pattern of *VND7* overlapped that of *VND2* to *VND5* in most of the differentiating vessels. Furthermore, a yeast two-hybrid assay revealed the ability of *VND7* to form homodimers and heterodimers with other VND proteins via their N-termini, which includes the NAC domain. Together these data suggested that *VND7* regulates the differentiation of all types of vessels in roots and shoots, possibly in cooperation with *VND2* to *VND5* (Yamaguchi et al., in press, *Plant J.*). Heterologous expression of *VND7* in tobacco BY-2 cells demonstrated that *VND7* stability could be regulated by protein degradation, suggesting that *VND7* is controlled by some regulatory proteins.

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#### **ICAR1150**

A CLASS ONE ADP-RIBOSYLATION FACTOR GTPASE-ACTIVATING PROTEIN IS CRITICAL FOR MAINTAINING DIRECTIONAL ROOT HAIR GROWTH IN *ARABIDOPSIS THALIANA*

Category: Developmental Mechanisms

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Membrane trafficking and cytoskeletal dynamics are important cellular processes that drive tip growth in root hairs. These processes interact with multitude signaling pathways that allow for the efficient transfer of information to specify the direction in which tip growth occurs. Here we show that AGD1, a class one ADP ribosylation factor-GTPase activating protein (ARF-GAP), is important for maintaining straight growth in *Arabidopsis* root hairs since mutations in the AGD1 gene resulted in wavy root hair growth. Live cell imaging of growing *agd1* root hairs revealed bundles of endoplasmic microtubules and actin filaments extending into the extreme tip. The wavy phenotype and pattern of cytoskeletal distribution in root hairs of *agd1* partially resembled that of mutants in an armadillo-repeat containing kinesin (*ARK1*). Root hairs of double *agd1/ark1* mutants were more severely disrupted compared to single mutants. Organelle trafficking as revealed by a fluorescent Golgi marker was slightly inhibited and Golgi stacks frequently protruded into the extreme root hair apex of *agd1* mutants. Transient expression of GFP-AGD1 in tobacco epidermal cells labeled punctate bodies that partially colocalized with the endocytic marker, FM4-64, while ARK1-YFP associated with microtubules. Brefeldin A, rescued the phenotype of *agd1* indicating that the altered activity of an AGD1-dependent ARF contributes to the defective growth, organelle trafficking and cytoskeletal organization of *agd1* root hairs. We propose that AGD1, a regulator of membrane trafficking and ARK1, a microtubule motor, are components of converging signaling pathways that impact cytoskeletal organization to specify growth orientation in *Arabidopsis* root hairs.

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**ICAR1151**

A DIVERGENT ROLE OF THE BROTHER OF FT AND TFL1 (*BFT*) GENE IN FLORAL DEVELOPMENT

Category: Developmental Mechanisms

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*BFT* is a member of *FLOWERING LOCUS T(FT)/TERMINAL FLOWER 1 (TFL1)* gene family, which contains important flowering time regulators in *Arabidopsis*. Despite high sequence homology within the *FT/TFL1* family members, their functions are different during floral transition, for instance, *FT* promotes flowering, whereas *TFL1* represses. Function of most members has been extensively studied; however, *BFT* is remained uncharacterized. In order to identify its function, we performed functional analyses of *BFT* by using gain-of-function alleles. Transgenic plants constitutively expressing *BFT* showed delayed flowering time and altered inflorescences, suggesting that *BFT* functions similarly as does *TFL1*. Semiquantitative RT-PCR revealed that expressions of *FT* and *SOC1* were downregulated in *35S:BFT* plants, whereas expressions of *TFL1* and *FLC* were not affected. Consistent with this, a *bft-1* allele, which is a T-DNA-tagged gain-of-function allele of *BFT*, showed delayed flowering time. *tfl1-1 bft-1* double mutants showed reduced number of auxiliary buds, implicating that the *bft-1* partially suppresses the *tfl1-1* phenotype. However, a loss-of-function allele, *bft-2*, did not show any obvious phenotype in floral development. Taken together, these results suggest that *BFT* controls flowering time and inflorescence meristem development and that it may act redundantly with other members of *FT/TFL1* family in *Arabidopsis*.

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**ICAR1152**

SD5, A HOMOLOGUE OF SPLICOSOME SUBUNIT, IS ESSENTIAL FOR PROLIFERATION IN POST-SEEDLING DEVELOPMENT.

Category: Developmental Mechanisms

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Plant body size tightly depends on both cell numbers and cell size comprising its organ, and this regulation is controlled genetically. To elucidate body size control, we analyzed *segregation distortion 5 (sd5)* originally identified as a marker non-mendelian inherited mutant from RIKEN *Ds* insertional mutant lines. *sd5* mutant consisted of small and abnormal shape leaves, and it showed various developmental arrest at post-seedling stage. Cell numbers were decreased and *CYCB1;1* expression was also down-regulated in *sd5* leaves, whereas *sd5* developed apparently normal shoot apical meristem. These indicate that cell proliferation was strongly affected in *sd5*.

Corresponding gene of *sd5* encodes a homologue of yeast *DIM1*, a component of U5 spliceosome. Animal and plants possess two *DIM1* homologues, although only *DIM1* was found in yeast genome. Loss of *DIM1* caused lethality as a result of defect of cell cycle in *S. pombe*. Interestingly, SD5 protein did not complement to the *pombe dim1-35* mutation when *SD5* was expressed under limited temperature. On the other hand *Arabidopsis* another homologue of *DIM1* could complement to this mutation. These results suggested SD5 plays different functions of authentic *DIM1* protein.

Here we will demonstrate the detail of *sd5* phenotypes and physiological functions of SD5.

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**ICAR1153**

SIGNALING OF ANTER DEVELOPMENT BY THE TPD1 SMALL PROTEIN AND EMS1 RECEPTOR KINASE IN ARABIDOPSIS

Category: Developmental Mechanisms

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Sexual reproduction requires specification of cells with distinct fates in both plants and animals. So far, little is known about the molecular mechanisms underlying cell fate determination during sexual reproduction in plants. Recently anther emerged as a prime model system for the study of cell fate determination and receptor-linked signaling, in addition to its central importance to plant reproduction and breeding. In flowering plants, a mature anther is usually a four-lobed structure. Each lobe contains five types of highly specialized cells, which are the epidermis, endothecium, middle layer, tapetum and microsporocytes (pollen mother cells). Microsporocytes are reproductive cells that generate pollen via meiosis, while somatic cells, particularly the tapetum, are required for the normal development and release of pollen. The *ems1* (also known as *exs*) mutant anthers lack the tapetum, but produce more microsporocytes at the expense of tapetal cells, suggesting that there is a trade off between somatic and reproductive cells. The *tpd1* mutant has a phenotype indistinguishable from that of *ems1*. The *EMS1* gene encodes a leucine-rich repeat receptor-like kinase (LRR-RLK), while *TPD1* encodes a small, putatively-secreted protein. Our new results show that ectopic expression of *TPD1* causes abnormal differentiation of tapetum and microsporocytes. In addition, ectopic *TPD1* activity requires functional *EMS1*. Yeast two-hybrid, pull-down and co-immunoprecipitation analyses further demonstrate that *TPD1* interacts with *EMS1* in vitro and in vivo. Moreover, *TPD1* induces *EMS1* phosphorylation in planta. Thus, our results provide several lines of evidence to strongly support that *TPD1* serves as a ligand of *EMS1*. A model for explaining the molecular mechanism of anther development is also proposed based on our work.

References:

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2. Jia, G., Liu, X., Owen, H.A. and Zhao, D. (2008) Signaling of cell fate determination by the *TPD1* small protein and *EMS1* receptor kinase. **PNAS** 105: 2220-2225.

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**ICAR1154****BIOCHEMICAL CHARACTERIZATION OF ARABIDOPSIS SPA-COP1 COMPLEXES IN LIGHT CONTROL OF PLANT DEVELOPMENT**

Category: Developmental Mechanisms

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COP1 (constitutively photomorphogenic 1) and the four partially redundant SPA (suppressor of phyA) proteins work in concert to repress photomorphogenic development in *Arabidopsis*. Accumulating evidence suggests that these proteins constitute an E3 ligase activity responsible for targeting several photomorphogenesis-promoting transcription factors and phytochrome A for degradation via the 26S proteasome. Here, we report a detailed biochemical characterization of the SPA-COP1 complexes. The four endogenous SPA proteins exhibit distinct expression profiles in different tissue types and light treatments. All four SPA proteins can form stable complexes with COP1 *in vivo* regardless of light conditions. The SPA proteins can either self associate or interact with each other, forming a heterogeneous group of SPA-COP1 complexes in which the exact SPA protein compositions vary among the individual complexes. The relative abundance of individual SPA-COP1 complexes depends on the abundance of the individual SPA proteins in a given tissue type under a defined light condition. The four SPA proteins could be divided into two functional groups depending on their interaction affinities, differential contributions in regulating HY5 degradation and their opposite effects on COP1 protein accumulation. Loss-of-function mutations in a predominant SPA protein may cause a significant reduction in the overall SPA-COP1 E3 ligase activity, resulting in partial constitutive photomorphogenic phenotype. This study thus provides an in-depth view of the biochemical nature of the SPA-COP1 E3 ligase complexes and offers new insights into the molecular basis for their distinct roles in light control of plant development.

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## CELL WALLS

### ICAR201

PATCHY ENCODES A PUTATIVE BETA-XYLOSIDASE/ARABINOFLUORANOSIDASE REQUIRED FOR PECTIN SIDECHAIN MODIFICATION IN ARABIDOPSIS MUCILAGE SECRETORY CELLS (MSC)

Category: Cell Walls

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In *Arabidopsis*, the epidermal cells of the outer ovule integument differentiate through a complex process into specialized cells that produce mucilage between the primary cell wall and plasma membrane. Upon imbibition the mucilage expands rapidly, breaking through the primary cell wall and enveloping the seed. A mutation in *PATCHY* (*PTY*) causes a peculiar phenotype where mutants have patchy and delayed mucilage release compared with wild type seeds. These mutants appear to undergo normal mucilage production and mucilage secretory cell development. Cloning of *PTY* by plasmid rescue revealed a T-DNA insertion in *AtBXL1*, a gene encoding a beta-xylosidase/arabinofuranosidase. Molecular complementation, and two independent Salk T-DNA knockout lines in the same locus producing a similar 'patchy' release phenotype, confirms the gene is involved in mucilage release. Expression analysis with real-time RT-PCR GUS shows expression of *PTY* in all tissues tested, including 7, and 10 day old seeds, consistent with its proposed role in mucilage release and cell wall modification. Chemical analysis suggests that pty mutants have an increase in arabinose, specifically (1,5)-linked alpha-D-arabinofuranose in their seed coat mucilage when compared to wildtype. Genetic analysis suggests *PTY* may act independently of known MSC regulators.

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### ICAR202

IDENTIFICATION AND CHARACTERIZATION OF ARABIDOPSIS MUTANTS FOR THE GLUCOSYL TRANSFERASES POTENTIALLY INVOLVED IN THE GLUCOSYLATION OF MONOLIGNOLS, THE LIGNIN PRECURSORS.

Category: Cell Walls

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In angiosperms, the lignin polymer is composed mainly of guaiacyl (G) and syringyl (S) units. These units are derived from coniferyl and syringyl alcohols, also named monolignols. These monolignols are thought to be glucosylated before their transport to the cell wall for polymerization into lignin *in muro*.

In *Arabidopsis*, the genes coding for glycosyltransferases (UDP-glucosyltransferase; UGTs) belong to a large multigene family. Two of them (*UGT72E2*, *UGT72E3*) were found to be responsible for the biosynthesis of 4-O-glucosides of coniferyl and sinapyl alcohols (Lim et al, 2001) and one (*UGT72E1*) was found highly specific to coniferyl and sinapyl aldehydes (Lim et al, 2005) *in vitro*.

Recently, it was shown that the coniferin (coniferyl alcohol 4-O-glucoside) content in continuous light grown roots of *Arabidopsis* was significantly reduced in *UGT72E2* knock-down mutant lines (Lanot et al. 2006).

The expression profiles of the *UGT72E1*, *UGT72E2* and *UGT72E3* genes were determined in different parts of *Arabidopsis* plants.

Knockout mutants for each of these three genes were identified in collections of *Arabidopsis* T-DNA insertion mutants. At the homozygous stage, these mutants do not present any specific phenotype when grown in the greenhouse and are not affected in their lignin amount and structure.

Since light induces phenylpropanoid metabolism in *Arabidopsis* roots (Hemm et al, 2004), these mutants were cultivated in permanent light conditions. The root size, the root content in soluble phenolics (flavonol glycosides and coniferin) and the metabolite profiles were determined in wildtype and mutant lines.

Characterization of double mutants is underway to test the possibility of redundancy of these genes.

#### References

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### ICAR203

A LARGE GENETIC SCREEN IN ARABIDOPSIS TO IDENTIFY GENES INVOLVED IN POLLEN EXINE PRODUCTION.

Category: Cell Walls

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Plants have evolved remarkable and unique pollen walls. Exine, the outside pollen wall, is important for pollen protection, dispersal, and species-specific pollen-stigma recognition. There is an astonishing variability in exine patterns across different plant species, yet the overall pollen wall architecture is conserved. Exine is made of sporopollenin, a tough and chemically inert biopolymer, whose chemical resistance makes it a formidable challenge to deduce its structure. Only a handful of the genes involved in the exine development have been isolated so far. We have performed a genetic screen that aimed to identify genes involved in exine development. We have used a simple yet effective approach of visual screening at low magnification that was applied to a random T-DNA insertion collection, as well as to a handpicked collection of mutations in the genes that were selected as possible exine gene candidates. We have been able to identify multiple mutants with abnormal exine that exhibited a large variety of phenotypes ranging from a complete lack of exine to a loss of the net-like structure, changes in the thickness of exine wall, abnormal distribution of exine on pollen surface, abnormal number and position of apertures, changes in the sizes of lacunae on the exine surface to exine that appeared morphologically normal yet demonstrated an altered autofluorescence spectrum, suggesting a change in its composition. We began characterization of 56 mutants isolated through a forward genetic screen and of 9 mutants isolated through a reverse genetic screen, and identified several genes that have not been previously implicated in exine production. We have complemented the analysis of these genes by a metabolomics approach that sought to look at the compounds produced in the anthers of these mutants at the time when exine development takes place.

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**ICAR204**

THE CELL WALL STRESS RESPONSE IN ARABIDOPSIS THALIANA IS MEDIATED BY MECHANOPERCEPTION, ATRBOH D AND JAR 1

Category: Cell Walls

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Maintaining the functional integrity of the plant cell wall during different biological processes like cell morphogenesis and plant pathogen interaction is crucial. Previously the existence of a mechanism monitoring and maintaining wall integrity has been postulated. By analysing the plant's response to cell wall stress caused by cellulose biosynthesis inhibition (CBI) in Arabidopsis seedlings a systematic analysis of the cell wall integrity maintenance mechanism was initiated.

Here we show that genes originally implicated in pathogen / drought response, mechano-perception, lignin and cell wall biosynthetic processes exhibit expression changes in response to CBI. Arabinose and uronic acids in seedling cell walls are increased by CBI. A promoter reporter construct for a putative xylose epimerase (UXE4) is activated specifically by CBI and activation is suppressed by osmotic support. 6h of CBI cause lignin deposition and lesion formation in the primary root elongation zone. Osmotic support and a mutation in ATRBOH D prevent lignin deposition and lesion formation while knocking out JAR 1 enhances them. The observed phenotypes are hexose dependent. Measurements of jasmonic (JA), salicylic (SA) and abscisic acid detect hexose dependent and osmo-sensitive JA concentration changes. CBI induced expression of mechano-sensitive and pathogen response genes is prevented by osmotic support and influenced by hexose availability.

ATRBOH D and JAR 1 apparently regulate CBI induced lignin deposition and lesion formation. The effects of osmotic support and hexose presence on lesion formation, lignin deposition, gene expression and JA production highlight the important role of osmo-perception and hexoses in regulating the plant stress response.

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**ICAR205**

TESTING FUNCTIONS OF ABC TRANSPORTERS EXPRESSED IN VASCULAR BUNDLES OF ARABIDOPSIS STEMS

Category: Cell Walls

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In Arabidopsis, the ATP-binding cassette (ABC) protein superfamily is one of the largest gene families. Plant ABC transporters are required for the export of diverse molecules (auxins, alkaloids, fatty acids, waxes and herbicides). The objective of this study was to test whether ABC transporters are required for normal lignin deposition in the primary stem of Arabidopsis, in vascular bundles and interfascicular fibres. Microarray studies demonstrate a correlation between expression of phenylpropanoid biosynthetic genes and ABC transporters genes during inflorescence stem development (*ABCB11/MDR8; ABCB14/MDR12; ABCB15/MDR13; ABCG33PDR5*). Promoter::glucuronidase reporter constructs for each of these genes indicated promoter activity in both phloem and xylem of primary stem. Homozygous TDNA insertional mutant lines (*abcb11/mdr8; abcb14/mdr12; abcb15/mdr13, and abcg33/pdr5*) had wild-type lignification patterns. However, in mutants of *ABCB14/MDR12*, vascular morphology was disorganized and these mutants also showed decreased polar auxin transport. These results indicate that, while ABCB-type ABC transporters are important for auxin transport in the stem, the overlapping expression of these proteins makes it difficult to draw conclusions about other transport functions such as monolignol export.

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**ICAR206**

UNRAVELING TRANSCRIPTIONAL REGULATORY NETWORK DURING SECONDARY WALL SYNTHESIS

Category: Cell Walls

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Secondary walls are synthesized only in cells of limited tissues such as stem, hypocotyl, anther and siliques. In the course of systematic functional

analysis of NAC transcription factors (TFs), we found that NST1, NST2 and NST3 (=SND1) TFs are key regulators for secondary wall synthesis. The *nst1 nst2* double T-DNA tagged line showed indehiscent anther due to loss of secondary wall in anther endoecium, while *nst1 nst3* double T-DNA tagged line could not stand erect and showed indehiscent siliques due to complete loss of secondary walls in stem, hypocotyl and siliques except in vascular vessels. On the contrary, ectopic expression of *NST*s induced ectopic secondary wall synthesis in various above-ground organs. We concluded that NST TFs are master regulators of secondary wall synthesis in plants. However, factors functioning in downstream and upstream of *NST*s are still obscure. We are now trying to find such TFs and identified several TFs as candidate. We will draw putative regulatory network during secondary wall formation in various tissues.

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### ICAR207

#### CHARACTERIZATION OF ATSBT1.7, A SUBTILISIN-LIKE SERINE PROTEASE ESSENTIAL FOR MUCILAGE RELEASE FROM ARABIDOPSIS SEED COATS

Category: Cell Walls

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During Arabidopsis seed development large quantities of mucilage composed of pectins are deposited into the apoplast underneath the seed coats' outer wall. Upon imbibition of mature seeds the stored mucilage expands through hydration, breaks the outer cell walls and encapsulates the whole seed. Mutant seeds carrying loss-of-function alleles of *AtSBT1.7* that encodes one of 56 *Arabidopsis thaliana* subtilisin-like serine proteases (subtilases) do not release mucilage upon hydration. Microscopic analysis of the mutant seed coat revealed no visible structural differences compared to wild-type seeds. Weakening of the outer primary wall using cation chelators triggered mucilage release from the mutant's seed coat. However, in contrast to mature wild-type seeds the mutants' outer cell walls did not rupture at the radial walls of the seed coat epidermal cells but opened at the chalazal end of the seed and were released in one piece. In *atsbt1.7* mucilage the total rhamnose and galacturonic acid contents, representing the backbone of mucilage, remained unchanged compared to wild-type seeds. Thus, extrusion and solubility but not the initial deposition of mucilage are affected in *atsbt1.7* mutants. AtSBT1.7 is localized in the developing seed coat and is processed through autocatalytic intramolecular cleavage. AtSBT1.7 expression is repressed by the active AtSBT1.7 protein. AtSBT1.7 is possibly involved in the accumulation and/or activation of cell wall modifying enzymes necessary either for the loosening of the outer primary cell wall or to facilitate swelling of the mucilage. This is supported by elevated pectin methylesterase activities in developing *atsbt1.7* mutant seeds. Strategies are presented to identify possible substrates for the subtilase by characterizing molecular interaction partners of AtSBT1.7.

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### ICAR208

#### ABCG11 AND ABCG12, ABC TRANSPORTERS WORKING TOGETHER AT THE EPIDERMAL SURFACE FOR WAX EXPORT

Category: Cell Walls

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ATP binding cassette (ABC) proteins are annotated as a gene superfamily of over 120 genes in Arabidopsis, the majority of which encode integral membrane protein transporters. The nomenclature of the ABC transporters has recently been unified and the largest subfamily, formerly known as the White-Brown Complex (WBC), was renamed the ABCG subfamily. The functions of the ABCG transporters are largely unknown, with the exceptions of ABCG11 and ABCG12 that are required for lipid export to the plant cuticle. Mutants lacking ABCG11 [*wbc11*] or ABCG12 [*cer5*] accumulate lipidic inclusions inside epidermal cells and have reduced wax at the plant surface. The ABCG subfamily transporters are predicted to be half-transporters, i.e. they must dimerize to function. Given the similarity of phenotypes between *wbc11* and *cer5*, it was postulated that they might act together in the transport of lipids to the cuticle. Double mutants of *wbc11* and *cer5* had the same phenotype as *wbc11* single mutants, supporting this hypothesis. Further work is underway to test the hypothesis that ABCG11 and ABCG12 form a heterodimer that is required for wax export.

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### ICAR209

#### RECEPTOR LIKE KINASES INVOLVED IN SECONDARY CELL WALL SYNTHESIS.

Category: Cell Walls

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Cellulose is central to plant development and is synthesized at the plasma membrane by an organized protein complex that contains three different cellulose synthase proteins (Taylor et al 2003). The ordered assembly of these three catalytic subunits is essential for normal cellulose synthesis. The way in which the cell regulates cellulose synthesis is currently unknown though it is clear that there must be some form of communication between the extracellular cell wall and the intracellular site of polymerization of glucan chains. Trans-membrane signaling by receptor like kinases (RLKs) is one mechanism by which this could occur. RLKs contain an extracellular 'sensing' domain linked by a trans-membrane domain to an intracellular kinase domain which is capable of phosphorylating proteins to regulate their activity, stability or to activate a signal transduction pathway. Recent work has shown that at least two of the cellulose synthases responsible for secondary cell wall cellulose synthesis in *Arabidopsis thaliana*, AtCesA4 and AtCesA7, are phosphorylated *in vivo*. Analysis of phosphorylation sites by mass spectrometry has identified some of these *in vivo* phosphorylation sites which occur in a region of hyper-variability between the CesA proteins (Taylor 2007). Current work is focused on the identification of the kinases that phosphorylate the cellulose synthase proteins, and the latest results from this work will be presented. The identification of the receptor like kinases involved in regulating cellulose synthesis would clearly represent a major step forward in our understanding of cellulose synthesis. The identification of regulatory mechanisms controlling cellulose synthesis will allow a unique opportunity to manipulate the content and composition of cell walls, a developmentally and economically important target for biotechnology.

Taylor et al (2003). PNAS 100:1450-1455.

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**ICAR2010**

MICROTUBULE ORGANIZATION INFLUENCES CELLULOSE CRYSTALLINITY AND THE VELOCITY OF CELLULOSE-SYNTHASE-COMPLEXES DURING CELL EXPANSION

Category: Cell Walls

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Plant shape is dependent on the mechanical properties of the cell wall. Cellulose microfibrils (CMFs) are synthesized at the plasma membrane and aligned in an ordered manner to restrict the direction of cell expansion. Microtubules (MTs) are considered the most likely candidates for influencing the organization of CMFs but the exact role MTs play is still unknown. A model for the role of MTs [Wasteneys 2004, Curr Opin Plant Biol 7(6): 651-660] suggests that well-organized MTs ensure that the synthesis and integrity of CMFs are maintained. Here, this hypothesis has been tested by analyzing cellulose properties in *mor1-1*, a mutant of *Arabidopsis thaliana* in which MT dynamics are greatly reduced and MTs become short and disorganized at a restrictive temperature of 29°C. X-ray diffraction analysis demonstrated that cellulose crystallinity in *mor1-1* remains high at 29°C, whereas cellulose crystallinity declines in wild-type plants under the same growth-promoting conditions. This finding suggests that dynamic MTs are required for the decline in cellulose crystallinity that normally accompanies increased cell expansion. We next compared the velocity of yellow fluorescent protein (YFP)-tagged CesA6 in *mor1-1* and wild-type hypocotyl cells. The motility of cellulose-synthase-complexes at the plasma membrane is driven by the polymerization and crystallization of cellulose, so the velocity of cellulose synthase complexes is expected to correlate with the rate of cellulose polymerization. YFP-CesA6 velocity increased at the higher (*mor1-1*-restrictive) temperature in both genotypes but was significantly faster in the *mor1-1* cells, suggesting that cellulose production is in fact slightly greater in *mor1-1*. Taken together, our findings suggest that the failure of cell walls to grow anisotropically in the *mor1-1* mutant is connected to an inability to reduce cellulose crystallinity during rapid growth at high temperatures.

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**ICAR2011**

ARABIDOPSIS MYB26/MALE STERILE 35 GENE REGULATES SECONDARY CELL WALL THICKENING IN THE ANTER ENDOTHECIUM AND CONTROLS ANTER DEHISCENCE.

Category: Cell Walls

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Secondary cell wall thickening is vital for many aspects of plant growth, typically for the production of mechanical tissues for water transport and support, but also when other aspects of mechanical force are required, for example anther dehiscence. Secondary thickening occurs in the anther endothecium and is vital for the physical forces needed for anther opening, as demonstrated by our *Arabidopsis* non-dehiscent *ms35* mutant.

The *ms35* mutation specifically prevents secondary thickening in the anther endothecium, resulting in the endothelial cells becoming flattened and distorted, the outward bending of the anther wall fails to occur, the endothelial cells collapse and the anther fails to open. The *ms35* mutation is due to a defect in the *MYB26* gene, which has homology to R2R3-type MYB transcription factors, which we have shown is nuclear localised and regulates endothelial development and secondary thickening in a cell specific manner in the anther (Yang *et al* 2007).

Microarray analysis performed using isolated, staged anthers from the *ms35* mutant and wild type, revealed a coordinated down-regulation of genes previously linked to secondary cell wall biosynthesis, including *IRREGULAR XYLEM 1 (IRX1), IRX3, IRX8, IRX12, NAC SECONDARY WALL PROMOTING FACTOR1 (NST1)* and *NST2*. This indicates that the down-stream mechanisms for secondary thickening are conserved, but that the trigger for such events is due to specific regulators, for example *MYB26*, which determines temporal and tissue specificity in the anther. Immunolocalisation of pectin and xylan has provided further evidence for the role of *MYB26* in the regulation of secondary wall development in the anther. The microarray analysis also indicates that a number of additional genes are involved, including several transcription factors and genes in ubiquitin-dependent protein degradation and phytohormone related pathways. This suggests that *MYB26* functions in a regulatory role in determining endothelial cell development and acts upstream of the lignin biosynthesis pathway. These data and the potential role for *MYB26* will be discussed.

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**References**

Yang, C.-Y., Zhengyao, X., Song, J., Conner, K., Vizcay-Barrena, G., and Wilson, Z.A. (2007) The Plant Cell 19, 534-548.

## **Evolution and Natural Variation**

### **ICAR301**

MITOCHONDRIAL MRNA POLYMORPHISMS IN DIFFERENT ARABIDOPSIS THALIANA ACCESSIONS

Category: Evolution and Natural Variation

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The systematic analysis of mitochondrial mRNAs in the three *Arabidopsis thaliana* accessions Columbia (Col), C24 and Landsberg erecta (Ler) revealed distinct transcript polymorphisms for seven mitochondrial genes. These transcript polymorphisms are predominantly attributed to variations at the 5' termini and were consistently observed in all tissues investigated. mRNA phenotyping of respective reciprocal F1 hybrids revealed the differing transcript patterns of ccmC, cox3 and atp6-2 to be inherited maternally suggesting these to arise from differences in the mitochondrial DNA. Bi-parental inheritance was observed for the polymorphic transcripts of nad4, nad9, ccmB and rpl5 indicating these differences to be caused by nuclear encoded trans-factors. Deviant transcript patterns were tested in further accessions and were indeed found in at least three additional accessions. The presence of the individual RNA polymorphisms is not linked among the analyzed accessions, suggesting that different nuclear loci are responsible for the mRNA variability. This study shows that natural genetic variation in *Arabidopsis thaliana* can also affect mitochondrial mRNA processing. Map-based cloning is presently under way to map the nuclear encoded genes responsible for the nad4 and nad9 polymorphisms. Preliminary results suggest that at least one of the genes encodes a pentatricopeptide repeat (PPR) protein, which play an important role in plant mitochondrial RNA metabolism.

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### **ICAR302**

ANALYSIS OF NATURAL-GENETIC VARIATION CONTROLLING THE TIMING OF GIGANTEA EXPRESSION IN ARABIDOPSIS

Category: Evolution and Natural Variation

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Daily rhythms in expression of clock-controlled genes vary between accessions of the same plant species, and these rhythmic variations can be implicated in adaptation to growth at different latitudes. Mathematical and experimental data strongly suggest that the GIGANTEA (GI) protein plays a major role in the regulation of the *Arabidopsis* circadian clock. GI expression follows a circadian rhythm, with a peak in the evening, and changes in the timing of GI expression have dramatic effects on biological programs such as flowering. GI is also involved in other fundamental biological programs such as red and blue light signaling, suggesting a broad role for this protein in the regulation of plant development. Despite the importance of GI in these processes, its biochemical function and how its expression is regulated are still unknown. The present work proposes to identify new regulators of the timing of GI expression. Eighty fives accessions were transformed with a construct expressing the luciferase gene under the control of the GI promoter (GI:LUC), and the rhythm of GI expression was monitored under five different photoperiods. In several accessions, the peak of GI expression occurs significantly later than in the reference ecotype Col0, and these differences are enhanced when plants are grown under long day conditions. Two of these ecotypes were crossed with Col0 and F2 mapping populations were generated. GI peak of expression was determined in approximately 150 F2 individuals grown in long and short days (LD and SD). Results showed a broad segregation of GI peak of expression with appearance of transgressive individuals in both mapping populations grown in LD. In contrast, populations grown in SD showed a narrow distribution of GI "time of peak", suggesting that the genetic basis underlying the control of GI expression is more complex under LD. To identify the regulators responsible for differences in GI timing of expression between the ecotypes, GI time of peak is being used as a quantitative trait to map QTL in both mapping populations.

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### **ICAR303**

A SYNTHETIC LETHAL COP PHENOTYPE RESULTING FROM ALLELE-SPECIFIC TWO-LOCI INTERACTION

Category: Evolution and Natural Variation

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Natural genetic variation within populations largely determines phenotypic variation, and natural allelic variants which by themselves are *a priori* not deleterious can have strong synthetic effects when combined. Despite their hypothesized importance, the extent and nature of such natural genetic interactions in multicellular eukaryotes remains largely unknown. We have found a synthetic lethal phenotype in *Arabidopsis thaliana* that arises from genetic incompatibility: small cop/fusca-like seedlings, named synthetic cops (sco). The sco phenotype was recovered in crosses between hy5 hyh double mutants (in the Ws background) and a brx mutant (an introgression of the natural UK-1 null allele into the Sav-0 background). Importantly, the high frequency of the novel sco phenotype suggests that it does not represent a double or triple mutant between the hy5, hyh or brx loci. Rather, initial genetic mapping suggests that the phenotype is due to genetic interactions involving two wild type background-specific alleles at novel loci. Our aim is to isolate these alleles by map-based cloning and use this information to decipher the molecular genetic basis of the observed phenotypes.

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### **ICAR304**

ASSOCIATION MAPPING OF SHADE AVOIDANCE RESPONSES IN ARABIDOPSIS THALIANA

Category: Evolution and Natural Variation

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In response to a decreased ratio of red to far red (R:FR) light, plants exhibit a series of developmental responses known as shade avoidance.

Shade avoidance responses include enhanced elongation growth and acceleration of flowering. Although phytochrome B has long been known to be the major photoreceptor sensing changes in R:FR, the downstream pathway that leads to shade avoidance responses remains largely unknown. Since *Arabidopsis thaliana* has been shown to have considerable natural variation in light responses, the goal of this work is to characterize the genetic basis for natural variation in shade avoidance responses in *Arabidopsis* and to use this variation to identify novel loci involved in the phytochrome B downstream signaling pathway. A genome-scan association mapping study using 95 accessions of *Arabidopsis thaliana* identified 171 markers positively associated with shade avoidance phenotypes. Current work is focusing on functional validation of two of these marker associations. The first marker of interest is in predicted linkage disequilibrium with a candidate gene that is upregulated in response to low R:FR light. The second marker of interest physically overlaps with a strong shade avoidance QTL on chromosome four. Analysis of linkage disequilibrium around this marker yielded two candidate genes, both of which have non-synonymous polymorphisms between the parental ecotypes used for QTL mapping. In order to confirm the roles of candidate genes for both markers in shade avoidance, as well as to identify polymorphisms that are causative for natural variation, we are examining T-DNA insertion line phenotypes and assessing the induction of these genes in response to low R:FR.

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**ICAR305**

## VARIATION IN SEED SIZE, YIELD AND HARVEST INDEX IN ARABIDOPSIS THALIANA

Category: Evolution and Natural Variation

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Continued improvement of seed crop yield is vital to feed a growing population and compensate for the use of land for biofuel production. One potential strategy to raise yield is to increase seed size. However in nature, due to the limited resources of the mother plant, enlarged seed size is often associated with reduced seed number. Despite this trade-off, there is still the potential to enhance yield through increasing seed size by maximising the resources allocated to seeds. We have sought to use natural variation in *Arabidopsis thaliana* to evaluate the impact of increased seed size on seed yield. To ensure agronomic relevance both harvest index (HI, the ratio of seed yield to biological yield) and gross yield were measured. We uncovered considerable variation in both parameters. Significantly, high seed weight was not associated with high yield or high HI, even in Cvi, which has an exceptionally high individual seed weight. In contrast, high seed number and reduced plant stature were revealed as important components of high yield and yield efficiency. Whilst several existing ecotypes of *Arabidopsis* have one or more features of a potentially ideal high yield ideotype, no ecotype currently combines high seed number and HI with high seed weight. I will discuss approaches to develop such a high yield *Arabidopsis* and its application to crop improvement.

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**ICAR306**

## COMPARATIVE STUDIES ON NON-DORMANT COL AND DORMANT CVI SEEDS: COMMON AND POLYMORPHIC REGULATIONS ON MOLECULAR MECHANISM OF SEED IMBIBITION IN ARABIDOPSIS

Category: Evolution and Natural Variation

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Seed imbibition triggers numerous biological responses. However, little is known about its molecular mechanism. In this study, gene expression profiles for *Arabidopsis* Col (non-dormant) and Cvi (dormant) seeds were analyzed at 15 min, 30 min, 1 h and 3 h after the onset of imbibition. Transcriptional profiles of imbibed seeds from Cvi and Col accessions were similar to those of dry seed within 1 h after imbibition. In contrast, a large number of genes showed changes in transcript levels at 3 h after imbibition, suggesting that de novo transcription is initiated after 1 h in both Col and Cvi seeds. We found that 1015 genes and 717 genes were up-regulated at 3 h in Col and Cvi, respectively. These genes were categorized into 3 groups: genes that were under control of 569 Col-polymorphic, 271 Cvi-polymorphic, and 446 common regulations based on 2-fold changes in transcript levels. Cvi-polymorphic and common genes were enriched in the gene ontology categories of energy metabolism and transport facilities, whereas, Col-polymorphic genes contained an overrepresentation of genes involved in protein synthesis. These results indicate that seed imbibition responses are comprised of common and polymorphic regulations. Interestingly, genes that were up-regulated at 3 h in both Col and Cvi included *ABA1* and *CYP707A2*, which are responsible for ABA signaling and catabolism, respectively. Furthermore, LC-MS/MS analysis revealed reduction in ABA levels initiated at 3 h after imbibition in both Col and Cvi. In addition we noticed that timing of changes in transcript levels of ABA-related genes coincided with that of the reduction in ABA levels. We are currently investigating whether the reduction of ABA levels is dependent on de novo transcription or translation after imbibition. We will discuss the molecular nature of common and polymorphic regulations in response to seed imbibition.

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**ICAR307**

## DEMOGRAPHY OF ARABIDOPSIS THALIANA AT ITS NORTHERN RANGE LIMIT IN FINLAND

Category: Evolution and Natural Variation

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Much is known about *Arabidopsis thaliana* development under controlled environments, but relatively little is known about the ecology of this herbaceous annual in natural habitats. As *Arabidopsis* is increasingly used as a model organism in studies of ecological genetics, understanding demographic processes in wild populations of the species will become increasingly important. To better understand the ecology of *Arabidopsis thaliana* at the edge of its range, we collected demographic data for two seasons in a natural population in Raahen, Finland at 65 degrees north. Data including germination timing, mortality, density, and bolting date were gathered at the population level. In addition, detailed repeated measurements were made on individuals within the population, including censuses of length and width of longest leaf, leaf number, and bolting and flowering date. These data show that Raahen *Arabidopsis thaliana* behaves as a winter annual, germinating in autumn, overwintering under the snow and bolting soon after

snowmelt. There is no evidence for a spring germinating cohort. We also observed substantial overwinter mortality, with this stage being most important in determining whether or not individuals survived to reproductive age. From these data we hope to gain a better idea of natural phenotypic and life-history variation in a northern population of *Arabidopsis thaliana*.

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**ICAR308**VARIABILITY OF FLOWERING TIME IN *ARABIDOPSIS* STRAINS IN RESPONSE TO FAR-RED LIGHT

Category: Evolution and Natural Variation

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The addition of far-red light reduces the red:far-red ratio and elicits a response collectively called the shade avoidance syndrome. These responses include petiole elongation, accelerated flowering, and changes in leaf angle. In natural *Arabidopsis* strains the flowering response to far-red light is highly variable. In contrast, we find little variation in responsiveness to leaf angle and petiole elongation. We have utilized the differential responsiveness to far-red light to map causative loci involved in the flowering response. While a major QTL over FLC is identified in multiple crosses, FT expression appears to be the major determinant in the far-red flowering response. The same crosses were grown outdoors in a partially shaded environment, and the QTL overlapped with our long-day plus far-red growth conditions, demonstrating that our experimental growth conditions, and the loci identified, are ecologically relevant.

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**ICAR309**

## QUANTITATIVE TRAIT ANALYSIS OF ARABIDOPSIS ROOT BEHAVIOR ON TILTED, HARD AGAR SURFACES

Category: Evolution and Natural Variation

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*Arabidopsis thaliana* roots grown on hard, tilted agar surfaces show characteristic behaviors. Two such behaviors are root growth away from the vertical, known as skewing, and alternating tip growth from right to left resulting in a wave-like pattern. These phenotypes are complex and vary somewhat between the roots of an individual *Arabidopsis* accession. They also differ greatly when multiple accessions are compared. To discern genetic and environmental components of these traits, quantitative trait loci (QTL) mapping and analysis has been undertaken. The goal of the study is to elucidate some of the genes contributing to the behaviors and their ecological and evolutionary significance.

Three trials have been completed using the recombinant inbred line (RIL) population created from the Cvi and Ler2 accessions. The trials show consistent QTL peaks for root length, root skewing angle, and root straightness. Fine mapping is underway using near isogenic lines (NILs) to discover some of the causative genes for the skewing behavior. Other current applications of the QTL approach to study root growth behavior include growing the roots on different agar concentrations as well as root morphology upon exposure to polyamines.

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**ICAR3010**

## GENOME-WIDE ASSOCIATION MAPPING IN ARABIDOPSIS THALIANA.

Category: Evolution and Natural Variation

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Recent advances in genotyping technology have made large scale genome-wide association studies feasible. The small genome size, range of linkage disequilibrium, selfing nature, and reproducibility of phenotypes make *A. thaliana* an excellent subject for such studies. Here we present a GWA study with a number of phenotypes related to flowering and disease resistance, and 192 *A. thaliana* accessions from a global sample, genotyped using a 250k Affymetrix SNP chip. Several association methods were applied to the data, both haplotype based and single-SNP methods. Confounding population structure continues to be a problem, underscoring the importance of controlling for it.

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**ICAR3011**

#### EVOLUTION OF FLOWER MONOSYMMETRY

Category: Evolution and Natural Variation

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Angiosperms are the most diverse group of the plant kingdom, comprising about 250.000 species in 350 families. Their flowers are normally all composed of four floral organs: sepals, petals, stamens and carpels. Particularly petals, involved in attracting pollinators, exhibit a large diversity in their shape, form, size and symmetry and thus contribute to the large floral diversity of angiosperms. Analysis of floral mutants unraveled the molecular mechanisms governing flower development. The organogenesis for the four floral whorls is controlled by MADS box transcription factors, which form a highly conserved regulatory network in the angiosperms. Once petal primordia are initiated, further morphogenesis can generate different symmetries in this whorl. Polysymmetrical flowers with petals adopting identical shapes dominate the basal angiosperms. It is assumed that during co-evolution with flower pollinators, a morphological novelty, namely flower monosymmetry, evolved. In the model organism *Antirrhinum majus*, flower monosymmetry is regulated by the activity of *TCP* transcription factors, such as *CYC* (*CYCLOIDEA*). Loss of *CYC* activity converts monosymmetrical *Antirrhinum* flowers into polysymmetrical ones. We wanted to investigate if and how *CYC*-like genes were recruited during evolution to establish monosymmetry in the angiosperms. Towards this goal, the Brassicaceae were chosen as a model system. This family is dominated by over 350 genera that form four equally sized petals and thus generate a polysymmetrical corolla. Only very few Brassicaceae genera exhibit a monosymmetrical corolla. The most dramatic effect is observed in the genus *Iberis*. *Iberis amara* produces two petal pairs of different sizes, the adaxial petals being much smaller than the abaxial ones. Analysis of an *Iberis TCP* gene and its function in establishing monosymmetry will be presented.

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## SIGNAL TRANSDUCTION

### ICAR401

TISSUE-SPECIFIC POSTTRANSCRIPTIONAL REGULATION OF *XIPOTL1* (PEAMT1)

Category: Signal Transduction

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Phosphatidylcholine (Ptd-Cho) is a major membrane lipid (40-60 %) in several cellular compartments in plants and, a precursor for the synthesis of major glycerolipids in plastid membranes. In *Arabidopsis thaliana*, *XIPOTL1* (At3g18000) encodes for an S-adenosyl-L-methionine: phosphoethanolamine N-methyltransferase (PEAMT1), enzyme that catalyzes the synthesis of Ptd-Cho. Knockout mutants of *XPL1* have severe effects on root architecture and responses to environmental stress, confirming the key roles of Ptd-Cho in plants. Bioinformatics analysis of full-length cDNA sequence collections reported a conserved upstream open reading frame (uORF) in the 5'\_untranslated region of *XPL1*. uORFs are regulatory elements that can affect the translational efficiency of downstream ORF. To study the possible role of this predicted uORF on *XPL1*, we produced transgenic Arabidopsis plants harboring constructs with the WT and mutated versions of the *XPL1*-uORF fused upstream of a double *GFP-GUS* reporter gene. When plants growing in a medium with choline chloride (Cho-Cl) at physiological concentrations ( $\mu$ M) were analyzed, an almost complete inhibition of both GUS-specific activity and GFP expression was observed in roots. Interestingly, only a partial reduction of the reporter genes was observed in shoots. RT-PCR analysis showed no changes in *GUS* and *XPL1* transcripts levels. Our results show that uORF-*XPL1* act only at translational level, and that this modulation is tissue-specific. A model of how choline, phosphocholine and Ptd-Cho levels could affect the XPL1 expression, through uORF, during plant development will be discussed.

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### ICAR402

ANALYSIS AND CHARACTERIZATION OF POLLEN-SPECIFIC MEMBERS OF THE ARABIDOPSIS PERK GENE FAMILY.

Category: Signal Transduction

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The PERK receptor kinase family is characterized by an extracellular domain that is proline-rich and extensin-like followed by transmembrane and kinase domains. The original member, PERK1, was isolated from *Brassica napus*, and 15 PERK1-related members have been subsequently identified in the Arabidopsis genome. The *AtPERK* gene family can be subdivided according to the gene expression patterns. In particular, six *AtPERKs* genes (AtPERK4-7, 11 and 12) are specifically expressed in mature pollen grains according microarray data and possibly in the pollen tube as suggested by transgenic GUS plants. To study the biological functions of the pollen specific *AtPERKs*, we have screened for T-DNA insertions in all the lines except *AtPERK6*. General growth assays have not shown any phenotypes associated with the knock-out plants, possibly due to functional redundancy. To address this issue, quadruple and quintuple mutants have been generated, and are being studied in detail for any changes in pollen development or pollen tube growth. In addition, microarray analyses are being conducted with pollen grains from the quadruple mutants to determine if other genes have been up-regulated as a compensatory mechanism. Finally, transgenic *Arabidopsis* overexpressing *AtPERK4* have been generated to examine the effects of over-expression on pollen grains and pollen tube growth.

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### ICAR403

OPTIMIZED POLYETHYLENE GLYCOL FRACTIONATION METHOD FOR ACCESSING LOW-ABUNDANCE AND PHOSPHORYLATED ARABIDOPSIS LEAF PROTEINS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

Category: Signal Transduction

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Detection of low-abundant proteins in two-dimensional gel electrophoresis (2-DE) is a common challenge. The presence of high abundant ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in plants further complicates this problem by limiting protein loading capacity in immobilized pH gradient (IPG) strips. As many low-abundant proteins are involved in numerous regulatory and signaling pathways in cells, yielding information about these proteins is critical for understanding complex cellular systems. Recently, polyethylene glycol (PEG) fractionation is used to deplete Rubisco in plant sample. Here, by using an optimized PEG fractionation method, we provide first results of identification of a number of phosphorylated proteins in *Arabidopsis* leaf sample using 2-DE technique. This optimized approach significantly improves protein resolution, and reduced streaking and background staining in 2-DE. As expected, Rubisco large and small subunits were precipitated predominantly in 10% and 20% PEG fractions, which allowed Rubisco depleted supernatant fraction to be analyzed separately. A total of 192 protein spots from three fractions: 10% PEG, 20% PEG and supernatant were excised from gels and analyzed by LC-MS/MS using a hybrid quadrupole-TOF (Q-TOF) instrument resulting identification of 185 proteins. Interestingly, a total of 71 proteins (38%) out of 185 were phosphorylated proteins with novel phosphorylation sites detected without further enrichment of phosphopeptides by affinity chromatography. Compared with non-fraction, PEG fractionation increased the number of phosphorylated proteins identified, and 20% PEG precipitant contained relatively higher number of phosphorylated proteins than other fractions. Surprisingly, the ion scores of each identified phosphopeptides were significantly very high, which suggests the usefulness of this method for phosphoproteomic analysis in plants. We have confirmed the reproducibility of this study by repeating this experiment. Taken together, our optimized PEG fractionation protocol coupled with 2-DE separation of proteins represents a simple, rapid and reproducible tool for the identification of phosphorylated proteins in green plant tissues.

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### ICAR404

DISTINCT AND OVERLAPPING ACTIVITIES OF ZTL, FKF1, AND LKP2 IN THE PROGRESSION OF THE ARABIDOPSIS CIRCADIAN CLOCK.

Category: Signal Transduction

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Targeted protein degradation mediated by ZEITLUPE (ZTL) and FLAVIN-BINDING KELCH REPEAT F-BOX1 (FKF1) is at the center of the circadian

clock progression as well as photoperiodic control of flowering. Blue-light activation of these F-box proteins and a succession of specific protein interactions have been shown to control the turn-over of key proteins involved in these two pathways. ZTL activity is believed to refine the circadian waveform of TIMING OF CAB1 EXPRESSION (TOC1) and PSEUDO-RESPONSE REGULATOR5 (PRR5) expression, two central components involved in clock progression, while KFK1 interaction with CYCLING DOF FACTOR1 alleviate CONSTANS repression, inducing a switch to reproductive growth in long-day conditions. A related F-box protein, LOV KELCH PROTEIN2 (LKP2) is able to affect the same pathways when over-expressed *in planta* but its real contribution in wild-type plants remains unknown. In order to gain further insights into the relative contribution of ZTL, KFK1, and LKP2 into clock progression, the phenotype of the corresponding single mutants was analyzed and compared with that of the double and triple mutant combinations. Use of the *CAB2::LUC* reporter revealed that, although *ztl* is the only single mutant affecting the period of the oscillator, introduction of *kfk1* in this context is enhancing this phenotype. In the triple mutant, the long period phenotype of *CAB2::LUC* oscillations is even increased compared to that of *ztl kfk1*, suggesting that all three F-box proteins are redundant but unequally involved in the determination of the period of the oscillator. We have performed the molecular phenotyping of the triple mutant with the systematic determination of the expression levels of the core clock genes by Q-PCR. Together with the results, the potential roles of these F-box proteins in the clock progression will be discussed.

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**ICAR405**

STUDIES ON DNA-BINDING SELECTIVITY OF WRKY TRANSCRIPTION FACTORS LEND STRUCTURAL CLUES INTO WRKY-DOMAIN FUNCTION

Category: Signal Transduction

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WRKY transcription factors have been shown to play a major role in regulating, both positively and negatively, the plant defense transcriptome. Nearly all studied WRKY factors appear to have a stereotypic binding preference to one DNA element termed the W box. How specificity for certain promoters is accomplished therefore remains completely unknown. In this study, we tested five distinct Arabidopsis WRKY transcription factor subfamily members for their DNA binding selectivity towards variants of the W box embedded in neighboring DNA sequences. These studies revealed for the first time differences in their binding site preferences, which are partly dependent on additional adjacent DNA sequences outside of the TTGACY-core motif. A consensus WRKY binding site derived from these studies was used for *in silico* analysis to identify potential target genes within the Arabidopsis genome. Furthermore, we show that even subtle amino acid substitutions within the DNA binding region of AtWRKY11 strongly impinge on its binding activity. Additionally, all five factors were found localized exclusively to the plant cell nucleus and to be capable of trans-activating expression of a reporter gene construct *in vivo*.

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**ICAR406**

N-ACYLETHANOLAMINE (NAE) METABOLISM IMPACTS SEEDLING GROWTH AND INTERACTS WITH ABA SIGNALING IN ARABIDOPSIS SEEDLINGS

Category: Signal Transduction

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In mammalian systems the metabolism of the N-acylethanolamine (NAE) group of lipids is part of the endocannabinoid signaling system where NAEs activate G-protein-coupled cannabinoid receptors, which in turn regulate an array of physiological and behavioral processes. Although, the occurrence and metabolism of NAEs is conserved among eukaryotes, the physiological functions of these lipids have been investigated mostly in animals. In mammals, the NAE hydrolyzing enzyme, fatty acid amide hydrolase (FAAH) terminates NAE signaling. Recently, we described an Arabidopsis FAAH, which when over-expressed or down-regulated in Arabidopsis seedlings, confers tolerance or hypersensitivity to the growth inhibitory effects of exogenous NAEs. NAEs, which are most abundant in seeds are depleted during imbibition concomitant with FAAH expression suggesting that the catabolism of NAEs is necessary for cell expansion that accompanies germination and seedling establishment. Transcript profiling of NAE-treated seedlings revealed that a number of genes regulated by NAE where also ABA responsive. Exogenous ABA and NAE together produced a more severe effect on seedling growth than either compound alone, suggesting a synergistic interaction between NAE and ABA. ABA insensitive (abi) mutants were insensitive to NAE and to the synergistic effects of NAE and ABA. AtFAAH over-expressing seedlings, although tolerant to high NAE, were hypersensitive to ABA. Our data indicate that NAE metabolism acts in concert with ABA to negatively regulate seedling development in Arabidopsis. Our results provide evidence that NAE metabolism, which has been implicated mostly in the regulation of animal physiology, impacts a major hormone signaling pathway in plants (Supported by DOE grant DE-FG02-05ER15647).

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**ICAR407**

MAPK PHOSPHATASE 2 IS A NOVEL POSITIVE REGULATOR IN ARABIDOPSIS TRICHOME DEVELOPMENT

Category: Signal Transduction

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Mitogen-activated protein kinase (MAPK) signaling pathways are involved in many important physiological processes in eukaryotic cells. There are 20 MAP kinases (AtMPKs) in *Arabidopsis* which have been shown to participate in diverse processes of plant growth, development and defense. As negative regulators of AtMPKs, MAPK phosphatases (AtMKPs) deactivate AtMPKs by dephosphorylating their activation domain. To date, five putative AtMKPs, including AtMKP1, AtMKP2, DsPTP1, PHS1 and IBR5, have been identified in the *Arabidopsis* genome, but the physiological roles of these AtMKPs still remain largely unknown. Previous studies indicate that AtMKP2 is a novel regulator of ozone stress responses, but the phenotype of loss-of-function AtMKP2 mutants suggests that it may also play a role in plant development. To help position AtMKP2 within developmental signaling systems, we used a range of reverse genetic approaches. By using AtMKP2 promoter:GUS reporter lines, we found that AtMKP2 was expressed in all stages of trichome development. This association was confirmed by a similar temporal and spatial expression pattern of AtMKP2-YFP fusion protein. To directly test the involvement of AtMKP2 in trichome development, we employed transgenic plants that conditionally expressed AtMKP2 interference RNA. Repression of AtMKP2 by RNAi resulted in defects in trichome branching and reduction in trichome density. The association of AtMKP2 with trichome development was further supported by RT-PCR analysis of the expression of several canonical trichome development regulator genes. The expression level of GL1, GL2 and EGL3 were down-regulated in AtMKP2 RNAi plants, whereas TRY and TTG1 were up-regulated. Taken together, our results collectively suggest that AtMKP2 may function as a novel positive regulator in trichome development.

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**ICAR408**

A TRANSCRIPTIONAL NETWORK REGULATES CYTOKININ SIGNALING IN *ARABIDOPSIS*

Category: Signal Transduction

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Cytokinins regulate many different 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 multi-step phosphorelay that culminates in transcriptional regulation by the type-B response regulators (type-B ARR family). We used a mutant-based approach to analyze the *Arabidopsis* transcriptional response to cytokinin. A type-B *ARR* triple mutant (*arr1,10,12*) shows a substantially reduced response to cytokinin based on physiological and molecular analysis. Based on microarray analysis, we identified 71 genes induced 3-fold or more in cytokinin-treated wild-type shoots. But the *arr1,10,12* mutant severely attenuated expression for the vast majority of cytokinin-regulated genes. Many of the genes whose cytokinin-induced expression is affected in the *arr1,10,12* mutant represent additional transcription factors, suggesting that the type-B ARRs act at the head of a transcriptional cascade to regulate the cytokinin response. Two families of the Cytokinin-regulated Transcription Factors (*CTFs*), *CTFA* and *CTFB*, were chosen for further study. Cytokinin-regulated expression and subcellular localization of family members will be presented.

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**ICAR409**

DYNAMIC MOLECULAR RESPONSES BY PHYTOCHROME B

Category: Signal Transduction

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Plants exhibit, as sessile photosynthetic organisms, particularly plastic development and growth response to their ever-changing environmental conditions. This is in part achieved by formulating and integrating the dynamic properties of light signaling components including photoisomerization, nucleo-cytoplasmic partitioning, nuclear body formation, dynamic protein complex, and propagation of gene regulatory networks. We are interested in understanding the role of various dynamic properties in light signal processing. The nucleocytoplasmic distribution and photoisomerization of phyB is a crucial mechanism for light signaling transduction. We discovered that Pr and Pfr photoisomers of phyB have opposite roles in light dependent BR responses and that phyB exerts clearly distinct effects in these responses depending on the cellular localization. To investigate the dynamic combinatorial complexes of phytochrome B under different light conditions, we are characterizing phyB interactomes and found that their composition may be dynamically changed dependent on the photophysiological status of phyB.

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**ICAR4010**

NETWORK BIO-DYNAMICS OF ARABIDOPSIS RESPONSE REGULATORS

Category: Signal Transduction

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Phytohormone cytokinins play essential roles in plant growth and development, such as cell division, cell proliferation, root and leaf differentiation and leaf senescence. In *Arabidopsis*, cytokinin signal is transduced via two-component phosphorelay comprised of sensor histidine kinases, histidine phosphotransfer proteins, and response regulators similar to bacterial two component signaling systems. The three cytokinin receptors, AHK2, AHK3, and AHK4/CRE1/WOL, perceive and transmit the signal to the response regulators via a His-to-Asp phosphorelay cascade. The *Arabidopsis* response regulators (ARRs) have been grouped into type-A and type-B ARRs. Type-A ARRs are primary transcriptional targets of cytokinin and type-B ARRs are transcription factors not induced by cytokinins.

It is expected that the combinations of response regulators show functional diversity in various cytokinin-mediated biological events. So, we have been trying to understand cytokinin signaling with systems biological view and to construct dynamic regulatory networks among the response regulators. Here, to evaluate the specificity of induction of type-A ARRs by type-B ARRs, we have examined the expression of type-A ARR genes in *ARR1*, *ARR2*, and *ARR10* overexpression lines and in *ARR7* inducible line. We found that these type-B ARR proteins differentially transactivate type-A ARR genes. Next, we also have been generating the other transgenic lines constitutively and transiently overexpressing response regulators. Based on the diverse cytokinin related phenotype, we will construct organ specific ARR-transcriptional network. Finally, using these systematic approaches, we will get better understanding in cytokinin signaling in *Arabidopsis*.

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**ICAR4011**

PARTICIPATION OF SNRK1 IN PHOSPHATE STARVATION SIGNALING IN ARABIDOPSIS THALIANA

Category: Signal Transduction

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Phosphorus (P) is an essential element required by plant growth and development. P concentration in soil solution is high but the assimilable form, inorganic phosphate (Pi), is always in limiting concentration; thus, plants are frequently growing in Pi limiting conditions. Several biochemical and molecular changes have been described in plants in response to Pi deficiency; however, the signaling pathways that conduct to those changes are not clearly known. Using different approaches, we identified genes that are regulated by Pi deficiency and some of them encode proteins involved in signal transduction. *Arabidopsis thaliana* SnRK1 protein kinase is a heterotrimeric protein formed by one catalytic subunit (alpha) and two regulatory subunits (beta and gamma). Characterization of SnRK1 during Pi deficiency, showed that the kinase activity was reduced, while gene expression remains constant during the same period of time. In *Arabidopsis* each subunit is encoded by multiple genes giving rise to a large variety of possible heterotrimeric combinations. To explain the reduction in activity during Pi deficiency, we developed *Arabidopsis* transgenic plants carrying the two

catalytic subunits (AKIN 10 and AKIN 11) as GFP fusion proteins. Experiments with the transgenic plants indicated that one of the catalytic subunit was activated by Pi deficiency, whereas the other was specifically degraded under the same conditions. In this work, we identified some of the subunits that are part of the active heterotrimeric complex during phosphate starvation and analyzed its possible role.

Research Grants PAPIIT IN202206, PAIP 6290-13, CONACyT 52072

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#### **ICAR4012**

CHEMICAL GENETIC IDENTIFICATION OF A NEW FAMILY OF ABA RESPONSE FACTORS

Category: Signal Transduction

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A new and complementary approach to genetic pathway dissection is to systematically identify small molecule "perturbagens" of the pathway in phenotype-based screens. These molecules can be then used as reagents to identify protein components of the pathway (i.e. the forward chemical genetic approach). The Cutler lab has an ongoing chemical genetic research program focused on identifying and characterizing new small molecule modulators of plant cell growth. This program has uncovered a new class of naphthalene sulfonamide growth regulators, called pyrabactins (for pyridyl containing ABA activators), which act as agonists (i.e. activators) of the seed ABA signal transduction pathway. A genetic screen for pyrabactin resistant mutants identified recessive alleles in a locus called Pyr1 (pyrabactin resistance 1) that encodes a Bet V protein family member. Structurally, this family is characterized by the presence of a hydrophobic ligand-binding pocket, otherwise their *in vivo* functions are poorly defined. An emerging theme in plant signal transduction is that plant hormones can induce downstream effects by modulating protein-protein interactions between receptors and effectors. In this context, the predicted ligand binding properties of Bet V proteins coupled to the requirement of PYR1 for pyrabactin activity, suggested that the binding of pyrabactin to PYR1 might trigger an ABA response by promoting a protein-protein interaction. Indeed, a yeast two-hybrid (Y2H) screen using PYR1 as bait identified a PP2C as an interactor. The PP2C-PYR1 interaction depends on the inclusion of pyrabactin in the yeast growth media, and thus behaves as a ligand induced protein-protein interaction. This interaction has been reconstituted *in vitro*, and is not triggered by an inactive analog of pyrabactin. Moreover, the interaction between PYR1 and the PP2C is triggered by ABA in both the Y2H and *in vitro* assays. Thus, we propose that PYR1 is a new ABA receptor. The Arabidopsis genome encodes ~65 Bet V family members, and 4 of the 12 closest relatives of PYR1 (named PYL 1 - 4) also possess ABA receptor activity in the Y2H assay. Thus, our combined chemical and genetic approach has identified a new family of putative ABA receptors, which we call the PYR/PYL family. Genetic and biochemical characterization of the PYR/PYL family is ongoing.

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#### **ICAR4013**

THE DELLA REPRESSORS INHIBIT CELL ELONGATION THROUGH PROTEIN INTERACTION WITH PIF4 (PHYTOCHROME INTERACTING TRANSCRIPTION FACTOR 4)

Category: Signal Transduction

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Seedlings undergo alternative developmental programmes depending on whether they germinate in the dark (known as de-etiolated growth) or in the light (known as photomorphogenesis). Dark grown seedlings exhibit etiolated growth, characterized by long hypocotyls, small and closed cotyledons with undifferentiated chloroplasts and repression of light-regulated genes. By contrast, during photomorphogenesis, light inhibits hypocotyl growth and promotes cotyledon opening and expansion, chloroplast differentiation and activation of light-regulated genes. These processes are also regulated by GAs through a crosstalk mechanism that has long been elusive.

To reveal the molecular mechanism underlying the coordinated regulation of plant development by light and GAs, we analyzed *in vivo* function of DELLA proteins, which are known repressors of GA-signaling. These proteins accumulate in the nucleus and are degraded in response to GA. In Arabidopsis, among the 5 DELLA, RGA (Repressor of ga1-3) and GAI (GA Insensistive) are the main repressors controlling hypocotyls growth and stem elongation. Mutations within DELLA domain render these proteins resistant to degradation, and result in a GA-insensitive dwarf phenotype. This domain binds the GA receptor GID1 in a GA-dependent manner, which promotes interaction with the F-box protein SLEEPY1 (SLY1), and polyubiquitination of the DELLA by the SCF<sup>SLY1/GID2</sup> ligase complex, thereby signaling their degradation by the 26S proteasome pathway.

The functional mechanism by which DELLA regulate gene expression and promote photomorphogenesis remains unclear. Attempts to demonstrate direct DNA-binding ability of DELLA have been unsuccessful, indicating that these repressors might exert their function interacting with other transcription factors.

We identified both *in vitro* and *in vivo* PIF4 (PHYTOCHROME INTERACTING FACTOR4) as DELLA-interacting factor. PIF4 is a bHLH transcription factor negatively regulated by the light photoreceptor phyB. Using transient expression system, we showed a positive regulatory function of PIF4 in cell elongation, that DELLA proteins repress PIF4-mediated gene expression, and that treatment with GA abrogates the DELLA-mediated inhibitory effect. ChIP analysis further supported the positive regulatory of PIF4, and showed that interaction of PIF4 with his targets was strongly reduced in seedlings accumulating the DELLA repressors (treated by Paclitaxel) whereas it was enhanced in seedlings treated with GAs to destabilize the DELLA. Over expression of PIF4 rescue the growth restraint induced by DELLA accumulation.

So, in the light, phyB negatively regulates PIF4 transcriptional activity by targeting degradation of this factor by the 26S proteasome pathway. In the absence of GA, DELLA proteins interact with PIF4 proteins and prevent them from binding to their target genes and regulating their expression. By contrast, in the presence of GA, DELLA proteins are degraded and PIF4-mediated cell elongation can occur. PIFs function as integration node for light and GA-signaling pathways, thereby providing a regulatory mechanism by which plants adapt their growth to changing environmental conditions.

\*de Lucas et al. *Nature* (2008) 451:480-84.

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#### **ICAR4014**

THE ARABIDOPSIS SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE (SERK) PROTEINS FUNCTION IN BRASSINOSTEROID DEPENDENT AND INDEPENDENT SIGNALING

Category: Signal Transduction

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Structurally related Receptor Like Kinase (RLKs) often function in similar signaling pathways. To determine whether this holds true for all 5 members of the Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) family we generated double, triple and quadruple mutants. One member of this family, SERK3, is also known as BAK1, the co-receptor of the brassinolide (BR) perceiving receptor BRI1. We show that only serk1 but not serk2, serk4 or serk5 mutant alleles enhance the BR insensitivity of serk3-1 mutant roots and hypocotyls. SERK1, together with SERK2 is also essential for male sporogenesis and tapetum formation, a function that is not controlled by BRI1 signaling. Likewise, SERK3 alone controls innate immunity and together with SERK4 can also mediate cell death control in a BR-independent manner. This shows that individual SERK proteins serve roles in different and independent signaling pathways, possibly through heterodimerization with different ligand-perceiving receptors and/or recruitment of different target proteins. We will discuss how in plant cells the same receptor protein can serve in different signaling pathways and activate specific downstream targets.

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#### **ICAR4015**

ARABIDOPSIS GLUTAMATE-LIKE RECEPTORS AND THEIR ROLE IN CELLULOSE BIOSYNTHESIS AND CARBON/NITROGEN BALANCE

Category: Signal Transduction

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On completion of the *Arabidopsis thaliana* sequencing project, twenty putative plant Glutamate-like receptor genes were identified and divided into three clades. The plant *GLR* genes are believed to be glutamate-gated channels involved in the movement of Na<sup>+</sup>, Ca<sup>2+</sup> and K across the plasma membrane. They have been implicated in regulation of carbon/nitrogen balance, cell elongation, sensing of mineral nutrient status and light-signal transduction. In order to identify genes involved in cell wall stress responses, a time course microarray experiment was carried out using isoxaben treated seedlings (a herbicide causing cellulose biosynthesis inhibition). This treatment causes changes in metabolic flux, lignin deposition, necrosis, transcriptional shutdown of photosynthetic and activation of biotic and abiotic stress response genes. Expression of *AtGLR2.5* in seedlings was shown to increase in a time dependant manner to treatment with isoxaben. In addition, expression of *AtGLR2.5* is also induced in mutants deficient in cellulose (*radially swollen 1, korrikan, detiolated 3*), suggesting *AtGLR2.5* transcriptional activation is inherently linked to cellulose biosynthesis. Staining of *atglr2.5* seedlings with phloroglucinol revealed a reduction of lignification within the cotyledons and altered germination rates when exposed to a range of nitrogen/carbon ratios within growth media. Etiolated *glr2.5* seedlings exhibit increased hypocotyl length. Preliminary studies suggest that *AtGLR2.5* is localised in the stomata and vasculature of cotyledons. Microarray based expression profiling experiments have identified fifteen candidate genes as possible down stream targets of *GLR2.5*. Ongoing research looks to establish the biological function of *AtGLR2.5* and its downstream targets in cellulose biosynthesis and carbon/nitrogen balance. Ultimately, our aim is to understand cell wall dynamics of Arabidopsis with the long term view of optimising the production of sustainable, efficient bio-energy from crop species.

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#### **ICAR4016**

**CHARACTERIZATION OF THE ARABIDOPSIS MUTANT *PIC30*, THAT IS SPECIFICALLY RESISTANT TO AUXINIC HERBICIDE PICLORAM.**

Category: Signal Transduction

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Auxin is a major plant hormone that regulates many aspects of plant growth and development. At molecular level, auxin controls the expression of many genes through the degradation of Aux/IAA proteins, a group of transcriptional repressors. While Indole acetic acid (IAA) is the major natural auxin in plants, there are many synthetic chemicals with auxinic activities that are being used in agriculture. While these synthetic auxins are structurally diverse, whether all of them function in a similar manner at molecular level is currently unknown. We recently used a biochemical approach to test some of these synthetic auxins and found that picloram, an auxinic herbicide, might function differently from other auxinic compounds such as IAA and 2,4-D. Interestingly, primary root growth of the auxin receptor mutant *tir7-1* was sensitive to picloram suggesting that TIR1 or its related proteins AFB1, AFB2 or AFB3 might not function as receptors for picloram. To identify new components in auxin signaling pathways, we screened EMS mutagenized Arabidopsis (Col-0) seedlings against picloram. We isolated 30 mutants that displayed altered responses to picloram. Of which, 16 were specific to picloram and the rest were resistant to both picloram and 2,4-D suggesting the existence of unique and common signaling components for different auxinic compounds. One of these mutants, *pic30* is a semi-dominant mutation and is specifically resistant to picloram. We recently mapped the mutant gene to chromosome 2 south. As evident from molecular genetic experiments, picloram induced gene expression as well as picloram induced Aux/IAA degradation are impaired in *pic30* background suggesting that *PIC30* may be a new gene involved in auxin response of plants.

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#### **ICAR4017**

ARABIDOPSIS ADP1 GENE IS REQUIRED FOR RESISTANCE AGAINST BACTERIAL PATHOGEN PSEUDOMONAS SYRINGAE

Category: Signal Transduction

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In order to understand the complex signaling mechanisms regulating defense against pathogens in plants, it is necessary to identify and characterize genes whose products are involved in initiation and regulation of the plant defense responses. Here we describe identification and

characterization of *ARABIDOPSIS DEFENSE PROTEIN 1 (ADP1)* gene. *ADP1* is induced faster and stronger in response to avirulent pathogen *Pst* DC3000 (*AvrRpm1*) compared to the virulent pathogen (*Pst* DC3000). *adp1* null mutants are compromised in activation of *PR1* gene in response to *Pst* DC3000 (*AvrRpm1*). Additionally, constitutive overexpression of *ADP1* leads to induction of *PR1*. These results suggest that *ADP1* positively regulates expression of *PR1* and possibly other defense-related genes. Furthermore, resistance against virulent bacterial pathogen *Pst* DC3000 is compromised in *adp1* mutants. *ADP1* expression in response to *Pst* DC3000 is compromised in *Arabidopsis* mutants defective in accumulation of and signaling mediated by salicylic acid. *ADP1* is a small protein and has no homology to any known protein in public databases. Taken together, our results suggest that *ADP1* represents a novel class of *Arabidopsis* proteins involved in pathogen defense signaling via SA-mediated defense pathway.

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**ICAR4018**

## REDUCTION IN ISOPENTENYLTRANSFERASE (IPT) RESULTS IN EARLY MERISTEM CESSION

Category: Signal Transduction

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Cytokinin influences many aspects of plant growth and development including seed germination, vasculature development, cell proliferation, apical dominance and leaf senescence. The current model for cytokinin signaling is a multi-step phosphorelay, similar to the prokaryotic two-component systems, consisting of membrane-bound hybrid sensor kinase receptors (AHKs), histidine phosphotransfer proteins (AHPs) and response regulators (ARRs). Extensive molecular and genetic analyses of loss-of-function mutants indicate that these two-component elements have redundant and overlapping functions in cytokinin signaling. The requirement for cytokinin in plant cell cultures and the inability of the triple *a/hk* mutant to respond appropriately to cytokinin in various assays raises the question whether cytokinin is required *in vivo* for plant development. To address this, we investigated the role of cytokinin biosynthesis in plant development by generating and analyzing loss-of-function and DEX-induced RNAi *ipt* plants. IPTs (isopentenyltransferases) catalyze the first and rate-limiting step of cytokinin biosynthesis- the transfer of an isopentenyl moiety from dimethylallyldiphosphate to the N<sup>6</sup> position of ATP and to ADP. Lowering endogenous levels of cytokinin via DEX application caused pleiotropic developmental changes, including delayed leaf initiation and expansion, reduced root elongation and early root and shoot meristem cessation. The introduction of a bacterial IPT or the application of exogenous cytokinin can partially rescue these plants suggesting that cytokinin is required for meristem maintenance.

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**ICAR4019**

## TOWARDS DOWNSTREAM COMPONENTS OF RECEPTOR TRAFFICKING

Category: Signal Transduction

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Cell surface receptor kinases serve as sensors for internal and external stimuli that trigger signaling cascades and are evidently important for the regulation of plant growth and development. Additionally, several receptor kinases are key components discriminating self from non-self, which is essential for pollination, symbiosis and immunity. Active defenses are initiated upon receptor-mediated perception of so-called pathogen-associated molecular patterns (PAMPs). One of the best-studied PAMPs is bacterial flagellin (flg22), which is recognized by the receptor kinase Flagellin Sensing 2 (FLS2). Upon flg22 sensing, FLS2 elicits an array of defense responses including MAP kinase signaling, which enhance plant immunity to prevent bacterial infection and proliferation. Concomitantly, activated FLS2 translocates to an endosomal pool reminiscent of receptor-mediated endocytosis (RME). Although PAMP signaling and RME became a focus of research in the past years, there is largely nothing known about downstream molecules of receptor signaling and regulatory components of receptor endocytosis.

To identify such components we employed yeast-two-hybrid and yeast-split-ubiquitin screens using FLS2 as bait. Yeast strains expressing either the cytoplasmic part or full-length FLS2 were found to be stable and suitable for interaction assays. We inspected cDNA libraries produced from control and flg22 treated plants for potential FLS2 Interacting Proteins (FIPs). At present, out of 2.10<sup>6</sup> screened, we could confirm 3 FIPs in yeast. A role for the most interesting candidates in the flg22/FLS2 pathway is explored by phenotypic characterization of respective mutant lines, and their subcellular localization. We will present data obtained with FIP1 and FIP2. Confocal microscopy revealed that FIP1 localizes to the plasma membrane and is internalized into endosomes upon flg22 treatment. Currently, we are addressing the relevance of FIP1 interaction for FLS2 signaling and endocytosis.

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**ICAR4020**

## LOCALIZATION VS. ACTIVITY: TWO MODELS FOR HOW THE PROTEIN PHOSPHATASE TYPE 2C PROTEINS, POL AND PLL1 REGULATE ASYMMETRIC CELL DIVISIONS.

Category: Signal Transduction

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The stem cell populations in the meristems must undergo precise regulation for proper plant development to occur. The CLAVATA (CLV) pathway plays an important role in controlling shoot meristem size by limiting the cell expressing the homeodomain transcription factor, WUSCHEL (WUS). WUS promotes stem cell specification while the CLV pathway negatively regulates *WUS* expression. Two CLV pathway components, the protein phosphatase type 2C proteins POLTERGEIST (POL) and PLL1, are functionally overlapping positive regulators of *WUS* expression. POL and PLL1 are negatively regulated by the other known CLV pathway components. Recent genetic studies have revealed that POL and PLL1 are also required for the proper specification of key asymmetric cell divisions during early embryogenesis. WUS and the other CLV pathway components are not required for these

processes revealing that POL and PLL1 are shared signaling components for multiple pathways. Consistent with this, *WUS* expression is also dependent on asymmetric cell divisions. Specifically, the apical daughters of L3 stem cells remain stem cells while the basal daughters differentiate and express *WUS*. One possibility then is that POL and PLL1 are required to set up asymmetric cell divisions in all of these pathways.

Here we present two potential models for how POL and PLL1 regulate asymmetric cell divisions: the localization model and activity model. In the localization model, POL and PLL1 are asymmetrically localized in the mother cell and only one of the daughter cells receives the proteins. In the activity model, POL and PLL1 are inactivated on one side of the parental cell which results in only one daughter cell receiving the active forms of the proteins. We are pursuing a number of experimental approaches to test the validity of these models. Our localization studies in yeast and *Arabidopsis* which have revealed that POL and PLL1 undergo modifications which direct the proteins to the plasma membrane. These localization studies are supported by western analysis of membrane fractions. *In vitro* phosphatase assays using POL-FLAG and PLL1-FLAG have revealed that the full-length proteins have phosphatase activity contrary to prior studies. Using these and other findings, we will discuss the different models for how POL and PLL1 regulate cell asymmetry *in planta*.

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**ICAR4021**

INS(1,4,5)P<sub>3</sub> AND NUTRIENT SIGNALING

Category: Signal Transduction

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Various inositol phosphate molecules function in signaling pathways as second messengers. One such second messenger, Ins(1,4,5)P<sub>3</sub>, signals to increase cellular calcium concentration which leads to many downstream physiological responses. In plants, the regulation of Ins(1,4,5)P<sub>3</sub> levels has been shown to be crucial for responses to ABA, salt, cold and gravity. Myo-inositol polyphosphate 5-phosphatases (EC 3.1.3.56; 5PTases) regulate the level of Ins(1,4,5)P<sub>3</sub>, and other inositol-containing molecules, by hydrolyzing the 5-phosphate from the inositol ring. In *Arabidopsis thaliana*, there are 15 genes that encode enzymes called At5PTases. All eukaryotes contain 5PTase enzymes, however plants and filamentous fungi are unique in that some 5PTase genes contain WD40 repeat sequences at their N-termini. To determine the function of plant 5PTase in signaling, we have examined a WD40 repeat-containing 5PTase (5PTase13). WD40 repeats are often involved in protein:protein interactions, so we used the WD40 region of 5PTase13 as bait in a yeast two hybrid assay. We identified the *Arabidopsis* SNF1-like kinase (SnRK1.1) as an interacting protein, suggesting that 5PTase13 and Ins(1,4,5)P<sub>3</sub> may be involved in SnRK regulation and nutrient signaling. Pull-down experiments with recombinant proteins confirmed the 5PTase13: SnRK 1.1 interaction, and the results of other assays suggest that 5PTase13 may be required to stabilize SnRK1.1 and protect it from degradation. To determine whether 5PTase13 is required for nutrient or stress signaling, homozygous T-DNA mutants were identified. *5ptase13* mutants are ABA- and glucose-insensitive, and the endogenous Ins(1,4,5)P<sub>3</sub> level is reduced in *5ptase13* mutants upon exposure to a 6% glucose stress. Ectopic expression of 5PTase13 mimics some of the known effects of SnRK overexpression, in that transgenic plants appear to grow faster under low nutrient conditions. Together these data suggest that 5PTase13 regulates the SnRK1.1 nutrient sensor, and that Ins(1,4,5)P<sub>3</sub> signaling may be an important component of this process.

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**ICAR4022**

SUBCELLULAR LOCALISATION AND OLIGOMERISATION OF THE *ARABIDOPSIS THALIANA* ETHYLENE RECEPTORS

Category: Signal Transduction

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The gaseous phytohormone ethylene regulates many developmental processes and responses to environmental conditions in higher plants. Ethylene perception and initiation of signalling in *Arabidopsis thaliana* are mediated by a family of five receptors which are related to prokaryotic two-component sensor histidine kinases.

Transient expression of fluorescence-tagged receptors in tobacco (*Nicotiana benthamiana*) epidermal leaf cells demonstrated that all ethylene receptors are targeted to the ER endomembrane network. To investigate the oligomerisation potential of the receptors *in planta* we developed a new method for interaction analysis - the Membrane Recruitment Assay (MeRA). With the implicit understanding that a full-length RFP-tagged receptor is ER-localized, we coexpressed it with a truncated GFP-labelled receptor that is unable to integrate in the ER-membrane. Interaction of the cytosolic parts of the ethylene receptors lead to overlapping RFP and GFP signals that signify a recruitment of the truncated protein by the membrane-bound receptor. Our comprehensive MeRA interaction analysis presents the first cell biological evidence that the ethylene receptors are capable of forming homomeric and heteromeric protein complexes at the ER. Furthermore the results point to a major function of the cytosolic GAF-domain in mediating this dimerisation. Supporting evidence for the bivalent binding capacities of the receptors was obtained by means of the mating-based Split-Ubiquitin System (mbSUS) in yeast. Transcript analysis of the ethylene receptors and examination of ETR1 and ERS1 promoter::GUS lines yielded overlapping but distinct expression patterns at different developmental stages and tissues of *Arabidopsis*.

With a variety of oligomerisation partners for the receptors, and their differential expression in space and time, a new dimension in signalling already opens on the receptor level. Considering different and non-redundant characteristics of all five receptors towards the two-component signalling network, our findings may contribute to understanding how ethylene sensing efficiency, induction of signalling, signal attenuation, fine-tuning and cross-talk with other signalling pathways is controlled.

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**ICAR4023**

## QUANTITATIVE ANALYSIS OF THE PUTATIVE ETR1-AHP1 PHOSPHORELAY MODULE INVOLVED IN ETHYLENE SIGNALLING BY FLUORESCENCE POLARISATION STUDIES

Category: Signal Transduction

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Previous studies by yeast two-hybrid analyses suggest that signal transfer from the membrane-localized sensor kinase ETR1 to the nucleus is mediated by a family of histidine-containing phosphotransfer proteins [1-3]. Direct interaction of full-length ETR1 with the histidine-containing transfer protein AHP1 was now confirmed by fluorescence polarisation studies [4]. In contrast to yeast two-hybrid analyses which provide no quantitative information on stability or affinity of the putative ETR1-AHP1 signalling complex the sensitive fluorescence technique applied in our studies offers quantification of these parameter. Moreover, it can provide information on the kinetics of the protein-protein interaction when analysis is extended from steady-state to time-resolved fluorescence polarisation measurements.

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### ICAR4024

#### AUTOKINASE ACTIVITY OF ETR1 IS CONTROLLED BY ETHYLENE AGONISTS AND ANTAGONISTS

Category: Signal Transduction

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The plant hormone ethylene controls many processes of plant growth and development. Reverse genetics have identified a family of membrane intrinsic proteins (ETR1, ERS1, ETR2, ERS2, EIN4) similar to bacterial two-component histidine kinases as receptors of the gaseous hormone [1-3]. The hydrophobic amino-terminal sensor domain of the ethylene receptor family shows high affinity ethylene binding when expressed on its own in *S. cerevisiae* [4]. Binding of ethylene is thought to be accomplished by a hydrophobic pocket in the transmembrane amino-terminal part of the receptor and critically depends on copper as a cofactor. The membrane-extrinsic carboxyl-terminal part of the receptor which is likely to play an important role in signal transduction, on the other hand, showed intrinsic kinase activity when expressed and purified on its own [5]. In a recent study, we have analysed autoposphorylation of purified full-length ETR1 and probed whether autoposphorylation occurs in response to perception of the ethylene signal [6]. Our experiments clearly demonstrate that autokinase activity of the purified receptor is controlled by ethylene or by ethylene agonists like the n-acceptor compound cyanide. In fact, both molecules were able to completely turn off the intrinsic kinase activity. Furthermore, the observed inhibition of autoposphorylation in ETR1 by ethylene or cyanide could be prevented when the ethylene antagonist 1-methyl-cyclopropene (MCP) was applied. These results demonstrate for the first time a strict correlation of signal perception and autokinase activity in the ethylene receptor and confirm functional interconnection of sensor and transmitting kinase domain in a plant hybrid-type histidine kinase.

### References

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### ICAR4025

#### AN INVESTIGATION OF THE EXOCYST COMPLEX, AND ITS ROLE IN COMPATIBLE POLLEN-STIGMA INTERACTIONS IN ARABIDOPSIS

Category: Signal Transduction

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Vesicle trafficking in plants is essential in numerous processes such as cell expansion, pollen tube elongation and immune response to pathogens. Exocytosis is a highly ordered vesicle trafficking process where vesicles are delivered to the plasma membrane and secreted to the cells exterior. The exocyst is a large eight subunit multimeric complex that is primarily involved in polarized or regulated exocytosis in eukaryotic cells where it functions to tether exocytic vesicles to the plasma membrane. Originally identified in *Saccharomyces cerevisiae*, the exocyst is composed of eight subunits: Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p, and putative homologs have been identified in both animal and plant genomes. In *Arabidopsis*, the majority of the exocyst subunit genes exist as single copies or duplicates, but interestingly, there has been an expansion of the *A1Exo70* gene to produce a 23-member gene family. One of the Exo70 members has been recently implicated in pollen-pistil interactions in *Brassica* and *Arabidopsis*. My current research goals are to study the role and localization of the exocyst during pollen-pistil interactions in *Arabidopsis*.

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**ICAR4026**

SUCROSE CONTROLLED BZIP TRANSCRIPTION FACTORS CONTROL PRIMARY METABOLISM

Category: Signal Transduction

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Sugars serve as a source for energy and carbon but are also potent signaling molecules that regulate physiology and development. One third of all expressed genes are influenced by the daily changes in sugar concentration in the plant. Several transcription factors responsible for sugar regulated transcriptional control have been identified, including the *Arabidopsis thaliana* bZip transcription factors that are translationally repressed by sucrose. bZip transcription factors bind DNA as dimers that are formed from a range monomers with different affinities for each other. This allows for the presence of a multitude of different dimers with an equally broad spectrum of putative targets.

The data presented here suggest that bZips are potent integrators of metabolism. Altered bZIP protein activities combined with direct measurements of target gene expression show that different bZip dimers differentially regulate messenger levels of key regulatory genes in metabolism. The bZip controlled gene expression patterns are confirmed and further characterized by *in vivo* induction of promoter-reporter constructs by transient expression of bZip transcription factors. What's more is that the metabolic status of plants is directly reflected by these changes in gene expression, as shown by unbiased metabolomic analysis. A broad range of metabolites including sugars and amino acids are shown to be affected by bZip transcription factors. Taken together the data presented show bZIP transcription factors to be central components in the control of the metabolic status of plants in response to sucrose signaling.

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**ICAR4027**

GA SIGNALING: CHARACTERIZING THE DYNAMIC EFFECTS OF PROTEIN-PROTEIN INTERACTIONS IN ARABIDOPSIS THALIANA SEEDS

Category: Signal Transduction

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This project investigates control of seed germination by gibberellin (GA) signaling gene, DELLA RGL2 (RGA-LIKE2). The plant hormone GA is required to stimulate stem elongation, flowering, and seed germination. All of these GA responses are negatively regulated by DELLA proteins. Of the five DELLA proteins, DELLA RGL2 is the main negative regulator of seed germination. Mutations in RGL2 rescue the failure of seed germination in the GA biosynthesis mutant *ga1-3* and the increased dormancy phenotype of the GA-insensitive F-box mutant *sly1*. One mechanism for the relief of DELLA repression of seed germination is DELLA protein destruction. DELLA destruction occurs in response to GA binding by the GA receptor GID1 via SCF<sup>SLY1</sup> and the ubiquitin-proteasome pathway. However, a recent study examining the germination defect of *sly1* mutants found that after-ripening can allow germination of *sly1* seeds and induction of GA-dependent transcripts in the absence of DELLA RGL2 and RGA destruction (Ariizumi and Steber, 2007). The goal of this research is to characterize the specific posttranslational modifications and protein-protein interactions involved in relieving DELLA RGL2 repression of seed germination. Preliminary methods used for these studies include: *in vitro* phosphatase assays and yeast 2-hybrid screens. RGL2-interacting proteins identified using the yeast 2-hybrid approach will be further characterized by comparing proteins that interact with RGL2 in dormant and after-ripened seeds. Currently, the majority of the research is taking place in the model system *Arabidopsis thaliana* (ecotype Landsberg erecta). However, long term goals also include taking the first steps to identify homologues of RGL2 in wheat (*Triticum aestivum* L.), and to investigate their role in control of preharvest sprouting.

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**ICAR4028**

PHOSPHOPROTEINS OF ARABIDOPSIS MITOCHONDRIA

Category: Signal Transduction

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The mitochondrion is one of the principle components involved in energy production within the plant cell. The strict control and regulation of energy production during plant growth and development and in response to external stimuli such as environmental stresses are vital for the survival of the organism. A number of studies have provided evidence to suggest that the regulation of mitochondrial processes, especially ATP production, is likely to occur through protein phosphorylation. While recent studies of both yeast and mouse mitochondria using phosphoproteomic techniques has identified over 100 phosphoproteins. Extensive studies on the proteome of Arabidopsis mitochondria have revealed few phosphoproteins. Consequently we have attempted to integrate multiple phosphoproteomic approaches to identify plant mitochondrial phosphoprotein. Utilizing techniques such as 2D-PAGE, phosphoprotein enrichment methods, phosphopeptide enrichment methods and the fluorescent phospho-stain Pro-Q Diamond we have created a set of approximately 20 mitochondrial phosphoproteins. A total of 5 of these proteins were validated through the identification of the phosphorylated residue using mass spectrometry. The results suggest that other than the well-documented phosphoprotein E1-alpha of the pyruvate dehydrogenase complex that controls the entry of carbon into the TCA cycle, the most prominent phosphoproteins of plant mitochondria are not directly involved in ATP production. Instead these phosphoproteins are derived from diverse functional groups such as a protease, a heat shock protein, metabolic pathways and a number of unknown proteins.

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**ICAR4029**

CROSSTALK BETWEEN NUCLEUS AND ORGANELLES BY DUALLY TARGETED TRANSCRIPTION FACTORS?

Category: Signal Transduction

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Research in model plants like *Arabidopsis* has shown that plant cell organelles like plastids and mitochondria are integrated into many stress response reactions and that the communication and interaction between these DNA-containing compartments and the nucleus play an important role in plant cells. Organellar and nuclear gene expression are tightly co-regulated through nuclear encoded but organelle-located proteins, on one hand, and retrograde signals from the organelles in the form of redox changes or intermediates of the chlorophyll biosynthesis pathway, on the other hand. However, the link between these retrograde signals and the changes in nuclear and organellar transcriptional activity, which depends on transcription factors and RNA polymerases, is not yet well understood.

Recently, a group of nuclear transcription factors, the Whirly proteins, was shown to be targeted also to chloroplasts or mitochondria. It seems feasible that such a factor might be involved in interorganellar communication. In order to find out whether or not the localisation of the Whirly proteins is unique among plant transcription factors, an *in silico*-based screening of all transcription factors from *Arabidopsis* for putative N-terminal chloroplast and mitochondrial targeting sequences was carried out. In order to reduce the amount of false positives, the individual predictions of several independent programs were combined to a consensus prediction using a naive Bayes method. This consensus prediction showed a higher specificity at a given sensitivity value than each of the single programs. With this prediction, a total of 90 transcription factors from a variety of protein families were identified that possess sequences that could putatively target them to either plastids or mitochondria as well as to the nucleus. The present study provides experimental data to test the predicted localization patterns. All candidates will be screened for their subcellular localization per se and the ability to be imported into different organelles. Candidates with a confirmed dual localization pattern will be tested for putative functions as integrators between nuclear- and organellar- genome for example in response to certain stresses.

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#### **ICAR4030**

ANALYSIS OF THE FUNCTION OF B-TYPE ARR IN THE CYTOKININ SIGNAL TRANSDUCTION USING A DOMINANT REPRESSOR

Category: Signal Transduction

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The cytokinin signal transduction is mediated via a multi-step phosphorelay system. In the current model the cytokinin signal is perceived by plasma membrane-bound sensor histidine kinases and transported via phosphotransmitter proteins to the nucleus. Here they activate the B-type response regulators, which act as transcription factors. The *Arabidopsis* genome encodes eleven B-type response regulators and genetic analyses have indicated functional redundancy. To study the extent of the contribution of B-type ARRs to cytokinin activities, we generated a dominant repressor version of the *Arabidopsis* response regulator ARR1 (ARR1-SRDX) using chimeric repressor silencing technology. In protoplast transactivation assays, the dominant repressor not only completely abolished the transactivation activity of ARR1, but also of all other B-type ARRs tested, including those of more distantly related subfamilies. Subsequently, we studied the consequences of the dominant repression of B-type ARR activity on short-term and long-term responses to cytokinin. Northern Blot analysis revealed a clear reduction in the induction of the primary cytokinin response genes *ARR5* and *ARR7*. A microarray analysis showed that 76% of those genes induced after cytokinin treatment in the wild type, were not induced in the 35<sup>o</sup> ARR1-SRDX plants. Thus the transcriptional response to cytokinin treatment was clearly dampened. Plants expressing the dominant repressor showed phenotypic changes reminiscent of those plants with a reduced cytokinin status, such as the triple cytokinin receptor mutants, or cytokinin oxidase overexpressors. In summary, these data indicate that most if not all of the cytokinin responses in *Arabidopsis* are mediated at least partially by B-type ARRs.

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#### **ICAR4031**

THE AGRON-OMICS CONSORTIUM

Category: Signal Transduction

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Post-genomic research using model organisms is characterized by renewed efforts to understand biological complexity through systems biology. The AGRON-OMICS Consortium (*Arabidopsis* GROWth Network integrating -OMICs technologies) focuses on the comprehensive study of the molecular mechanisms involved in plant growth and development. This partnership between 14 European laboratories aims at (i) surveying systematically the molecular components driving growth; (ii) explaining and classifying leaf growth phenotypes at the molecular level; and (iii) building coordinated molecular networks between the functional modules involved. Our core experimental plan relies on combined profiling techniques including the characterization of genome, transcriptome, proteome and metabolome, in conjunction with automated phenotyping of a common set of leaf samples (different genotypes grown under several controlled environmental conditions). These samples are obtained at one location and distributed to the profiling platforms for processing and analysis. In parallel, the consortium is contributing to the development of novel high-throughput methods for the functional analysis of plant genes and proteins, including cell-based assays. The large amount of data generated in the project requires dedicated repositories to access and analyze data series. In this context, the consortium is participating to the improvement of existing standards and ontologies and aims to develop robust methods for data integration. In its final phase, the consortium will also construct analytical, mathematical and visualization tools for processing functional genomics data and for building models of leaf developmental scenarios.

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#### **ICAR4032**

THE *ARABIDOPSIS THALIANA* RESPONSE REGULATOR ARR22 IS A PUTATIVE AHP PHOSPHO-HISTIDINE PHOSPHATASE EXPRESSED IN THE CHALAZA OF DEVELOPING SEEDS

Category: Signal Transduction

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The *Arabidopsis* Response Regulator 22 (ARR22) is one of two members of a recently defined group of two-component system (TCS) elements. In plants, TCS regulators are involved in hormone response pathways, such as those for cytokinin and ethylene. While the functions of other TCS elements in *Arabidopsis*, such as histidine kinases (AHKs), histidine-containing phosphotransfer proteins (AHPs) and A- and B-type ARRs are becoming evident, the role of ARR22 is poorly understood.

Transient expression of fluorescence-tagged ARR22 demonstrated that ARR22 is preferentially a cytoplasmic protein which specifically interacts with AHP2, AHP3 and AHP5 in yeast and living plant cells. Analysis of promoter::GFP transgenic lines revealed that the gene is exclusively expressed in the chalaza of developing seeds. Two new loss-of-function alleles of ARR22, *arr22-2* and *arr22-3*, were isolated and characterized. With respect to their morphology and metabolite status, no significant difference in the developing seeds of the *arr22* mutants was observed compared to wild type. However, the genetic complementation of the *arr22* mutants with a genomic *ARR22* fragment resulted in plants which exhibited a pleiotropic phenotype of different penetrance. This phenotype resembles those of *P<sub>35S</sub>::ARR22* transgenic lines and those of multiple *ahk*, *ahp* and B-type *arr* mutants. Moreover, this phenotype was not observed when the phosphorylatable Asp74 of ARR22 was changed to either a dominant active Glu or a dominant inactive Asn. The observation, that the mis-expression of *ARR22*, even from its native promoter, causes dramatic developmental defects, indicates that the chromatin status might play a crucial role in maintaining the chalaza-restricted expression of the response regulator. Our results favor the model that, at least in non-chalaza tissues, ARR22 acts as a potent phospho-histidine phosphatase on specific AHPs establishing a strong phosphate sink within the bidirectional TCS signalling network. The lack of any aberrant morphological and metabolite phenotype in the seeds of the *arr22* mutants indicates that the ARR22 function in the chalaza might be attenuated *via* an unknown mechanism or by TCS-unrelated phosphatases.

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#### **ICAR4033**

PROTEOLYTIC PROCESSING OF A PLANT PEPTIDE HORMONE BY A SUBTILASE IN ARABIDOPSIS

Category: Signal Transduction

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Phytosulfokines (PSKs) are secretory peptides with sulfated tyrosine residues known to promote cell proliferation in tissue culture (Matsubayashi and Sakagami, 2006, Ann. Rev. Plant Biol. 57, 649-674.) AtPSK4 from Arabidopsis is a five-residue peptide derived from a 79 residue prepropeptide precursor. We demonstrated that AtPSK4 is released from a precursor protein by AtSBT1.1 (At1g01900), one of the 56 subtilisin-like serine proteases (subtilases) encoded in Arabidopsis genome. Proteolytic processing was demonstrated by cleavage of a proAtPSK4-myc transgene product to AtPSK4-myc *in vivo*. The precursor was not processed in sbt1.1-1 and sbt1.1-2, T-DNA knockout mutants in AtSBT1.1, indicating that AtSBT1.1 is required for proAtPSK4 processing. Proteolytic processing was also not observed in intact roots but was induced by explanting roots and placing them into tissue culture. The gene encoding AtSBT1.1 was upregulated when roots were explanted suggesting that activation of the proteolytic machinery which processes proAtPSK4 is dependent on AtSBT1.1 induction. A fluorogenic peptide representing the putative subtilase recognition site in proPSK4 was cleaved *in vitro* by an affinity-purified AtSBT1.1. Alanine scans through the recognition site peptide indicated that AtSBT1.1 is highly specific for the AtPSK4 precursor. MALDI-TOF analysis of the reaction products revealed that the preferential site of cleavage was three residues upstream from the presumed N-terminus of the mature peptide indicating that further processing at the N-terminus may be required for activation. Thus, a peptide growth factor that promotes callus formation in culture is proteolytically processed by a specific plant subtilase encoded by a gene upregulated during the process of explanting tissue for culture. (Supported by NSF IBN0420015)

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#### **ICAR4034**

ATCIPK8, A CBL-INTERACTING PROTEIN KINASE, REGULATES THE PRIMARY NITRATE RESPONSE IN THE LOW-AFFINITY PHASE

Category: Signal Transduction

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Nitrate, the major nitrogen source for most plants, is not only a nutrient source, but also a signaling molecule. For almost two decades, it has been known that nitrate can rapidly induce the transcriptional expression of several nitrate-related genes, a process referred to as the primary nitrate response. However, little is known about how plants actually sense nitrate and how the signal is transmitted in this pathway. In this study, a calcineurin B-like-interacting protein kinase (CIPK) gene, CIPK8, was found to be involved in early nitrate signaling. *CIPK8* expression was rapidly induced by nitrate. Analysis of two independent T-DNA mutants and mutant complemented with constitutive expressed CIPK8 showed that CIPK8 positively regulated the nitrate-induced expression of primary nitrate response genes, including nitrate transporter genes and genes required for assimilation. Kinetic analysis of nitrate induction levels of these genes in wild-type plants indicates that there are two response phases: a high-affinity phase with  $K_m$  of ~30  $\mu\text{M}$  and a low-affinity phase with  $K_m$  ~0.9 mM. As *cipk8* mutants are defective mainly in the low-affinity response, the high-affinity and low-affinity nitrate signaling systems are proposed to be genetically distinct, with CIPK8 involved in the low-affinity system. In addition, CIPK8 was found to be involved in long-term nitrate-modulated primary root growth and nitrate-modulated expression of a vacuolar malate transporter, indicating that CIPK8 participates in multiple nitrate-related metabolic pathways. Taken together, our results report that CBL-CIPK networks are responsible not only for stress responses and potassium shortage, but also for nitrate sensing.

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**ICAR4035**IDENTIFICATION OF PROTEINS THAT FUNCTION IN CO<sub>2</sub> SENSING AND SIGNAL TRANSDUCTION

Category: Signal Transduction

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Stomatal guard cells sense many stress and environmental signals and regulate CO<sub>2</sub> influx into leaves for photosynthetic carbon fixation in exchange for plant water loss. The continuing rise in atmospheric CO<sub>2</sub>, as well as photosynthesis and respiration activities within the plant, regulate leaf stomata to close at high [CO<sub>2</sub>] and to open at low intercellular [CO<sub>2</sub>]. Although atmospheric [CO<sub>2</sub>] is predicted to double during this century and this CO<sub>2</sub> response affects global CO<sub>2</sub> intake and water use efficiency of plants, the signal transduction mechanisms that control CO<sub>2</sub>-induced stomatal movements remain largely unexplored. Recently, initial CO<sub>2</sub>-signaling mutants, such as *gca2*, *ht1* and *slac1*, have been described (1, 2, 3, 4), but the upstream mechanisms mediating CO<sub>2</sub> sensing remain unknown. Here we show that the expression of genes (named CORP for CO<sub>2</sub>-interacting Response Protein) is required for [CO<sub>2</sub>] regulation of gas exchange in Arabidopsis. The *corps* knock-out mutant plants exhibit a dramatic reduction in CO<sub>2</sub> regulation of plant gas exchange and stomatal movements, but are functional in response to other physiological stimuli, including abscisic acid- and dark-induced stomatal closing, and blue-light induced stomatal opening. Genomic DNA complementation of with *CORP* genes can restore the CO<sub>2</sub> response phenotype (impaired CO<sub>2</sub> response), indicating these genes function in CO<sub>2</sub> perception. Genetic epistasis analyses with the upstream *ht1* kinase mutant (2) further suggest that CORPs function upstream of HT1 in very early CO<sub>2</sub> signaling and signal transduction.

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**ICAR4036**

## GENETIC ANALYSES OF NOVEL COP9 SIGNALOSOME (CSN) MUTANTS SUGGEST MULTIPLE ROLES FOR THE CSN IN AUXIN SIGNALING

Category: Signal Transduction

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The COP9 signalosome (CSN) is an evolutionarily conserved multisubunit protein complex that regulates a variety of signaling and developmental processes. The only known biochemical activity of the CSN is an isopeptidase activity that cleaves the ubiquitin-like protein RUB/NEDD8 off of the cullin subunits of cullin-based ubiquitin-ligases (deneddylation). In the Arabidopsis auxin response pathway, CSN deneddylyase activity is required for optimal activity of the SCFT<sup>TIR1</sup> complex: the ubiquitin-ligase responsible for targeting Aux/IAA proteins for proteolysis in response to auxin. In a genetic screen for enhancers of the *tir1-1* auxin response defect, we identified two recessive mutant alleles of *CSN1* and *CSN3*, designated as *eta6* and *eta7*, respectively. Although both *eta6* and *eta7* display similar auxin response defects, RUB-modified CUL1 accumulates only in the *eta6* mutant, suggesting that the deneddylyase activity of the CSN complex is unaffected by the *eta7* mutation. Genetic interactions between these CSN mutants and other auxin response mutants also suggest that *eta6* and *eta7* affect distinct aspects of CSN function. Additionally, the Aux/IAA reporter protein AXR3NT-GUS is stabilized in *eta6* but not *eta7* seedlings, indicating the SCFT<sup>TIR1</sup> activity is unaffected by the *eta7* mutation. The contradiction between the quantitatively similar auxin response defects of *eta6* and *eta7* but opposing effects of these two mutations on SCFT<sup>TIR1</sup> suggest that the CSN plays a second role in addition to CUL1 deneddylylation in the auxin response pathway.

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**ICAR4037**

## STALLED RIBOSOMES CAUSE SUCROSE DEPENDENT TRANSLATIONAL REPRESSION OF S1-CLASS BZIP GENES

Category: Signal Transduction

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Translational control of gene expression is important for growth and development of plants as well as other organisms. Eukaryotic ribosomes generally translate only one open reading frame (ORF) per mRNA. Therefore, it is surprising that nearly 4000 *Arabidopsis thaliana* genes harbor upstream open reading frames (uORFs) preceding main ORFs. Previous reports have shown that the 5'leader of *bZIP11* is necessary for the sucrose dependent translational repression of the bZIP11 protein. This 5'leader harbors four uORFs of which only uORF2 is required for the sucrose dependent translational repression. uORF2 encodes the Sucrose Control (SC) peptide which is translated in vivo. uORF2 is also sufficient for the sucrose dependent translational control shown by transplanting the sequence to an independent 5'leader sequence. Most scanning ribosomes do not recognize the AUG of uORF2 due to the weak AUG context, i.e leaky scanning. As a result *bZIP11* is translated by ribosomes that passed the uORF2 initiation codon. Sucrose dependent translational control relies on specific conserved amino acids of the SC-peptide and the stop codon position of uORF2 as shown by mutagenesis and in vivo testing. Based on these results a mechanistic model is proposed in which translating ribosomes stall at uORF2 on the mRNA in response to sucrose, efficiently blocking *bZIP11* translation.

These results indicate that the translational apparatus changes in response to sucrose, thereby regulating *bZIP11* translation. The sucrose mediated signaling pathway acts cell autonomously as shown by experiments in protoplasts and active in all cell types studied. The *bZIP11* gene encodes a S1 class bZip transcription factor that controls e.g. amino acid metabolism. Further characterization of the signaling pathway will shed light on the regulation of metabolic processes in response to starvation and excess nutrient availability.

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**ICAR4038**CHARACTERIZATION OF ENHancers OF THE *AUXIN-RESISTANT4* MUTATION

Category: Signal Transduction

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The *axr4* mutation causes defects in gravitropism and lateral root formation as well as resistance to actively-imported auxins, and has been shown to result in mislocalization of the auxin import carrier protein AUX1 in certain cell types. However, the biochemical function of the AXR4 protein is unclear. To address this problem, we have identified second-site mutations that enhance the auxin resistant root elongation phenotype of *axr4-2* mutants. Initial characterization of two such enhancers showed that, when they are combined with *axr4-2*, they confer enhanced auxin resistance to imported auxins, but no change in resistance to an imported auxin or in aerial morphology. The enhancer mutations are allelic, auxin-sensitive in the absence of the *axr4-2* mutation, and map to the long arm of chromosome 5. Molecular characterization of these mutants could help reveal the function of the AXR4 protein and identify other components of membrane protein targeting pathways.

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**ICAR4039**BIOTIC AND ABIOTIC STRESS-INDUCED STROMAL  $\text{Ca}^{2+}$  TRANSIENTS IN CHLOROPLASTS

Category: Signal Transduction

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$\text{Ca}^{2+}$  plays a key role in intracellular signal transduction in plant cells. Various biotic and abiotic stresses have been shown to cause transient  $\text{Ca}^{2+}$  oscillations in cytoplasm. For example, pathogen-derived elicitors are recognized by specific receptors and induce rapid responses, including intracellular  $\text{Ca}^{2+}$  variations and associated oxidative burst in a few minutes. Recently, it has been shown that elicitors may directly decrease photosynthesis activity in chloroplasts. However, little is known how stress signals are transmitted to chloroplasts and affect photosynthesis. Here we provide direct evidence that stromal  $\text{Ca}^{2+}$  levels are up-regulated by various biotic and abiotic stresses, including pathogen-derived elicitors,  $\text{H}_2\text{O}_2$ , high NaCl and high osmolarity. Stromal  $\text{Ca}^{2+}$  variations may be involved in the regulation of photosynthesis and/or photosynthesis genes expression in chloroplasts. In order to measure stromal free  $\text{Ca}^{2+}$  concentrations, we used aequorin selectively targeted to stroma. Aequorin luminescence indicated that steady-state  $\text{Ca}^{2+}$  levels are relatively low in stroma, suggesting that  $\text{Ca}^{2+}$  is sequestered in the thylakoid lumen or unidentified  $\text{Ca}^{2+}$  stores in chloroplasts. Elicitors like flagellin 22 (flg22) and chitin oligosaccharides induced a rapid cytosolic  $\text{Ca}^{2+}$  increase within 2-3 min. Subsequently, slower stromal  $\text{Ca}^{2+}$  transients were evoked by the elicitors within 10-15 min and continue for several dozens of minutes, suggesting that the stromal  $\text{Ca}^{2+}$  responses might be involved in the plant defense responses against pathogens. Similarly,  $\text{H}_2\text{O}_2$  caused a slower stromal  $\text{Ca}^{2+}$  transients, preceded by a rapid cytosolic  $\text{Ca}^{2+}$  elevation.

Furthermore, we found that various abiotic stresses rapidly evoked the stromal  $\text{Ca}^{2+}$  transients in a stress-dependent manner. High NaCl caused a transient elevation of both cytosolic and stromal  $\text{Ca}^{2+}$  within 2-3 min. On the other hand, slower and sustained stromal  $\text{Ca}^{2+}$  transients were evoked by osmotic stresses. Possible roles of stromal  $\text{Ca}^{2+}$  signaling in biotic and abiotic stress responses will be discussed.

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**ICAR4040**

## PROMoter ANALYSIS OF A SUBFAMILY OF CALMODULIN-LIKE GENES

Category: Signal Transduction

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$\text{Ca}^{2+}$  ions participate as second messengers in many stress-response and developmental pathways. Among eukaryotes, plants possess a remarkable diversity of  $\text{Ca}^{2+}$ -binding proteins ( $\text{Ca}^{2+}$  sensors) such as calmodulin (CaM) and CaM-related proteins (CMLs) that regulate downstream targets and coordinate signal transduction events in response to stimuli. We have been studying a small subfamily of *CMLs* (*CML37*, *CML38*, *CML39*) in *Arabidopsis* that show a dramatic induction of expression in response to environmental stress. In order to understand the underlying regulatory mechanisms of these genes, we have been conducting promoter analysis experiments using 5'-upstream regions of the *CMLs* to drive GUS reporter expression. This empirical approach is a critical complement to algorithm-based prediction methods. Delineation of the relevant *cis*-elements should lay the groundwork to identify the transcriptional regulators that direct stress-responsive *CML* gene expression. Our data reveal clear developmental, tissue and stimulus-specific patterns of expression among these three *CMLs*. *CML37* and *CML38* respond very strongly to mechanical wounding but not herbivory by cabbage moth larvae (*Pieris rapae*) whereas *CML39* is less responsive to wounding but is induced significantly by jasmonate. We have identified negative regulators in the promoter regions of *CML37* and *CML38* and analysis is ongoing to determine whether these are the major elements controlling wound response or whether positive regulatory elements also contribute.

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**ICAR4041**

## MAP KINASES IN ROS-MEDIATED ABA SIGNALING IN GUARD CELLS

Category: Signal Transduction

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Abscisic acid (ABA) plays an essential role in protection of plants from a variety of environmental stresses such as drought, salt, and cold. Guard cells form stomatal pores in the leaf surface and are responsible for controlling CO<sub>2</sub> uptake and water loss by regulating the size of stomatal pores. Reactive oxygen species (ROS) have been suggested to function in guard cell ABA signaling. Despite extensive studies, molecular components working downstream of ROS in ABA signaling remain to be elucidated. In order to identify and characterize MAPK cascades mediating guard cell ABA/ROS signaling, we identified two MAPK genes, GCMAPK3 and GCMAPK4, that are preferentially and highly expressed in guard cells. To provide direct genetic evidence, RNAi-based gene silencing plant lines were generated in which both genes are silenced. In parallel, Arabidopsis single and double mutants carrying deleterious point mutations in these genes were identified. ABA-induced stomatal closure was strongly impaired in the RNAi lines in which both GCMAPK3 and GCMAPK4 transcripts were significantly silenced. Consistent with this result, the Arabidopsis mutants carrying point mutations in both genes showed an enhanced transpirational water loss and ABA- and H<sub>2</sub>O<sub>2</sub>-insensitive response in stomatal movement assays, whereas mutants carrying a mutation in one of these genes did not show any altered phenotype. A GCMAPK4-YFP fusion construct rescued the double mutant phenotype in ABA-induced stomatal movements, demonstrating that the mutations in these genes caused the phenotype. Together, these results provide genetic evidence that GCMAPK3 and GCMAPK4 function in guard cell ABA signaling, and there is functional redundancy in these genes. A further biochemical characterization of these proteins is being pursued.

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#### **ICAR4042**

THE ROLE OF CML42 IN TRICHOME BRANCH FORMATION IN ARABIDOPSIS

Category: Signal Transduction

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Trichomes are branched, unicellular structures that serve as a model for the study of cell morphogenesis. The current literature suggests that there are multiple genes that regulate trichome branching. We have been studying CML42, a Ca<sup>2+</sup>-binding Arabidopsis protein related to the prototypical Ca<sup>2+</sup> sensor, calmodulin (CaM). A *CML42* T-DNA insertion line (*cml42*) exhibits a mutant trichome phenotype with increased branch numbers compared to wild-type plants. Various biochemical analyses demonstrated that recombinant CML42 binds Ca<sup>2+</sup> which results in a conformational change and the exposure of hydrophobic regions. CML42 promoter analysis using a GUS reporter demonstrated that *CML42* is widely expressed, most notably in specialized support cells found at the base of trichomes. Yeast-2-hybrid and GST-pull down analysis suggest that CML42 interacts with a protein termed KIC (kinesin interacting calcium-binding protein). Consistent with our findings, previous work has implicated KIC in trichome morphogenesis through its regulation of the motor-domain protein, kinesin (KCBP). Like *CML42* mutants, both *KIC* and *KCBP* mutants show mutant trichome phenotypes. Our genetic studies imply that CML42 is a negative regulator of trichome branching. It is possible that CML42 interacts with KIC under certain Ca<sup>2+</sup> concentrations to form a multiprotein complex with KCBP that mediate trichome morphogenesis. Current studies are examining the interaction between CML42 and KIC to determine the peptide region of physical binding, affinity, and the role of Ca<sup>2+</sup> in the association. In addition, we recently isolated a *KIC* T-DNA insertion line and are assessing it for altered trichome morphology. Collectively our data support the important role of Ca<sup>2+</sup> signaling in trichome branching and structure.

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#### **ICAR4043**

THE PHENYLPROPAENOID PATHWAY IS DIFFERENTIALLY INDUCED BY AUXIN AND ETHYLENE

Category: Signal Transduction

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Phenylpropanoid biosynthesis is a tightly regulated and important metabolic pathway, producing flavonoids such as quercetin (Q) and kaempferol (K) as well as other phenolics. The induction of flavonoid synthesis by light and wounding are well characterized and coupled to the regulation of plant growth and development by environmental factors. The hormones auxin and ethylene are involved in many responses of plants to the environment. Mutants that make no flavonoids show enhanced auxin transport, suggesting these metabolites are negative regulators of auxin transport. We have examined the relationship between auxin, ethylene, and the regulation of phenylpropanoid metabolism using fluorescent measurement of flavonoid accumulation and real time qRT-PCR of genes encoding the enzymes of this pathway. IAA and the ethylene precursor, ACC, increase flavonoid levels *in vivo*, perhaps by transcriptional enhancement of genes encoding the pathway enzymes. While ACC increases both K and Q accumulation equivalently, IAA induces Q accumulation significantly more than K. This result suggests auxin differentially regulates enzymes whose activity increases the relative abundance of Q, and may lead to fine tuning of auxin transport by changing the K to Q ratio. We are currently examining expression of genes encoding key pathway enzymes, such as chalcone synthase (CHS), the first enzyme in flavonoid biosynthesis and flavonoid 3'-hydroxylase, which converts dihydroxykaempferol to dihydroxyquercetin, precursors of K and Q, respectively. *CHS* is upregulated by IAA and ACC, consistent with enhanced accumulation of flavonols. The induction of negative regulators of auxin transport by IAA represents feedback regulation of auxin movement. Finally, experiments are underway to identify the signal transduction pathways necessary for induction of flavonoid biosynthesis by auxin and ethylene utilizing hormone receptor and signal transduction mutants, including *tir1*, *tir3*, *axr4*, *ein2*, and *etr1*.

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#### **ICAR4044**

ATCDPK1, AN ARABIDOPSIS CALCIUM-DEPENDENT PROTEIN KINASE, IS INVOLVED IN RESPONSE TO BOTH BIOTIC AND ABIOTIC STRESSES

Category: Signal Transduction

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Growing evidence indicates that Calcium-dependent protein kinases (CDPKs) regulate many aspects of plant growth, development and hormone

signaling as well as plant adaptation to biotic and abiotic stresses. However, the biological roles of most CDPKs in stress signaling remain unclear so far. Here we report that transcript of *AtCDPK1* was induced by both virulent and avirulent *Pseudomonas syringae*. The T-DNA insertion line with comprised *AtCDPK1* expression (*cdpk1-1*) leads to slightly decreased level of defense marker genes *PR1*, *PR2* and *AIG1* after inoculation with *Pst* *avrRpt2*. The stomata of the *cdpk1-1* plants was kept open at 1hr after *Pst DC3000* infection, while closed in the wild-type at the same time point. Histochemical staining of GUS fused with *AtCDPK1* promoter showed that GUS activity is mainly expressed in root tip, lateral root primodia and trichome under normal growth conditions, and in guard cells as well as pavement cells after infection. Constitutively active *AtCDPK1* transgenic Arabidopsis plants formed spontaneous small lesions in leaf and were more resistant to salt stress compared with the wild type during seed germination and seedling growth stage. Further analysis showed that expression of *AtCDPK1* were also induced by saline, drought and cold treatments, and by plant hormones such as SA, MeJA and ABA. Taken together, these results suggested that AtCDPK1 may positively regulate Arabidopsis defense response and salt stress signaling pathways.

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**ICAR4045**

SOI1 IS REQUIRED FOR EARLY CYTOKININ SIGNALING

Category: Signal Transduction

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The cytokinin signal transduction pathway includes a two-component system. Binding of cytokinin by histidine kinase receptors leads to the activation of type-B response regulators (ARRs), transcriptional activators that promote cytokinin responses. Type-B ARR activation is mediated by histidine phosphotransfer proteins and also leads to the induction of type-A ARR genes that encode repressors of cytokinin responses. To identify new loci that control the cytokinin response pathway, we screened for mutations that suppress the rosette phenotype of the cytokinin overproducing transgenic line *ipt-161*. The *soi1* (*suppressor of ipt-161*) mutant has a strong suppression phenotype. When introduced into the wild-type background, this mutation causes decreased cytokinin sensitivity in growth response assays, indicating that the corresponding gene product controls cytokinin responses rather than biosynthesis or metabolism. In addition, the cytokinin induction of type-A ARR genes was suppressed, suggesting a defect in early signaling. The *soi1* mutation maps to a region of the Arabidopsis genome that does not contain a currently known cytokinin response locus. We propose that SOI1 is a novel regulator of the primary cytokinin response pathway.

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**ICAR4046**

EXPRESSION OF HEXOSE TRANSPORTER (HXT2) FROM *SACCHAROMYCES CEREVISIAE* IN ARABIDOPSIS PRODUCE GLUCOSE TOLERANCE.

Category: Signal Transduction

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Environmental conditions can affect negatively plant performance. As a result, the amount of sugars available to support growth and development can be severely reduced. Plants have a system to regulate the distribution of photosynthates. In this context, the effect of glucose as inhibitor of plant growth, can be easily understood as a part of a mechanism needed to prevent the accumulation of photosynthates in few organs. However, the basis of it are not clear. We used the expression of hexose transporter (*hxt2*) from *S. cerevisiae* in Arabidopsis as an alternative to increase glucose uptake. The HXT2 transporter expressed in Arabidopsis has low affinity for glucose and no difference in growth was observed in plants cultivated at low glucose concentration (0.1-1.0 %). At 3 % of glucose, wild type plants showed a reduced size and accumulation of anthocyanins. Plants expressing HTX2 were able to continue growing at 3 % glucose and no signs of stress were observed. The results suggest that the uptake may generate signals that regulate some of the plant responses to glucose.

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**ICAR4047**

NEW MUTANTS WITHIN THE ETHYLENE SIGNALING PATHWAY IN *ARABIDOPSIS*

Category: Signal Transduction

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The plant hormone ethylene is an important regulator of plant growth and development, including senescence, abscission, fruit ripening, and responses to biotic and abiotic stresses. We have performed a mutagenesis screen to isolate new players in the ethylene signaling pathway. Ethylene is perceived by a family of five receptors in *Arabidopsis thaliana*, which negatively regulate the response to the hormone. The ethylene-insensitive *etr1-2* is dominant gain-of-function allele within the ETR1 ethylene receptor. We mutagenized *etr1-2* seeds with the goal of identifying suppressors of this mutation. The screen was carried out by searching for mutants that exhibited the triple response, a phenotype characteristic of wild-type etiolated seedlings grown in the presence of ethylene. Here, we present two mutants isolated from the *etr1-2* suppressor screen. The first mutant, *etr1-10*, is an intragenic mutation within *ETR1*, a unique missense mutation that apparently eliminates ETR1-2 signaling. The second mutation, *rte3* (*reversion to ethylene-sensitivity3*), is extragenic and is located within a previously uncharacterized protein. There is no known function for *RTE3* in *Arabidopsis*. Current work focuses on the characterization of the *rte3* and *etr1-10* mutants.

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**ICAR4048**

SENSITIVITY TO FREEZING 6 (SFR6) HAS A CONDITIONAL CIRCADIAN PHENOTYPE THAT IS DEPENDENT UPON SUCROSE.

Category: Signal Transduction

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The *sensitive to freezing 6 (sfr6)* mutant of *Arabidopsis thaliana* is late flowering in long days. This can be attributed to reduced expression of components in the photoperiodic flowering pathways, including *FKF1*, *GIGANTEA*, *CONSTANS* and *FLOWERING LOCUS T*, in long day photoperiods.

Microarray analysis of gene expression showed that a circadian clock-associated motif, the evening element, was overrepresented among genes down-regulated in *sfr6* plants. We investigated the effect of *sfr6* upon clock gene expression using QRT-PCR on timecourses collected from plants grown in free-running and entrained conditions. Expression of the morning clock component *CCA1* was reduced in *sfr6* seedlings: rhythmic expression of the evening clock genes *GIGANTEA* and *TOC1* showed damped amplitude and delayed phase.

We therefore measured the effects of the *sfr6* mutation upon behaviour of the free-running circadian clock. We found *sfr6* plants showed a significant long period phenotype that was dependent upon the presence of sucrose in the growth medium. The circadian period of wild type plants shortened when they were grown with sucrose; this was not so for *sfr6* seedlings, suggesting that this mutation alters the clock's ability to respond to sucrose.

Analysis of *sfr6* circadian behaviour and gene expression imply that, contrary to the predictions of the current model, large changes in level and timing of clock gene expression may have little effect upon clock outputs. Moreover, our data suggest that sucrose, although causing only relatively minor changes in clock gene expression, acts upon the *Arabidopsis* clock. The implications for clock regulation will be discussed.

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#### **ICAR4049**

VISUALIZING ACID GROWTH

Category: Signal Transduction

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Growing *Arabidopsis* roots are characterized by spontaneous and complex surface pH fluctuations. Using a combination of confocal vertical-stage and inverted-stage microscopy, extracellular pH was monitored in real time along the root longitudinal axis and around the cross-sectional perimeter. Based on these measurements, we are constructing a model of the three-dimensional surface pH pattern of the vertically growing root. The spatial-temporal properties of this pattern will be analyzed to identify origin(s) of pH fluctuations as well as direction and speed of propagation. Preliminary analysis has revealed an acropetal component seemingly originating in the central elongation zone, which may be mechanically regulated. Further analysis will show if additional, migrating or stationary pH fluctuations are superimposed on this acropetally moving pattern. Of particular interest are modulations of surface pH reflecting basipetal transport of auxin. To identify auxin-dependent components of the root surface pH pattern, extracellular pH will also be monitored in roots of auxin-transport mutants.

This work is supported by NSF.

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#### **ICAR4050**

POSSIBLE INVOLVEMENT OF CAS IN CA<sup>2+</sup> COMMUNICATION BETWEEN CYTOPLASM AND CHLOROPLASTS IN ARABIDOPSIS

Category: Signal Transduction

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Ca<sup>2+</sup> has been implicated as a central second messenger in a wide variety of cellular processes in plant cells. Intracellular Ca<sup>2+</sup> signaling involves primarily entry of Ca<sup>2+</sup> across plasma membrane, while intracellular mobilization appears to play a crucial role in cell signaling. Vacuole and endoplasmic reticulum are the major Ca<sup>2+</sup> stores in plant cells. The chloroplast may also serve as a potential Ca<sup>2+</sup> sink. However, the role of chloroplasts in the intracellular Ca<sup>2+</sup> signaling is at present unclear. In addition to the potential role of chloroplasts in sequestering Ca<sup>2+</sup>, it has been proposed that free Ca<sup>2+</sup> concentration in the stroma is maintained at low levels (sub-μM) and transiently elevated in response to the light-to-dark transition in tobacco (Sai and Jonson, Plant Cell, 2002). Interestingly, dark-induced stromal Ca<sup>2+</sup> elevation precedes the generation of cytosolic Ca<sup>2+</sup> transients, suggesting the presence of Ca<sup>2+</sup> communication between chloroplasts and cytoplasm. However, the underlying molecular mechanisms are largely unknown.

In this study, we confirmed that stromal Ca<sup>2+</sup> levels are also transiently up-regulated after the onset of darkness in *Arabidopsis*. Stromal Ca<sup>2+</sup> transients were evoked by the light-to-dark transition within 10 min and continue for several dozens of minutes. CAS is a putative Ca<sup>2+</sup> sensor protein localized mainly in the chloroplast thylakoid membrane. We recently demonstrated that CAS is involved in external Ca<sup>2+</sup>-induced cytosolic Ca<sup>2+</sup> elevation and stomatal closure (Nomura et al., Plant J. 2008). Interestingly, the dark-induced stromal Ca<sup>2+</sup> transients were significantly reduced in cas-1 mutant. CAS likely forms a 200 kDa protein complex associated with the thylakoid membrane and is known to be phosphorylated in a light-dependent manner. Taken together, these results suggest that CAS is involved in the dark-induced release of Ca<sup>2+</sup> from the thylakoid membrane and the regulation of stromal Ca<sup>2+</sup> levels in chloroplasts.

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#### **ICAR4051**

BRASSINOSTEROIDS INDUCED INHIBITION OF ROOT GROWTH IS MEDIATED BY ETHYLENE PRODUCTION IN *ARABIDOPSIS THALIANA*

Category: Signal Transduction

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It is known that both brassinosteroids and ethylene have inhibitory effect on root growth of plants. Brassinosteroids are also known to activate

ethylene production in root of plants, suggesting that brassinosteroids induced inhibition of plant root growth is mediated by ethylene. To confirm the possibility, effects of ethylene and its biosynthetic inhibitors on brassinosteroids induced inhibition of root growth of *Arabidopsis thaliana* were investigated. Simultaneous application of brassinosteroids and ethylene showed no additive or synergistic effect on root growth. AVG or Co<sup>2+</sup> applied with brassinosteroids exhibited reduction of these inhibitory effect on root growth than that induced by only brassinosteroids. Application of brassinosteroids with AVG showed shorter roots than that induced by application of AVG alone. Application of brassinosteroids with Co<sup>2+</sup> showed no difference in root growth compared to application of Co<sup>2+</sup> alone. These suggest that brassinosteroids induced inhibition of root growth is mediated by ethylene production, especially conversion of ACC to ethylene. In fact, an ACC oxidase, *AtACO1*, was up-regulated by application of brassinosteroids. *AtACO1* expression was increased in dominant mutant of brassinosteroids transcription factor, *bes1-D*. In addition, promoter-GUS system revealed that *AtACO1* is mainly expressed in tip and maturation zone of *Arabidopsis* roots. In this presentation, more details about molecular evidence for involvement of *AtACO1* in brassinosteroids induced inhibition of root growth in *Arabidopsis* are discussed.

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**ICAR4052**

MEMBRANE-BOUND TRANSCRIPTION FACTORS PLAY A CRITICAL ROLE IN REGULATING STRESS RESPONSES IN ARABIDOPSIS

Category: Signal Transduction

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Controlled release of membrane-tethered, dormant precursors is an intriguing activation mechanism that regulates diverse cellular functions in eukaryotes. An exquisite example is the proteolytic activation of membrane-bound transcription factors (MTFs). Regulated activation of preexisting dormant MTFs is considered to be an adaptive strategy that ensures prompt responses to environmental changes. The MTFs are activated by controlled proteolytic cleavage through either one of two distinct, but biochemically related pathways. In regulated ubiquitin (Ub)/proteasome-dependent processing (RUP), they are ubiquitinated and partially degraded by the 26S proteasome. In regulated intramembrane proteolysis (RIP), active forms are released by specific membrane-associated proteases. Genome-wide analysis revealed that at least thirteen members of the Arabidopsis NAC transcription factor family contain -helical transmembrane motifs (TMs) in their C-terminal regions and thus are membrane-associated. Interestingly, most of the NAC MTF genes are up-regulated under stress conditions. Membrane release of the NAC MTFs is also activated by diverse environmental factors, suggesting that they are involved in stress responses. Transgenic plants overexpressing partial-size NAC constructs devoid of the TMs, but not those overexpressing full-size constructs, showed distinct phenotypic changes, including dwarfed growth, delayed flowering and seed germination, and alterations in leaf senescence. Furthermore, a number of plant transcription factors are predicted to be anchored to the intracellular membranes, thus indicating that proteolytic activation of MTFs is a regulatory scheme that occurs widely in plant genomes.

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**ICAR4053**

REGULATING THE ACTIVITY AND EXPRESSION OF AUXIN RECEPTORS IN ARABIDOPSIS

Category: Signal Transduction

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The Arabidopsis TIR1/AFB family of F-box proteins act as cellular receptors for the plant hormone auxin [1]. The TIR1, AFB1, AFB2 and AFB3 members of this family exhibit 60% at the amino acid level. We show through genetic studies that each of these proteins has slightly different roles in development. Most interesting is the more significant role that the TIR1 and AFB2 proteins play in the response to auxin.

Using *in vitro* translated TIR1/AFB proteins we show that TIR1 and AFB2 interact with AuxIAA proteins with higher affinity than AFB1 or AFB3. Further we have recently developed a Y2H system to investigate the interactions between each auxin receptor, different auxins and proteins from each clade of the AuxIAA family [2]. We present data that demonstrates the activity of each TIR1/AFB protein in this system.

We have created transgenic Arabidopsis plants expressing either transcriptional or translational GUS fusions of each TIR1/AFB promoter or coding regions. These lines showed that TIR1, AFB2 and AFB3 undergo significant post-transcriptional regulation and that expression in the translational fusions is restricted to the meristem regions. In contrast, both promoter and protein fusions of AFB1 are expressed throughout the plant. The microRNA *miR393* has been shown to bind to *TIR1/AFB* mRNA [3] so we have investigated the role of the *miR393* in the regulation of TIR1/AFB expression. We have created transgenic lines to investigate the activity and expression of *miR393* and also to assess the significance of altering the ability of the *miR393* to bind to *TIR1/AFB* mRNAs. Our findings suggest that *miR393* plays a role in fine-tuning the activity of the auxin receptors.

[1]- Dharmasiri *et al* (2005), Dev Cell v9 pg109.

[2]- Overvoorde *et al* (2005), Plant Cell v17 pg3282

[3]- Navarro *et al* (2006), Science v312 pg436

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**ICAR4054**

ANALYSIS OF JAZ REPRESSOR ACTIVITY AND PROTEIN TURNOVER IN CELL CULTURE BASED SYSTEMS

Category: Signal Transduction

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Jasmonic acid (JA) and its derivatives are small signaling molecules that regulate many aspects of plant defense and development. In the presence of JA-Ile, JAZ proteins interact with the F-box protein COI1, marking them for degradation by the 26S proteasome. JAZ proteins negatively regulate JA

responses, probably by interacting with the MYC2 (At1g32640) transcriptional activator and inhibiting its function. JAZ gene expression is rapidly induced by JA. Here, we show that the JAZ1 promoter (PJAZ1) is activated by transiently overexpressing MYC2. Co-expression of JAZ1 (At1g19180) represses MYC2 activation potential, both on an endogenous promoter (PJAZ1) and a heterologous promoter when MYC2 is fused to the GAL4DBD. Moreover, we show that GAL4DBD fused JAZ proteins are able to repress transcription, revealing the presence of an intrinsic repressor activity. We also present the development of a cell culture based cycloheximide chase system for quantitative measurements of protein degradation. With Gateway compatible cloning, a gene of interest can be fused to the firefly luciferase gene and stably expressed in *Arabidopsis* cells, driven by a promoter of choice. We were able to quantify the JA-induced JAZ degradation of several JAZ proteins. This assay can be used to investigate the influence of other hormones or signaling molecules on JA-mediated JAZ degradation.

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## ICAR4055

### SIGNALING AT THE MEMBRANE: LARGE SCALE IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS

Category: Signal Transduction

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*Arabidopsis* genome is predicted to encode about 6,000 membrane proteins, including transporters, receptors, enzymes and structural proteins, and about 20,000 proteins that are either soluble or attached to the membrane. Membrane-localized events like transport or signal perception are coordinated to cytosolic events mediated by soluble proteins. The regulation of many transporters and membrane receptors through interaction with soluble proteins is still poorly documented, albeit the function and the role of these membrane proteins are well known. Examples in plants and other organisms show that kinases, phosphatases, together with ubiquitin ligases, are likely to be general intermediates that transduce signals to and from the membrane. In a common effort to functionally annotate *Arabidopsis* genome, we set up a large-scale project aiming to determine the protein-protein interaction network of *Arabidopsis* membrane and soluble proteins.

We designed a list of 8,500 genes to be studied. This list contains 5,800 membrane proteins that are not targeted to the mitochondrion or chloroplast. We included 2,700 soluble proteins likely to interact with membrane proteins: they are involved in signal transduction (900), trafficking (300) or ubiquitylation (1,500) processes. Pairwise protein-protein interactions are tested in yeast using a special two-hybrid system: the split-ubiquitin system. This technique allows to identify interactions between membrane proteins or between membrane and soluble proteins. Upon interaction of the two proteins, a transcription factor translationally fused to one of the two partners is released, and is able to migrate into the nucleus. In order to make correct protein fusions, the number of transmembrane domains and the location of the C- and N-termini of each partner were determined manually using prediction algorithm, bibliography and similarity searches. Pairwise interactions are scored according to yeast growth on agar plates.

Taking into account the cloning success rate and system limitations due to protein topologies, about 43 millions interactions will be tested. Presently, about 87,000 have been performed. Yeast growth is quantitatively scored by automated image analysis. The identification and the validation of these interactions are in progress.

The interaction maps will be used to build an interaction network. The analysis of the network will enable to identify new membrane protein complexes, regulatory pathways and signal transduction cascades. These data will also enable to annotate unknown genes according to their relationship with genes of known function. The data will eventually be made available for public searches on a web-based interface.

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## ICAR4056

### ANALYSIS OF MAPK SIGNALING NETWORKS USING ARABIDOPSIS THALIANA PROTEIN MICROARRAYS

Category: Signal Transduction

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We are generating ATEC, a high quality collection of *Arabidopsis* TAP-tagged ORFs cloned in a plant binary vector. ATEC has been designed to contain a significant proportion of genes with functions in transcription, signal transduction, protein degradation and stress response, and also genes with other known or unknown molecular functions. The current composition of ATEC allows comprehensive studies of transcription regulation, signal transduction, and stress-response. More than 5000 unique *Arabidopsis* TAP-tagged clones have been already deposited into the public *Arabidopsis* Resource Center database. In the next 2 years the collection is estimated to include half of the predicted *Arabidopsis* ORFs. In our laboratory, ATEC is employed in generating protein microarrays by expressing tagged ORFs in a homologous system and then purifying recombinant proteins. Protein microarrays containing 60% of known and predicted transcription factors, a large group of kinases and other proteins were produced with the goal of analyzing mitogen-activated protein kinase (MAPK) signaling pathways. Specifically, we have performed a large scale analysis of the *Arabidopsis thaliana* MAPK signaling pathways using a hierarchical approach that reconstructs multiple levels of the cellular signaling network. First, MAPKK-MAPK functional modules were selected by combinatorial pairing of nine MAPKs and ten MAPks. Subsequently, phosphorylation substrates were identified for all ten MAPks by probing the protein microarrays with *in vivo* activated MAPks.

We identified known and novel signaling modules encompassing more than 600 MAPK phosphorylation substrates enriched in transcription factors involved in the regulation of developmental processes and stress response. Subsequent *in planta* re-constitution experiments validated selected MAPK pathways and effectors. We show that several WRKY and TGA transcription factors involved in defense responses were phosphorylated when co-expressed with specific MAPKK/MAPK modules.

The reconstructed phosphorylation network exhibits modularity and redundancy of signal transduction pathways. Our analysis shows complex interactions between signaling modules with highly specific combinations of transcription factor targets. The predicted MAPK phosphorylation network constitutes the first comprehensive resource to understand the function and architecture of MAPK signaling systems.

#### References

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Popescu, S. C., Snyder, M., Dinesh-Kumar, S.P. (2007). "Arabidopsis protein microarrays for the high-throughput functional characterization of proteins" *Journal of Plant Signaling & Behavior*, 2(5) Sept/Oct.:415-19.

Popescu S. C. et al. – "MAPK signaling networks in *Arabidopsis thaliana*", submitted.

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#### ICAR4057

SIGNAL TRANSDUCTION IN GUARD CELLS OF ARABIDOPSIS IN RESPONSE TO ELEVATED CO<sub>2</sub>: REACTIVE OXYGEN SPECIES PRODUCTION IS AN EARLY EVENT DURING BICARBONATE INDUCED STOMATAL CLOSURE IN ABAXIAL EPIDERMIS

Category: Signal Transduction

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*Arabidopsis thaliana* is an ideal plant to study various aspects of plant function. This paper reports our observations made with Arabidopsis that are relevant to not only signal transduction phenomenon but also responses to elevated CO<sub>2</sub> (simulated by raising bicarbonate in the medium). During the past two decades, extensive work has been done on stomatal closure induced by abscisic acid (ABA). In contrast to studies with ABA, only limited information is available on the mechanism of perception and participation of various signaling components during stomatal closure induced by CO<sub>2</sub>. We have used external bicarbonate as a source of CO<sub>2</sub>. This paper reports our observations on an increase in reactive oxygen species (ROS) production in relation to bicarbonate-induced stomatal closure in abaxial epidermis of Arabidopsis. The presence of 2 mM bicarbonate in the incubation medium induced stomatal closure and elevated markedly the levels of ROS in guard cells within 5 min, as indicated by the fluorescent probe, dichlorofluorescein diacetate (H<sub>2</sub>DCF-DA). Bicarbonate induced stomatal closure as well as ROS production were restricted by exogenous catalase or diphenylene iodonium (DPI, an inhibitor of NAD(P)H oxidase). The reduced sensitivity of stomata to bicarbonate and ROS production in homozygous *atrbhD/F* double mutant of Arabidopsis confirmed that NAD(P)H oxidase is involved during bicarbonate induced ROS production in guard cells. The production of ROS was quicker and greater with ABA than that with bicarbonate. Such pattern of ROS production may be one of the reasons for ABA being more effective than bicarbonate, in promoting stomatal closure. Our results demonstrate that ROS is an essential secondary messenger during bicarbonate induced stomatal closure in Arabidopsis. Thus, increase in ROS production in guard cells, appears to be a common event during stomatal closure in response to ABA or MJ. Obviously, rise in ROS levels, is one of the early steps on exposure to elevated CO<sub>2</sub> in plant cells. We suggest that ROS could be an important secondary messenger during the transduction of CO<sub>2</sub> signal in not only guard cells but also other plant tissues.

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#### ICAR4058

GENETIC DISSECTION OF HORMONAL RESPONSES IN THE ROOTS OF ARABIDOPSIS GROWN UNDER CONTINUOUS MECHANICAL IMPEDANCE

Category: Signal Transduction

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We investigated the role of ethylene and auxin in regulating the growth and morphology of roots during mechanical impedance by developing a new growing system and using the model plant *Arabidopsis thaliana*. The Arabidopsis seedlings grown horizontally on a dialysis membrane-covered agar plate encountered adequate mechanical impedance as the roots showed characteristic ethylene phenotypes: 2-fold reduction in root growth, increase in root diameter, decrease in cell elongation and ectopic root hair formation. The root phenotype characterization of various mutants having altered response to ethylene biosynthesis or signaling, the effect of ethylene inhibitors on mechanically impeded roots and transcription profiling of the ethylene responsive genes led us to conclude that enhanced ethylene response plays a primary role in changing root morphology and development during mechanical impedance. Further, the differential sensitivity of horizontally and vertically grown roots toward exogenous ethylene suggested that ethylene signaling plays a critical role in enhancing the ethylene response. We subsequently demonstrated that the enhanced ethylene response also affects the auxin response in root. Taken together, our results provide a new insight into the role of ethylene in changing root morphology during mechanical impedance.

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#### ICAR4059

DIRECT TARGETS OF THE ABA ACTIVATED TRANSCRIPTION FACTORS ABI4 AND ABI5

Category: Signal Transduction

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The plant hormone abscisic acid (ABA) is a key regulator of seed development. In addition to promoting seed maturation, ABA can also act to

inhibit seed germination and seedling growth. Many components involved in the ABA response have been identified, including the transcription factors ABA insensitive 4 (ABI4) and ABI5. The genes encoding these factors are expressed predominantly in developing and mature seeds, and are positive regulators of ABA mediated inhibition of seed germination and growth. The direct effects of ABI4 and ABI5 in the ABA response remain largely undefined. To address this question, microarrays were used to identify ABA dependent transcriptional targets of ABI4 and ABI5. In contrast to previous mutant based assays, plants overexpressing ABI4 or ABI5 were used to allow identification of direct transcriptional targets. Under these conditions, ABI4 and ABI5 each induced a small set of genes (95 and 59, respectively), with only 16 shared targets. In addition to effector genes involved in seed maturation and storage, several signaling proteins and transcription factors not previously known to be involved in the ABA signaling pathway were identified as targets of ABI4 and/or ABI5. Interestingly, the promoters for many of the ABI4 target genes do not contain previously characterized ABI4 binding sites (including CE-1 or the S box), suggesting that a different mechanism may activate some ABI4 regulated genes.

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**ICAR4060**

ALTERNATE METHODS ARE REQUIRED TO ASSESS ABA BINDING TO CANDIDATE RECEPTORS.

Category: Signal Transduction

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The phytohormone abscisic acid (ABA) plays an integral role in plant growth and development. ABA regulates dormancy, seed maturation and germination, as well as maintaining osmotic homeostasis. Numerous components of the ABA biosynthetic and signal transduction pathway have been identified, but until recently receptors of this important hormone have remained elusive.

In the last two years three ABA receptors have been identified in *Arabidopsis thaliana*. These are the nuclear flowering-time protein Flowering Time Control Protein A (FCA) (Razem *et al.*, (2006) *Nature* **439**), the plastid-associated Mg-chelatase H subunit (CHLH) (Shen *et al.*, (2006) *Nature* **443**) and a protein originally identified as a membrane-bound G-protein coupled receptor 2 (GCR2) (Liu *et al.*, (2007) *Science* **315**).

To further characterize the ABA binding site and potentially identify other ABA receptors we initially focused our studies on FCA, and later on GCR2. FCA binds FY and the presence of ABA was reported to disrupt this interaction. However, we did not observe any effect of ABA on the FCA:FY interaction. Direct binding assays also failed to show binding of ABA to FCA. We were also unable to show an ABA:GCR2 interaction. This is consistent with other studies that show that GCR2 is not an extracellular ABA receptor (Gao *et al.*, (2007) *The Plant Journal* **52** and Johnstone *et al.*, (2007) *Science* **318**). The ABA-binding assays used in all three ABA receptor papers are highly reliant on the quality of the protein used and we believe this gives rise to the variable binding results. As such we propose that improved methods are required to assess ABA binding and to validate the published results.

JR received a PhD scholarship from Enterprise NZ.

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**ICAR4061**

ARABIDOPSIS RTE1 SPECIFICALLY PROMOTES ETR1 ETHYLENE RECEPTOR SIGNALING VIA THE ETHYLENE-BINDING DOMAIN

Category: Signal Transduction

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Ethylene is an important regulator of plant growth, development and responses to environmental stresses. *Arabidopsis* perceives ethylene through five homologous receptors, which negatively regulate ethylene responses. *RTE1*, a novel gene of unknown molecular function conserved in plants, animals and some protists, was recently identified as a positive regulator of the ethylene receptor ETR1. *RTE1* is required for wild-type ETR1 signaling and over-expression of *RTE1* confers reduced ethylene sensitivity that partially depends on *ETR1*. Here, we show that ETR1 co-localizes with RTE1 in the ER and unexpectedly the Golgi membrane. Consistent with this, the pattern of *RTE1* gene expression to a certain extent correlates with previously described sites of *ETR1* expression and ethylene responses. Furthermore, we investigate the ability of the *rte1* mutant to suppress a variety of *etr1* ethylene-binding domain missense mutations, all of which confer dominant ethylene insensitivity due to constitutive signaling. We uncover two classes of *etr1* alleles, *RTE1*-dependent and *RTE1*-independent, neither of which correlate with the positions of the mutations in the ethylene-binding domain nor the ethylene binding ability of the mutant ETR1 forms. We find that *ETR1* is distinct from the other four ethylene receptor genes because *RTE1*-dependent mutations only confer insensitivity in *ETR1* and not in the other ethylene receptors when the same mutations are introduced. In contrast, the *RTE1*-independent *ETR1* insensitive mutations do give insensitivity in the closest receptor to *ETR1*, *ERS1*. Based on the existing structure/function model of ETR1 signaling, we deduce that *RTE1* specifically promotes ETR1 signaling via conformational changes in a unique way that does not occur in other ethylene receptors. These results highlight the importance and uniqueness of ETR1 signaling conformation(s) with respect to the other ethylene receptors.

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**ICAR4062**

ANALYSIS OF STO AND STH FUNCTION IN A. THALIANA.

Category: Signal Transduction

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The Salt Tolerance protein (STO) has been recently described as a major modulator of light signalling during photomorphogenesis (Indorf *et al.*, 2007). STO and its homologue STH, are double B-box Zn finger proteins and interact with COP1 in the yeast 2-hybrid system (Holm *et al.*, 2001).

Analysis of STO gain- and loss-of-function mutants revealed that STO functions as negative regulator in light dependent inhibition of hypocotyl elongation under continuous red, far-red, and blue light (Indorf *et al.*, 2007). STO is tightly regulated at the mRNA and protein level by light and, in addition, COP1 is responsible for degradation of the protein in the dark. The function of STH in *A. thaliana* is still an open question. To uncover its function we have isolated STH T-DNA insertion lines and generated overexpressor Arabidopsis plants. In this work we are presenting new insights in the function of both proteins and also aspects on their light dependent regulation.

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**ICAR4063**

## INTEGRATION OF PLANT STRESS, SUGAR AND ENERGY SIGNALING BY THE ENERGY SENSOR PROTEIN KINASES KIN10/11

Category: Signal Transduction

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The control of energy homeostasis is vital to plant growth and survival, but how exactly plants sense and adapt to diurnal darkness and unpredictable environmental conditions that compromise photosynthesis and respiration, is still largely unknown. Using a combination of cellular and functional genomics screens, we identified the *Arabidopsis thaliana* SnRK1 protein kinases KIN10 (At3g01090) and KIN11 (At3g29160) as evolutionarily conserved energy sensors, controlling convergent reprogramming of transcription in response to seemingly unrelated stress conditions that deplete sugar and energy supplies. These kinases target a broad array of genes to promote catabolic and suppress anabolic activities, at least in part through specific G-box binding bZIP transcription factors. Transgenic KIN10 overexpression confers enhanced starvation tolerance and lifespan extension, and alters architecture and developmental transitions, while double kin10 kin11 deficiency abrogates the transcriptional switch in darkness and stress signaling, and impairs starch mobilization and growth. In conclusion, our studies uncovered surprisingly pivotal and conserved roles for KIN10/11 in linking stress, sugar and developmental signals to regulate plant metabolism, energy balance, growth and survival. Future studies will focus on elucidating the exact molecular mechanisms involved in KIN10/11 signalling.

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**ICAR4064**

## CONTROL OF CIRCADIAN FUNCTION AND PHOTOPERIODIC FLOWERING BY COP1 AND ELF3

Category: Signal Transduction

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COP1 is a RING-type E3 ubiquitin-ligase that targets positive regulators of photomorphogenesis to degradation through the 26S proteasome in the dark. However, *Arabidopsis cop1* weak mutants exhibit not only constitutive de-etiolation but also other developmental abnormalities, including defects in circadian gene expression and flowering time, suggesting that COP1 functions as a general developmental regulator. Thus, *cop1* mutants shorten the period of clock-controlled gene expression and, unlike wild type plants, flower with the same number of rosette leaves in short day (SD) as in long day (LD), indicating that COP1 plays an important role in photoperiodic flowering by repressing early flowering in SD. Accordingly, it has been recently shown that, under SD conditions, COP1 mediates dark-triggered degradation of CO protein, a key inducer of the floral transition. Here, we show that COP1 control of flowering time is also related to its function in the regulation of light-resetting of the clock. This process allows plants to perceive seasonal changes in photoperiod and is negatively regulated by ELF3. In this context, we present genetic data showing that *COP1* acts close to *ELF3* to mediate day length signaling within the photoperiod flowering pathway. In line with this, we found that COP1 physically interacts with ELF3. Moreover, we show that ELF3 allows COP1 interaction with GI, which positively controls clock resetting by light and photoperiodic flowering. Indeed, both COP1 and ELF3 induce degradation of GI in vivo. We propose a model in which ELF3 acts as a substrate adaptor that enables COP1 to modulate light input signal to the circadian clock through targeted destabilization of GI.

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**ICAR4065**

## PHOSPHOLIPID-DERIVED SIGNALING MEDIATED BY PHOSPHOLIPASE A IN PLANTS

Category: Signal Transduction

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Evidence is rapidly mounting that phospholipid-derived molecules act as secondary signal messengers in plant signaling. Recent studies have significantly advanced our understanding of phospholipase A (PLA) signaling in plant growth and development, and abiotic and biotic stress responses. The PLA superfamily includes a broad range of enzymes defined by their ability to catalyze the hydrolysis of the ester bonds of their substrate phospholipids. The hydrolysis products of these reaction (free fatty acids and lysophospholipids) have recently been found to perform many important

roles. Here, I describe the current classification of various PLAs that have been identified in plants, including *Arabidopsis*, and recent advances in our understanding of how these PLAs are involved in various cellular signaling pathways.

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**ICAR4066**

## LONG DISTANCE SIGNALING IN SYSTEMIC ACQUIRED RESISTANCE

Category: Signal Transduction

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The activation of systemic acquired resistance (SAR), which confers resistance against a broad-spectrum of pathogens, requires the translocation of a factor(s) from the pathogen-inoculated organ to the distal organs where SAR is manifested. The identity of this long distance translocated SAR activating factor has been elusive. Our studies indicate that petiole exudates (PeXs) collected from *Arabidopsis thaliana* leaves inoculated with an avirulent (Avr) pathogen contain a SAR promoting activity that enhances resistance against bacterial and fungal pathogens in *Arabidopsis*, wheat and tomato plants (Chaturvedi et al. 2008). Activation of SAR requires this SAR promoting activity in *Arabidopsis* Avr PeX in conjunction with the DIR1 lipid transfer protein. Genetic studies demonstrate that a plastid glycerolipid is required for accumulation of this SAR promoting activity in Avr PeX. Plastid glycerolipids provide polyunsaturated fatty acids for oxylipin synthesis, and a recent study implicated a role for the oxylipin jasmonic acid (JA) in SAR (Truman et al. 2007). However, our studies indicate that JA is not a constituent of the SAR promoting activity in *Arabidopsis* Avr PeX. Thus, JAs involvement in SAR may be subsequent to the perception of this SAR activating factor present in Avr PeX by the distal leaves.

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**ICAR4067**

## IDENTIFICATION OF KINASE-SUBSTRATE PAIRS INVOLVED IN PLANT INNATE IMMUNE RESPONSES

Category: Signal Transduction

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Plants defend themselves from a majority of potential pathogens. Although genetic screens have been successful in identifying proteins involved in the perception of microbial elicitor molecules, little is known about how perception is translated via plant signal transduction pathways to elicit plant defense responses. Protein phosphorylation plays a critical role in this process. Previous phosphoproteomic screens have identified many proteins differentially phosphorylated after treatment of *Arabidopsis thaliana* with bacterial elicitors. The goal of the present work is to identify kinases phosphorylating these putative substrates. We started with targeted in vitro kinase studies using eight kinases known to be activated during/involved in microbial elicitation responses: the receptor of flagellin, AtFLS2; another receptor-like kinase required for perception of multiple microbial elicitors, AtBAK1; three MAP kinases AtMPK3, AtMPK4, and AtMPK6; the AGC kinase AtOXI1; and two other kinases AtPDK1 and AtPTI1-2. One of the nine substrates used in the screen is a new substrate of AtMPK3 and AtMPK6 and none are substrate of AtMPK4. We are in the process of testing the remaining kinases. Proteins which are not substrates of these kinases will be used for traditional protein purification to isolate the kinase activity. Establishing of kinase-substrate pairs will be followed by identification of the phosphorylation site(s) of the substrate and determination of residues surrounding the phosphorylation site contributing to kinase specificity. Further characterization of the novel kinase(s) will involve reverse genetic studies using T-DNA tagged lines for biological characterization of their role in defense signaling pathways.

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**ICAR4068**

## 26S PROTEASOME-DEPENDENT CONTROL OF CYTOKININ SIGNALING

Category: Signal Transduction

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Early cytokinin signaling involves the activation of type-B *Arabidopsis* response regulators (ARRs) that promote cytokinin responses. Type-B ARRs also promote the up-regulation of type-A ARR transcripts that encode repressors of cytokinin action, thus providing feedback-inhibition control. The 26S proteasome mutant *rpn12a-1* has reduced cytokinin sensitivity in various growth response assays suggesting the stabilization of a repressor of cytokinin action. However, type-A ARR induction is enhanced in this mutant, indicating that loss of proteasome function also leads to the stabilization of an activator of early cytokinin signaling. This was confirmed by the identification of ARR1, a type-B ARR, as a proteasome target. The ARR1 protein is destabilized by cytokinin treatments, a feature common to the activation of many transcriptional activators. In addition, the effects of increased ARR1 expression on type-A ARR induction are enhanced in *rpn12a-1*. The enhanced activity of ARR1 in a proteasome mutant background as well as its proteasome dependent proteolysis implies the existence of a ubiquitin ligase that catalyzes ARR1 degradation. Progress in the identification of the ligase that targets ARR1 for degradation will be presented at the meeting.

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**ICAR4069**

## TANDEM AFFINITY PURIFICATION OF MAP KINASES INVOLVED IN DEFENCE SIGNALLING

Category: Signal Transduction

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The mitogen-activated protein (MAP) kinases play a central role in the signal transduction and control of different outputs in response to diverse stimuli (Ichimura 2000, Zhang 2006, Tena 2001). They are organised in distinct networks, which can share multiple signalling components. Pathway specificity is realised by structures like scaffolds, anchors or adaptor proteins (Burack and Shaw 2000 Park 2003, Schwartz and Madhani 2006). In our laboratory, we analyse such protein-kinase interactions with mass spectrometry (MS) and Tandem Affinity Purification (TAP, Puig 2001) of tagged MAP kinases involved in plant-pathogen interactions.

The existence of pre-formed MAP kinase complexes in *Arabidopsis thaliana* was indicated by size exclusion chromatography and could be demonstrated for both untagged and tagged MAP kinases, MPK3 and MPK6. The TAP-MAP kinases also exhibit full protein functions. The dwarf phenotype of *mpk4* (Petersen 2000) could be complemented with a TAP-tagged MPK4 fusion protein. In an activity assay, MPK6 and MPK3 exhibited phosphorylation activity after flg22 treatment. These data indicate integration of the tagged kinases into physiologically relevant pathways. The Tandem Affinity Purification was performed with leaf material from stable transgenic plants. It could be optimised for the proteins MPK3-TAP and MPK6-TAP. RuBisCO and additional abundant contaminants could be reduced by prefractionation. The purified proteins are being analysed by 1D-PAGE MALDI-TOF-MS or shot-gun LC-MS/MS.

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## ICAR4070

### DISSECTION OF THE ATMYB61 REGULATORY CIRCUIT BY CHEMICAL GENETICS

Category: Signal Transduction

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As sessile organisms, plants have evolved mechanisms that enable the developmental plasticity that allows them to respond to fluctuations in their environment. *Arabidopsis thaliana* MYB61, a member of the R2R3-MYB family of transcription factors, is involved in the regulation of phenotypic plasticity in response to environmental cues, such as light and sucrose availability. AtMYB61 regulates several key aspects of plant growth and development, including vascular architecture, secondary root formation, stomatal aperture, and flowering time. Though AtMYB61 has been shown to play an important role in the control of these traits, the components of the regulatory circuitry that reside upstream and downstream of this transcription factor remain to be elucidated. One method that can be used to dissect a regulatory circuit is to disrupt the pathway using small molecules, and then to relate this disruption to the action of genes in the circuit. This approach, known as chemical genetics, is particularly amenable to high throughput analysis, where thousands of different chemicals are screened with relative ease. Recently, we employed a chemical genetics approach involving a screen of over 2000 different compounds that differentiated between wild-type *A. thaliana* seedlings relative to seedlings containing a myb61 loss-of-function mutation. Interestingly, five different chemicals belonging to the sulfonamide family of compounds had a deleterious effect on dark-mediated elongation growth in wild-type seedlings, while the myb61 T-DNA insertion mutants were relatively unaffected by the presence of the sulfonamides. Here we report on the initial chemical screen, and the progress we have made toward using the screen to characterise additional components of the AtMYB61 regulatory circuit. We propose that sulfonamide insensitivity found in the myb61 mutant seedlings can be used as a starting point to dissect the molecular components involved in both the upstream regulation and downstream manifestation of AtMYB61 activity

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## ICAR4071

### FUNCTIONAL CHARACTERIZATION OF GTP-BINDING PROTEINS OF OBG-ERA FAMILY IN ARABIDOPSIS

Category: Signal Transduction

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The assembly and maturation of ribosomes is an important step in translational regulation and involves various non-ribosomal proteins. Small GTP-binding proteins belong to *Obg-Hflx* and *TrmE-Era* superfamilies define a class of non-ribosomal proteins that are involved in several steps of ribosomal maturation. These small GTP-binding proteins are conserved among bacteria, archaea and eukaryotes. *Arabidopsis* genome contains 18 *Obg-Hflx* and *TrmE-Era* superfamily genes. The number of these genes is significantly higher in plants compared to the bacteria, yeast and human, suggesting their divers roles in plant cells. Subcellular localization and bioinformatics analyses revealed that the archaea-derived Nog1 and DRG proteins target to nucleus and cytoplasm, respectively, while the bacteria-derived proteins function as GTPases in chloroplasts and/or mitochondria. The increase in the number of the *Obg-Era* family genes may have some relation to the evolution of plant organelles. In yeast, Nog1 has been reported to be involved in the ribosome biogenesis mediated by TOR. Interestingly, NOG1 is a nucleolus protein that forms a complex with other nucleolus proteins in

*Arabidopsis*, suggesting that Nog1 may also regulate ribosome maturation in plant nuclei. Furthermore, we found that chloroplast Obg is also localized in the chloroplast nucleoids and its localization is regulated in a cell- and tissue-specific manner.

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**ICAR4072**

HIGH-THROUGHPUT DETECTION OF UBIQUITINATION AND POLY-UBIQUITINATION BASED ON WHEAT CELL-FREE PROTEIN PRODUCTION SYSTEM  
Category: Signal Transduction

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Protein ubiquitinations play crucial roles for numerous cellular processes such as cell growth, development, and response to diverse biotic and abiotic stresses. The ubiquitination is mainly mediated by three enzymes, ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2) and ubiquitin ligase (E3). The *Arabidopsis* genome research found >1,500 proteins concerned with the ubiquitination. However, the function of their proteins on the ubiquitination process has been obscure. The comprehensive biochemical analysis of these proteins therefore would be conducive to understanding of biological regulation by the protein ubiquitination. Here we developed a novel method for *in vitro* ubiquitination analysis based on high-throughput wheat cell-free protein expression system and luminescent detection system (AlphaScreen<sup>TM</sup>). For the analysis, 30 E2 and 11 E3 (9 RING and 2 HECT) proteins in Riken *Arabidopsis* full length (RAFL) cDNAs were prepared by the wheat cell-free system with biotinylated or FLAG-tagged form on N terminal and used for *in vitro* analysis without any purification. By using the method, activity of 29 E2 proteins and ubiquitination of a HECT E3 protein were detected. In addition, poly-ubiquitin chain formation of AtUBC22 in E3 independent manner and of CIP8, a RING-type E3 ligase, was detected by luminescent assay using biotinylated and FLAG-ubiquitins. Furthermore, the method found poly-ubiquitination of a novel clone by screening in a RING group including CIP8. Interestingly, the *in vitro* ubiquitination assay were carried out without addition of exogenous E1 and/or E2 proteins, suggesting that ubiquitin pathway of at least plant could utilize endogenous E1 and E2s in the wheat cell-free system. In conclusion, we successfully developed the novel method for analysis of a large number of ubiquitination and poly-ubiquitination.

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**ICAR4073**

THE DOF TRANSCRIPTION FACTOR DAG1 REPRESSES PHYB-DEPENDENT SEED GERMINATION THROUGH REGULATION OF GA BIOSYNTHESIS  
Category: Signal Transduction

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Light-mediated seed germination is a complex process where a key role is also played by GAs and ABA. It's known that phytochrome B acts on this process through transcriptional regulation of both GA biosynthetic genes (*AIGA3ox1-2*) and GA deactivation genes (*AIGA2ox2*). Few factors involved in PHYB- and GA-mediated seed germination have been already identified. Among these, the bHLH transcription factors PIL5 (PHYTOCHROME INTERACTING factor LIKE 5) is known to play a pivotal role in this process. PIL5 is a PHYB- and PHYA-interacting protein, characterized as a negative regulator of seed germination in *Arabidopsis*. *PIL5* inactivation can complement to a certain extent the lack of germination of the *phyB* null mutant under R light. In seeds, PIL5 activates transcription of GAI and RGA, two of the five DELLA proteins that function as negative regulators of GA responses. Previous data showed that inactivation of the *Arabidopsis* transcription factor DOF AFFECTING GERMINATION 1 (DAG1), makes seed germination more sensitive to both phytochrome B (PHYB) and gibberellins (GA). *dag1* mutant seeds require less red fluence and a lower GA concentration than wild type to germinate. In order to unravel the role of DAG1 in the phyB/PIL5 signaling to seed germination, we produced double mutant of *dag1* with mutants of several regulators of this pathway. The phenotypic and molecular analysis of these lines is being performed. Seeds of these double mutant lines are being analyzed for phyB-mediated seed germination, as well as for other processes regulated by phyB, as hypocotyl elongation and cotyledon expansion. Data arising from these analyses and a putative model of DAG1 function will be presented.

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**ICAR4074**

BRASSINOSTEROIDS AND AUXIN WORK TOGETHER TO PROMOTE GROWTH

Category: Signal Transduction

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Plant development involves the integration of many diverse signals, and plant hormones play a critical role in regulating responses to these stimuli. We are studying the striking interconnection between the hormones brassinosteroids (BRs) and auxin during seedling development. Our recent work on Auxin Response Factor 2 (ARF2) suggests a potential molecular mechanism for BR:auxin interaction. Yeast 2-hybrid analysis and immunoprecipitation indicate that ARF2 can interact with BIN2, a BR-regulated kinase. Using *in vitro* DNA binding assays, we have shown that phosphorylation of ARF2 by BIN2 greatly reduces DNA binding by ARF2. In addition, dark-grown *arf2* mutants show resistance to brassinazole (BRZ), a BR biosynthetic inhibitor. Based on these results, we propose that ARF2 negatively regulates auxin:BR-responsive genes and contributes to the synergistic increase in gene expression observed when both hormones are present.

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**ICAR4075**

DISCRETE AND ESSENTIAL ROLES OF THE MULTIPLE DOMAINS OF ARABIDOPSIS FHY3 IN MEDIATING PHYTOCHROME A SIGNAL TRANSDUCTION  
Category: Signal Transduction

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Phytochrome A (phyA) is the primary photoreceptor for mediating various far-red light induced responses in higher plants. We recently showed that Arabidopsis FHY3 and FAR1, a pair of homologous proteins sharing significant sequence homology to *Mutator*-like transposases, act as novel transcription factors essential for activating the expression of *FHY1* and *FHL*, whose products are required for light-induced phyA nuclear accumulation and subsequent light responses. FHY3, FAR1 and *Mutator*-like transposases also share a similar domain structure, including an N-terminal C2H2 zinc-finger domain, a central putative core transposase domain, and a C-terminal SWIM motif. In this study, we performed a promoter-swapping analysis of *FHY3* and *FAR1*. Our results suggest that the partially overlapping function of *FHY3* and *FAR1* entails divergence of their promoter activities and protein sub-functionalization. To gain a better understanding of the molecular mode of FHY3 function, we performed a structure-function analysis, using site-directed mutagenesis and transgenic approaches. We show that the conserved N-terminal C2H2 zinc-finger domain is essential for direct DNA binding and biological function of FHY3 in mediating light signaling, whereas the central core transposase domain and C-terminal SWIM domain are essential for the transcriptional regulatory activity of FHY3 and its homodimerization or hetero-dimerization with FAR1. Further, the ability to form homo- or hetero-dimers correlates with the transcriptional regulatory activity of FHY3. Together, our results reveal discrete roles of the multiple domains of FHY3 and provide functional support for the proposition that FHY3 and FAR1 represent transcription factors derived from a *Mutator*-like transposase(s).

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#### ICAR4076

#### SLAC1 IS REQUIRED FOR PLANT GUARD CELL S-TYPE ANION CHANNEL FUNCTION IN STOMATAL SIGNALLING

Category: Signal Transduction

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Stomatal pores, formed by two surrounding guard cells in the epidermis of plant leaves, allow gas exchange in leaves and control transpirational water loss to the atmosphere. Stomata restrict the entry of ozone – the major air pollutant with increasingly negative impact on crop yields. The aperture of stomatal pores is regulated by transport of osmotically active ions and metabolites across guard cell membranes. Despite the vital role of guard cells in controlling plant water loss and ozone sensitivity, genes encoding some of the major regulators of stomatal movements remain unknown. Guard cell anion channels have been proposed to function as important regulators of stomatal closure and to be essential in mediating stomatal responses to physiological and stress stimuli. However, genes encoding membrane proteins mediating guard cell anion efflux have not been identified to date. Here we report the mapping and characterization of an ozone sensitive Arabidopsis mutant, *slac1*. We show that *SLAC1* (*SLOW ANION CHANNEL-ASSOCIATED 1*) is preferentially expressed in guard cells and encodes a distant homolog of fungal and bacterial dicarboxylate/malic acid transport proteins with 10 predicted transmembrane domains. *SLAC1* is essential for stomatal closure in response to ABA, ozone, light/dark transitions, humidity change, Ca<sup>2+</sup>, H<sub>2</sub>O<sub>2</sub>, and NO. Mutations in *SLAC1* abolish cytosolic Ca<sup>2+</sup>- and ABA-activated slow (S-type) anion channel currents, but do not affect rapid (R-type) anion channel or ABA-activated Ca<sup>2+</sup> channels. A low homology of SLAC1 to bacterial and fungal malate transporters and the disruption of S-type malate-permeable anion channels in *slac1* mutants suggest a vital role for the plasma membrane protein SLAC1 in the function of S-type anion channels.

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#### ICAR4077

#### TEMPORAL SEPARATION OF FREE SA SYNTHESIS FROM ITS MODIFICATION AND ACTIVATION OF GENE EXPRESSION ALLOWS FOR DISSECTION OF SA METABOLISM AND RESPONSE

Category: Signal Transduction

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The phytohormone salicylic acid (SA, 2-hydroxybenzoic acid) is best known for its role as a key regulator of plant defense against pathogens. It is synthesized in response to pathogens and required for the widespread transcriptional reprogramming of the plant, resulting in local and systemic acquired resistance responses. Furthermore, SA is synthesized in response to abiotic stresses such as ozone, UV-C, cold, and heat, and it impacts the timing of developmental transitions such as flowering and senescence. Understanding the mechanisms of action of SA requires knowledge of SA homeostasis – controls over the active form of SA – and temporal or spatial resolution of free and modified forms of SA. We have developed an inducible Arabidopsis system that allows us to separate free SA synthesis from formation of the dominant modified form of SA, its glucose conjugate, and from the expression of SA-dependent genes. Using this system, we observe a biphasic pattern of gene expression for the SA biosynthetic gene *AtICS1*, with AtICS1 protein expression paralleling that of the transcript. The initial AtICS1 peak of expression corresponds with free SA synthesis, whereas formation of the SAG conjugate and expression of SA-dependent genes is associated with the second peak in expression. We are using this system to dissect the complexity of SA metabolism and response by identifying components and processes associated with these distinct phases.

Supported in part by NSF2010 grant MCB-0420267 to MCW.

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**ICAR4078**

ARABIDOPSIS PLANT U-BOX (PUB) E3 UBIQUITIN LIGASES HAVE DIVERSE REGULATORY ROLES DURING PLANT GROWTH AND DEVELOPMENT

Category: Signal Transduction

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The ability of plants to sense and respond to environmental and endogenous signals is essential to their growth and development. Ubiquitin-mediated proteolysis has emerged as an important process in regulating plant signaling pathways and responses to environmental or developmental cues. Many of the components required for this process have been expanded in plant genomes, including the substrate targeting E3 ubiquitin ligases. The focus of this research is on PUB-ARM repeat E3 ligases for which a 41-member family exists in *Arabidopsis*. The U-box family of E3 ligases is one of the more recently identified players in this ubiquitination process, and as such, there is little knowledge on the precise regulatory roles of these proteins in *Arabidopsis*. In plants, roles for this family have been identified in self-incompatibility, plant defense responses, and hormone responses.

A number of PUB-ARM T-DNA insertion lines were screened for altered plant growth and development, and no readily discernable changes were observed. With closer inspection of selected insertion lines, interesting phenotypes emerged. Insertion lines for *PUB44* showed altered growth during seed germination and seedling growth. While *pub44* seedlings grew very poorly, seeds from *pub44/+* plants had altered germination in the presence of ABA. Interestingly, insertion lines for the closely related *PUB43* did not show altered seedling growth, but *pub43* seeds showed similar germination effects in the presence of ABA. As well, crosses between insertion lines for the closely related *PUB19* and *PUB18* uncovered a distorted segregation ratio. No *pub18/pub19* plants were ever observed among the F<sub>2</sub> generation and selfed *pub18/+ pub19/+* plants never yielded *PUB18/+ PUB19/+* progeny, but instead showed a ratio that did not reflect independent gene assortment. Thus, while *PUB44* appears to have a role in regulating cell death and ABA responses, *PUB19* and *PUB18* appear to have a role during gametophytic transmission.

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**ICAR4079**

IDENTIFICATION OF FUNCTIONALLY IMPORTANT AMINO ACIDS FOR ARABIDOPSIS CYCLIC NUCLEOTIDE-GATED ION CHANNELS USING THE CHIMERIC ATCNGC11/12 GENE

Category: Signal Transduction

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The chimeric *Arabidopsis* cyclic nucleotide-gated ion channel, ATCNGC11/12, was utilized in a structure-function study of plant cyclic nucleotide-gated ion channels (CNGCs). ATCNGC11/12 was identified as the cause of multiple pathogen resistance responses in the *Arabidopsis* mutant constitutive expresser of PR genes22 (*cpr22*) (Yoshioka et al., 2006). A genetic screen for mutants that suppress *cpr22*-conferred phenotypes identified two intragenic mutants (#58 and #73) with substitutions in the cyclic nucleotide binding domain (CNBD) of ATCNGC11/12. #58 possesses an arginine to cysteine (R557C) substitution in the alpha-C helix, while #73 contains a glutamate to lysine substitution (E519K) at the beginning of the eighth beta-sheet of the cyclic nucleotide binding domain in ATCNGC11/12. Both #58 and #73 mutants are morphologically identical to wild type plants and have lost all *cpr22*-related phenotypes including spontaneous cell death formation and enhanced pathogen resistance. Heterologous expression using K<sup>+</sup> and Ca<sup>2+</sup>-uptake deficient yeast mutants revealed that E519 is important not only for ATCNGC11/12 channel function, but also for its wild type channel protein, ATCNGC12. Interestingly, both assays showed that the R557C mutation does not abolish channel function, suggesting that R557 is not essential for basic channel function itself, but may be involved in the constitutive active nature of ATCNGC11/12. Computational structural modeling and in vitro cAMP binding assays suggest that E519 is a key residue for structural stability of ATCNGCs that contributes to the interaction of the CNBD and the C-linker domain, rather than for binding to cAMP. On the other hand, R557 is predicted to be involved in intersubunit interactions between the CNBD and the N-terminal domain of CNGCs. Furthermore, a mutation in the alpha-subunit of the human cone receptor CNGA3 (a human CNGC) that causes total color blindness aligned well to the position of E519 in ATCNGC11/12. This suggests that the suppressor screening of ATCNGC11/12 is a useful tool to discover important residues not only for plant CNGCs, but also for CNGCs in general.

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**ICAR4080**

A MITOGEN-ACTIVATED PROTEIN KINASE CASCADE POSITIVELY REGULATES LATERAL ROOT FORMATION IN ARABIDOPSIS

Category: Signal Transduction

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Although they are sessile, plants show considerable plasticity during postembryonic development, enabling them to adjust their overall structure in response to different environmental conditions. Proper root architecture is important not only to provide the physical anchoring of plants, but to efficiently absorb water and nutrients as well. Signaling molecules are therefore essential to transmit the environmental signals to the plants, leading to the appropriate formation of discrete plant organs, such as lateral roots. Here we used reverse genetic approaches to study the function of mitogen-activated protein kinases, a protein family conserved through all eukaryotes, in lateral root formation in *Arabidopsis*. By using RNA interference technique to conditionally reduce the expression of *Arabidopsis* Mitogen-Activated Protein Kinase Kinase 6 (AtMKK6) and Mitogen-Activated Protein Kinase 13 (AtMPK13), we found that the reduction of the transcripts of these two genes was correlated with the reduction of the lateral root formation. Consistent with these observations, we found that *Promoter<sub>AtMKK6</sub>::GUS* and *Promoter<sub>AtMPK13</sub>::GUS* transgenic reporter lines showed high expression in the vascular tissue that connects the primary and lateral roots. In addition, semi-quantitative RT-PCR showed that *AtMKK6* and *AtMPK13* share similar expression pattern throughout various *Arabidopsis* tissues, while yeast-two hybrid assays revealed a physical interaction of AtMKK6 with AtMPK13. Taken together, we propose that AtMKK6-AtMPK13 cascade positively regulates lateral root formation in *Arabidopsis*.

# **20th** International Conference on Arabidopsis Research

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## BIOTIC INTERACTIONS

### ICAR501

#### ANALYSES OF ARABIDOPSIS RESPONSE TO THIRPS FEEDING

Category: Biotic Interactions

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We analyzed the interaction between Arabidopsis and western flower thrips (*Frankliniella occidentalis*), which are one of the most serious insect pests of cultivated plants. Thrips use their mouthparts to pierce plant cells and suck out the contents. This feeding mode differs from those of the well-studied caterpillars or aphids, and thrips can also act as virus vectors. Because the emergence of insecticidal resistance, it is difficult to control this species with insecticides. Therefore, elucidation of the molecular mechanism of the plant response to the feeding of western flower thrips is important for the development of new methods to prevent damage.

We focused on the function of the immunity-related plant **hormones jasmonate (JA), ethylene (ET) and salicylic acid (SA) in the plant's response to thrip feeding**. Expression of the marker genes for each hormone response was induced by thrip feeding in wild-type (WT) plants. Further analyses in the hormone-related mutants *coi1-1* (JA insensitive), *ein2-1* and *ein3-1* (ET insensitive) and *eds16-1* (SA deficient) suggested the importance of these hormones in the plant response to feeding. Comparative transcriptome analyses suggested a strong relationship between thrip feeding and JA treatment, but not ET or SA treatment. The JA content of WT plants was significantly increased after thrip feeding. Moreover, *coi1-1*, but not *ein2-1*, showed lower feeding tolerance against thrips than the WT. Application of JA to WT plants before thrip feeding enhanced the plants' feeding tolerance. JA modulates several defense responses in cooperation with ET, but application of the ET precursor 1-aminocyclopropane-carboxylic acid had a marked negative effect on feeding tolerance. Our results indicate that JA plays an important role in Arabidopsis in terms of response to, and tolerance against, thrip feeding.

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### ICAR502

#### NAC TRANSCRIPTION FACTORS PLAY A NUMBER OF ROLES DURING AGE-RELATED RESISTANCE IN *ARABIDOPSIS THALIANA*

Category: Biotic Interactions

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As *Arabidopsis thaliana* matures it becomes more resistant to virulent *Pseudomonas syringae* pv. *tomato* (*Pst*) and *Hyaloperonospora parasitica*. This is known as Age-Related Resistance (ARR). ARR requires intercellular accumulation of salicylic acid (SA), and it is associated with flowering. To identify genes that are differentially expressed during ARR, microarray technology was employed to compare gene expression in leaves of mature *Arabidopsis* plants ecotype Columbia-0 (Col-0) that were *Pst*-inoculated and mock-inoculated (10 mM MgCl<sub>2</sub>) (Neumann and Cameron, unpublished). Mature T-DNA insertion mutants in some of the most highly up-regulated genes in the microarray, including two *NO APICAL MERISTEM CUP-SHAPED COTYLEDONS* (*NAC*) transcription factors, a *CYTIDINE DEAMINASE* (*CDA*), and a *UDP-GLUCOSYL TRANSFERASE* (*UGT*) were more susceptible to *Pst* to varying degrees than wild type Col-0. This suggests that *NAC1*, *NAC2*, *CDA*, and *UGT* are required for ARR. RT-PCR analysis demonstrated that expression of *NAC1* and *NAC2* was up-regulated more rapidly during ARR in mature plants compared to young plants that do not display ARR. To determine if *NAC1* and *NAC2* function in the same pathway during ARR, *nac1nac2* double mutant plants were produced. Mature *nac1nac2* plants were as susceptible to *Pst* as *nac1* and *nac2* single mutants, suggesting that the *NAC* genes function in the same pathway during ARR. Expression of *NAC7* was reduced in mature *nac2* plants during ARR, suggesting that *NAC2* is upstream and a positive regulator of *NAC7*. Weekly ARR assays and flowering data revealed that the ARR deficiency exhibited by *nac1* plants was associated with delayed flowering. After *nac1* plants underwent the transition to flowering they displayed enhanced resistance to *Pst* compared to Col-0. Taken together, this data suggests that *NAC1* may be a positive regulator of the onset of ARR and flowering, but a negative regulator of ARR after flowering.

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### ICAR503

#### CATERPILLAR SALIVA INFLUENCES PLANT DEFENSE RESPONSES VIA THE SYSTEMIC ACQUIRED RESISTANCE PATHWAY

Category: Biotic Interactions

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Plants target their defense responses against the herbivore which is feeding upon it. Caterpillars, on the other hand, may undermine these induced plant defenses. During feeding, caterpillar labial saliva is believed to affect cellular redox status, activating the systemic acquired resistance (SAR) pathway which antagonizes the induced resistance (IR) pathway. To further understand the basis of this mechanism, molecular and biochemical markers of IR were analyzed in *Arabidopsis thaliana* (L.) Heynh. genotypes limited in their ability to mount either IR or SAR. These plants were subject to herbivory by 4th instar caterpillars of the beet armyworm, *Spodoptera exigua* Hübner which either had intact or impaired salivary secretions. Transcript expression of genes encoding laccase-like multicopper oxidase (AtLMCO4 (polyphenol oxidase)) and defensin (AtPDF1.2) and activity of octadecanoid-associated anti-nutritive proteins, such as LMCO and trypsin inhibitor, showed salivary-specific patterns which were disrupted in the SAR-mutant plants. These results support the model that caterpillar saliva interferes with jasmonate-dependent plant defenses by activating the SAR pathway.

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### ICAR504

#### FUNCTIONAL ORIGIN OF RPW8-MEDIATED RESISTANCE

Category: Biotic Interactions

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**Abstract:** The *RPW8* gene isolated from *Arabidopsis thaliana* confers broad-spectrum disease resistance to *Erysiphe* spp. that cause powdery mildew disease on many plant species. Despite having an atypical R protein structure, *RPW8* activates a conserved salicylic acid (SA)-dependent

defense pathway leading to hypersensitive cell death in response to powdery mildew challenge. All of the tested *Arabidopsis* accessions to date contain three closely linked homologs of *RPW8* named *HR1*, *HR2*, and *HR3* at the same locus that do not seem to contribute to powdery mildew resistance. Recent evolutionary analyses revealed that *RPW8* most likely originated from an *HR3*-like progenitor gene by gene duplication and functional diversification. To understand how the resistance function of *RPW8* originated, we have recently conducted genetic analyses and found that 1) overexpression of *HR1*, *HR2* or *HR3* individually in susceptible accession Col-0 resulted in enhanced resistance to powdery mildew; 2) knocking out these genes individually appeared to cause enhanced susceptibility; and 3) unexpectedly silencing these three genes simultaneously results in enhanced resistance to the pathogen and some developmental phenotypes that resemble those caused by overexpression of 14-3-3 lambda, whose product interacts with *RPW8* in the yeast-two-hybrid. Our genetic data support the hypothesis that these *RPW8* homologs, particularly *HR3*, may play an important role in basal resistance via the conserved SA pathway against powdery mildew and perhaps other pathogens and suggest that there may be complicated genetic interaction between *HR1*, *HR2*, *HR3* and other defense components for regulation of plant basal resistance.

**Broader Impacts:** Control of crop diseases by using naturally evolved resistance mechanisms is crucial to developing a more sustainable agriculture and protecting our living environment. Findings from this work may provide a novel avenue to fine-tune broad-spectrum disease resistance in plants, thereby maximizing benefits and minimizing costs both associated with disease resistance.

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### **ICAR505**

#### CHARACTERISATION OF *ARABIDOPSIS* MUTANTS INVOLVED IN CUTICLE FORMATION

Category: Biotic Interactions

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The plant cuticle, composed of cutin, a lipid derived polyester, and cuticular wax, is a hydrophobic extracellular layer of lipidic nature that covers the aerial organs of the plants and protect them from biotic and abiotic stresses. *pec1* and *bre1* mutants of *Arabidopsis* (for *permeable cuticle 1* and *botrytis resistance 1*) were isolated in screens adapted from phenotypes of cutinase expressing plants. These mutants have characteristics of mutants having a permeable cuticle, as a high transpiration rate under drought conditions, internalisation of chemicals through the cuticle, and organ fusions. Those two mutants showed also an increased resistance to *Botrytis cinerea* due to a rapid production and release of antifungal compounds. *BRE1* was identified as *LACS2*, a gene that was previously postulated to be involved in cutin formation. In *bre1* the quantity of dicarboxylic acids, the major monomer components of the cutin in *Arabidopsis*, is reduced by five-fold and the TEM pictures show that the cuticular structure is missing. *PEC1* was identified as *AtPDR4*, a gene coding for an ABC transporter of the Pleiotropic Drug Resistance family. *pec1* had no changes in the composition in monomers of the cuticle, but in TEM the structure of the cuticle showed alterations. *AtPDR4* is strongly expressed in the epidermis of tissues in expansion, but also in specific other location during plant development. The nature of the substrat(s) of this transporter is unknown yet, but it seems to have an important role in the formation of the cuticle.

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### **ICAR506**

#### LOCALIZATION OF DIR1, A PUTATIVE LIPID-TRANSFER PROTEIN INVOLVED IN LONG DISTANCE SIGNALING DURING SYSTEMIC ACQUIRED RESISTANCE.

Category: Biotic Interactions

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Systemic Acquired Resistance (SAR) is induced by an initial priming infection in one leaf resulting in broad non-specific resistance throughout the plant to normally virulent pathogens. A key feature of SAR is the movement of mobile signals via the phloem and/or cell-to-cell, from the induced leaf to distant leaves where these signals are perceived and the plant becomes primed, such that upon subsequent infection the plant responds in a resistant manner. Studies in our lab using *dir1-1*, a SAR-defective mutant, indicate that *dir1-1* can perceive the SAR signal present in phloem sap exudates from wild type SAR-induced plants, but *dir1-1* exudates do not contain this signal. Western analysis demonstrated that DIR1 protein is present in phloem sap of SAR-induced wild type, but not *dir1-1* plants suggesting that DIR1, which encodes a putative lipid transfer protein, may be involved in transporting a lipid signal to distant tissues to establish SAR. Expression of DIR1:GFP in one leaf of *dir1-1*, using Agrobacterium-mediated transient transformation, followed by SAR induction, was sufficient to rescue the *dir1-1* SAR defect, suggesting that expression of functional DIR1 in the induced leaf is sufficient for a successful SAR response. If DIR1 is a SAR long distance signal, it should move from the induced leaf to distant leaves during SAR. This question is being addressed using Agrobacterium-mediated transient transformation experiments to observe DIR1:GFP movement during SAR. We are also developing a cucumber-Arabidopsis SAR model combining the excellent genetics of Arabidopsis and the robust SAR response of cucumber. Preliminary experiments indicate that phloem sap collected from SAR-induced cucumber leaves can rescue the *dir1-1* SAR defect, suggesting that similar SAR mobile signals exist in cucumber and Arabidopsis.

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### **ICAR507**

#### AN IMPORTANT ROLE OF A BAHD ACYL TRANSFERASE-LIKE PROTEIN IN PLANT INNATE IMMUNITY

Category: Biotic Interactions

Zheng, Zuyu Qualey, Anthony Fan, Baofang Dudareva, Natalia \*Chen, Zhixiang [zhixiang@purdue.edu](mailto:zhixiang@purdue.edu)

Salicylic acid (SA) is an important regulator of plant resistance to biotrophic and hemibiotrophic microbial pathogens. The enhanced susceptibility to *pseudomonas 1* (*esp1*) mutant in *Arabidopsis thaliana* exhibited enhanced susceptibility to both virulent and avirulent strains of the hemibiotrophic bacterial pathogen *Pseudomonas syringae*. Through positional cloning, the *ESP1* gene was isolated and found to encode a novel member of the BAHD CoA-dependent acyl transferase superfamily. Pathogen-induced accumulation of SA and expression of Pathogenesis-related (PR) genes were compromised in the *esp1* mutant. Application of exogenous SA could induce PR1 gene expression and restore disease resistance in the *esp1* mutant. These results suggest that *ESP1* functions upstream of SA and may be involved directly in the synthesis of a precursor or a regulatory molecule for SA

biosynthesis. Mutations of ESP1 or other genes important for SA accumulation or signaling conferred enhanced resistance to necrotrophic fungal pathogens *Botrytis cinerea* and *Alternaria brassicicola* in the Nossen-0 background but had little effect in the Columbia-0 background. These results suggest that there is natural variation among *Arabidopsis* ecotypes in the antagonistic crosstalk between defense signaling pathways against different types of microbial pathogens.

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**ICAR508****CONCERTED REGULATION OF *SCLEROTINIA SCLEROTIORUM CUTINASEA* AND *POLYGLACTURONASE1* DURING INFECTION**

Category: Biotic Interactions

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During infection, *Sclerotinia sclerotiorum* releases enzymes that sequentially degrade and penetrate different layers of the plant including the cuticular wax, middle lamella and cell wall. The leaf cuticular wax is comprised of C16 and C18 hydroxy fatty acids linked through ester bonds.

*Sclerotinia sclerotiorum CutinaseA* (*SscutA*) hydrolyses the ester bond releasing fatty acids and allowing wax layer penetration. *Polygalacturonase1* (*Sspg1*) degrades pectin in the subsequent middle lamella and cell wall releasing galacturonic acid.

*SscutA* and *Sspg1* are regulated in a concerted manner that is related to plant architecture. *SscutA* is expressed during the earliest stages of penetration to degrade the wax but not during infection. After cuticle penetration *Sspg1* is expressed as penetration of the middle lamella and cell wall is required. The concerted regulation of *SscutA* and *Sspg1* is partially due to surface sensing where contact with the leaf surface triggers high level of *SscutA* and to a less extent *Sspg1* expression. Pectin and low pH strongly induce the expression of *Sspg1* while galacturonic acid or neutral pH inhibit expression. Pectin, galacturonic acid, glucose or leaf wax do not induce the expression of *SscutA*. Only contact with the hard surface appears to be essential for the induction of *SscutA* expression. Calcium flux and cellular cAMP levels are involved in the regulation of *SscutA* and *Sspg1*. Blocking of calcium channels using lanthanum chloride or elevating the intracellular cAMP levels abolished expression of *SscutA* and *Sspg1* and led to a decrease in *S. sclerotiorum* pathogenicity. *SscutA* and *Sspg1* regulation is dependent upon the interplay between various stimuli and signaling pathways to ensure they are expressed at the right stage. Very little is known about these pathways though we have shown that cAMP and Ca<sup>2+</sup> signalling are possibly involved.

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**ICAR509****A RAPID BIOSENSOR-BASED QUANTIFICATION OF FREE AND TOTAL SALICYLIC ACID FROM PLANTS**

Category: Biotic Interactions

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The plant signaling molecule salicylic acid (SA) is involved in several processes including heat production, flowering, germination, and pathogen resistance. The most common methods for measuring plant SA employ HPLC and GC/MS. However, both are time consuming and require organic extraction resulting in the partial recovery of SA. Recently Huang *et al.* (1) developed an SA biosensor, named ADPWH<sub>-lux</sub>. This strain is derived from *Acinetobacter* sp. ADP1, and contains a salicylate-inducible *luxCDABE* operon in the chromosome, providing both substrate and catalyst for SA-induced luminescence. ADPWH<sub>-lux</sub> responds to SA, methyl-SA, and the synthetic SA derivative acetylsalicylic acid, but not to other structurally similar compounds or to glucose-conjugated SA (SAG). Quantification of free SA from crude extracts of TMV-infected Tobacco using ADPWH<sub>-lux</sub>, HPLC, and GC/MS yielded similar results, confirming the accuracy and precision of the biosensor (2). Here, we use ADPWH<sub>-lux</sub> to measure total plant SA after enzymatic hydrolysis of SAG in the crude extract, allowing the simultaneous measurement of free and total SA from small amounts of tissue. We also present a streamlined protocol that allows the measurement of free and conjugated SA from hundreds of samples per day. These modifications make SA quantification with ADPWH<sub>-lux</sub> a rapid and inexpensive alternative to HPLC and GC/MS. Application of this approach to the study of plant immunity is discussed.

1. Wei E. Huang, Hui Wang, Hongjun Zheng, Linfeng Huang, Andrew C. Singer, Ian Thompson and Andrew S. Whiteley. (2005) Environ Microbiol. 7: 1339-1348.

2. Wei E. Huang, Linfeng Huang, Gail M. Preston, Martin Naylor, John P. Carr, Yanhong Li, Andrew C. Singer, Andrew S. Whiteley and Hui Wang. (2006) Plant J. (2006) 46: 1073–1083.

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**ICAR5010****THE NANOVIRUS-ENCODED CLINK PROTEIN AFFECTS PLANT CELL CYCLE REGULATION THROUGH INTERACTION WITH THE RETINOBLASTOMA-RELATED PROTEIN**

Category: Biotic Interactions

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Nanoviruses, multicomponent single-stranded DNA plant viruses, encode a unique cell cycle link protein, Clink, that interacts with retinoblastoma-related (RBR) proteins. We have established transgenic *Arabidopsis thaliana* lines that conditionally express Clink or a Clink variant deficient in RBR-binding. By controlled induction of Clink expression we demonstrated the capacity of the Clink protein to alter the RBR function in vivo. We showed that transcription of both S-phase-specific and G2/M-phase-specific genes was upregulated depending on the RBR-binding proficiency of Clink. Concomitantly, ploidy levels increased in a substantial fraction of leaf cell nuclei. Also, leaf epidermis cells of transgenic plants producing Clink were smaller and more numerous, indicative of additional cell divisions in this tissue. Furthermore, cytogenetic analyses following induction of Clink

expression in mature leaves revealed the presence of metaphasic and anaphasic nuclei, clear evidence that Clink-mediated RBR inactivation is sufficient to induce quiescent cells to re-enter cell cycle progression and, at least for a fraction of them, to pass through mitosis. Expression of Clink had no effect on genes transcribed by RNA polymerase I and III suggesting that, in contrast to its mammalian homologue, *A. thaliana* RBR is not involved in the repression of polymerase I and polymerase III transcription. The results of these *in vivo* analyses firmly establish Clink as a member of the diverse class of multifunctional cell cycle modulator proteins encoded by small DNA viruses.

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**ICAR5011****TURIP MOSAIC VIRUS REQUIRES ARABIDOPSIS THALIANA CLASS II POLY(A)-BINDING PROTEINS FOR EFFICIENT MULTIPLICATION.**

Category: Biotic Interactions

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The poly(A)-binding protein (PABP) is an important translation initiation factor that binds to the polyadenylated 3' end of mRNA. We have previously shown that PABP2 interacts with the RNA-dependent RNA polymerase (RdRp) and VPg-Pro of *Turnip mosaic virus* (TuMV) within virus-induced vesicles. At least eight PABP isoforms are produced in *Arabidopsis thaliana*, three of which (PABP2, PABP4 and PABP8) are highly and broadly expressed, and probably constitute the bulk of PABP required for cellular functions. Upon TuMV infection, an increase in protein and mRNA expression of *PAB2*, *PAB4*, and *PAB8* genes was recorded. *In vitro* binding assays revealed that RdRp and VPg-Pro interact preferentially with PABP2 but are also capable of interaction with one or both of the other class II PABPs (i.e. PABP4 and PABP8). To assess if PABP is required for potyvirus replication, *A. thaliana* single and double *pab* knockouts were isolated and inoculated with TuMV. All lines showed susceptibility to TuMV. However, when precise monitoring of viral RNA accumulation was performed it was found to be reduced by 2.2 and 3.5 fold in *pab2 pab4* and *pab2 pab8* mutants, respectively, when compared to wildtype plants. PABP levels were most importantly reduced in the membrane-associated fraction in both of these mutants. TuMV mRNA levels thus correlated with cellular PABP concentrations in these *A. thaliana* knockout lines. These data provide further support for a role of PABP in potyvirus replication.

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**ICAR5012****ARABIDOPSIS TAO1 IS A TIR-NB-LRR PROTEIN THAT CONTRIBUTES TO DISEASE RESISTANCE INDUCED BY THE PSEUDOMONAS SYRINGAE EFFECTOR AVR B**

Category: Biotic Interactions

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Many phytopathogenic bacteria infect host plants using a type III secretion system for the purpose of promoting virulence. Type III effectors are injected into host cells, likely to contribute to pathogen virulence by modifying host targets. To counter pathogen challenge, plants have evolved R (Disease Resistance) proteins that are activated after recognizing the modification of these targets. The action of R proteins restricts bacterial growth and frequently triggers the Hypersensitive Response (HR), a type of localized programmed cell death. AvrB is an extensively studied type III effector that activates the R-protein RPM1. RPM1 is a nucleotide-binding site-Leucine rich repeat protein (NB-LRR) that has domain similarity to animal proteins of the NOD/CARD class. *TAO1* (Target of AvrB Operation) was initially isolated in a conditional screen for host gene products that interact genetically with AvrB in an *rpm1* background. *TAO1* encodes a nucleotide-binding-Leucine rich repeat protein of a different R-protein sub-class than RPM1. Interestingly, two recent studies have shown that there are likely separate mechanisms of AvrB perception by RPM1 and TAO1. Activation of both R-proteins is required for a full defense response to *Pto* DC3000(*avrB*). Therefore, our findings show that multiple NB-LRR proteins can perceive a single pathogen effector and that the function of these NB-LRR proteins additively contributes to disease resistance.

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**ICAR5013****RHIZOBACTERIA INCREASE ARABIDOPSIS THALIANA ROOT HAIR LENGTH VIA A MECHANISM REPRESSED BY THE ETHYLENE SIGNALLING PATHWAY.**

Category: Biotic Interactions

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Several plant growth-promoting rhizobacteria (PGPR) stimulate plant root hair elongation (Lopez-Bucio *et al.*, 2007 MPMI, 20:207). To decipher this mechanism, we inoculated *Arabidopsis thaliana* with various rhizobacteria. They were all able to increase root hair length (Desbrosses *et al.*/unpublished). As ethylene stimulates root hair elongation, we tested the implication of ethylene and its signalling pathway during *Arabidopsis*-rhizobacteria interaction.

We measured both ethylene emission and the expression levels of the *ACC synthase* (*ACS*) gene family upon rhizobacteria inoculation. Ethylene emission and *ACS* gene expression levels were not changed suggesting that ethylene is not directly involved. Consistent with that, all the *Arabidopsis* ethylene mutants tested present a root hair elongation in response to rhizobacteria inoculation. However, ethylene insensitive mutant *ein2* displays enhanced root hair elongation while ethylene mutants with a constitutive triple response (*ctr1*, *eto1*) have a reduced root hair elongation in response to rhizobacteria. These observations suggest that the ethylene signalling pathway indirectly represses root hair elongation induced by the rhizobacteria.

The ethylene signalling pathway is negatively regulated by two F-box proteins EBF1 and EBF2 that target EIN3 and EIL1 to the 26S proteasome (Potuschak *et al.*, 2003 Cell 115:679). We speculated that rhizobacteria could repress the ethylene signalling pathway by increasing the abundance of

EBF1 and EBF2 proteins. First results indicate that at least one of the bacteria increase the expression of *EBF1* and *EBF2* confirming the repression of the ethylene signalling pathway.

Future works will focus on the protein levels of *EBF1* and *EBF2* of *Arabidopsis thaliana* in response to the different rhizobacteria.

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#### ICAR5014

A PHAGE-DISPLAY-BASED METHOD FOR SELECTION OF MICROBE-BOUND PROTEINS REVEALS NEW FUNCTIONS OF ARABIDOPSIS PR PROTEINS

Category: Biotic Interactions

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The *phage-display* strategy has been extensively used for selection of high-affinity antibodies. This strategy allows for the expression of a large number of antibody variants on a phage surface and their selection on the bases of their physical affinity for a substrate. Here we took advantage of this technology to express *Arabidopsis thaliana* transcriptome under pathogen attack and to select plant proteins that were able to interact with microbes as a substrate.

By using a phage-display vector, we constructed cDNA libraries from *A. thaliana* following infection with *Pseudomonas syringae* pt DC3000 and *P. aeruginosa* PA14. Libraries represent 4x104 and 8x106 plant cDNAs that can be expressed as functional proteins fused to the capsid of a bacteriophage. The phage-displayed proteins and their corresponding genes were rescued by the ability of phagemic particles to bind living *Pseudomonas* cells, in a so-called "biopanning" selection. By performing several rounds of biopanning, the original library was enriched in *Pseudomonas*-bound clones that were isolated and sequenced. Comparison against TAIR transcripts database revealed several previously classified as pathogenesis-related proteins. One of the clones encodes for ATERF1, which has been annotated as a transcription factor able to "sense" ethylene pathway in response to pathogen attack. Our results argue for a role of ATERF1 as a direct target for the pathogen. The potential of this phage-display based strategy for wide exploration of plant-pathogen interactions is discussed.

This work has been supported by grants from the Spanish MEC (BIO2006-01299) and Basque Government (PI-2006-10).

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#### ICAR5015

SRFR1, A SUPPRESSOR OF EFFECTOR-TRIGGERED IMMUNITY, IS A CONSERVED TETRATRICOPEPTIDE REPEAT PROTEIN WITH SIMILARITY TO TRANSCRIPTIONAL REPRESSORS

Category: Biotic Interactions

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Plant immunity is mediated in large part by specific interactions between a host resistance gene and a pathogen avirulence (*avr*) gene. *avr* genes encode effectors for pathogen fitness, so this immune response is now named effector-triggered immunity (ETI). ETI needs to be controlled both positively and negatively, because excessive ETI is detrimental to the host. In previous work, we reported that mutations in *SUPPRESSOR of rps4-RLD1* (*SRFR1*), identified in a suppressor screen, enhanced *avrRps4*-triggered immunity. These mutants were fully susceptible to virulent *Pseudomonas syringae* pv. tomato strain DC3000 and did not constitutively express the *PATHOGENESIS-RELATED1* gene (1). Consistent with this, the response of *srfr1-1* plants to flagellin, an elicitor of basal resistance, was unaltered. In contrast, resistance in *srfr1-1* was dependent on the known positive ETI regulator *ENHANCED DISEASE SUSCEPTIBILITY1*, suggesting that *SRFR1* is a negative regulator of *avrRps4*-dependent ETI in *Arabidopsis*. *SRFR1* encodes a pioneer tetratricopeptide repeat (TPR) protein conserved between plants and animals. The SRFR1 TPR domain has significant sequence similarity to those of the *Saccharomyces cerevisiae* Ssn6 and *Caenorhabditis elegans* OGT (*O*-linked N-acetylglucosamine transferase) proteins, which function as transcriptional repressors. We therefore propose that SRFR1 functions in a transcriptional repressor complex that balances plant resistance activation and suppression. Characterization of *SRFR1* is on-going, and recent results will be presented.

(1) Kwon S.I., Koczan J.M. and Gassmann W. (2004) Plant J. 40, 366-375.

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#### ICAR5016

THE 14-3-3 NU PROTEIN REGULATES THE FLG22-INDUCED OXIDATIVE BURST

Category: Biotic Interactions

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Plants respond to pathogen attack by inducing multiple defense genes. To identify genes involved in defense responses, we used a reverse-genetics approach involving the inactivation of pathogen-induced plant genes. We found that plants with mutations in a gene encoding a 14-3-3 protein (nu isoform) allowed more growth of *Pseudomonas syringae* pv. *maculicola* ES4326 (*Pma* ES4326) than wild-type plants, demonstrating that the 14-3-3 nu protein has a role in defense. The 14-3-3 proteins play key functional roles in many critical physiological pathways including responses to several biotic and abiotic stresses. 14-3-3 proteins are known to regulate biological reactions by binding to phosphorylated client proteins. Some components of defense are triggered by recognition of Pathogen Associated Molecular Patterns (PAMPs) by plant receptor-like kinases. A peptide derived from bacterial flagellin, flg22, is recognized by the receptor FLS2. We found that the oxidative burst that occurs in response to flg22 perception is greatly reduced in 14-3-3 nu mutants, indicating a role for 14-3-3 nu in PAMP signaling. However, other defense responses tested appeared normal. The NADPH oxidase AtrobohD is responsible for the flg22-induced oxidative burst. AtrobohD mutants did not show enhanced susceptibility to *Pma* ES4326, indicating that the defect in the oxidative burst in 14-3-3 nu mutants is not the cause of the enhanced susceptibility phenotype. We are investigating proteins controlling AtrobohD function that may be clients of 14-3-3 nu.

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**ICAR5017**

ARABIDOPSIS DAP1, AN ASPARTYL PROTEASE PROMOTES DISEASE DEVELOPMENT DURING PATHOGEN ATTACK

Category: Biotic Interactions

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Plant defense involves coordinate activity of several defense genes. Here we describe preliminary characterization of *DEFENSE-ASSOCIATED PROTEASE 1 (DAP1)* gene of Arabidopsis, which encodes for an aspartic protease. *DAP1* is expressed at low levels and is not significantly induced in response to virulent and avirulent bacterial pathogens. However, several lesion mimic mutants constitutively express *DAP1* at high levels. *dap1* knockout plants are more resistant to virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*/DC3000) compared to the wild type Col-0. Transgenic plants overexpressing *DAP1* are more susceptible to *Pst*/DC3000. *DAP1* protein appears to localize to the apoplastic space. Our results suggest that *DAP1* is a negative regulator of defense and a likely target of pathogen virulence factors.

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**ICAR5018**

DUAL FUNCTION RECEPTOR KINASES INVOLVED IN DEVELOPMENT AND IMMUNITY

Category: Biotic Interactions

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Leucine-rich-repeat (LRR)-type receptors constitute key perception elements in innate immunity in plants and animals. From a total of 235 *Arabidopsis* LRR-RLK genes, 49 are transcriptionally up-regulated upon either pathogen infection and/or PAMP elicitation. Two of them were previously identified to contribute to plant developmental processes. One is the brassinosteroid receptor BRI1-associated kinase, BAK1, the second the phytosulphokine receptor PSKR1.

T-DNA insertions in the Col-0 *BAK1* gene resulted in a semi-dwarf growth phenotype due to its action in brassinosteroid signaling but also in stronger symptom development after infection with necrotrophic fungi and virulent bacterial pathogens.

BAK1-deficient plants develop spreading necrosis upon infection and this phenomenon is not dependent on brassinosteroid signaling. We proposed a novel, BL-independent function of BAK1 in plant cell death control that is distinct from its BL-dependent role in plant development. The dual function of BAK1 is most likely based on differential interaction with independent ligand-binding receptor molecules. This hypothesis is supported by the work of Chinchilla et al. who show that BAK1 interacts with FLS2 after flg22 perception. Our recent work focuses on the identification of BAK1 interacting proteins that are necessary for the regulation of plant cell death control. Potential interactors were identified by a yeast-two-hybrid screen and co-immunoprecipitations of stimulus dependent *in vivo* BAK1 complexes. For details on the biochemical and phytopathological analyses of these BAK1 interaction partners please refer to the presentation by Sandra Postel.

A second receptor kinase PSKR1 which perceives phytosulphokines, disulfated pentapeptides derived from a family of 5 precursor proteins, was previously identified to be involved in callus growth regulation. Receptor mutants show severe alterations in defense responses against bacterial and fungal pathogens. We are currently figuring out how PSKR1 can serve this dual role in development and immunity and how specificity of the downstream signal transduction is maintained.

- Kemmerling B, et al. Curr Biol. 2007;17:1116-22

- Chinchilla D, et al. Nature. 2007;448:497-500

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**ICAR5019**

JASMONATE SIGNALING DURING *PSEUDOMONAS SYRINGAE* PATHOGENESIS

Category: Biotic Interactions

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We are interested in the mechanisms by which plant pathogens modulate normal host cell processes to promote tissue colonization and disease development. Successful pathogen infection involves entry into host tissue, suppression of general antimicrobial host defenses, and growth to high levels in the plant. The phytotoxin coronatine (COR), a virulence factor of the bacterial plant pathogen *Pseudomonas syringae* is required at multiple steps during *P. syringae* pathogenesis, including: 1) entry into host tissue, 2) suppression of salicylic acid-mediated defenses subsequent to entry, and 3) promotion of disease symptom development. COR is a functional analog of the endogenous plant hormone jasmonic acid (JA), most closely resembling the active JA amino acid conjugate JA-isoleucine. Molecular and genetic studies indicate that COR stimulates JA signaling during *P. syringae* infection, and that an intact JA signaling pathway is required for full disease susceptibility in both *Arabidopsis* and tomato. We are working to further elucidate the roles of COR and JA-mediated processes in *P. syringae* pathogenesis, and are focusing on the AtMyc2/JIN1-mediated branch of the JA signaling pathway required for susceptibility to *P. syringae*. This includes utilizing a combination of forward and reverse genetic approaches to identify and characterize novel JA signaling components and to investigate their roles during pathogenesis of *Arabidopsis*. We have recently isolated a mutant that defines a gene encoding a novel component of the JA signaling pathway that negatively regulates JA signaling, COR sensitivity and susceptibility to *P. syringae*. Our progress towards characterizing this gene and its role in regulating JA-mediated responses will be presented.

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**ICAR5020**

THE ROLE OF AUTOPHAGY-ASSOCIATED PROTEINS IN THE PLANT INNATE IMMUNE RESPONSE

Category: Biotic Interactions

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Autophagy is an evolutionary highly conserved mechanism that was first identified as a cellular response to nutrient limitation. Thereby, cytosol and organelles are sequestered within double-membrane vesicles called autophagosomes that are transported to the vacuole. Fusion of the completed autophagosome with the vacuole results in the delivery of an inner vesicle, the so called autophagic body, into the lumen of the degradative compartment. Subsequent breakdown of the vesicle membrane allows the degradation of its cargo by resident vacuolar hydrolases and eventual recycling to survive periods of nutrient deficiency. Autophagy has further been implicated in disposal of protein aggregates, removal of damaged proteins and organelles during normal growth conditions, senescence and oxidative stress, but also in the regulation of programmed cell death. In *Arabidopsis thaliana*, it was shown that plants lacking ATG6, which is important for autophagy, were impaired in the restriction of programmed cell death after treatment with *Pst* DC3000 bacteria containing the AvrRpm1 effector protein.

In this project we investigate the role of autophagy genes in the plant innate immune response. Database searches based on homology to yeast revealed 33 ATG genes in *Arabidopsis thaliana*. In microarray analysis, 13 of these 33 genes, such as ATG7 and ATG18 were transcriptionally upregulated upon pathogen infection. For these induced genes, we collected T-DNA insertion and RNAi lines to silence whole gene families. Knock outs were analysed with respect to their defense response after infection with different *Pseudomonas syringae* strains or necrotrophic fungi, like *Alternaria brassicicola* and *Botrytis cinerea*. We will present data for *atg5*, *atg7*, *atg10* and *atg18* knock-out lines, which show an increased resistance to biotrophic pathogens, as well as a decreased resistance to necrotrophic fungi compared to wild type plants. Moreover, responses to cell death-inducing elicitors such as NLPPp and to different abiotic stress conditions, such as osmotic, salt or drought stress will be investigated in mutant plants and compared to those in wild type plants.

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## ICAR5021

### IDENTIFYING ARABIDOPSIS TARGETS OF THE HOPZ FAMILY OF PSEUDOMONAS SYRINGAE TYPE III EFFECTOR PROTEINS

Category: Biotic Interactions

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The plant pathogen *Pseudomonas syringae* uses the type III secretion system to secrete and translocate effector proteins into the plant. The primary function of many of these effectors is believed to be the suppression of host defense signaling; nevertheless, recognition of these effectors by resistance (R) proteins induces a defense response. The YopJ / HopZ family of effectors is a common and widely distributed class found in both animal and plant pathogenic bacteria. The *P. syringae* HopZ family includes the closely related allelic variants HopZ1a, HopZ1b and HopZ1c, as well as HopZ2 and HopZ3, which have been brought into this species via horizontal gene transfer. Ma et al (2006) recently showed that HopZ diversification was driven by the host defense response. Our goal is to identify *Arabidopsis* targets of the five HopZ members, as well as the previously unidentified and RIN4-independent R protein that recognizes the effector; and to determine how evolutionary adaptation has molded these interactions. We are using two complementary interaction screens, and constructing transgenic *Arabidopsis* plants expressing the effector proteins to identify effector-host protein complexes, and examine effects on global gene expression. We have characterized virulence and defense induction phenotypes that are strongly allele-specific, and are using selection analysis to identify specific polymorphisms associated with these phenotypes. This work will address how differences in host target specificity within one family of type III effectors contributes to the host specificity in this important pathogen.

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## ICAR5022

### ARABIDOPSIS THALIANA- GREEN PEACH APHID (*MYZUS PERSICA*E SÜLZER) INTERACTION: ROLE OF LIPIDS IN PLANT DEFENSE

Category: Biotic Interactions

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Aphids are phloem feeding insects that utilize their slender stylets to consume copious amounts of photoassimilates from plants. The green peach aphid (GPA; *Myzus persicae* Sulzer) is the most polyphagous of all aphids. We have utilized *Arabidopsis thaliana* to characterize host interaction with GPA, in particular plant defense mechanisms against GPA. Previously, we had demonstrated that the Arabidopsis *PHYTOALEXIN DEFICIENT4* (*PAD4*) gene, which exhibits homology to lipases, is an important modulator of antixenosis (feeding deterrence) and antibiosis (affect aphid fecundity) against GPA (Pegadaraju et al. 2005, 2007). The *MPL1* (*MYZUS PERSICA*E INDUCED LIPASE1) gene is another critical component of Arabidopsis defense against GPA. *MPL1* expression is induced in response to GPA infestation. Furthermore, *MPL1* transcript level is elevated in the Arabidopsis *ssi2* (*suppressor of salicylic acid insensitivity2*) mutant, which exhibits heightened antibiosis to GPA. *ssi2*-conferred resistance to GPA was attenuated in the *ssi2* *mpl1* double mutant plant suggesting that *MPL1* promotes the antibiosis mechanism that is hyperactive in *ssi2*. No-choice tests confirmed the involvement of *MPL1* in Arabidopsis defense against GPA. GPA numbers were higher on *mpl1* single mutant plant than the wild type (WT) plant. Furthermore, in comparison to the WT plant, petiole exudates (enriched in phloem sap) collected from the *mpl1* mutant lacked the antibiosis activity that restricted GPA growth on an artificial diet. However, choice test experiments showed no difference between GPA preference for the WT and *mpl1* mutant plants. Moreover, electrical penetration graph (EPG) analysis did not reveal significant differences in the total duration spent by GPA in the sieve element phase (SEP) on the *mpl1* and WT plants, confirming that *MPL1* is not required for antixenosis. Since, *MPL1* encodes a putative lipase and *SSI2* is a stearoyl ACP-desaturase, we propose that a lipid(s), or a product thereof, is involved in Arabidopsis antibiosis to GPA.

References cited:

**Pegadaraju, V., Knepper, C., Reese, J., and Shah, J. (2005)** Premature leaf senescence modulated by the *Arabidopsis thaliana* *PAD4* gene is associated with defense against the phloem-feeding green peach aphid. *Plant Physiol.* 139, 1927-1934.

**Pegadaraju, V., Louis, J., Singh, V., Reese, J.C., Bautor, J., Feys, B.J., Cook, G., Parker, J.E. and Shah, J. (2007)** Phloem-based resistance

to green peach aphid is controlled by Arabidopsis *PHYTOALEXIN DEFICIENT4* without its signaling partner *ENHANCED DISEASE SUSCEPTIBILITY1*.  
Plant J 52, 332–341.

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**ICAR5023**

EXPLORATION OF DEFENSE-DEPENDENT DWARFISM OF *ACD6-1* IN A GENETIC SCREEN TO IDENTIFY NOVEL DEFENSE GENES IN ARABIDOPSIS  
Category: Biotic Interactions

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Pathogen infection activates complicated defense signaling networks in plants. However, it remains challenging to identify defense genes and understand the mechanisms of action of these genes. Accelerated Cell Death 6 (ACD6) is an important positive regulator of Arabidopsis defense that acts through the key defense signaling mediated by salicylic acid (SA). A gain of function mutant, *acd6-1*, has hallmarks of extremely small size, constitutive resistance, cell death, and accumulation of high levels of SA and the phytoalexin camalexin. In a comprehensive genetic analysis to study the regulation of *acd6-1* conferred phenotypes, we found that the plant size was in reverse proportion to the SA levels in the *acd6-1* background. SA also modulated the severity of cell death and camalexin accumulation. Interestingly, ethylene (ET) and jasmonic acid (JA) mediated signaling pathways were highly suppressed in *acd6-1* and further blocking these two pathways did not affect *acd6-1* conferred phenotypes. Together these data suggest that SA but not ET/JA mediated signaling is required for *acd6-1* conferred phenotypes and SA antagonizes ET/JA in *acd6-1*.

We exploited the unique defense-dependent dwarfism of *acd6-1* in a large-scale genetic screen to identify novel defense genes. From screening 36,000 T-DNA mutagenized *acd6-1* lines, we isolated 20 putative *acd6-1* suppressor (*sup*) mutants and cloned two *SUP* genes. *sup1* was an allele of *SA INDUCTION-DEFICIENT 2 (SID2)* which is involved in SA biosynthesis. *SUP6* encoded an uncharacterized novel metalloprotease. Like the original suppressor *sup6-1*, the second allele, *sup6-2*, suppressed *acd6-1* conferred phenotypes. In addition, in the absence of *acd6-1*, both *sup6* alleles showed enhanced disease susceptibility to *Pseudomonas syringae* infection. Therefore, we have validated the potential of this genetic screen in identifying novel defense genes. Further investigation of these novel defense genes will advance our understanding of the mechanisms of plant defense.

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**ICAR5024**

IDENTIFICATION AND CHARACTERIZATION OF PUTATIVE ADENYLYL CYCLASE GENES IN ARABIDOPSIS THALIANA

Category: Biotic Interactions

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**Adenyl cyclases (AC)** are a diverse group of enzymes that are responsible for the production of 3',5'-cyclic AMP (cAMP) from ATP. cAMP is an important universal secondary messenger involved in mediating responses to external stimuli. In animal systems, the role of cAMP is often associated with the G-protein signal transduction pathway, which is responsible for mediating responses to sensory stimuli. In plants, current research indicates the existence of both AC and cAMP; however, so far no adenyl cyclase from Arabidopsis has been characterized. Recently our research group has shown the importance of cyclic nucleotide-gated channels (CNGCs) in pathogen resistance as well as programmed cell death development (Yoshioka et al., 2006, Urquhart et al., 2007). This research has provided some evidence for the involvement of cAMP in the gating of CNGC11 and CNGC12, suggesting that ACs may play an important role in the pathogen defense response. Therefore, a bioinformatics analysis was conducted and three genes were identified in Arabidopsis that possess an AC domain, designated as AtPAC1, AtPAC2, and AtPAC3. Homozygous T-DNA insertion knockout mutants were generated for all three genes and a characterization of these knockout mutants was performed. AtPAC1 and AtPAC2 knockout mutants exhibited enhanced susceptibility against the bacterial pathogen, *Pseudomonas syringae* as well as the oomycete pathogen, *Hyaloperonospora parasitica* isolate Emwa1. Interestingly, although knockout mutants developed significantly higher pathogen growth compared to wild type, a greater degree of hypersensitive response in true leaves was observed, indicating a strong resistance response. Additionally, the infected knockout mutants showed reduced growth after infection. These results indicate that these putative AC genes may play important roles in pathogen defense as well as development in Arabidopsis.

Yoshioka et al., (2006) Plant Cell 18, 747-763, Urquhart et al., (2007) Plant Mol. Biol. 65, 747-761.

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**ICAR5025**

MAC3 AND MAC4 FUNCTION REDUNDANTLY IN PLANT IMMUNITY

Category: Biotic Interactions

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Plant cells are equipped with an arsenal of sensory molecules and receptors to detect the presence of microbial pathogens and activate defense responses at multiple levels. One of the most effective defense responses is mediated by plant Resistance (R) proteins that trigger a cascade of physiological changes to restrict pathogen growth (1). Although many R-genes have been cloned, the signaling pathways downstream of R-protein activation remain elusive.

The unique gain-of-function mutant *snc7* has proven to be very useful as an auto-immune model to identify components of R-protein mediated

signaling networks. The *snc1* mutation causes constitutive activation of the RPP5-like R-protein SNC1, leading to constant stimulation of immune responses, even in the absence of pathogens (2). A screen for *snc1* suppressors revealed several MODIFIER OF *snc1* (MOS) proteins as key players in defense. One of these proteins, MOS4, forms a nuclear complex with the atypical Myb-transcription factor AtCDC5L and the pleiotropic regulator PRL1 (3). This complex, which we named the MOS4-Associated Complex (MAC), plays an important role in NPR1-independent disease resistance signaling.

Proteomics analysis in other eukaryotes suggested that the MAC may contain additional components. To identify other MAC proteins in Arabidopsis, we conducted a pull down assay using HA-tagged MOS4 in complementing *mos4* transgenic lines, followed by protein sequencing using mass spectrometry. Two of the precipitated proteins, MAC3 and MAC4, encode nuclear proteins with 83% sequence identity at the amino acid level. While loss-of-function *mac3* and *mac4* single mutants do not seem to have any aberrant phenotypes, double *mac3 mac4* mutant plants are compromised in basal and R-mediated signaling, and are able to suppress the auto-immunity phenotypes associated with *snc1* to the same level as *mos4*. These and other data strongly suggest that MAC3 and MAC4 function redundantly in immunity signaling.

(1) *Ann Rev Phytopath*, 2004, 42: 185-209, (2) *Plant Cell*, 2003, 15: 2636-46, (3) *Genes Dev*, 2007, 21: 1484-1493, (4) *EMBO*, 2000, 19, 23: 6569-6581

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#### **ICAR5026**

EXTRACELLULAR PYRIDINE NUCLEOTIDES INDUCE *PR* GENE EXPRESSION AND DISEASE RESISTANCE IN *ARABIDOPSIS*

Category: Biotic Interactions

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The pyridine nucleotides NAD and NADP play an important role in regulation of intracellular signaling processes. NAD serves as substrate for protein modification including ADP-ribosylation and deacetylation. NAD and NADP are precursors of the intracellular  $\text{Ca}^{2+}$ -mobilizing agents cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), respectively, which release  $\text{Ca}^{2+}$  from intracellular stores. Interestingly, recent evidence from animal and human studies suggests that extracellular NAD(P) [eNAD(P)] can either directly bind to plasma membrane receptors or be metabolized by ectoenzymes to produce cADPR and NAADP and/or to ADP-ribosylate cell-surface receptors, resulting in activation of transmembrane signaling. In this study, we report that in plants, eNAD(P) induces the expression of pathogenesis-related (*PR*) genes and resistance to the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326. Chelation of extracellular  $\text{Ca}^{2+}$  by EGTA significantly inhibits the induction of *PR* genes by eNAD(P), suggesting that eNAD(P) induces *PR* genes through a pathway that involves  $\text{Ca}^{2+}$  influx. We show that exogenous application of NAD(P) causes accumulation of the defense signal molecule salicylic acid (SA), and induces both SA/NPR1-dependent and -independent *PR* gene expression and disease resistance. Furthermore, we demonstrate that NAD(P) leaks into the plant extracellular compartment after mechanical wounding and pathogen infections. We propose that NAD(P) leakage from damaged tissues caused by environmental stress may function as an elicitor to activate plant defense responses. Our data provide the first evidence that eNAD(P) functions in plant signaling, and illustrate the potential importance of eNAD(P) in plant innate immunity.

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#### **ICAR5027**

INTERACTIONS BETWEEN PLANT AND PEST GENOMES: *ARABIDOPSIS*/TWO SPOTTED SPIDER MITE *TETRANYCHUS URTICAE*, NOVEL MODEL FOR PLANT-HERBIVORE INTERACTIONS

Category: Biotic Interactions

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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 survival. The two-spotted spider mite *Tetranychus urticae* is a generalist herbivore and major agricultural pest that sucks out the plant cell's contents leaving small light-coloured punctures or spots on their leaves. Furthermore, the spider mite is a newly developed model organism whose whole genome is currently being sequenced and in the near future, will provide tools for molecular analysis of its development, physiology and metabolism. Along with the molecular and genetic tools available in *Arabidopsis thaliana*, studies analyzing *Arabidopsis* and spider mite interactions will provide a unique opportunity to perform novel genetic and genomic studies on both sides of this plant-pest interaction. The main objective of my research is to characterize damage on *Arabidopsis* plants upon spider mite feeding. This includes characterization of the differential resistance among natural *Arabidopsis* accessions to spider mite damage, the mapping of the genes responsible for the variation in susceptibility between Ler and Col, and profiling the transcriptome of naturally resistant and susceptible *Arabidopsis* accessions. A genetic dissection of mite-plant interactions could provide insight into the signalling and transcriptional basis of plant defences used against herbivores. In addition, genome-wide sequences of spider mites will lend themselves to the analysis of the transcriptome's response to host-plant defensive compounds. Ultimately, this research will aid in our understanding of plant-pest interactions, and provide us with opportunities to control pests in an environmentally-friendly and energy-efficient manner.

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#### **ICAR5028**

A BAK1-INTERACTING RECEPTOR FAMILY

Category: Biotic Interactions

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Plants possess a powerful innate immune system to defend themselves against invading pathogens. It has already been shown that some leucine-rich repeat receptor-like kinases (LRR-RLKs) are involved in the recognition of certain pathogen-specific structures called PAMPs and in the activation of immune response reactions in plants. One example is the flagellin receptor FLS2. BAK1, the BRI1-associated kinase 1, is also a member of the large plant LRR-RLK family. Despite its function in brassinosteroid signaling it was shown that it is involved in plant defense reactions and pathogen induced cell death control. BAK1 interacts directly with the brassinosteroid receptor BRI1 and in addition with the PAMP receptor FLS2. Therefore BAK1 seems to be a protein that can interact with diverse receptor proteins in different signaling pathways.

In co-immunoprecipitation experiments a new BAK1-interacting receptor, BAK1-interacting protein BIP89, was identified. According to microarray data (ATGenExpress) the expression of this gene is induced upon treatment with non-pathogenic bacteria and the bacterial PAMP HrpZ. This mirrors a tight coregulation of BIP89 with its interactor BAK1. BIP89 belongs to a small subfamily of LRR-RLKs (BIP89-like, BIL) which shows interesting expression patterns indicating that these proteins might be involved in defense signaling regulated by BAK1. While BIP89 and BIL1 expression is enhanced BIL3 expression is suppressed by pathogen or PAMP treatment. Interaction of BIL2 and 3 with BAK1 was shown in co-immunoprecipitation experiments and/or in yeast-two-hybrid assays. Further analysis of the interactions of BAK1 with BIP89 and its closest homologs will reveal how these RLKs interfere with the diverse BAK1 signaling processes. The phenotypic analysis of knockout lines in these genes will reveal which BAK1 regulated pathways are influenced by these additional receptor proteins. We hypothesize that differential interaction with the members of this gene family might influence the complex formation of BAK1 with its ligand-binding receptors.

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### ICAR5029

NETWORK ANALYSIS OF ARABIDOPSIS DEFENSE SIGNALING

Category: Biotic Interactions

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In order to robustly execute defense responses against a multitude of pathogens with various defense suppressors, plants have developed a complex and highly interconnected signaling network. To dissect the *Arabidopsis* defense signaling network, we have used gene expression profiles to characterize *Arabidopsis* mutants in detail. For this purpose, we have manufactured a custom small-scale microarray "the miniaarray" (Sato *et al.* (2007) Plant J. 49: 565–577). We used the miniaarray to assay pathogen-inducible gene expression in a collection of twenty-one *Arabidopsis* mutants whose defects were predicted to perturb various points in the signaling network. Plant mutants were infected with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 carrying *AvrRpt2*, and gene expression profiles were analyzed using non-linear dimensionality reduction method (RepEdLEGG). By identifying the similarities in expression profiles between mutants using RepEdLEGG, we developed a signaling network model that infers interactions between genes corresponding to the mutations. Using this model, we have developed and tested hypotheses about signal flow through the network.

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### ICAR5030

INTERACTION BETWEEN SALICYLIC ACID AND JASMONIC ACID SIGNALING IN ARABIDOPSIS AND WHEAT IMPACTS MACROCONIDIA GERMINATION OF THE FUSARIUM HEAD BLIGHT FUNGUS, *FUSARIUM GRAMINEARUM*.

Category: Biotic Interactions

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*Fusarium* head blight (FHB), also known as Scab, is an important disease of wheat and barley that limits grain productivity and quality. *Fusarium graminearum* is the principal causative agent of FHB. The absence of gene-for-gene type resistance against this disease has hampered the development of FHB resistant wheat and barley varieties. Previously, we had demonstrated that constitutive overexpression of *Arabidopsis NONEXPRESSER OF PR GENES 1 (NPR1)* enhanced FHB resistance in transgenic wheat, suggesting that an NPR1-modulated mechanism is important for controlling *F. graminearum*. To further evaluate the role of this mechanism in controlling *F. graminearum*, we have conducted experiments in wheat and *Arabidopsis thaliana*. Since NPR1 regulates the activation of salicylic acid (SA) signaling and also impacts activation of jasmonic acid (JA) signaling, we tested the role of both these mechanisms in wheat and *Arabidopsis* interaction with *F. graminearum*. SA application enhanced resistance in wheat and *Arabidopsis*. Genetic studies in *Arabidopsis* confirmed an important role for SA/NPR1 signaling in defense against this fungus. In particular, activation of SA/NPR1 signaling correlated with the reduction in efficacy of fungal macroconidia germination. In contrast, a JA and *JASMONATE RESISTANT 1 (JAR1)*-dependent mechanism, although activated in response to *F. graminearum* infection, attenuated SA accumulation in fungus-challenged *Arabidopsis* leaves and promoted germination of fungal macroconidia. Furthermore, pretreatment of *Arabidopsis* and wheat plants with methylJA (MeJA) attenuated NPR1 overexpression-conferred resistance to *F. graminearum*. However, analysis of the *Arabidopsis jar1 npr1* double mutant indicates that JA signaling also contributes to resistance: the double mutant plant was more susceptible to the fungus than the *npr1* single mutant. These results suggest a complex interaction between SA and JA signaling in plant interaction with *F. graminearum*. We hypothesize that during the early stages of infection a SA and NPR1-dependent mechanism controls macroconidia germination and during the later stages of infection a JA and JAR1-dependent mechanism controls fungal growth/development.

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### ICAR5031

TGA1 CLADE TRANSCRIPTION FACTORS ARE POSITIVE REGULATORS OF DISEASE RESISTANCE AGAINST *PSEUDOMONAS SYRINGAE*, AND REGULATE BOTH NPR1-DEPENDENT AND NPR1-INDEPENDENT GENES.

Category: Biotic Interactions

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Upon pathogen recognition, *Arabidopsis* initiates transcriptional reprogramming, which is mediated in large part through the activity of NPR1 (NON-EXPRESSOR OF PATHOGENESIS GENES1). NPR1 does not contain any known DNA-binding domain, but interacts with members of the TGA family of bZIP transcription factors to enhance their binding to cognate *as-1*-like promoter elements. It is postulated that NPR1 mediates its function through interactions with TGA factors. However, the contributions made by different TGA factors to the transcriptome during the induction of disease defense responses have yet to be determined. Here we report on the characterization of multiple T-DNA insertion mutants within members of the TGA1 clade (*TGA1* and *TGA4*). Monogenic mutants of every allele display enhanced susceptibility to virulent strains of *Pseudomonas syringae*, but maintain intact systemic acquired resistance against this pathogen. The digenic *tga1-1 tga4-1* mutant is more susceptible than either of the monogenic mutants, suggesting partial functional redundancy between members of the TGA1 clade. Unlike *npr1*, these mutants show no enhanced susceptibility to SA toxicity, but display growth and developmental abnormalities, such as curled leaves and delayed flowering. We used microarray analysis to examine changes in global patterns of gene expression in leaves of *tga1-1*, *tga4-1*, and *npr1* mutant plants. Our results indicate that NPR1 and clade I TGA factors (TGA1 and TGA4) regulate largely distinct sets of genes. TGA1 and TGA4 also contribute to the regulation of genes involved in processes distinct from disease resistance, potentially acting in both a positive and negative regulatory capacity.

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### ICAR5032

#### WRKY27-DEPENDENT COMMUNICATION BETWEEN THE PHLOEM AND XYLEM DETERMINES TOLERANCE TOWARDS *RALSTONIA SOLANACEARUM*

Category: Biotic Interactions

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WRKY transcription factors play a key role in modulating the plant defense transcriptome. Here we show that an *Arabidopsis* mutant *wrky27-1* lacking a functional WRKY27 transcription factor delayed symptom development toward the bacterial wilt pathogen *Ralstonia solanacearum*. Additionally, *wrky27-1* plants failed to express *PR* marker genes upon infection, similar to what is observed in resistant Nd-1 plants. Spatial expression of *WRKY27* correlated well with the route of bacterial infection within the vasculature and propagation *in planta*. Complementation experiments however revealed that both the early wilting phenotype of wild-type Col-1 plants and the activation of *PR* genes can be restored not only when the *WRKY27* cDNA is expressed under the control of the native promoter, but also with the *SUC2* promoter, suggesting that *WRKY27* exerts its function in phloem companion cells. In contrast, expression of *WRKY27* under the control of the xylem-specific *4CL-2* promoter failed to restore the early wilting phenotype. Tolerance to *R. solanacearum* was not correlated with decreased bacterial titers. Increased sensitivity of *wrky27-1* plants to the nitric oxide donor sodium nitroprusside (SNP) indicates that these plants may be impaired in NO homeostasis. Our results show that *WRKY27* negatively influences symptom development of a vascular pathogen possibly by impinging on signaling or trafficking between the phloem and the xylem. *WRKY27* expression was also observed in certain floral tissue hinting towards an additional function of this gene. Plants ectopically expressing *WRKY27* under the strong *CaMV 35S* promoter displayed visible phenotypes including delayed anther development and perianth dehiscence as well as strongly reduced pollen viability.

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### ICAR5033

#### THE REGULATORY ROLE OF ARABIDOPSIS NUDIX HYDROLASE NUDT7 IN INNATE IMMUNITY

Category: Biotic Interactions

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Nudix hydrolases belong to a large family of enzymes that are present in organisms from viruses to humans and cleave a variety of nucleoside diphosphate substrates. The *Arabidopsis thaliana* Nudix hydrolase NUDT7 acts as a negative regulator of plant immunity and programmed cell death. *Nudt7* mutants display enhanced basal resistance, exhibit retarded growth and spontaneous initiation but not spread of leaf cell death. Also, the mutants have elevated levels of salicylic acid (SA), a key molecule of plant resistance, and exhibit a hyper susceptibility towards oxidative stress, i.e. to hydrogen peroxide induced by methyl viologen (MV) in the chloroplasts. Genetic epistasis analysis shows that all of these defects in *nudt7* plants require *ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)*, an intracellular regulator of plant basal and systemic immunity. Pathogen-induced expression of *NUDT7* mRNA and *NUDT7* protein is dependent on *EDS1*. *NUDT7* protein has a cytosolic localization in healthy plants and after infection with avirulent *Pseudomonas syringae* expressing *avrRPS4*. Genetic analysis revealed that *NUDT7* acts on an SA-independent pathway downstream of *EDS1* that we are now characterizing. We provide evidence that the *nudt7* growth phenotype is not due to hyperresponsiveness to PAMPs or to perturbation of auxin signalling. Our results point to a deregulated defence pathway in *nudt7* plants that constitutively activates *EDS1*. MV application causes massive H<sub>2</sub>O<sub>2</sub> production and eventual cell death in leaves of both wild type and *nudt7* but not in *eds1* or *nudt7/eds1* mutants indicating that *EDS1* acts as an early promoter of chloroplastic derived ROS accumulation. We present a model in which *EDS1* functions to promote cell death by modulating ROS, we are testing now whether this is at the level of conditional interfering with ROS scavenging systems.

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### ICAR5034

## THE ARABIDOPSIS TDP1 GENE IS REQUIRED FOR RESISTANCE TO PSEUDOMONAS SYRINGAE

Category: Biotic Interactions

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When a pathogen invades a host plant there are two possible outcomes: the plant may succumb to the infection and develop disease, or it may mount a resistance response that prevents growth of the pathogen. Successful host resistance depends on the plant's ability to recognize the pathogen and initiate complex defense mechanisms. Our understanding of plant-pathogen interactions depends on our ability to identify genes involved in the defense response and characterize their functions. Here we describe preliminary characterization of *TOLB-RELATED DEFENSE PROTEIN 1 (TDP1)*, a novel Arabidopsis gene of unknown function which is predicted to contain TolB and WD40 domains. Expression of this gene is induced in response to virulent and avirulent bacterial pathogens, oxidative stress, salt stress, and wounding. It is also expressed at high levels in several lesion mimic mutants and during senescence. *tdp1* null mutants are compromised in resistance against the virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000, and preliminary analysis indicates that *TDP1* overexpression is lethal. *TDP1* expression in response to virulent *Pseudomonas syringae* is significantly reduced in several mutants defective in salicylic acid biosynthesis or signaling, indicating that it may play a role in salicylic acid-mediated defenses.

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## ICAR5035

### INTERPLAY BETWEEN MAMP-TRIGGERED AND SA-MEDIATED DEFENSE RESPONSES

Category: Biotic Interactions

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Plants respond to pathogen infection using an innate immune system with at least two distinct recognition mechanisms. One mechanism recognizes microbe-associated molecular patterns (MAMPs). The other is based on resistance (R) genes and specifically recognizes specific pathogen virulence factors including type III effector proteins of bacteria. R-gene mediated defenses require salicylic acid (SA) -mediated pathways, however it was unclear if MAMP-triggered pathways interact with SA-mediated signaling. Here, we report the interaction between MAMP-triggered and SA-mediated signaling pathways. We found that SA accumulated in the Col-0 wild-type plants after we triggered MAMP pathways using either flg22 or a mutant of *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) deficient in the type III secretion system (Tsuda et al. (2008) Plant J. 53: 763-775). Expression profile analysis of six Arabidopsis defense-related mutants (*sid2*, *pad4*, *ein2*, *dde2*, *col1*, *mpk3*) revealed the importance of SA signaling on MAMP-triggered gene expression changes. Clustering analysis suggests mutual inhibition between SA signaling pathways and ethylene, jasmonic acid and MAP kinase signaling pathways. The effects of flg22 pretreatment on resistance against a bacterial pathogen, *Pst* DC3000, are partially dependent on SA signaling. However, expression of activated MAP kinase pathway members, which are known to be activated by flg22, did not result in SA accumulation. These results suggest that the SA production triggered by flg22 is a component of the flg22-triggered, MAP kinase 3/6-independent signaling mechanism leading to resistance.

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## ICAR5036

### FUNCTIONAL CHARACTERIZATION OF *ARABIDOPSIS* CYCLIC NUCLEOTIDE-GATED ION CHANNELS WITH RESPECTS TO BASAL AND R-GENE MEDIATED PATHOGEN DEFENSE SIGNALING

Category: Biotic Interactions

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Cyclic nucleotide-gated ion channels (CNGCs) are a family of non-selective ion channels that have been recently identified in plants. The *Arabidopsis* CNGC gene family is a relatively large family with twenty members. The large family size of ATCNGCs indicates divers and functionally important roles for these *Arabidopsis* channels. Regardless of their perceived importance, the biological roles of these twenty ATCNGC members have only started to be identified. Several ATCNGCs, ATCNGC2, 4, 11, and 12 may function within the R-gene mediated resistance pathway. This pathway is responsible for recognizing isolate specific pathogen infections, resulting in a rapid activation of defense response. One of these rapid responses is the hypersensitive response (HR) which is a localized programmed cell death around the site of infection. In addition to the R-gene mediated response, ATCNGC2 has been implicated in the basal defense signaling pathway which is a broad acting defense to general pathogen infection. Our laboratory's findings that the resistance to the avirulent *Hyaloperonospora parasitica* strain Emw1, is attenuated in ATCNGC11 and 12 T-DNA insertion knockout mutants suggests that *CNGC11* and *12* may play a role in the R-gene mediated response pathway. We have continued to utilize T-DNA insertion knockout mutant lines to elucidate the importance of ATCNGCs in both the basal and R-gene mediated resistance pathways. To date, we have demonstrated the importance of several ATCNGCs to the development of HR conferred by a number of R-genes. We are also currently investigating the contribution of ATCNGCs to the basal defense signaling pathway where pathogen growth and callose formation will be specifically addressed. This investigation will ultimately lead to a better understanding of ATCNGC function, while possibly placing targeted ATCNGCs into functional roles within the basal and/or R-gene mediated resistance pathways.

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## ICAR5037

### MOLECULAR MECHANISM BEHIND CROSS-TALK BETWEEN SALICYLIC ACID- AND JASMONIC ACID-DEPENDENT DEFENSE PATHWAYS IN ARABIDOPSIS

Category: Biotic Interactions

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Plants have developed several ways to defend themselves against attack by pathogens or insects. The signaling molecules salicylic acid (SA) and jasmonic acid (JA) play major roles in these defense responses. SA is needed for protection of the plant against biotrophic pathogens, while JA is required for protection of plants against attack by necrotrophic pathogens and insects. The SA and JA signaling pathway can cross-communicate to fine-tune the plants defense reaction in response to the type of invader that is encountered. In *Arabidopsis thaliana* SA was observed to repress expression of JA-induced genes, among which *PDF1.2* and *VSP2* (Spoel et al., 2003; Plant Cell 15: 760-770). Here, we aim to unravel how SA can exert its repressive effect on JA responsive gene expression and where in the JA signaling pathway SA exerts its antagonistic action. We found that plants impaired in JA biosynthesis were still able to show SA-mediated suppression of JA-responsive genes, suggesting that SA targets the JA signaling pathway downstream of JA biosynthesis. Promoter analysis of JA-induced genes that are suppressed by SA revealed an overrepresentation of the GCC box in their promoters. In addition, using plants that carry the GUS reporter gene under control of a minimal 35S promoter and four copies of the GCC box, we found that the GCC box is a sufficient element for SA induced suppression of JA induced gene expression. AP2/ERF-domain proteins form a family of transcription factors that are able to bind the GCC box. The AP2/ERF transcription factor ORA59 was found to function as an important activator of the JA responsive gene *PDF1.2* (Pré et al., 2006: Ph.D thesis, Leiden University, Leiden, The Netherlands). Based on these findings we speculate that SA can repress JA responsive genes via interference with the function of ORA59. Future research will focus on the role of ORA59 and the GCC box in the antagonism between the SA and JA signaling pathway.

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#### ICAR5038

Category: Biotic Interactions

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The non-protein amino acid  $\beta$ -amino-butyric acid (BABA) induces resistance in *Arabidopsis*, which can function independently of salicylic acid (SA), against an exceptionally wide range of pathogens. BABA-induced resistance (BABA-IR) is not based on direct defense activation after induction treatment, but on a stronger and faster defense response after the plant is exposed to pathogen attack. Such an enhanced defensive capacity is commonly referred to as priming.

To gain more insight into the ecological function of priming, we performed a lab study on the costs and benefits of priming in *Arabidopsis*. This study revealed that the benefits of priming-mediated resistance outweigh the costs of priming under conditions of disease pressure. Hence, faster and/or **stronger activation of defenses increases the plant's ability to survive in hostile environments**. In agreement with this, we recently found natural variation among *Arabidopsis* accessions in responsiveness to the phytohormones SA and/or JA, which correlated with basal resistance to various pathogens. In order to further assess the ecological value of priming, we are currently evaluating the performance of primed plants under field conditions.

To gain more insight in the molecular mechanisms behind priming, we screened for *Arabidopsis* mutants impaired in SA-independent BABA-IR. So far, this screen has yielded one mutant that is specifically impaired in BABA-induced priming for callose-rich papillae upon inoculation by pathogenic fungi and oomycetes. Additionally, we have investigated the role of transcription factors in BABA-induced priming for SA/NPR1-dependent defenses, using a qPCR-based transcription profiling approach. This screen revealed a novel promoter element that is strongly over-represented in promoters of BABA-inducible WRKY transcription factor genes, which may serve as a key element for BABA-induced priming.

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#### ICAR5039

MECHANISMS OF CLE PEPTIDE MIMICRY IN CYST NEMATODE PATHOGENESIS

Category: Biotic Interactions

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Species of cyst nematodes (*Heterodera* and *Globodera* species) have evolved sophisticated parasitic relationships with the roots of host plants. These nematodes parasitize roots and feed as obligate sedentary endoparasites; an evolutionarily advanced form of plant parasitism that requires modulation of host cellular developmental pathways to form enlarged, multinucleate feeding cells that serve as the sole nutritive source for the nematode. Secretory proteins encoded by parasitism genes expressed within the nematode's esophageal gland cells provide the primary signals in the formation of feeding cells. We have isolated parasitism genes from two species of *Heterodera*, including *H. schachtii*, a parasite on *Arabidopsis*, encoding small secreted peptides sharing similarity with plant CLAVATA3/ESR (CLE) signaling peptide ligands. We have demonstrated functional similarity between the nematode and plant CLEs in overexpression, complementation, and exogenous synthetic peptide application studies that suggests they mimic the function of endogenous CLE peptides. Our data indicates that nematode CLEs secreted from a parasite and non-parasite of *Arabidopsis* signal through different host receptors. In addition, our data implies that sequence outside the conserved CLE motif may be important for nematode CLE function in planta and play a role in host-specific control of nematode CLE peptide recognition that could explain the specific adaptation of cyst nematodes to parasitize a limited number of plant species. Recent discoveries are providing novel insights into the signal exchange mediating plant-nematode interactions.

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#### ICAR5040

THE CALMODULIN-BINDING PROTEIN ATCBP60G CONTRIBUTES TO MAMP-INDUCED SA ACCUMULATION

Category: Biotic Interactions

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Salicylic acid (SA) is one of the major plant hormones that are induced upon pathogen attack. It is particularly important for resistance against hemi-biotrophic pathogens. A recent study indicated that SA accumulates upon sensing microbe-associated molecular patterns (MAMPs), and is required for MAMP-induced resistance. We show that a member of the *Arabidopsis* CBP60 gene family, *AtCBP60g*, contributes to MAMP-triggered SA accumulation. *AtCBP60g* is inducible by both pathogen infection and MAMP treatment. Mutants with defects in *AtCBP60g* support more *Pseudomonas syringae* growth than wild-type plants. Expression profiles of an *atcbp60g* mutant after MAMP treatments show similarity to those of *sid2* and *pad4*. Relative to wild-type plants, *atcbp60g* mutants accumulate less SA after treatment with flg22 or a *Pseudomonas syringae* hrcC strain. *AtCBP60g* encodes a bona fide calmodulin binding protein with a calmodulin-binding domain located at the N-terminus. We are testing whether abolishing **ATCBP60G's ability to bind calmodulin by site-specific mutagenesis** leads to disrupted ATCBP60G function. Our results indicate that ATCBP60G functions in disease resistance. The reduction in MAMP-induced activation of SA accumulation may account for the enhanced disease susceptibility of *atcbp60g* mutants.

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#### ICAR5041

##### INFLUENCE OF BACTERIAL EFFECTOR PROTEINS ON PLANT SECRETION

Category: Biotic Interactions

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Plant cells sense the presence of bacteria through cell-surface receptors that recognize conserved features intrinsic to the life-style of the microbe called MAMPs (microbial-associated molecular patterns). Binding of a MAMP to its receptor initiates signaling pathways leading to defense responses that restrict the growth of invading microbes. To repress the plant's MAMP response, bacteria such as *Pseudomonas syringae* use a type three secretion system (T3SS) to inject an array of effector proteins into the plant cell. For many/most of these proteins, the mechanism by which the effectors impede host defenses is not known.

Recent work indicates that protein secretion may be a major determinant of the plant defense against microbes. To gain a better understanding of the changes in the extracellular proteome (secretome) during bacterial infection, we performed a proteomic analysis of the secretome after infection of *Arabidopsis* cell cultures with either virulent or avirulent *Pseudomonas* strains as well as strains that no longer contain a functional T3SS. These results indicated that the effector proteins indeed suppress the extracellular accumulation of plant proteins secreted during basal defense responses. However, we also found that virulent bacteria cause the extracellular accumulation of plant proteins lacking secretory signal sequences. We have produced antibodies against proteins from the different accumulation classes observed in the proteomic analysis to be used as biomarkers in a screen to determine which effector proteins mediate these effects on secretion of plant proteins. Ultimately, the goal of the project is to investigate the mechanism(s) by which these effectors influence protein secretion in the host.

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## GENETIC AND EPIGENETIC MECHANISMS

### ICAR601

FUNCTIONAL REDUNDANCY AND SPECIFICITY OF THE MIR159 GENE FAMILY IN *ARABIDOPSIS*.

Category: Genetic and Epigenetic Mechanisms

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In both plants and animals loss-of-function mutants are rare for microRNA genes. It has been postulated that functional redundancy may in part account this. Here using T-DNA insertional mutants, we show this to be the case for the *Arabidopsis* miR159 family, where single *miR159a* and *miR159b* mutants show no phenotype, in contrast to pleiotropic phenotypes found in a *miR159ab* double mutant. Although bioinformatics and overexpression studies have predicted miR159 to regulate at least seven MYB genes in *Arabidopsis*, only the redundant gene pair of MYB33 and MYB65 was found to be deregulated in *miR159ab*. This can be explained by the transcriptional domains of the miRNAs and their targets; using GUS reporter constructs we show the transcriptional domains of miR159a and miR159b overlap with MYB33 and MYB65 in all tissues except anthers, where MYB33 and MYB65 are expressed. In contrast the other five MYB genes appear to have reciprocal transcriptional domains to miR159a and miR159b, only being transcribed in anthers. Consequently the pleiotropic defects of *miR159ab* can be suppressed in a *myb33myb65* background. This demonstrates that the functional specificity of a plant microRNA is narrower than predicted by overexpression or bioinformatics, which accords with similar findings in animals.

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### ICAR602

GENOME-WIDE HIGH-RESOLUTION MAPPING OF EXOSOME SUBSTRATES REVEALS HIDDEN FEATURES IN THE ARABIDOPSIS TRANSCRIPTOME

Category: Genetic and Epigenetic Mechanisms

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The exosome complex plays a central and essential role in RNA metabolism. However, comprehensive studies of exosome substrates and functional analyses of its subunits are lacking. Here, we demonstrate that, as opposed to yeast and metazoans, the plant exosome core possesses an unexpected functional plasticity, as well as present a genome-wide atlas of *Arabidopsis* exosome targets. We find that the exosome in plant kingdom has two distinctive features not present in the respective complexes in other phyla: (i) at least one of the RNA binding subunits of the plant exosome core is dispensable for viability, as well as for many (but not all) exosome functions, and (ii) plant exosome core possesses an intact catalytically active site. The functional significance of such plant kingdom-specific conservation of this active site is currently being tested by site directed mutagenesis and the results will be presented.

The results of the genome-wide mapping of the exosome substrates provide evidence for widespread polyadenylation- and exosome-mediated RNA surveillance in plants, and further experiments reveal a complex network of unconventional poly(A) polymerases that cooperate with the exosome in RNA quality control. The atlas of *Arabidopsis* exosome targets also reveals hitherto unknown aspects of stable structural RNA metabolism and uncovers numerous novel exosome substrates, including a select subset of mRNAs and miRNA processing intermediates. 3'-RACE mapping of these intermediates, as well as additional results of ultradeep sequencing suggest an alternative model of miRNA biogenesis in plants.

Finally, our data reveal upregulation of hundreds of noncoding RNAs, the vast majority of which have not been previously described and belong to a "deeply hidden" layer of the transcriptome that can only be visualized upon inhibition of exosome activity. One prominent component of the exosome-regulated fraction of the transcriptome consists of hundreds of polyadenylated heterochromatic transcripts that exhibit highly nonrandom associations with repeated sequences in the genome and small RNA-producing loci, suggesting a general role of exosome in regulating heterochromatic transcripts. Taken together, these first genome-wide maps of exosome substrates illuminate new fundamental components and regulatory mechanisms of eukaryotic transcriptomes.

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### ICAR603

CRYSTAL STRUCTURE OF A WHIRLY PROTEIN BOUND TO SINGLE-STRANDED DNA

Category: Genetic and Epigenetic Mechanisms

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Members of the Whirly family of proteins are found throughout the plant kingdom. In *Arabidopsis thaliana*, three genes coding for Whirly proteins were identified. The products of those genes are targeted to the mitochondrion (*AtWhy2*) or to the chloroplast (*AtWhy1* and *AtWhy3*). Members of the Whirly family were shown to bind single-stranded DNA both *in vitro* and *in vivo*. The Whirly domain, the most conserved part of the Whirly proteins, is necessary and sufficient for high affinity single-stranded DNA binding. Our group elucidated the crystal structure of the Whirly domain of StWhy1, an homolog of AtWhy1, by X-ray crystallography. The structure reveals that the Whirly proteins assemble into tetramers. Each protomer comprises two anti parallel beta-sheets packed together and protruding out from an alpha-helical core. Although the crystal structure of StWhy1 was elucidated, the mechanism of single-stranded DNA binding remains elusive. In order to understand the structural determinants of the single-stranded DNA binding of the Whirly family of proteins, we have solved the crystal structure of a Whirly protein in complex with single-stranded DNA.

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**ICAR604**

PARALLEL MICROARRAY AND PROTEOMIC ANALYSIS OF A SEED-SPECIFIC ACETYL-COA CARBOXYLASE MUTANT REVEALS ASSORTED CHANGES IN METABOLISM

Category: Genetic and Epigenetic Mechanisms

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The biosynthetic pathways responsible for the major seed storage components are now largely known, although much less is known about the regulation of these pathways. To better understand the regulatory networks governing metabolism in developing seed, two independent lines for an *Arabidopsis thaliana* plastid heteromeric acetyl-CoA carboxylase (ACCase; Thelen and Ohlrogge (2002) *Plant J.* 32:419-431) mutant which has a seed-specific, low oil phenotype were further analyzed. Microarray and two complementary proteomic approaches were applied to compare the transcriptome and proteome during seed filling. Conclusions from this systems biological study can be summarized as follows: 1) over-expression of the biotin-containing subunit to ACCase biotin carboxyl carrier protein 2 (BCCP2) did not affect the expression of the three other subunits to ACCase; 2) all four subunits to the plastid pyruvate dehydrogenase complex were significantly down-regulated at the protein but not transcript level; 3) lipid modifying enzymes downstream of ACCase were transcriptionally up-regulated; 4) storage proteins (CRA1, 2S albumin), but not cognate transcripts, were up-regulated while oleosin protein (but not transcript) was down-regulated; 5) the biotin biosynthesis pathway was transcriptionally up-regulated. These observations indicate that there are several tiers of regulatory mechanisms that the plant employs to respond to reduce fatty acid synthesis in developing seeds and that transcript profiling alone can not account for these changes. It is also apparent that biotin synthesis is closely matched to the principal seed protein that requires this cofactor, since over-expression of BCCP2 induced the entire biotin synthesis pathway in response to the accumulation of apo-BCCP2. Reduced fatty acid synthesis also induced complex feed-back and starvation responses at steps before (glycolysis) and after (fatty acid synthesis) ACCase, respectively. Confirmation of these results by immunoblot analyses and a model of transcript and protein changes will be presented.

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**ICAR605**

SINE RNA INDUCES SEVERE DEVELOPMENTAL DEFECTS IN ARABIDOPSIS THALIANA AND INTERACTS WITH HYL1 (DRB1) A KEY MEMBER OF THE DCL1 COMPLEX

Category: Genetic and Epigenetic Mechanisms

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The proper temporal and spatial expression of genes during plant development is governed, in part, by the regulatory activities of various types of small RNAs produced by the different RNAi pathways. Here we report that transgenic *Arabidopsis* plants constitutively expressing the rapeseed SB1 SINE retroposon exhibit developmental defects resembling those observed in some RNAi mutants. We show that SB1 RNA interacts with HYL1 (DRB1), a double-stranded RNA-binding protein (dsRBP) that associates with the Dicer homologue DCL1 to produce microRNAs. RNase V1 protection assay mapped the binding site of HYL1 to a SB1 region that mimics the hairpin structure of microRNA precursors. We also show that HYL1, upon binding to RNA substrates, induces conformational changes that force single-stranded RNA regions to adopt a structured helix-like conformation. *Xenopus laevis* ADAR1, but not *Arabidopsis* DRB4 binds SB1 RNA in the same region as HYL1, suggesting that SINE RNAs bind only a subset of dsRBPs. Consistently, DCL4-DRB4-dependent miRNA accumulation was unchanged in SB1 transgenic *Arabidopsis*, whereas of DCL1-HYL1-dependent miRNA and DCL1-HYL1-DCL4-DRB4-dependent tasiRNA accumulation was decreased, which correlated with increased accumulation of their corresponding targets. We propose that SINE RNA can modulate the activity of the RNAi pathways in plants and possibly in other eukaryotes.

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**ICAR606**

A NEW IMPRINTED GENE IN *ARABIDOPSIS THALIANA*: THE ROLE AND REGULATION OF *MPC*

Category: Genetic and Epigenetic Mechanisms

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Genomic imprinting is critical for the normal development of offspring and has been found in just two taxa, mammals and flowering plants. It is the system of epigenetic modification that leads to mono-allelic expression and depends on the parent-of-origin. In flowering plants, imprinting occurs in the endosperm and greatly affects seed development. Only four imprinted genes have been described in *Arabidopsis* to date, compared to approximately 100 in mammals. We have recently discovered a fifth imprinted gene, *MPC* (*Maternally expressed PAB C-terminal*), which is paternally silenced in endosperm. This gene encodes a functional C-terminal domain (CTC) of a poly(A) binding protein (PABP). PABPs are fundamental in the regulation of translation and protection of RNA from de-adenylation and subsequent decay. *MPC* binds to CTC-Interacting Domain (CID) proteins in a yeast two-hybrid assay, suggesting that *MPC* may also bind CID proteins *in vivo*. There are three other maternally-expressed, imprinted genes, *FIS2*, *MEDEA* and *FWA*. Both *FIS2* and *FWA* are regulated by DNA methylation led silencing on the paternal side, mediated by MET1. In a *met1* mutant background, *MPC* is ectopically expressed in stamen and leaf. The normally silent paternal allele of *MPC* was also ectopically expressed in siliques when transmitted by a *met1* pollen parent. Demethylation mediated by the DNA glycosylase DME, is also known to be important for the regulation of *MEDEA* and *FWA* imprinting. Experiments to assess *MPC* expression in a *dme* mutant background are underway. To identify the function of *MPC* an RNAi approach was used to knock-down expression. This revealed that *MPC* plays an important role in seed development, with seeds from RNAi plants showing delayed embryogenesis, altered embryo morphology, increased chalazal endosperm growth and an overall reduction in seed size. Characterization of this gene will improve our understanding of seed development and the mechanism and importance of imprinting in plants.

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**ICAR607**

## CHARACTERISATION OF ARGONAUTE5 IN ARABIDOPSIS THALIANA

Category: Genetic and Epigenetic Mechanisms

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The Argonaute (AGO) family is a ten member protein family in *Arabidopsis thaliana*. The family divides into three different phylogenetic clades of which the first includes AGO1, AGO10 (Pinhead/Zwille) and AGO5, the second has AGO7, AGO2 and AGO3 and the last comprises AGO4, AGO8, AGO9 and AGO6. Each AGO protein binds 21 nt to 26 nt short RNAs (sRNA) and forms an RNA-induced silencing complex (RISC). RISCs are involved in multiple RNA interference pathways involving micro RNA (miRNA) trans-acting siRNAs or RNA-directed chromatin silencing.

We have carried out a comparative analysis of AGO1 and AGO5. Our prediction was that AGO5 would bind 21nt small RNAs, as has been shown recently for AGO1. However we find, unexpectedly, that AGO5 binds all the major size classes: 21, 22 and 24nt of siRNA. AGO5 has a strong preference for small RNAs with a 5' cytosine whereas AGO1 has a U. In addition, and also unlike *ago7*, the *ago5* mutants have no visible phenotype. It is therefore likely that AGO1 and AGO5 are functionally distinct. Characterisation of the AGO5 target RNAs will be informative about the biological role of this protein.

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**ICAR608**

## PHYSICAL AND GENETIC INTERACTION BETWEEN HC-PRO AND A MEMBER OF THE RAV FAMILY OF TRANSCRIPTION FACTORS

Category: Genetic and Epigenetic Mechanisms

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Helper component proteinase (HC-Pro) is a potent suppressor of RNA silencing encoded by potyviruses. In our lab, the Tobacco Etch potyvirus (TEV) HC-Pro has been shown to interact with several endogenous *Nicotiana tabacum* proteins in the yeast two-hybrid system. One of these tobacco proteins is termed ntRAV due to its homology to the *Arabidopsis thaliana* RAV/EDF family of proteins, which are ethylene-inducible transcription factors. To study the interaction of these proteins in *Arabidopsis*, we used Turnip Mosaic potyvirus (TuMV) HC-Pro, which is a strong suppressor of silencing in *Arabidopsis*, and AtRAV2 which is closely related to ntRAV. Using pulldown methods, we show that HC-Pro and RAV interact directly and *in planta*. *In vitro* experiments using a purified epitope tagged TEV HC-Pro and *in vitro* translated ntRAV verify a direct interaction between these two proteins, while experiments with transgenic plants expressing epitope tagged AtRAV2 and TuMV HC-Pro indicate that the proteins interact *in vivo*. Phenotypic analysis supports the interaction between RAV2 and TuMV HC-Pro in *Arabidopsis*. A parental line expressing TuMV HC-Pro exhibits serrated leaves and decreased fertility while a *rav2* knockout parental line has a wild-type phenotype. In contrast, F2 progeny that express TuMV HC-Pro in a *rav2* knockout background display an intermediate phenotype, marked by reduced serration of leaves and increased fertility. Because the RAV family of proteins is ethylene-inducible, we tested the effect of RAV2 and HC-Pro on ethylene-induced senescence in isolated leaves. Senescence in *rav2* knockout plants was comparable to that of wildtype plants. In contrast, HC-Pro-expressing plants displayed a significant delay in senescence which was dependent on the presence of wildtype levels of RAV2. Our results show a genetic and physical interaction between RAV2 and HC-Pro and suggest that some aspects of HC-Pro function are mediated by RAV2.

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**ICAR609**

## INTRA-SPECIFIC VARIATION OF VEGETATIVE EXPRESSION OF IMPRINTED FWA GENE

Category: Genetic and Epigenetic Mechanisms

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Heritable epigenetic variation is an enigmatic genetic phenomenon, which is known both in mammals and plants. *FWA* gene in *Arabidopsis thaliana* has a potential to cause epigenetic variation in flowering time, because loss of DNA methylation induced by DNA hypomethylation mutations causes heritable late-flowering phenotype. Here we report intra-specific variation of *FWA* expression in natural strains of *A. thaliana*, *Arabidopsis lyrata*, *Arabidopsis halleri*, and *Arabidopsis kamchatica*. Comparison of variations of epigenetic states and nucleotide sequences suggest that the epigenetic change is much more frequent. In addition, *FWA* expression level often changed in artificially generated inter-specific hybrids. Possible contribution of such metastable epigenetic variation to the reversible phenotypic diversity is discussed.

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**ICAR610**

## COMPARING ILLUMINA-BASED METHODS TO STUDY THE ARABIDOPSIS METHYLLOME

Category: Genetic and Epigenetic Mechanisms

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DNA methylation plays an important role both in gene regulation and genome defense. Plants display a rich repertoire of methylation patterns, yet they are remarkably tolerant to dramatic alterations in DNA methylation. This makes them a valuable model system to study methylation. New-generation sequencing has provided the opportunity to expand the current scope of methylation analysis and characterize whole-genome methylation patterns. We used *Arabidopsis thaliana* to compare 3 different techniques that utilize Illumina high-throughput sequencing to study genome-wide methylation: McrBC digestion, bisulfite conversion, and Methylated DNA immunoprecipitation. Each method was assessed both for its sensitivity, by comparing the results with publicly available data, and efficiency, by estimating the number of runs a given technique requires to reach a certain sensitivity level. We also address the advantages and limitations of using each technique.

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**ICAR6011**

ANALYSIS OF EMBRYO-LIKE STRUCTURE FORMATION IN *CLF SWN* DOUBLE MUTANT OF *ARABIDOPSIS THALIANA*.

Category: Genetic and Epigenetic Mechanisms

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In *Arabidopsis thaliana*, CURLY LEAF (CLF) and SWINGER (SWN) are known as Polycomb complex Group (PcG) factor that play key roles by modulating histone methylation. It is suggested that PcG regulates repression of embryonic traits by the fact that *clf swn* forms embryo-like structure (ELS) after germination, but the molecular mechanisms are still unclear.

We found that the formation of ELS in the aerial part of the *clf swn* was suppressed by low temperature (10°C). The LEC1 was mis-expressed in a whole part of the *clf swn* plants at 23°C. By contrast, the LEC1 expression in the aerial part of the *clf swn* was reduced at 10°C, consistent with the suppression of the formation of somatic embryos. To identify developmental stage for repressing embryonic traits, the *clf swn* was transferred from 21°C to 10°C at various time after sowing. ELS were formed when the *clf swn* was transferred after the stage of cotyledon expansion. On the other hands, ELS formation was induced at the low temperature when the germinated seedlings were treated with extremely low concentration of 2,4-D. These results suggest that auxin should be involved in ELS formation in *clf swn* at the stage of cotyledon expansion.

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**ICAR6012**

ACTIVATION OF *ECL1* PROMOTES FLOWERING BY SUPPRESSING THE *FLC* IN *ARABIDOPSIS*.

Category: Genetic and Epigenetic Mechanisms

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A dominant mutant, which showed early flowering and curly leaves phenotype in long day (LD) conditions, was isolated from activation tagging pool. We re-named it *early flowering and curly leaves1-1D* (*ecl1-1D*). Expression studies showed that *FLOWERING LOCUS T* (*FT*) was up regulated, whereas *FLOWERING LOCUS C* (*FLC*) was down regulated in *ecl1-1D*. Loss of function mutant of *ECL1* using artificial miRNA showed high expression of *FLC*, thereby inducing late-flowering phenotype. *ecl1-1D* showed additive effect with photoperiod mutants. Moreover *ecl1-1D* suppressed strong repression effect of *FRI/FLC*. These data suggest that *ECL1* promotes flowering via a suppression of *FLC* in *Arabidopsis*.

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**ICAR6013**

ESSENTIAL ROLES OF THE RNASE PH-LIKE RING PROTEINS OF THE RNA-PROCESSING EXOSOME COMPLEX IN ARABIDOPSIS.

Category: Genetic and Epigenetic Mechanisms

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The exosome is a protein complex that is required for processing, quality control and degradation of a wide variety of RNA species in the nucleus as well as in the cytoplasm. Its core structure, including 6 RNase PH-like subunits and 3 RNA-binding domain subunits, is remarkably conserved in all Eukaryotes and even Archaea. Although in the yeast and human exosome, each subunit seems to have a unique and essential place in the complex, this seems to be true only of the members of the RNase PH-like ring in the Arabidopsis exosome. While knockouts of the RNA-binding domain genes *AtRRP4* and *AtCSL4* produce embryo lethal and no developmental phenotypes, respectively, mutant alleles of the RNase PH-like *AtRRP41* show a female gametophytic arrest at the 2- to 4-nucleate stage of embryo sac development. We have now investigated the effects of loss of other RNase-PH like genes in Arabidopsis, and found that they also produce phenotypes similar to that observed for *Atrrp41* mutants. We will present our observations of the *Atrrp43* null alleles that show female gametophyte arrest mainly at the 2-nucleate stage of embryo sac development, as well as our investigations into partial loss-of-function phenotypes produced by the transformation of *Atrrp43* null mutants with a series of promoter-truncated genomic constructs. Our results suggest that many aspects of plant development, from the female gametophyte onwards, require the presence of all 6 members of the exosome's RNase PH-like ring.

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**ICAR6014**

HAIRPIN-INDUCED GENE SILENCING IN ARABIDOPSIS

Category: Genetic and Epigenetic Mechanisms

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Hairpin constructs are designed to produce double stranded RNA and can be used to target silencing to endogenous genes. The Chalcone Synthase (*CHS*) gene of Arabidopsis codes for the first step of the biosynthesis of the purple pigment anthocyanin. Transgenic plants containing hairpin constructs directed to either the promoter or the reading frame of the *CHS* gene show an anthocyanin deficient (green) phenotype. Over 50 mutants impaired in gene silencing, DNA methylation or RNA processing have been described in Arabidopsis. These mutants have been crossed to appropriate homozygous *CHS* hairpin containing lines to look for second site modifiers of silencing in the F2 generation plants. Using strongly silencing *CHS* hairpin reading frame or promoter constructs, silencing suppressor mutations can be identified. Using a weakly silencing *CHS* hairpin reading frame line the same mutants are being tested as possible second site enhancer mutations of hairpin silencing.

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**ICAR6015**

A NOVEL CLASS OF HISTONE METHYLTRANSFERASES IS REQUIRED FOR HETEROCHROMATIN FORMATION IN ARABIDOPSIS

Category: Genetic and Epigenetic Mechanisms

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Arabidopsis constitutive heterochromatin is marked by repressive chromatin modifications including DNA methylation, histone 3 di-methylation at lysine 9 (H3K9me2), and mono-methylation at lysine 27 (H3K27me1). In contrast to DNA methylation and H3K9me2, the enzyme(s) responsible for methylating H3K27 at the heterochromatin remain unknown. Here we show that ARABIDOPSIS TRITHORAX-RELATED PROTEIN 5 (ATXR5) and ATXR6 exhibit H3K27 mono-methyltransferase activity *in vitro* and *in vivo*. *atxr5 atxr6* double mutants show reduced H3K27me1, partial heterochromatin decondensation, and transcriptional activation of repressed heterochromatic elements. Our results also indicate that the PHD domains of ATXR5 and ATXR6 might be responsible for targeting the proteins to heterochromatin by interacting with histone 3 proteins lacking trimethylation at lysine 4 (H3K4me3). ATXR5 and ATXR6 have previously been shown to be specifically expressed during S-phase of the cell cycle and to interact with the DNA replication factor PCNA. Together, these results suggest that ATXR5 and ATXR6 play a role in the epigenetic inheritance of heterochromatin in Arabidopsis

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**ICAR6016**

CHARACTERIZATION OF AN EARLY FLOWERING MUTANT, SUPPRESSOR OF FRIGIDA5

Category: Genetic and Epigenetic Mechanisms

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Winter-annual accessions requiring vernalization have both functional *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) in Arabidopsis. *FRI* increases the transcript level of *FLC* which encodes a floral repressor. In addition to *FRI*, other factors such as *FRIGIDA LIKE1* (*FRL1*), *FRIGIDA ESSENTIAL1* (*FES1*), and *SUPPRESSOR OF FRIGIDA4* (*SUF4*) are required for *FLC*-specific activation. We isolated another early flowering mutant, *suppressor of FRIGIDA5* (*SUF5*). Analysis of *SUF5p::SUF5-GUS* transgenic line showed that temporal and spatial expression pattern of *SUF5* is similar to that of *FLC*. Previously we showed that *SUF4* interacted with *FRI* and *FRL1*. Through yeast two hybrid assay, the interaction among *FRI*, *FRL1*, *FES1*, *SUF4*, and *SUF5* were checked. We found that *FRI* can interact with all the other factors, which indicates that *FRI* functions as a scaffold for complex formation. The pivotal role of *FRI* in a complex may be related with the natural variants of *fri* in summer-annual Arabidopsis.

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**ICAR6017**

DUAL ROLES OF THE NUCLEAR CAP BINDING COMPLEX AND SERRATE IN PRE-MRNA SPLICING AND MICRORNA PROCESSING IN ARABIDOPSIS

THALIANA

Category: Genetic and Epigenetic Mechanisms

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The processing of Arabidopsis thaliana microRNAs (miRNAs) from longer primary transcripts(pri-miRNAs) requires the activity of several proteins, including DICER-LIKE1 (DCL1), the double stranded RNA binding protein HYPONASTIC LEAVES1 (HYL1), and the zinc finger protein SERRATE (SE). It has been noted before that the morphological appearance of weak se mutants is reminiscent of plants with mutations in *ABH1/CBP80* and *CBP20*, which encode the two subunits of the nuclear cap-binding complex. We report that, like SE, the cap-binding complex is necessary for proper processing of pri-miRNAs. Inactivation of either *ABH1/CBP80* or *CBP20* results in decreased levels of mature miRNAs accompanied by apparent stabilization of pri-miRNAs. Whole genome tiling array analyses reveal that *se*, *abh1/cbp80* and *cbp20* mutants also share similar pre-mRNA splicing defects, leading to the accumulation of many partially spliced transcripts. This is unlikely to be an indirect consequence of improper miRNA processing or other mRNA turnover pathways, since introns retained in *se*, *abh1/cbp80* and *cbp20* mutants are not affected by mutations in other genes required for miRNA processing or for non-sense-mediated mRNA decay. Taken together, our results uncover dual roles in splicing and miRNA processing that distinguish SE and the cap-binding complex from specialized miRNA processing factors such as DCL1 and HYL1.

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**ICAR6018**

MET2A, A DNA METHYLTRANSFERASE REGULATED BY MIR773, REGULATES ARABIDOPSIS GROWTH AND DEVELOPMENT

Category: Genetic and Epigenetic Mechanisms

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Epigenetic regulation is mediated by changes in DNA methylation and/or chromatin modifications. Dynamic changes in DNA methylation status and/or histone modification patterns may be key elements in the plasticity of plant genome as it responds to developmental and environmental cues. Small RNAs, and particularly microRNAs, have been implicated in several developmental processes and stress responses in plants through post-

transcriptional regulation of a variety of genes. The microRNA MIR773 targets two *MET2* (*MET2a* and *MET2b*) genes (DNA methyltransferases) which are linked to the mammal DNMT1 family. MIR773 is regulated by oxidative stress and preferentially accumulated in roots, suggesting its implication in adaptive modifications of root architecture. Transgenic lines overexpressing this microRNA or affected in its production (such as *dcf1* mutants) demonstrated that *MET2* transcripts are regulated by MIR773. A MIR-resistant version of the *MET2a* transcripts has been expressed in Arabidopsis leading to strong accumulation of *MET2a* transcripts. The growth and development of three independent homozygous lines are affected in these MET2 over-expressors. In particular, quantitative modifications of root architecture (primary root length and lateral root densities) were detected in these plants without any major change in root meristem structure. The impact of MET2a in genome regulation is currently being analysed using DNA methylome approaches. Our results raise the possibility that MIR773 and MET2 are novel elements of the regulation of DNA methylation in plants.

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**ICAR6019**

## ROLE OF A HISTONE H3 LYSINE METHYLTRANSFERASE IN CONTROL OF ARABIDOPSIS DEVELOPMENT

Category: Genetic and Epigenetic Mechanisms

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The growth and development of eukaryotic organisms is determined by differential gene expression. Histone methylation has emerged as an important post-translational modification involved in determining chromatin states associated with gene expression or silencing. Knockout mutation (2 T-DNA insertion lines) of an Arabidopsis SET domain gene abolishes trimethylation of H3 lysine 36 in vivo and has pleiotropic effects on plant development, including increased shoot branching and altered anther development. The recombinant protein from the SET domain gene can trimethylate histone H3 in vitro. To identify the genes whose expression is regulated by this H3 methyltransferase, microarray expression profiling were conducted on mRNA from the knockout mutant and wild type plants. Numerous genes were found to be differentially expressed between the mutant and wild-type plants. The differential expression of the genes identified by microarray was further confirmed by quantitative real-time PCR analysis. Furthermore, chromatin immunoprecipitation assay indicated that some of the differentially expressed genes associated with histones that had altered H3 lysine 36 methylation patterns between the mutant and wild-type plants. The possible role of this histone H3 methyltransferase in plant development and growth will be discussed.

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**ICAR6020**

## PROFOUND INFLUENCE OF MITOCHONDRIAL GENOME INSTABILITY ON PLANT DEVELOPMENT

Category: Genetic and Epigenetic Mechanisms

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We have identified nuclear-encoded components of the mitochondrial recombination surveillance apparatus. Disruption of these components allows manipulation of illegitimate recombination activity, an effective means of altering mitochondrial genome behavior in Arabidopsis and several other plant species. Subsequent cytoplasmic sorting of derived mitochondrial variants revealed several altered plant phenotypes including male sterility, leaf variegation, modified leaf morphology, stunted plant growth and enhanced response to heat. Remarkably, these phenotypes were associated with distinct mitochondrial genome configurations and produced dramatic changes in the gene expression profile of the plant. Cross-species redundancy of these types of plant phenotypes in response to mitochondrial genetic perturbation argues the mitochondrial intersection of distinct and conserved cellular pathways influencing plastid developmental functions, cell division processes and plant stress response.

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**ICAR6021**

## OVEREXPRESSION OF MTDNA-ASSOCIATED ATWHY2 COMPROMISES MITOCHONDRIAL FUNCTION

Category: Genetic and Epigenetic Mechanisms

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In *Arabidopsis thaliana*, members of the Whirly family of single-stranded DNA-binding proteins have recently been shown to be primarily localized in organelles. Two representatives of the family, AtWhy1 and AtWhy3 are imported into plastids while AtWhy2 localizes to mitochondria. To understand the role of mitochondrial Whirlies in higher plants, we produced *A. thaliana* lines with altered expression of the *AtWhy2* gene. Overexpression of *AtWhy2* in plants perturbs mitochondrial function by causing a diminution in transcript levels and mitochondrial DNA (mtDNA) content which translates into a low activity level of respiratory chain complexes containing mtDNA-encoded subunits. This lowered activity of mitochondria yields plants that are reduced in size and have distorted leaves that exhibit accelerated senescence. Inactivation of the *AtWhy2* gene did not affect plant development and had no detectable effect on mitochondrial morphology, activity of respiratory chain complexes, transcription or the amount of mtDNA present. This lack of phenotype upon abrogation of *AtWhy2* expression suggests the presence of functional homologues of the Whirlies or the activation of compensating mechanisms in mitochondria. Organellar DNA immunoprecipitation experiments also demonstrated that AtWhy2 binds to mtDNA.

This report constitutes the first evidence of a function for the Whirlies in organelles. We propose that AtWhy2 could play a role in the regulation of the gene expression machinery of mitochondria.

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**ICAR6022**

## THE MECHANISMS OF MOBILE RNA SILENCING

Category: Genetic and Epigenetic Mechanisms

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Small RNAs are important regulators of plant growth and function. It is believed that small RNAs can move from cell to cell, but the function and mechanism of small RNA movement is poorly understood. A reporter system to assay small RNA movement was developed in *Arabidopsis* using a phloem specific promoter to silence the *Phytoene Desaturase (PDS)* gene, producing small RNAs in the phloem which spread and cause photobleaching around the vein. Several RNA silencing mutants were found to affect photobleaching and were hypothesized to be involved in small RNA production or movement. In particular, mutants in the heterochromatic RNA silencing pathway were found to either increase or decrease photobleaching spread in the transgene background. In the research presented here, double mutants were made between heterochromatic RNA silencing proteins to better understand why proteins in this pathway can cause enhancement or reduction of silencing. Furthermore, to characterise the requirements of RNA silencing proteins in either the production or movement of small RNAs in the *PDS* silencing system, a mislocalisation study was undertaken. RNA silencing proteins were expressed in a null background under various promoters. Transformed plants were then analysed for levels of *PDS* silencing. Initial results from these studies suggest that RNA silencing machinery works in modules, and that several RNA silencing proteins are required for the production and reception of small RNAs.

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### **ICAR6023**

#### MIR171A TRANSCRIPTION AND ACTIVATION

Category: Genetic and Epigenetic Mechanisms

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We have previously shown with miR171a, that plant miRNA genes are modular independent transcription units in which the fold-back pre-miRNA is sufficient for miRNA processing. This processing depends on flanking sequences within the miRNA stem-loop precursor rather than the miRNA sequence itself. We also showed that the upstream region of plant miRNA genes contains highly tissue specific promoter elements.

Our work aims at identifying the cis and trans-acting elements that modulate expression of miRNAs in time and space. To achieve this goal we are using two approaches: First, we are using phylogenetic shadowing to identify regulatory elements that account for the tissue specificity and basal activity of the miR171a transcription unit. Second, we are screening for mutants with altered expression pattern of miR171a. A miR171a promoter-GFP fusion line was mutagenized by EMS. M2 seeds were screened for altered GFP expression. Also, using transposons, a collection of insertion mutants of the selected miRNA promoter regions (p171a::DsRed) has been generated *in-vitro*. Molecular and phenotypic analysis of selected mutants will be presented.

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### **ICAR6024**

#### CHARACTERIZATION OF THE INTERACTION BETWEEN THE DSRNA-BINDING PROTEIN DRB4 AND THE DICER-LIKE PROTEIN DCL4

Category: Genetic and Epigenetic Mechanisms

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Double-stranded RNAs (dsRNAs) are signal molecules in both RNAi and microRNA (miRNA) pathways, and consequently dsRNA-binding proteins, which contain one to three conserved dsRNA-binding motifs (dsRBMs), play important roles in both pathways. RNaseIII enzymes, Dicer, which have one or two dsRBMs, process dsRNAs into small RNAs (small interfering RNAs (siRNAs) and miRNAs), and they interact with dsRNA-binding proteins. In *D. melanogaster*, Dicer-1 and Dicer-2 interact with Loquacious and R2D2, respectively, to function in miRNA and RNAi pathways. In *C. elegans*, DCR-1 associates with RDE-4, which is essential for RNAi.

*Arabidopsis thaliana* encodes four DICER-like (DCL) proteins and five RDE-4/R2D2-like dsRNA-binding (DRB) proteins. Previously, we have demonstrated that DCL4 specifically interacts with DRB4 *in vitro*. Here, we report the interaction between DCL4 and DRB4 *in vivo*. In immunoprecipitation experiments with either anti-DCL4 or anti-DRB4 antibody from crude extracts of wild-type *Arabidopsis* plants, co-immunoprecipitation of DCL4 and DRB4 was detected. This interaction was confirmed by immunoprecipitation experiments with transgenic plants expressing HA-tagged DCL4 (DCL4-HA) or DRB4 (DRB4-HA). Less accumulation of the TAS3 *trans*-acting siRNA (tasiRNA) and over accumulation of its target mRNAs (*ARF3* and *ARF4*) in both *drb4-1* and *dcl4-2* mutant plants indicate that DRB4 together with DCL4 functions in the tasiRNA pathway. These results suggest that a Dicer protein functions with a dsRNA-binding protein in a plant (*Arabidopsis*) as well as animals.

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### **ICAR6025**

#### GENIC AND GLOBAL FUNCTIONS FOR PAF1C IN CHROMATIN MODIFICATION AND GENE EXPRESSION IN ARABIDOPSIS

Category: Genetic and Epigenetic Mechanisms

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Modification of nucleosomal histone H3 within its N-terminal domain provides transcriptional regulatory information superimposed on nucleotide sequence. In budding yeast, H3 modifications are linked to transcriptional elongation through the conserved regulator Paf1C. In *Arabidopsis*, Paf1C influences various aspects of development including flowering time and floral development. To investigate Paf1C-related function in higher eukaryotes, we mapped genomic and genic patterns of histone H3 density and H3 methylated at K4, K27, and K36 at high resolution in the *Arabidopsis* genome,

analyzed the effects of loss of Paf1C on these patterns, and integrated this data with existing gene expression information. We found that Paf1C is required globally to maintain H3 density and appropriate intragenic domains of H3K4me3 and H3K36me2, especially within the most highly transcribed genes. Genes regulated by plant Paf1C showed dual enrichment by H3K4me3 and H3K27me3, a signature marking key developmental regulators in human embryonic stem cells. Consistent with this, the subset of genes targeted by Arabidopsis Paf1C was enriched in known developmental regulators including MADS-box genes. At the Paf1C- and Polycomb-Group (PcG)-regulated gene *FLC*, transcriptional silencing and loss of H3K4me3 and H3K36me2 in Paf1C mutant plants was accompanied by expansion of H3K27me3 into the promoter and transcriptional start regions and further enrichment of H3K27me3 within the transcribed region. These results highlight both genic and global functions for plant Paf1C in histone modification and gene expression, and link transcriptional activity with cellular memory.

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**ICAR6026**

TERMINAL FLOWER2, THE ARABIDOPSIS HETEROCHROMATIN PROTEIN1 HOMOLOGUE, IS A FACTOR CONTRIBUTING TO AUXIN HOMEOSTASIS

Category: Genetic and Epigenetic Mechanisms

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The *Arabidopsis terminal flower2 (tfl2)* mutant has a pleiotropic phenotype displaying dwarfism, early flowering, reduced photoperiod sensitivity and terminating inflorescences. Though this is not an obvious auxin-related phenotype, preliminary microarray studies indicated that the *tfl2* mutation might affect auxin-regulated processes.

The auxin biosynthesis rate and levels of endogenous auxin are significantly decreased in the *tfl2* mutant. This is seen specifically over time in aerial tissues, while roots remain unaffected. After measuring the relative transcription levels of the genes involved in the complex biosynthetic pathway of auxin, several differences were found between *tfl2* and wt. Interestingly, we found a reduction in the relative mRNA levels of specific *YUCCA* genes, which are believed to regulate the rate limiting step in the tryptophan dependent auxin biosynthesis pathway.

The mutation in *TFL2* also affects the response to exogenously supplied auxin in both root and shoot tissues. We have analysed the induction of auxin response genes in *tfl2* and wt plants as well as auxin regulated developmental responses. The results show that *tfl2* plants are less sensitive to exogenously applied auxin since the expression of early auxin induced genes is substantially reduced in *tfl2* compared to wt.

AUX/IAA proteins are powerful transcriptional repressors in auxin signal transduction and we show that TFL2 interacts with three AUX/IAA proteins, both in yeast and *in planta*. We are currently investigating the possible interaction between TFL2 and the remaining AUX/IAA proteins. Furthermore we are examining a possible mechanism by which TFL2 participates in the regulation of genes downstream of the AUX/IAA transcriptional repressors. Based on our results we suggest a model where TFL2 has dual functions related to auxin, in maintaining auxin homeostasis and in the response to auxin.

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**ICAR6027**

THE ARABIDOPSIS SUVR1, SUVR2 AND SUVR4 PROTEINS DEFINE A SUBGROUP OF SET DOMAIN PROTEINS ASSOCIATED WITH THE NUCLEOLUS

Category: Genetic and Epigenetic Mechanisms

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Chromatin is a dynamic DNA-protein structure that can exist as either transcriptionally repressive heterochromatin or permissive euchromatin. This chromatin organization is in part regulated by different posttranslational modifications such as methylation. Proteins containing the evolutionary conserved SET domain have the ability to methylate lysine residues on the histone tails, and are as such involved in the regulation of eukaryotic gene expression and chromatin structure through this histone lysine methyltransferase (HKMTase) activity. The position of the methylated lysine residue on the histone tail and the level of methylation (mono-, di, or tri) are of outmost importance, and determine downstream chromatin organization. The SET domain is evolutionary conserved and named after three *Drosophila* proteins SUPPRESSOR OF VARIEGATION 3-9 [SU(VAR)3-9], ENHANCER OF ZESTE [E(Z)] and TRITHORAX (TRX). SU(VAR)3-9 has been associated with heterochromatinization and gene repression. In *Arabidopsis*, there are 10 SUVH and 5 SUVR genes coding for proteins of that kind.

We have focused on the closely related SUVR1, SUVR2 and SUVR4 proteins. These proteins all contain a novel N-terminal domain that we called the WIYLD domain which we show is a protein-protein interaction domain. GFP-SUVR fusion proteins preferably localize to the nucleolus and it is therefore suggested that the SUVR proteins are involved in the regulation of rRNA expression.

Of the three proteins SUVR1, SUVR2 and SUVR4, it is only SUVR4 that has an *in vitro* HKMTase activity, and with specificity for monomethylated H3K9. In a position that has shown to determine the number of attached methyl groups (product specificity), called the tyrosine/phenylalanine switch, wild-type SUVR4 contain the amino acid tryptophane. We have shown that this amino acid is critical for the activity and not the product specificity of SUVR4. To better understand the *in vivo* function of SUVR1 SUVR2 and SUVR4, we are currently analyzing single and double knock-out mutants for molecular and morphological phenotypes.

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**ICAR6028**

GENETIC DISSECTION OF DNA HYPER-METHYLATION INDUCED BY *DDM1* (DECREASE IN DNA METHYLATION) MUTATION.

Category: Genetic and Epigenetic Mechanisms

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In the genome of Arabidopsis, DNA methylation is mainly found in repeated sequences. The methylation in the repeated sequences depends on a chromatin remodeling factor, DDM1 (decrease in DNA methylation). The *ddm1* mutation induces various types of developmental abnormalities through derepression of the repeats and transposons. On the other hand, characterization of one of the *ddm1*-induced phenotypes, called *bonsai* (*bns*), revealed that it is due to DNA hyper-methylation and silencing of a gene (*BNS* gene), which encodes a protein similar to the cell cycle regulator APC13 (Saze and Kakutani 2007). The silencing and hypermethylation is associated with small RNA for the *BNS* locus. To understand factors necessary for this enigmatic DNA hyper-methylation in the *BNS* locus, we combined the *ddm1* mutation with mutations in various DNA methylation machineries, such as DNA methyltransferases, RNAi components, and histone modifiers. Interestingly, mutations of known components in the RNA-directed DNA methylation (RdDM) pathway, such as RDR2, DCL3, AGO4, DRM2 and pol IV, didn't suppress hyper-methylation in the *BNS* locus. Results suggesting possible involvement of other factors will be presented.

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### **ICAR6029**

#### STUDIES ON FACTORS INVOLVED IN RNA SILENCING IN ARABIDOPSIS THALIANA

Category: Genetic and Epigenetic Mechanisms

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In eukaryotes, small RNAs play important roles in RNA silencing pathways which are involved in many biological processes, e.g. developmental regulation and stress responses. The most-well studied endogenous small RNAs are microRNAs (miRNAs). In *Arabidopsis thaliana*, miRNAs are excised from primary miRNA transcripts forming stem-loop structures by the RNase III enzyme DICER-LIKE 1 (DCL1). This enzyme works coordinately with HYL1, a double-stranded RNA binding protein, and SERRATE, a C2H2 zinc finger protein. In this study, to better understand the mechanisms and factors involved in miRNA pathway, a genetic screen was performed to identify second mutations that suppress *hy1* phenotype. We have identified a dominant suppressor of *hy1* in which the level of many kinds of miRNAs recovered to some extent, suggesting that the responsible mutation promoted the miRNA production without HYL1. However, in the single mutant of the responsible gene, miRNAs were slightly reduced unexpectedly. These results showed the intricate regulation of miRNA processing machinery.

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### **ICAR6030**

#### EPIGENOMIC PROFILING OF ARABIDOPSIS CELL SUSPENSION CULTURE AND THE PATTERNS OF EPIGENETIC CHANGES IN DE-DIFFERENTIATED PLANT CELLS

Category: Genetic and Epigenetic Mechanisms

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Plant cells grown in culture exhibit genetic and genomic instability, exemplified by reactivation of transposable elements and heritable somaclonal variation. Epigenetic mechanisms have been implicated in these processes, which parallels observations in animal cell cultures and clones. Using a combination of microarray profiling and high throughput sequencing technologies, we profiled gene expression patterns as well histone and DNA modifications and small RNA molecules creating extensive epigenomic maps of *Arabidopsis* rosette leaves and a rapidly dividing, de-differentiated cell suspension culture. The combined analysis showed that the bulk of euchromatic DNA methylation correlates with H3K4me2 and that a fraction of genic DNA becomes hypermethylated in cell culture. In contrast, we found striking DNA hypomethylation and loss of H3K9me2 histone modification and transcriptional activation patterns specific to transposable element families. Additionally we showed that a combination of the epigenetic changes in cell culture can impact gene expression through epiallele establishment. High throughput sequencing of small interfering RNA revealed a shift in abundance and size of TE siRNAs which correlates with the patterns of changes in DNA and histone methylation as well as with the transcriptional reactivation. This is accompanied by changes in expression of the Argonaute gene family, implicating the role of RNAi and targeting of chromatin modification in the activation of TEs and heritable changes of gene expression in plant cell culture. The resulting epigenetic changes resemble those in animal cell lines and may reflect a common targeting mechanism.

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### **ICAR6031**

#### NUCLEOSOME POSITIONING IN ARABIDOPSIS

Category: Genetic and Epigenetic Mechanisms

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Epigenetic variation is defined as mitotically and/or meiotically heritable changes in gene function not associated with changes in genomic DNA sequence. Mechanisms regulating epigenetic variation include changes to chromatin structure, where: alterations are brought about by modifications to DNA and histone proteins associated with DNA in chromatin. Nucleosomes consist of approximately 147 bp DNA wrapped around a histone octamer, separated by linker DNA and a linker-associated histone. While no consensus DNA sequence exists for nucleosome positioning, sequence preferences influence nucleosome position. Repeating dinucleotide sequences with 10 bp periodicity confer curvature and flexibility to a DNA molecule and are often found in nucleosomal DNA sequences. Since genome architecture and sequence composition differs between species, it is likely that nucleosome positioning sequence preferences do also.

The aims of this study are to obtain a set of nucleosome positions in *Arabidopsis* and test the data to determine sequence biases, and to use genome

tiling microarrays to investigate global patterns of nucleosome occupancy. The hypotheses being tested are: 1. Arabidopsis has specific sequence biases for nucleosome positioning. 2. Nucleosome positioning in plants differs from other taxa due to differences in sequence composition and genome architecture.

Dinucleosome DNA libraries have been constructed and approximately 500 clones sequenced by conventional Sanger methods. This has enabled the distribution of estimated linker lengths to be determined. Fourier analysis indicates that there may be bias of periodicity in Arabidopsis (of 7-8 bp) which differs from previously established linker lengths in yeast, chicken and human chromatin.

Cross-linked mononucleosome DNA fragments have successfully been hybridised to the Affymetrix Arabidopsis GeneChip® 1.0R tiling array, and analyses are currently underway. Initial findings indicate differences in the binding patterns between nucleosome and genomic DNA fragments for the nuclear chromosomes, which are not observed for chloroplast or mitochondrial chromosomes.

Ongoing work involves high-throughput sequencing in order to identify translational variation in nucleosome positions within Arabidopsis dinucleosomes.

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### **ICAR6032**

#### MOLECULAR CHARACTERIZATION OF SAGA FACTOR 29 (SGF29) MUTATIONS IN ARABIDOPSIS

Category: Genetic and Epigenetic Mechanisms

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The post-translational modification of histones plays an important role in chromatin regulation, a mechanism that insures the fidelity of gene expression and other DNA processes. Among these modifications, acetylation is mediated by histone acetyltransferase (HAT) complexes such as the Spt-Ada-Gcn5-Acetyltransferase (SAGA), a multiprotein coactivator, containing approximately 14 polypeptides in yeast. Although components of this complex are well conserved through evolution, plant orthologs of the yeast SGF29, a recently described component of SAGA, has not yet been identified, and its function is still unknown even in yeast. Arabidopsis genome encodes two orthologs of SGF29 (At3g27460 and At5g40550), designated SGF29a and SGF29b displaying 91% identity at amino acid sequence. To examine their biological role in Arabidopsis thaliana, we isolated T-DNA insertion mutants from the SALK collection. Homozygous mutations were identified for both genes, one with a T-DNA insertion in the seventh exon of SGF29a and the other in the 5' UTR of SGF29b. RT-PCR analysis of homozygous sgf29a plants confirmed the absence of full length SGF29a transcript, while a reduced amount of SGF29b transcript was observed in sgf29b mutants. Although sgf29b mutants were indistinguishable from wildtype plants, sgf29a plants showed less and smaller rosette leaves and late flowering in short days. Moreover, sgf29a mutants were resistant to salt stress in seed germination and root growth assays, indicating that SGF29a may be involved in regulation of flowering time in short days and in responses to salt stress. The double mutant sgf29a;ada2b-1 displayed an ada2b-1 phenotype indicating that there is no synthetic lethality. However the double sgf29a-/-;ada2b-1/+ resulted in an intermediate phenotype with short stamens, small siliques and increased number of secondary inflorescences, suggesting that ADA2b may require SGF29a function.

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### **ICAR6033**

#### T-DNA-MEDIATED TRANSFER OF AGROBACTERIUM CHROMOSOMAL DNA SEQUENCES INTO PLANTS

Category: Genetic and Epigenetic Mechanisms

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Our current view of plant transformation by Agrobacterium is that the T-DNA segment is the only piece of Agrobacterium DNA stably inserted into the plant genome. The exceptions observed were Ti plasmid vector backbone sequences found in some transgenic plants. Here we reveal that large fragments of Agrobacterium chromosomal DNA (AchromoDNA) are present in several transgenic Arabidopsis thaliana T-DNA insertion lines. The integrated AchromoDNA fragments are physically linked to plant genomic DNA and about 0.2 to at least 18 kb in length. They contained up to 18 complete bacterial ORFs, originated from either the linear or circular bacterial chromosomes, and are evident from several independent T-DNA insertion sequence databases. AchromoDNAs are often associated to the right border (RB) of the T-DNA and are derived from defined regions of the bacterial chromosomes. We estimate that about one out of every 250 transgenic plants may carry AchromoDNA fragments. We postulate that chromosomal nicks and breaks generated for example by IS-element transpositions in Agrobacterium along with micro homologies between T-DNA borders and the *A. tumefaciens* chromosomal DNAs facilitates the insertion of T-DNA into bacterial chromosomal DNA, and that functional border-like sequences around the insertion sites occasionally but regularly enable transfer of such sequences into plants.

Our findings have revealed novel insights into some of the sequential events that occur during the natural infections process of plants by Agrobacterium.

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## ABIOTIC INTERACTIONS

### ICAR701

IDENTIFICATION OF NOVEL ABIOTIC STRESS DETERMINANTS IN ARABIDOPSIS THALIANA THROUGH A REVERSE GENETICS APPROACH

Category: Abiotic Interactions

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Abiotic stresses, including extreme temperatures, drought, salinity and oxidative stress, have become a serious threat to the agricultural practice, greatly reducing crop yield well below the genetic potential. As a model species, *Arabidopsis thaliana* has been fundamental in the major progress in identifying plant abiotic stress tolerance determinants. A consistent strategy in the identification of stress tolerance determinants relies on the use of mutant analysis, centred on the characterization of a visible phenotype, and based on forward and reverse genetic techniques which are associated with gain and loss-of-function studies. The expression analysis of abiotic stressed *Arabidopsis* plants using publicly available Affymetrix ATH1 microarray data led to the identification of several genes of unknown function that are putatively involved in abiotic stress resistance. We have initialized the functional characterization of these genes, by isolating *Arabidopsis* loss-of-function mutant lines for each gene. Subsequent screens for stress-associated phenotypes are currently underway.

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### ICAR702

INTEGRATION OF SALT STRESS AND NITRATE CONTROL OF ARABIDOPSIS LATERAL ROOT PROLIFERATION

Category: Abiotic Interactions

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Plant roots exhibit remarkable developmental plasticity in response to local soil conditions such as drought and nutrient supply. Here we show that mild salt stress stimulates proliferation of lateral roots in *Arabidopsis*, with a concomitant reduction in lateral root length. Exposure of roots to  $\text{NO}_3^-$ -rich nutrient medium dramatically enhances salt stress stimulation of lateral root proliferation, thereby compensating for the decreased lateral root length and maintaining overall lateral root surface area. Increased lateral root proliferation is specific to salt stress and is due to the progression of more lateral root primordia into mature lateral roots in salt-stressed plants. In salt-stressed roots, greater numbers of lateral root primordia exhibit expression of a reporter gene driven by the auxin-sensitive *DR5* promoter than in unstressed roots. Moreover, in the auxin transporter mutant *aux1-7*,  $\text{NO}_3^-$ -enhanced salt stress stimulation of lateral root proliferation is completely abrogated. The results suggest that salt stress promotes auxin accumulation in developing primordia thereby preventing their developmental arrest. Examination of ABA and ethylene mutants revealed that ABA synthesis and a component of the ethylene signaling pathway are also required for  $\text{NO}_3^-$ -enhanced salt stress stimulation of lateral root proliferation. We propose that during root colonization of  $\text{NO}_3^-$ -rich soil patches in saline soils, salt stress signaling and nitrate signaling are integrated to stimulate lateral root proliferation thereby facilitating exploitation of soil resources under sub-optimal conditions.

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### ICAR703

THE NODULE INCEPTION-LIKE PROTEIN 7 IS AN IMPORTANT REGULATOR OF NITRATE SENSING AND METABOLISM IN *ARABIDOPSIS*

Category: Abiotic Interactions

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Nitrate is essential for plant growth and crop productivity<sup>1</sup>. However, no regulatory genes controlling its assimilation have so far been identified in higher plants. The first *NIN* gene was identified as being defective in a nodule inception mutant of the legume *Lotus japonicus*<sup>2</sup>. Recently the homologue *NIT2* protein from *Chlamydomonas* was found to regulate nitrate assimilation by controlling the expression of the nitrate reductase gene<sup>3</sup>. Both *NIN* and *NIT2* encode putative transcription factors and are involved in processes dependent of the nitrogen status of the organism. Interestingly, the *NIN* gene family is found in all higher plants which suggests a more general role of this gene family in the regulation of nitrogen management. We therefore decided to investigate the functions of the *NIN* Like Proteins (NLPs) in the model plant *Arabidopsis* which carries 9 *NLP* genes<sup>4</sup>. Here we report a role for the *NIN* Like Protein 7 (NLP7) in the regulation of nitrogen metabolism in *Arabidopsis*. *nlp7* knockout mutants constitutively display several traits of nitrogen starved plant and were affected in both nitrate assimilation and response to the nitrate signal. *In situ* hybridisation and reporter gene expression studies demonstrated *NLP7* expression in cell types related to sites of intensive assimilate transport, suggesting also a function in the sensing of N-metabolite fluxes. We showed that the NLP7 protein is indeed localized in the nucleus which is consistent with its function as a transcription factor. Altogether, we propose NLP7 as the first higher plant regulatory protein specific for N assimilation described so far.

<sup>1</sup> D. Lawler, G. Lemaire, F. Gastal, *Plant Nitrogen: Nitrogen, plant growth and crop yield*. P. Lea, J. F. Morot-Gaudry, Eds. (Springer, Berlin, 2001) pp.343-368.

<sup>2</sup> L. Schäuser, A. Roussis, J. Stiller, J. Stougaard, *Nature* 402, 191 (1999).

<sup>3</sup> A. Camargo, et al., *Plant Cell* 11, 3491 (2007).

<sup>4</sup> L. Schäuser, W. Wieloch, J. Stougaard, *J. Mol. Evol.* 60, 229 (2005).

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### ICAR704

OVEREXPRESSION OF ARABIDOPSIS TRYPTOPHAN SYNTHASE BETA 1 (ATTSB1) IN ARABIDOPSIS AND TOMATO CONFER TOLERANCE TO

## CADMUM STRESS

Category: Abiotic Interactions

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Tryptophan is an essential amino acid in humans, and in plants, it plays a major role in the regulation of plant development and defense responses. However, little is known about tryptophan (Trp)-mediated cadmium (Cd) tolerance. Gene expression analysis showed that *Arabidopsis thaliana* tryptophan synthase beta 1 (*AtTSB1*) is upregulated in plants treated with Cd; hence, we investigated whether this gene is involved in Cd tolerance. Exogenous application of Trp to wild-type *Arabidopsis* enhances Cd tolerance. Cd tolerance in the Trp-overproducing mutant *trp5-1* was associated with high chlorophyll levels and low lipid peroxidation, as indicated by malondialdehyde 4-hydroxyalkenal (MDA) level, whereas the wild-type developed symptoms of severe chlorosis. Moreover, the Trp-auxotroph mutant *trp2-1* was sensitive to Cd. CaMV 35S promoter-driven *AtTSB1* enhanced Trp accumulation and improved Cd tolerance in transgenic *Arabidopsis* and tomato plants without increasing the level of Cd. Moreover, RT-PCR confirmed that enhanced level of Trp in *AtTSB1* transgenic *Arabidopsis* plants affected the expression of *AtZIP4* and *AtZIP9* metal transporters, which interfered with Cd ion trafficking, a mechanism of transcriptional regulation that does not exist in wild-type plants. Overexpression of *AtTSB1* in transgenic tomato also produced higher TS-β enzyme activity than that in wild-type plants. These results implicate that Trp could be involved in Cd defense.

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## ICAR705

A HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP) PROTEIN, ATHB12, MAY REGULATE GROWTH OF THE INFLORESCENCE STEM IN ARABIDOPSIS.

Category: Abiotic Interactions

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*ATHB12* is a homeobox gene inducible by abscisic acid (ABA) and water stress. Previous studies revealed that *ATHB12* was expressed strongly in stem, petiole and leaves in response to ABA. Induction by ABA was analyzed using transgenic tobacco plants harboring serial 5' deletions of the *ATHB12* promoter fused to the B-glucuronidase (GUS) gene. Enzyme activity was measured by fluorometric assay and real-time quantitative PCR. The longest promoter (2.1-kb) produced the highest GUS activity about 3-fold higher than the 1.5-kb and other promoter fragments, indicating that the promoter region between 2.1-kb and 1.5-kb contains an essential element(s) required for ABA-induction of *ATHB12*. A T-DNA insertion mutant of *ATHB12* was isolated and found to contain an insertion -293 base pairs upstream of the transcription start site. The mutant had lower expression of *ATHB12* in the stems and longer inflorescence stems than wild type. T-DNA insertion mutant also had a higher germination rate on ABA-containing media. The phenotype of this mutant is the reversal of that of the *ATHB12* overexpressor showing the retardation of stem elongation. Moreover, ABA treatment induced *ATHB12* expression in inflorescence stem and inhibited the stem growth, similarly in the *ATHB12* overexpressor. Our data suggest that *ATHB12* which is induced by ABA and water deficit might have a negative role in the regulation of stem growth against the stresses and *ATHB12* could down-regulate the pathway of the other plant growth hormone related to the stem growth.

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## ICAR706

THE NAC DOMAIN TRANSCRIPTION FACTOR ANAC102 IS REQUIRED FOR FULL GERMINATION OF ARABIDOPSIS THALIANA SEEDS AFTER EXPOSURE TO LOW OXYGEN.

Category: Abiotic Interactions

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Low-oxygen stress imposed by field waterlogging is a serious impediment to plant growth and germination. An *Arabidopsis thaliana* NAC domain transcription factor, *ANAC102*, was found to be induced >2.5-fold under low oxygen stress within 30 minutes in both roots and shoots as well as in low oxygen treated imbibed seeds. Global transcriptome analysis of lines over-expressing *ANAC102* revealed altered expression of 63 genes under normal growth conditions, 51 of which had been previously identified as being low-oxygen responsive. These 51 low-oxygen responsive genes do not contain members of the glycolysis or fermentation pathways, suggesting that *ANAC102* is involved in regulation of low-oxygen responses outside of primary metabolism. Under both normal and low oxygen conditions, adult plants of *Arabidopsis* lines with knocked-out expression of this NAC domain transcription factor showed no difference in global gene expression. Increasing or decreasing the expression of *ANAC102* did not yield a visible phenotype in unstressed plants and did not affect adult plant survival under low oxygen stress, possibly due to redundancy as some NAC domain transcription factors closely related to *ANAC102* also appear to be induced under low-oxygen conditions. Decreased expression of *ANAC102* did result in germination efficiencies half that of wildtype following a low oxygen treatment, but increased expression had no effect on germination. Gene expression responses to hypoxia were found to be different in seeds than in adult plants and between seeds of *Arabidopsis* lines with knocked-out expression of this NAC domain transcription factor and wild-type. This protective role during germination appeared to be specific to low oxygen stress, implicating *ANAC102* as an important regulator of seed germination under low oxygen conditions. To the best of our knowledge, this is the first *Arabidopsis* transcription factor implicated in low-oxygen stress responses at the seed developmental stage.

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## ICAR707

COMMUNICATION OF WATER SHORTAGE OF ROOTS TO THE PLANT SHOOT

Category: Abiotic Interactions

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Communication of water shortage of roots to the plant shoot

Plant roots act as sensors for soil water status. According to a widely accepted model, water shortage sensed by the root leads to ABA synthesis. ABA is then thought to be translocated from the root to the shoot where it initiates stomata closure. We used reciprocal grafts between wild type and ABA-deficient *Arabidopsis* and an ABA-indicative reporter system to test this model. We found that ABA responses in the shoot are elicited in the absence of

root-generated ABA and that shoot-derived ABA is necessary and sufficient for stomata closure. Root water stress induced an immediate turgor decline in mesophyll cells pointing to the rapid propagation of a hydraulic signal to the shoot. Consistently, attenuating a hydraulic signal in the shoots of root water-stressed plants attenuated the turgor decline in mesophyll cells and abrogated stress-related shoot responses such as stomata closure and induction of ABA-dependent gene expression. Our findings disprove the model whereby ABA acts as a long-distance signal relaying water stress from the root to the shoot, but argue in favour of a hydraulic signal instead.

Reference:

Christmann, A., Weiler, E. W., Steudle, E. and Grill, E. (2007) A hydraulic signal in root-to-shoot signalling of water shortage. *Plant J.* 52, 167-174.

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### ICAR708

#### SUMO PROTEASES OTS1 AND OTS2 ARE REQUIRED FOR SALT TOLERANCE IN *ARABIDOPSIS*

Category: Abiotic Interactions

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High salinity is a major factor impairing plant growth and viability. However, the molecular basis underlying salt tolerance is still poorly understood.

Covalent protein modification by Small Ubiquitin-like Modifier (SUMO) is emerging as an important mechanism of signalling regulation in eukaryotes. SUMOylation affects protein target localization, activity and binding properties. Attachment of SUMO onto substrates is reversible and SUMO-proteases which specifically cleave the SUMO-substrate linkages play a vital regulatory role in the regulation of signalling. We have identified two nuclear-localised SUMO proteases *OTS1* and *OTS2* (*OVERLY TOLERANT TO SALT* 1 and 2) that act redundantly to regulate salt stress responses in *Arabidopsis*. *ots1 ots2* double mutants show a strong sensitivity to salt. However, during non-salt conditions, *ots1 ots2* double mutants are phenotypically similar to wild-type plants. Salt stress induces a dose dependant accumulation of AtSUMO1/2-conjugated proteins in *Arabidopsis*. OTS1 and OTS2 activities are required for de-conjugation of AtSUMO1/2 in vivo as *ots1 ots2* double mutants display an increased accumulation of AtSUMO1/2-conjugated proteins compared to wild type in both normal and salt-supplemented growth conditions. Transgenic lines over-expressing *OTS1* have increased salt tolerance and a concomitant reduction in the levels of SUMOylated proteins. Conversely, the ectopic expression of the mutant *ots1(C526S)* protein lacking SUMO protease activity fails to produce a salt tolerant phenotype. We find that salt induces a rapid OTS1 protein turn-over, thus providing a mechanism by which salt may negatively affect OTS1-dependant signalling in plants. Our results suggest a direct requirement for OTS1 deSUMOylation activity to mount salt tolerance responses and provide evidence that OTS1 target proteins are key determinants of salt tolerance in *Arabidopsis*.

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### ICAR709

#### SALT TOLERANCE HOMOLOG 3 (STH3)/ LZF1, A B-BOX PROTEIN IN ARABIDOPSIS INVOLVED IN LIGHT-DEPENDENT DEVELOPMENT AND GENE EXPRESSION, UNDERGOES COP1-MEDIATED UBIQUITINATION.

Category: Abiotic Interactions

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Recently B-box containing proteins have been shown to play an important role in light signaling in plants. Here we identify *STH3*, a B-box encoding gene, that genetically interacts with two key regulators of light signaling, HY5 and COP1. *STH3* physically interacts with HY5 both in yeast and in plant cells. While *STH3* is uniformly nuclear by itself, when co-expressed with COP1 it shows a COP1 dependent localization to speckles. We identified a T-DNA insertion mutant *sth3*, which is hyposensitive to high fluence blue and far-red light and has elongated hypocotyls under short days. Analyses of double mutants between *sth3* and *sth2*, another B-box protein in *Arabidopsis*, suggest that *STH3* and *STH2* have partially overlapping functions. Additive effects seen in the double mutants between *sth3* and *hy5* indicate that *STH3* and *HY5* might function independently. Highly elongated hypocotyls in the triple mutants between *sth2*, *sth3* and *hy5* suggest a synergistic relationship between the three genes. Interestingly, functional assays in protoplasts suggest that *STH3* can activate transcription both independently and together with *STH2* through the G-box promoter element. Furthermore, *sth3* partially suppresses the hypocotyl phenotype of dark grown *cop1* seedlings in an allele-specific manner. Under constant white light accumulation of anthocyanin is reduced in *sth3* and together with *STH2* it can suppress the high levels of anthocyanin in light grown *cop1-4* seedlings indicating a genetic relationship between the B-box proteins and COP1. Additionally, our in-vitro ubiquitination data suggests that COP1 ubiquitinates *STH3*. In conclusion, we have identified *STH3* as a positive regulator of photomorphogenesis acting in concert with HY5 and *STH2*, while at the same time being a target of COP1-mediated ubiquitination.

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### ICAR7010

#### THE ARABIDOPSIS THALIANA GCN2 KINASE IS ESSENTIAL FOR GROWTH IN STRESS CONDITIONS AND IS ACTIVATED IN RESPONSE TO WOUNDING

Category: Abiotic Interactions

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Phosphorylation of the *e* subunit of eukaryotic translation initiation factor 2 (eIF2 $\alpha$ ) provides a key mechanism for down-regulating protein synthesis in response to nutrient starvation or stress in mammalian and yeast cells. Although mammals use four different eIF2 $\alpha$ -kinases (PKR, PERK, HRI and GCN2) to respond to various stresses, plants only possess one type (a GCN2-like enzyme). Although *Arabidopsis* GCN2 was shown to

complement a yeast gcn2 mutation in the presence of inhibitors of amino acids biosynthesis, the mode of action of plant GCN2 is unknown and may differ as that of GCN2 in yeast. We show here that in response to several stresses, including amino acid and purine starvations, UV, cold shock and wounding, Arabidopsis GCN2 kinase is activated and phosphorylates eIF2a. We show that GCN2 is essential for plant growth in stress situations and that its activity is linked to a strong reduction in global protein synthesis. Our results suggest that a general amino acid control response is conserved between yeast and plants but that the plant enzyme evolved to fulfil a much more general function as an upstream sensor and regulator of very diverse stress-response pathways. The strong activation of GCN2 following wounding further suggests that this enzyme evolved to play a role in plant defence responses to herbivore or insect pathogens and may represent a key but yet uncharacterized player linking biotic and abiotic stresses

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**ICAR7011**

BRASSINOSTEROID INTERACTIONS WITH OTHER HORMONES IN PROMOTING TOLERANCE TO ARABIDOPSIS SEEDLINGS AGAINST HEAT STRESS  
Category: Abiotic Interactions

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Brassinosteroids (BRs) are a group of steroidal plant hormones that are essential for proper plant development. Without BRs, plants are dwarfs and infertile. In addition to their pivotal role in plant development, BRs also promote stress tolerance in plants. We have demonstrated that Arabidopsis thaliana and Brassica napus seedlings grown in the presence of 24-epibrassinolide (EBR) are more resistant to a variety of abiotic stresses (heat, drought, cold and high salt) and show an increased expression of several stress-responsive genes. The mechanisms by which BRs control plant stress responses and regulate the expression of stress response genes are not known. Since BRs interact with other plant hormones, we used a collection of Arabidopsis mutants that were either deficient or insensitive in hormone signaling pathways (salicylic acid, ethylene, jasmonic acid and abscisic acid) to study the effects of BR on thermotolerance of these mutants. With the exception of the npr1 mutant, EBR treatment enhanced thermotolerance of all mutant seedlings studied. Measurements of thermotolerance and transcript levels of hormone marker genes in treated and untreated mutant seedlings indicated that EBR exerts effects both independently as well as involving salicylic acid, abscisic acid and ethylene. Some genes previously thought of as salicylic acid and jasmonic acid marker genes were also induced by short-term treatment with EBR, which tentatively qualifies them as BR primary response genes.

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**ICAR7012**

IDENTIFICATION AND CHARACTERIZATION OF GENES REGULATED BY UV-C AND POTENTIALLY INVOLVED IN PROGRAMMED CELL DEATH IN ARABIDOPSIS THALIANA

Category: Abiotic Interactions

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In plants, programmed cell death (PCD) is an active process, controlled genetically, which occurs during the development but also in response to biotic (pathogens) or abiotic (ozone, UV-C...) stress. As for apoptosis, PCD is necessary to regulate plant homeostasis by eliminating damaged or unwanted cells in a targeted way. In comparison to apoptosis where many factors have been identified, several aspects of PCD in plants remain to be elucidated. Our experimental approach was to screen a T-DNA collection of Arabidopsis thaliana under conditions leading to PCD. This collection includes random insertion of 20,261 lines transformed with pTluc vector whose T-DNA contains the luciferase gene, without promoter. Expression of the luciferase gene occurs when it is integrated close to promoter sequences. This T-DNA collection was screened after induction of PCD using UV-C and analyzed using a CCD camera to detect luminescence. We have identified 42 lines which present a variation of luciferase expression following treatment with UV-C. We used TAIL-PCR and inverse PCR to locate the insertions. Genes identified as regulated by UV-C are currently being characterized to assess their potential involvement in PCD.

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**ICAR7013**

DROUGHT INDUCTION OF ATNCED3 OCCURS IN VASCULAR PARENCHYMA CELLS

Category: Abiotic Interactions

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Abscisic acid (ABA) is an essential hormone for plant adaptation to drought stress. In Arabidopsis, induction of *9-cis-epoxycarotenoid dioxygenase 3* (*AtNCED3*) is the regulatory step in ABA biosynthesis under drought stress conditions. However, its regulatory mechanism is largely unknown. In the present study, we investigated the expression and localization of AtNCED3 in turgid and dehydrated plants using the specific antibody. Immunohistochemical analysis showed that AtNCED3 was localized in the vascular parenchyma cells of dehydrated plants. In addition, AtABA2 and AAO3 were also localized in the similar tissues. *In situ* hybridization experiment confirmed that drought-induced *AtNCED3* mRNA was also detected in the vascular bundles. These results indicate that drought induced ABA production occurs in vascular systems. In addition, we observed the expression pattern of other stress-responsive genes in vascular cells and mesophyll cells by using laser microdissection technique. Among nine drought-responsive genes, early induction of most genes was detected in vascular tissue after 1 hour of dehydration and some gene expressions were increased in mesophyll cells after 3 hours of dehydration. This result indicates that vascular tissue plays an important role not only as the tissue for ABA production but also for systemic drought-stress response.

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**ICAR7014**

A NEW GSTF8 STRESS-RESPONSIVE PROMOTER ELEMENT

Category: Abiotic Interactions

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Plant glutathione S-transferases (GSTs) are detoxifying enzymes that play a key role in defending plants against xenobiotic compounds. One well-studied GST is *GSTF8*, whose expression is increased in response to a range of stresses. The *GSTF8* promoter is the best characterized of plant GSTs, and it is known to contain an ocs element that regulates gene expression in response to stressors such as salicylic acid (SA), auxin, and hydrogen peroxide. *GSTF8* expression is also induced by the auxin-type herbicide dicamba, suggesting that *GSTF8* has a role in detoxifying this compound. Interestingly, deletion of the ocs element does not completely abolish the dicamba-mediated induction of *GSTF8*, indicating that other stress-responsive elements are present in the promoter. An *in silico* analysis of the promoter suggested the presence of a cis-element similar to the auxin-responsive element LS10 in the promoter of *PR1*, a component of the SA-signalling pathway. Deletion of this new stress-responsive element significantly decreased *GSTF8* promoter activity in response to abiotic stresses. Four copies of the stress-responsive element 1 (SRE1) were fused with the luciferase gene and this construct was stably transformed into Arabidopsis. Using this cis-element tetramer, we established that SRE1 is sufficient to drive luciferase expression in response to dicamba, heat, and salicylic acid treatments. The luciferase expression increases as the seedling develops and is predominately localized to the root. The SRE1-tetramer is also to a lesser degree responsive to hydrogen peroxide ( $H_2O_2$ ), but not to the plant hormones ethylene and methyl jasmonic acid (meJA) or to the pathogen *Pseudomonas syringae*. In order to further define the SRE1 region, a series of mutations were made within a 10 nucleotide region of the putative SRE1 element that have identified nucleotides necessary for gene expression. Future work aims to identify the transcription factor that binds to this newly identified stress-responsive element of the *GSTF8* promoter.

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### ICAR7015

IDENTIFICATION AND IN SILICO PROMOTER ANALYSIS OF SINAPIS ALBA ABIOTIC STRESS RESPONSIVE GENES USING ARABIDOPSIS MICROARRAY

Category: Abiotic Interactions

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Genus Brassica has been domesticated for many oilseed, vegetable and fodder crops. It has close evolutionary relationship with Arabidopsis, that belongs to the same family Brassicaceae. This relationship is manifested by the high level of sequence identity and conserved structures shared by homologues gene regions. To exploit the conserved microsynteny, the Arabidopsis chips (ATH-12501) were used to scan the candidate genes responsible for drought in Brassica (cv Varuna) and its allied genera (Sinapis alba). The Brassica crop is often grown under rainfed ecosystems. The limitation of water availability due to inadequate and erratic rainfall has contributed towards unpredictable oilseed Brassica production. Therefore, crucial step towards understanding the molecular basis of drought tolerance in Brassica is identification of genes and understanding their role in drought tolerance using functional genomics tools.

In vitro and in vivo scanning, revealed that S.alba was highly tolerant whereas B. juncea cv. RLM-619 was the most susceptible line. Microarray analysis was performed using S. alba and B. juncea cv Varuna leaf samples. Total RNA was isolated from stress and non stressed leaves in three replicates and hybridization with Arabidopsis chips was carried out following the instructions provided in the user manual (Affymetrix). Only 6-7% hybridization signals were observed for both varuna and S. alba. Out of 22,800 oligonucleotide probes that were spotted on the chip, 100 genes showed more than **1.5 fold change at p ≤ 0.05 and from these only 41 genes showed more than two fold change at p ≤ 0.01 that were either up or down regulated. The differential expression of these 41 genes was confirmed by real time PCR**.

The analysis of promoter sequences of these candidate genes revealed many known stress responsive regulatory elements in the promoters in A. thaliana that included various transcription factor protein families. Positional specificity of stress responsive regulatory elements in the promoter sequences of these 41 genes revealed higher frequency of many well characterized regulatory elements involved in Abscisic acid (ABA) signaling pathway like CBF/DRE, AREB and AP2-EREBP in the 200 to 500 bp promoter regions. BLAST of these 41 genes was done against 514 complete BAC sequences of B. rapa, out of which twenty seven showed homology to the promoter sequences of B. rapa with 95 to 98% sequence homology at bit score of 500. Further it was seen that position specificity is conserved particularly in the 200 to 600 bp promoter regions of both these species. It reflected that similar mechanism of gene regulation might be occurring in A. thaliana and B. rapa under drought stress.

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### ICAR7016

ER STRESS SENSOR/TRANSDUCERS PERCEIVE AND MITIGATE ENVIRONMENTAL STRESSES

Category: Abiotic Interactions

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Stress tolerance is a highly desirable trait in crop plants given the demands on agriculture for supplying food, feed and fuel in the face of greater environmental extremes. A process in plant cells that is highly sensitive to environmental conditions is the folding of proteins in the endoplasmic reticulum. The accumulation of misfolded proteins in the ER can lead to a condition called ER stress. This form of stress is perceived and acted upon by stress sensor/transducers located in the ER. We have identified three ER-localized bZIP transcription factors in Arabidopsis that are candidates for ER stress sensors/transducers. They are type II proteins with a cytosol-facing bZIP domain, a transmembrane segment, and a lumen-facing canonical site-1 protease (S1P) cleavage site. We found that one of the candidate sensor/transducers, AtbZIP17 (At2g40950), is activated by salt stress (Liu et al. Plant Journal 51: 897, 2007) while another, AtbZIP28 (At3g10800), is activated in the typical manner of an unfolded protein response (UPR) triggered by stress agents, such as tunicamycin (TM) or DTT (Liu et al., Plant Cell 19: 4111, 2007). Following different ER stresses, the transcription factors are proteolytically processed, releasing their N-terminal bZIP domains, which are then translocated to the nucleus. Truncated forms of these stress sensor/transducers, lacking the transmembrane segment and lumen-facing domains, are constitutively active. When placed under the control of the 35S promoter the truncated form of AtbZIP17 causes chronic stress and delays plant growth and development. However, when placed under the

control of the RD29A stress-inducible promoter, the construct confers salt tolerance and permits normal development under unstressed conditions.  
(Supported by NSF IBN0420015)

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**ICAR7017****THE RELATIONSHIP OF DROUGHT-RELATED GENE EXPRESSION IN ARABIDOPSIS THALIANA TO VARIOUS HORMONAL AND ENVIRONMENTAL FACTORS**

Category: Abiotic Interactions

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Almost 2000 drought responsive genes were identified in *Arabidopsis thaliana* under progressive soil drought stress using whole genome oligonucleotide microarrays. Most of the drought regulated genes recovered to normal expression levels by 3h after rewetting. We have previously shown that the abscisic acid (ABA) analog (+)-**8'**-acetylene-ABA (PBI425) hyper-induces many ABA-like changes in gene expression to reveal a more complete list of ABA regulated genes (Huang et al, 2007, *Plant J* 50:414-428) and we demonstrate here that PBI425 produced a correspondingly increased drought tolerance. About two thirds of drought responsive genes (1310 out of 1969) were regulated by ABA and/or ABA analog PBI425. Analysis of promoter motifs suggests that most of the remaining drought responsive genes are also ABA-responsive. Concentrations of endogenous ABA and its catabolites significantly increased under drought stress and either completely (ABA) or partially (ABA catabolites) recovered to the normal levels by 3h after rehydration.

Detailed analyses of drought transcript profiles and in silico comparisons with other studies revealed that the ABA dependent pathways are predominant in the drought stress responses. These comparisons also showed that other plant hormones including jasmonic acid, auxin, cytokinin, ethylene, brassinosteroids and gibberellins also affected drought-related gene expression of which the most significant was jasmonic acid. There is also extensive crosstalk between responses to drought and other environmental factors including light and biotic stresses. These analyses demonstrate that ABA-related stress responses are modulated by other environmental and developmental factors.

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**ICAR7018****FUNCTIONAL DIVERGENCE OF ARABIDOPSIS GATA TRANSCRIPTION FACTORS GNC AND CGA1: UNIQUE INTEGRATION OF NITROGEN, CYTOKININ, AND LIGHT**

Category: Abiotic Interactions

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The GATA family of transcription factors are highly conserved in eukaryotes, but are over-represented in higher plants as a result of chromosome duplication. Paralogs GNC and CGA1 show circadian expression that is regulated by light, nitrate, and cytokinin; yet despite these similarities, functional divergence was predicted based on expression differences and co-expression analysis. Promoter:GUS expression displays over-lapping expression in green tissues but specific differences in the stem and root. Analysis of expression in cytokinin receptor mutants reveals differences in their induction capacity, with CGA1 exhibiting a degree of cytokinin specificity. Expression of GNC has a direct influence on chlorophyll levels, whereas CGA1 results in alterations to the growth rate of plants. Manipulation of these genes alters inflorescence biomass, especially under limiting nitrogen conditions. RNAi-cga1/ SALK01778-gnc pseudo-double mutants are drastically reduced in size and show similarities to cytokinin receptor ahk2/3 mutant, but also display an extreme lack of chlorophyll, reduced stem size and poor seed production. Further characterization of GNC protein provides evidence for direct modulation of a circadian-controlled, senescence-associated gene (SAG26). Though GNC and CGA1 exhibit some level of redundancy, divergence in function is evident. Our results indicate that GNC may influence nitrogen partitioning by regulating genes important for the chloroplast, while CGA1 alters the rate of life cycle progression.

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**ICAR7019****ANNEXIN 1 AND ANNEXIN 4 PLAY A NEGATIVE ROLE IN DROUGHT AND SALT STRESS TOLERANCE IN *ARABIDOPSIS***

Category: Abiotic Interactions

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Annexins are  $\text{Ca}^{2+}$  and phospholipids binding proteins that form an evolutionary conserved multigene family with members being expressed throughout animal and plant kingdoms. Annexins have been extensively studied in animal cells. Annexin proteins (AnnAt1 and AnnAt4) were identified by comparative proteomic analysis of root tissue in *Arabidopsis thaliana*. To study biological functions of annexins, annexin-overexpressing transgenic *Arabidopsis* plants were generated. Wild type (Col-0), T-DNA insertion mutants (*annAt4-1*, and *annAt4-2*), and annexin-overexpressing transgenic (*AnnAt4-3-16* and *AnnAt4-10-7*) plants were tested for their responses to environmental stresses. Also we crossed *annAt1-1* and *annAt4-1*, double mutants(*annAt1-1 annAt4-1*) were generated. The double mutants(*annAt1-1 annAt4-1*) were tested for their responses to environmental stresses. The results suggest that annexin proteins play important roles in stress responses in plants.

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**ICAR7020****STO AND STH1 INTERACTS WITH BOTH COP1 AND HY5 TO REGULATE PHOTOMORPHOREGULATION**

Category: Abiotic Interactions

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CONSTITUTIVE PHOTOMORPHOGENOC1 (COP1) and ELONGATED HYPOCOTYL5 (HY5) are two major regulators of light signaling in plants. SALT TOLERANCE (STO) and SALT TOLERANCE HOMOLOG1 (STH1) are two B-box containing proteins in *Arabidopsis* previously shown to interact with COP1 in yeast through a C-terminal Val Pro pair. Both proteins have two tandem repeated Zn<sup>2+</sup> binding B-boxes located at the N-terminal part of the protein sharing high amino acid homology.

Here we identify physical and genetic interactions between STO and STH1 with HY5. Both STO and STH1 were identified in a Yeast Two Hybrid screen using HY5 as bait. These interactions were mapped to the second B-box of both STO and STH1. Expression of the proteins in onion, tagged with CFP, gave a uniform nuclear fluorescence. However, when coexpressed with YFP tagged HY5 we were able to detect FRET, showing that STO and STH1 interacts with HY5 in plant cells as well as yeast. We then identified *Arabidopsis* T-DNA insertion lines of both *STO* and *STH1*. Seedling phenotypes of these plants indicate a hypersensitivity to light. Since STO and STH1 have an overall 70% amino acid similarity we created double mutants in order to investigate a possible overlapping function. These double mutants show a dramatic reduction in hypocotyl length and an increase in anthocyanine levels.

Introducing *sto* and *sth1* into a *hy5* mutant also showed that both these mutations are able to rescue the elongated hypocotyl phenotype of the *hy5* mutant.

Further analyzes of these two proteins should give us a greater understanding about the essential role of COP1 and HY5 in light signaling and give further insight to the role of the B-box domain in plants.

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### **ICAR7021**

FUNCTIONAL CHARACTERIZATION OF *RCI5*, A NEW GENE FROM ARABIDOPSIS INVOLVED IN FREEZING AND SALT TOLERANCE

Category: Abiotic Interactions

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We have identified a new *Arabidopsis* *Rare Cold Inducible* gene, *RCI5*, that encodes a member of the Flavin-Containing-Monoxygenase (FMO) family of proteins. RNA-blot analysis revealed that the induction of *RCI5* expression in response to low temperature is through ABA- and CBF-independent pathways. In addition, *RCI5* expression is also induced by salt stress but not by dehydration or exogenous ABA treatment. The characterization of *Arabidopsis* transgenic plants containing *RCI5* promoter::GUS fusions allowed to establish that the induction of *RCI5* in response to cold and salt stress is regulated at the transcriptional level and mainly restricted to vascular tissues. Biochemical analysis demonstrated that *RCI5* has monooxygenase activity in vivo. A T-DNA line containing one insertion in *RCI5* resulted to be null or highly hypomorphic for *RCI5* expression. Morphologically, *rci5-1* mutant plants were undistinguishable from wild types. These plants were also as wild-types when analyzed for their tolerance to different abiotic stresses, including freezing temperatures, dehydration and salt stress. Likely, the absence of mutant phenotypes in *rci5-1* plants reflects the existence of functional redundancy between *RCI5* and other members of the *Arabidopsis* FMO family. To overcome this problem, *RCI5* was constitutively overexpressed in *Arabidopsis*. Transgenic plants overexpressing *RCI5* are not different from wild types at the morphological level, however they are significantly more tolerant to freezing and salt stress. Interestingly, these tolerant phenotypes correlate with increased levels of transcripts corresponding to different stress-regulated genes. On the basis of these results, the role of *RCI5* in freezing and salt tolerance will be discussed.

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### **ICAR7022**

THELLUNGIELLA HALOPHILA (SALT CRESS), A HALOPHYtic RELATIVE OF ARABIDOPSIS, TOLERATES NITROGEN-LIMITING CONDITIONS BY MAINTAINING GROWTH, NITROGEN UPTAKE AND ASSIMILATION

Category: Abiotic Interactions

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A comprehensive knowledge of mechanisms regulating nitrogen use efficiency (NUE) is required to reduce excessive input of nitrogen fertilizers and maintaining acceptable crop yields under limited nitrogen supply. Studying plant species which are naturally adapted to low nitrogen conditions could facilitate identification of novel regulatory genes conferring better NUE. *Thellungiella halophila* has recently emerged as a halophytic *Arabidopsis* Relative Model System, having 90-95% identity with *Arabidopsis* at the cDNA level. Here we present that *Thellungiella* can also efficiently tolerate nitrogen-limiting conditions. *Thellungiella* grow better than *Arabidopsis* under moderate (1 mM nitrate) and severe (0.4 mM nitrate) N-limitation conditions. *Thellungiella* exhibited a lower carbon to nitrogen ratio than *Arabidopsis* under N-limitation which was due to *Thellungiella* plants possessing higher nitrogen content, total amino acids, total soluble protein and lower starch content compared to *Arabidopsis*. Furthermore, *Thellungiella* had higher amounts of several metabolites such as soluble sugars and organic acids under N-sufficient conditions (4 mM nitrate). Nitrate reductase activity and *NR2* gene expression in *Thellungiella* displayed less of a reduction in response to N-limitation than in *Arabidopsis*. *Thellungiella* shoot *GS1* expression was more induced by low-N than in *Arabidopsis*, while in roots, *Thellungiella* *GS2* expression was maintained under N-limitation whereas it decreased in *Arabidopsis*. Up-regulation of *NRT2.1* and *NRT3.7* expression was higher and repression of *NRT1.1* was lower in *Thellungiella* roots under N-limiting conditions compared to *Arabidopsis*. Differential transporter gene expression was correlated with higher nitrate influx in *Thellungiella* at low <sup>15</sup>NO<sub>3</sub><sup>-</sup> supply. Taken together, our results suggest that *Thellungiella* is tolerant to N-limited conditions and could act as a model system to unravel the mechanisms for low nitrogen tolerance.

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### **ICAR7023**

ARABIDOPSIS THALIANA: A VALUABLE TOOL TO STUDY ABIOTIC STRESS TOLERANCE IN PLANTS

Category: Abiotic Interactions

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The main research objective of our laboratory is to study abiotic stress tolerance in plants. Effects of salinity and drought as well as some environmental factors, related to human activities, on plants are being investigated. We are particularly interested in identifying and characterizing key genes governing tolerance to these environmental constraints. cDNAs encoding various proteins such as vacuolar pyrophosphatase, sodium antiporter and dehydrin were isolated from wheat. These genes were cloned in plant expression vectors and introduced into *Arabidopsis thaliana* by the flower dipping method. These genes were shown to enhance salinity and drought tolerance of transgenic *Arabidopsis* plants when compared to their wild type counterparts. As extension to this work, we evaluated the potential of some of these transgenic *Arabidopsis* lines to tolerate other abiotic stresses. To this end, they were planted in pots containing phosphogypsum (PG): a by-product of the phosphate fertiliser industry. Transgenic *Arabidopsis* lines showed some potential for phytostabilisation of this by-product. For all the above-mentioned studies, the use of *Arabidopsis* was instrumental in conducting our investigations due to its small size, availability of seeds and its short life cycle.

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#### **ICAR7024**

ATHMA1 CONTRIBUTES TO DETOXIFICATION OF EXCESS ZN(II) IN ARABIDOPSIS

Category: Abiotic Interactions

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P-type heavy metal ATPases ( $P_{1B}$ ATPases; HMAs) have been identified in a wide range of organisms and implicated in transport of essential and also potentially toxic heavy metals across the cell membrane. In *Arabidopsis thaliana*, there are eight members of  $P_{1B}$ ATPase. AtHMA1 is localized on chloroplast envelope and imports Cu(II) into chloroplasts. AtHMA2 and AtHMA4 are important for Zn(II) homeostasis in the plant. AtHMA6 (PAA1) and AtHMA8 (PAA2) transport Cu(II) into chloroplasts. AtHMA3 is localized in vacuole membrane when expressed in yeast and complements Cd(II)- and Pb(II)-sensitive phenotype of *delta-ycf1* mutant.

We studied whether AtHMA1 is important for Zn transport since it has high sequence similarity with HMA2 and HMA4, and has poly-His motifs that are commonly found in Zn(II) binding proteins. We found that AtHMA1 is expressed mainly in the shoot, but induced in roots under Zn(II) excess and omitted growth conditions. We also found that three independent alleles of T-DNA insertional mutant plants of AtHMA1 are more sensitive to high concentrations of Zn(II), and accumulate more Zn than wild type. This Zn(II)-sensitive phenotype of AtHMA1 knockout plants is complemented by expression of AtHMA1 gene using its own promoter. Together, the results show that AtHMA1 contributes to Zn detoxification in *Arabidopsis* plants.

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#### **ICAR7025**

SALT TOLERANCE OF ARABIDOPSIS THALIANA REQUIRES MATURATION OF N-GLYCOSYLATED PROTEINS IN THE GOLGI APPARATUS

Category: Abiotic Interactions

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Protein *N*-glycosylation in the endoplasmic reticulum (ER) and in the Golgi apparatus is an essential process in eukaryotic cells. While the *N*-glycosylation pathway in the ER has been shown to regulate protein quality control, salt tolerance, and cellulose biosynthesis in plants, no biological roles have been linked functionally to *N*-glycan modifications that occur in the Golgi apparatus. Herein, we provide evidence that mutants defective in *N*-glycan maturation, such as *complex glycan 1* (*cgl1*), are more salt-sensitive than wild type. Salt stress caused growth inhibition, aberrant root tip morphology, and callose accumulation in *cgl1*, which were also observed in an ER oligosaccharyltransferase mutant, *staurosporin and temperature sensitive 3a* (*stt3a*). Unlike *stt3a*, *cgl1* did not cause constitutive activation of the unfolded protein response. Instead, aberrant modification of the plasma membrane glycoprotein KORRIGAN 1/RADially SWOLLEN 2 (KOR1/RSW2) that is necessary for cellulose biosynthesis occurred in *cgl1* and *stt3a*. Genetic analyses identified specific interactions among *rsw2*, *stt3a*, and *cgl1* mutations, indicating that the function of KOR1/RSW2 protein is dependent on complex *N*-glycans. Furthermore, cellulose deficient *rsw1-1* and *rsw2-1* plants were also salt-sensitive. These results establish that plant protein *N*-glycosylation functions beyond protein folding in the ER, and is necessary for sufficient cell wall formation under salt stress.

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#### **ICAR7026**

EGY3, A PUTATIVE SALT AND OSMOTIC STRESS DETERMINANT IN ARABIDOPSIS THALIANA

Category: Abiotic Interactions

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It is predicted that by the year 2050, 50% of all arable lands will be facing a serious salinization problem. This abiotic stress is already a major **limiting factor in plant growth and will soon become more severe, as desertification increasingly covers more of the world's** terrestrial area. Major breakthroughs in the identification of salt/osmo-tolerance determinants have occurred due to the use of Arabidopsis as a molecular genetic model plant system. Phenotype-centred forward and reverse genetic approaches are screened in the Arabidopsis biological model, usually through loss-of-function mutant analysis. Based on this approach, the expression analysis of salt and osmotic stressed Arabidopsis plants made available through the Affymetrix microarray database (NASC), led to the identification of one gene of unknown function (*EGY3*). This gene, putatively involved in conferring tolerance to abiotic stress, codes for a predicted chloroplastidial metalloprotease. We are proceeding to the functional characterization of the gene.

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### **ICAR7027**

HSP90-2 REGULATES THE HEAT SHOCK RESPONSE IN ARABIDOPSIS THALIANA

Category: Abiotic Interactions

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The molecular chaperone Hsp90 plays key roles in signal transduction, protein degradation and trafficking, chromatin remodeling, epigenetic regulation, and morphological evolution. Two key features regarding the mechanism of hsp90 action are: 1) hsp90 operates as part of a multichaperone complex, facilitating the folding of client proteins into their stable or active conformations, and 2) hsp90 functions are driven by the hydrolysis of ATP. The Hsp90 protein family in *Arabidopsis* consists of seven members, of which Hsp90.1 through Hsp90.4 constitute the cytoplasmic subfamily. The *Arabidopsis* mutant *lra2-3*, characterized by the change D80N in the ATP-binding domain of hsp90.2, causes loss of RPM1 (a disease resistance protein)-specific hypersensitive response and disease resistance. Using *lra2-3* and other hsp90 mutants in thermotolerance assays, we demonstrate that Hsp90.2 plays a role in the heat stress response of *Arabidopsis*. These results are consistent with the results of a recent study (J. Biol Chem. 282: 37794) and support the idea that Hsp90.2 negatively regulates the heat shock response by suppressing the function of heat shock transcription factor.

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### **ICAR7028**

GENOME-WIDE CHARACTERIZATION OF THE ARSENATE RESPONSE IN ARABIDOPSIS

Category: Abiotic Interactions

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The presence of arsenic (As) in soils and water is a major public concern of environmental impact. In plants, arsenate [As(V)], the most bioavailable form of As, is assimilated through phosphate transporters. Previously, we had identified in *Arabidopsis*, a semidominant mutation at the high affinity phosphate transporter AtPHT1. pht1-1 shows reduced phosphate accumulation and enhanced arsenic content indicating that lowering the uptake rate leads to a more efficient acclimation to arsenate, thus providing increased accumulation capacity. This observation has been confirmed using other Pi transport mutants available in the laboratory. In addition we have shown that As(V) represses more efficiently than Pi the expression of genes involved in Pi uptake while induces others specifically regulated by As(V). Here we will report the transcriptome analysis of the As(V)/Pi interacting responses which allowed the identification of genes potentially involved in these converse signalling pathways that may be relevant in As perception.

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### **ICAR7029**

HIGH-RESOLUTION TRANSCRIPTIONAL PROFILING REVEALS TWO NOVEL REGULATORS OF IRON DEFICIENCY RESPONSE IN ARABIDOPSIS ROOTS

Category: Abiotic Interactions

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Anemia caused by iron deficiency is one of the most common and widespread nutritional disorders in the world. In many populations plants are the main source of dietary iron, however, poor soil conditions decrease iron availability for important crop species in many parts of the world. Plants undergo a host of developmental, biochemical and genetic alterations in response to iron deficiency. Understanding how plants respond to iron deficient conditions is important for efforts to feed the increasing global population. To better understand how plants respond to this condition we are using a systems approach to characterize the spatio-temporal expression pattern of genes within *Arabidopsis thaliana* roots exposed to iron deficient conditions. Our results showed that hundreds of genes are transcriptionally activated or repressed within hours of exposure to iron deficient growth conditions. Interestingly, these changes in gene expression occur at specific longitudinal and radial zones within plant roots. Clustering analysis reveals that many co-regulated genes belong to specific Gene Ontology functional categories and are associated with specialized cis regulatory elements. To identify potential key players in the iron deficiency response we screened *Arabidopsis* T-DNA insertions lines from the set of transcriptionally responsive genes described above, and looked for alterations in iron deficiency responsiveness. Two previously uncharacterized genes displayed altered responsiveness to iron deficiency and other metal deficiencies, cadmium toxicity, and high pH. In addition, these mutants exhibit changes in growth and development, and alterations in metal ion content under iron poor conditions. Microarray analysis of these loss-of-function mutants reveals alterations in gene expression which may explain their physiological responses. We have created transgenic lines containing transcriptional and translational fusions, and overexpression constructs to characterize the dynamics of these proteins in response to environmental stimuli. In addition, we are using Yeast-2-Hybrid analysis and CHIP-Q-PCR to identify interactors of these proteins. This study will allow us to better understand the correlations between transcriptional alterations and developmental and biochemical responses, and to determine how plant responses to iron deficiency are controlled transcriptional and post-transcriptional.

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### **ICAR7030**

ARABIDOPSIS TRANSCRIPTOME ANALYSIS UNDER DROUGHT, COLD, HIGH-SALINITY AND ABSCISIC ACID TREATMENT CONDITIONS USING A TILING ARRAY

Category: Abiotic Interactions

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Plants respond and adapt to drought, cold and high-salinity stresses in order to survive. In this study, we applied Arabidopsis Affymetrix tiling arrays to study the whole genome transcriptome under drought, cold, high-salinity and ABA treatment conditions. The bioinformatic analysis using the tiling array data showed that 7,719 non-AGI transcription units (TUs) exist in the unannotated "intergenic" regions of Arabidopsis genome. These include 1,275 and 181 TUs that are induced and downregulated, respectively, by the stress- or ABA treatments. Most of the non-AGI TUs are hypothetical non-protein-coding RNAs. About eighty percent of the non-AGI TUs belong to pairs of the fully-overlapping sense-antisense transcripts (fsATs). High-correlation between the expression ratios (treated/untreated) of the sense transcripts and the ratios of the antisense transcripts was observed in the SATs of AGI code/non-AGI TU. We studied the biogenesis mechanisms of the stress- or ABA-inducible antisense RNAs and found that the expression of sense TUs is necessary for the stress- or ABA-inducible expression of the antisense TUs in the fsATs (AGI code/non-AGI TU). We also showed that one component, NRPD2b is involved in the accumulation of several ABA- or stress-inducible antisense transcripts.

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### ICAR7031

#### URANIUM AFFECTS SEVERAL MORPHOLOGICAL AND BIOCHEMICAL RESPONSES INDUCED BY PHOSPHATE STARVATION IN ARABIDOPSIS THALIANA

Category: Abiotic Interactions

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Uranium is a heavy metal with known chemical and radiological toxicity, which is naturally present in the plant environment. Due to its high affinity for phosphate, insoluble uranium-phosphate precipitates are formed in soils as well as in contaminated plant cells. To date, consequences of such interactions on uranium toxicity and on phosphate availability and metabolism in plants are unknown. This study aims at evaluating in which extent uranium-phosphate interactions have an effects i) on physiological and molecular mechanisms involved in plant responses to an uranium contamination and ii) on physiological and molecular mechanisms involved in plant responses to phosphate availability. In *Arabidopsis thaliana*, mechanisms involved in phosphate homeostasis regulation are well known and have allowed identification of genetic and molecular tools useful to respond to the objectives of this work.

We have first characterized effect of uranium contamination on *Arabidopsis thaliana*. Due to the drastic decrease of uranium bioavailability with phosphate supply, macroscopic effects appeared particularly in medium containing no phosphate. A growth stimulation at low level of contamination (2µM U) and a reduced biomass at higher concentrations (50 and 500 µM U) have been quantified. Surprisingly, we have observed that root architecture modification induced in phosphate starvation condition was deregulated with 2 or 10 µM uranium inputs, with a continuous growth of the primary root. Other phosphate-starvation induced responses appears to be deregulated by uranium presence as phosphate uptake and inorganic phosphate distribution to organs (ratio [Pi] in roots / [Pi] in shoots). *Arabidopsis* mutants deregulated in phosphate transport and distribution are currently tested for their response to an uranium contamination to determine precise mechanisms involved in uranium mobility and toxicity.

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### ICAR7032

#### INVESTIGATION OF ABIOTIC STRESS RESPONSES IN THE PATHOGEN RESISTANT MUTANT *CPR22*

Category: Abiotic Interactions

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The *cpr22* mutant is an *Arabidopsis* lesion mimic mutant exhibiting spontaneous cell death. The *cpr22* plant constitutively expresses the pathogenesis related genes *PR-1*, *PR-2* and *PR-5*, has elevated levels of salicylic acid (SA) and displays an enhanced resistance to *Hyaloperonospora parasitica* Emco5. *cpr22* has a deletion in a cluster of cyclic nucleotide-gated ion channel genes, resulting in an in-frame chimeric fusion of the genes *AtCNGC11* and *AtCNGC12*. Interestingly, when *cpr22* plants were grown in high relative humidity, all the above *cpr22*-related phenotypes were suppressed. To investigate this environmental sensitivity further, genome wide transcriptome analyses were conducted to assess changes in gene expression after a 24 hour shift from a high relative humidity of 95 percent to a lower relative humidity of 65 percent. Interestingly, abscisic acid (ABA) related gene expression was altered in *cpr22* after humidity shift. Hormone analysis also revealed that the amount of ABA accumulation in *cpr22* is two fold greater than in wildtype after the humidity shift. Furthermore, we have shown that *cpr22* has an impaired ability to respond to ABA induced dormancy in germination assays compared to wildtype. In addition, *cpr22* has an accelerated loss of fresh weight during dehydration compared to wildtype. Taken together, these results suggest that *cpr22* has an attenuated response to abiotic stress, possibly due to altered ABA related signaling.

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### ICAR7033

#### ANALYSIS OF SEAWEED EXTRACT INDUCED TRANSCRIPTOME LEADS TO IDENTIFICATION OF A NOVEL NEGATIVE REGULATOR OF SALT TOLERANCE IN ARABIDOPSIS THALIANA

Category: Abiotic Interactions

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Organic components in the extracts of a brown seaweed, *Ascophyllum nodosum* was found to elicit tolerance in *Arabidopsis thaliana* against NaCl stress. To dissect the genetic basis of this induced salt tolerance, a global transcriptome analysis was conducted by comparing plants stressed with 150 mM NaCl in the presence or absence of the brown seaweed extract. Interestingly, a number of genes were significantly down regulated by the *A. nodosum* extract treatment suggesting that these genes are possible negative regulators of salt tolerance in Arabidopsis. T-DNA insertion lines in the ten most significantly down regulated genes predicted to function in cellular organization and biogenesis, signal transduction and transcription were screened for salt tolerance to 125 mM NaCl using the root bend bioassay method. T-DNA insertions in one of the ten selected genes exhibited tolerance to NaCl. The root growth was 68% more in this mutant under 125 mM salt as compared to Col-0 wild type plants. Analysis of root growth under different salt stress conditions revealed an increase of 13% and 13.93% in total root length in 75 mM NaCl and 100 mM NaCl respectively 3 days after transfer to MS media supplemented with different concentrations of NaCl. Additional phenotypic and molecular characterizations of this mutant will be presented. Taken together, our results demonstrate the potential use of physio-modulatory chemicals to decipher novel function(s) of genes in plant stress responses.

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### **ICAR7034**

TOWARDS IDENTIFYING TARGETS OF FARNESYLATION INVOLVED IN ABSCISIC ACID SIGNALING AND DROUGHT TOLERANCE.

Category: Abiotic Interactions

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A number of years ago era1 was discovered, which modulates ABA signaling through negative regulation (Cutler, Ghassemian et al. 1996). ERA1 encodes the β-subunit of a farnesyltransferase (FTase) and mutant plants are deficient in protein farnesylation. era1 was initially identified by screening for the inability to germinate on low concentrations of exogenous abscisic acid (ABA) and was later found to show a hypersensitive ABA response at the level of stomatal closure, thereby conferring drought tolerance (Pei, Ghassemian et al. 1998). Further analysis of era1 mutants revealed additional pleiotropy. For example, meristem and flower development are altered as well the process of meiosis. To date, relatively few proteins have been shown to be farnesylated in plants. These include the first plant protein shown to be farnesylated, the molecular chaperone ANJ1 from Atriplex nummularia, as well as ATFP3, Apetala1, ROP10, AtNAP1 and AtRAC7 from Arabidopsis. Unfortunately, the identification of these targets has not given much insight into how farnesylation regulates plant development and signaling. For example, there is still no clear understanding of how loss of farnesylation can confer ABA hypersensitivity. As previously mentioned, the simplest interpretation is that farnesylation acts as a negative regulator of ABA signal transduction. However, with the lack of a molecular target this interpretation remains to be proven.

In an effort to identify the molecular target/s of farnesylation in Arabidopsis, a high-throughput genetic approach has been conducted. Of the 582 putative farnesylation targets in Arabidopsis, 290 homozygous T-DNA knockout lines representing 217 unique genes have been screened for ABA hypersensitivity at the level of germination as well as drought tolerance. Current results will be presented.

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### **ICAR7035**

THE PLASMA MEMBRANE LIPOCALIN ATTIL PROTECTS ARABIDOPSIS AGAINST OXIDATIVE STRESS

Category: Abiotic Interactions

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Plant lipocalins accumulate in response to environmental stresses such as cold stress, however their cellular functions are still unknown. Here we demonstrate that the Arabidopsis AtTIL lipocalin is involved in modulating tolerance to oxidative stress. AtTIL knock-out plants are very sensitive to sudden drops in temperature and paraquat treatment, and dark-grown plants die shortly after transfer to light. Complementation restores the normal phenotype and overexpression enhances tolerance to the oxidative stress caused by freezing, paraquat and light. Moreover, the accumulation of AtTIL delays flowering and senescence. Microarray analyses identified several differentially-regulated genes encoding components of oxidative stress and energy balance. These findings are in agreement with the recent findings that animal lipocalins are involved in tolerance to oxidative stress and modulation of life span. Lipocalins thus share a conserved function in development and stress responses among species.

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### **ICAR7036**

CYTOCHROME P450S AS REPORTERS FOR CIRCADIAN-REGULATED PATHWAYS

Category: Abiotic Interactions

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Cytochrome P450 monooxygenases play important roles in the synthesis of diverse secondary compounds in *Arabidopsis thaliana*. Comparison of four datasets analyzing seedlings harvested over a two-day period of constant conditions after growth with varying photoperiods and thermocycles recorded a total of 98 P450 loci as circadian-regulated for at least one of the four conditions. Here, we further describe the circadian-regulated pathways using, as reporters, individual P450 loci that are likely to be rate-limiting in secondary metabolic pathways. RT-PCR gel blot analyses have confirmed circadian regulation of P450s in phenylpropanoid, carotenoid, oxylipin, glucosinolate and brassinosteroid biosyntheses and shown that P450 and non-P450 genes in the many branches of the phenylpropanoid pathway have similar circadian patterns of expression. *In silico* analyses of the subsets of co-regulated promoters have identified over-represented promoter elements in various biosynthetic pathway genes including MYB and MYB4 elements that are significantly more abundant in promoters for the core and lignin branch of phenylpropanoid metabolism. Interactions with these elements important for circadian regulation do not involve the MYB transcription factor PAP1 as previously proposed since the expression patterns of circadian-regulated P450s are the same in *pap1-D* mutant seedlings as in wildtype seedlings. Further analysis of circadian-regulated promoters in other

biochemical pathways will provide us with the opportunity to functionally characterize novel promoter motifs that might be important in P450 circadian regulation.

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**ICAR7037**

## MOLECULAR AND SYSTEMIC MECHANISMS OF PLANT PHOSPHATE HOMEOSTASIS

Category: Abiotic Interactions

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Inorganic phosphate (Pi) often limits plant growth and development due to its low bio-availability. The identification of a large number of Pi responsive genes and *Arabidopsis* mutants defective in Pi acquisition and homeostasis suggest that highly regulated Pi signaling cascades also exist in plants. Yet the fundamental molecular and systemic mechanisms of plant Pi signaling are still largely unknown. We identified the molecular lesion of *pho2*, a Pi hyper-accumulating mutant of *Arabidopsis thaliana*, as an E2 ubiquitin conjugase. *PHO2* gene harbours five target sites for microRNA399 (miR399). We found that miR399s respond strongly and specifically to Pi-starvation. miR399 negatively regulates *PHO2* by transcript degradation and most possibly by translational inhibition. *MIR399* genes are regulated by *PHR1*, which is a MYB related transcription factor. Reciprocal micro-grafting experiments using *pho2* and WT revealed that the *pho2* root genotype is necessary and sufficient for the *pho2* phenotype, indicating that the *pho2* mutant is either insensitive to the Pi-saturation feedback signal or the impact of the low Pi derived signal is continuously present in the *pho2* root. This, along with exquisitely high miR399 expression in shoots compared to roots, gave rise to the hypothesis of miR399 being a long-distance shoot to root transmissible Pi starvation signal. Accordingly, the mature miR399 is present in the phloem sap of rapeseed and pumpkin, and strongly and specifically increases there during Pi limitation. Furthermore, micrografted plants (miR399OE<sub>shoot</sub>-WT<sub>root</sub>) constitutively over-expressing miR399 in their shoots accumulated mature miR399 in their WT roots, while corresponding miR399 primary transcripts or fragments thereof were absent, revealing shoot to root movement of miR399. The same chimeric plants had reduced miR399 target (*PHO2*) level in roots, and accumulated high levels of Pi in their shoots, demonstrating biological activity of transported miR399 molecules. However, root to shoot translocation of miR399 does not take place. To our knowledge this is the first demonstration of the systemic control of a biological process by a phloem mobile miRNA. Therefore, we discovered a plant Pi signaling pathway involving *PHR1*, miR399 and *PHO2*, and demonstrated miR399 as a systemic long distance signal regulating plant Pi homeostasis.

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**ICAR7038**

## METABOLIC – CIRCADIAN INTERACTIONS

Category: Abiotic Interactions

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The ability of living beings to measure time is attributed to biochemical oscillations. Of these, Transcriptional-Translational Oscillations (TTOs) have been investigated extensively and hold a central place within circadian systems. Metabolic oscillations could in theory also provide time-keeping information. This possibility we investigated in *Arabidopsis thaliana*. We found that chemical perturbation of metabolic traits results in circadian-periodicity changes, and further, that these changes are modified by external application of sucrose. The identity of interactions between metabolic oscillations and TTOs was investigated by genetic means and these findings will be described. Collectively, we propose that basal metabolism is a controlling feature of the circadian oscillator.

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**ICAR7039**GENETIC ANALYSIS OF HIGH-AFFINITY K<sup>+</sup> TRANSPORT SYSTEM IN ARABIDOPSIS

Category: Abiotic Interactions

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Potassium (K<sup>+</sup>) is one of the major nutrients required for plant growth, development and reproduction. It is generally accepted that plants absorb K<sup>+</sup> through separate uptake systems operating at low (high-affinity transport) and/or high external concentrations (low-affinity transport). Since many plant K<sup>+</sup> transporters have been identified, the high-affinity K<sup>+</sup> transport system may consist of multiple transporters, which have not yet been clearly defined. To understand the molecular basis of the high-affinity K<sup>+</sup> transport system in *Arabidopsis*, we focus on the physiological function of AtHAK5 and AKT1. Compared with wild-type, *athak5* mutants exhibited growth defects at 10 μM K<sup>+</sup> in the absence of NH<sub>4</sub><sup>+</sup>. However, the *athak5* mutants were indistinguishable from wild-type at K<sup>+</sup> concentrations higher than 20 μM. We generated a double mutant, lacking both AtHAK5 and AKT1. The *athak5 akt1* double mutants were unable to germinate on low K<sup>+</sup> media. Determination of K<sup>+</sup>(Rb<sup>+</sup>) uptake kinetics in wild-type and mutant roots using <sup>86</sup>Rb<sup>+</sup> as a tracer for K<sup>+</sup> revealed that high-affinity K<sup>+</sup>(Rb<sup>+</sup>) uptake is abolished in double mutants and impaired in single mutants. These results strongly indicate that both AtHAK5 and AKT1 are the major molecular entities mediating high-affinity K<sup>+</sup> uptake in *Arabidopsis* roots.

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**ICAR7040**

## HIGH ENDOGENOUS LEVEL OF ABA MIGHT REGULATE DROUGHT TOLERANCE OF NHR1 MUTANT OF ARABIDOPSIS THALIANA COL-0

Category: Abiotic Interactions

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The *nhr1* is a no hydrotropic response mutant, which cannot sense moisture gradients(Eapen, 2004; Eapen et al., 2005). Because of the *nhr1* mutant was isolated in an *in-vitro* system with low water potential and ABA is an important factor in the response of plants to drought stress led us to investigate whether *nhr1* could have alterations in ABA signaling or in ABA biosynthesis. *nhr1* mutants displayed a significant reduction in both the number and length of lateral roots in water stress and also in optimal conditions. *nhr1* was also hypersensitive to ABA (50 nM in MS medium) on lateral root inhibition, but growth of its primary root was not affected; on the contrary, it was promoted under water stress. On the other side, *nhr1* presented tolerance for drought stress and water transpiration. Interestingly, the no hydrotropic phenotype of *nhr1* was inhibited by abamine (an inhibitor of ABA biosynthesis, Kitahata et al. 2006). We suggest that the lack of hydrotropism is an adaptive response to drought stress.

#### Acknowledgment

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#### ICAR7041

ANALYSIS OF ARABIDOPSIS MUTANTS REVEALS THE INTERACTION OF BRASSINOSTEROID WITH ABSCISIC ACID AND CALCIUM.

Category: Abiotic Interactions

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Brassinosteroids (BRs) are a class of hormones that are essential for growth and development of plants. BRs also protect plants from various abiotic and biotic stresses, but the underlying mechanism by which BRs induce this broad-range stress tolerance is poorly understood. We have demonstrated that exogenous treatment of *Arabidopsis thaliana* and *Brassica napus* seedlings with 24-epibrassinolide (EBR) confers tolerance to a range of abiotic stresses like high and low temperatures, high salt and drought. Microarray analysis of global gene expression in EBR-treated and untreated seedlings indicated that EBR upregulates the expression of several genes encoding calmodulin-like calcium-binding proteins, genes involved in abscisic acid (ABA) biosynthesis, and genes responsive to ABA. To further address the relationship between BR and ABA, we studied the effect of EBR on heat stress responses of *A. thaliana* ABA-deficient (*aba1-1*) and ABA-insensitive (*abi1-1*) mutants. Our results indicate that EBR treatment promotes basic thermotolerance in both ABA-deficient and insensitive mutants, and promotes higher accumulation of heat shock proteins in *aba1-1* as compared to wild type seedlings. Since Ca<sup>2+</sup>/calmodulin appear to play a role in ABA signaling, BR biosynthesis, as well as sensing and transducing environmental stimuli, we are studying the stress phenotypes of T-DNA insertion mutant lines of a subset of calmodulin-like Ca<sup>2+</sup>-binding protein genes identified in the microarray screen. These mutants displayed altered phenotypes as compared to wild type in ABA inhibition of germination, salt and mannitol-induced osmotic stresses. Ongoing studies are addressing the roles of these genes in relation to BR and abiotic stress signaling in *A. thaliana*.

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#### ICAR7042

ASCOPHYLLUM NODOSUM EXTRACTS IMPART FREEZING TOLERANCE IN ARABIDOPSIS THALIANA

Category: Abiotic Interactions

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The ability of the plant species to tolerate chilling to freezing temperatures is a major determining factor in the productivity and geographical distribution of many agricultural crops in temperate regions. In North America, especially in Canada, freezing temperature during early spring and late fall is one of the main causes of crop damage. Even in areas considered suitable for the cultivation for a given species, crop failures frequently occur due to hasty unpredicted frosts. Despite the attempts to minimize frost damages, significant economic losses are incurred annually in a wide variety of agricultural crops. *Ascophyllum nodosum*, a marine brown algae, is dominant perennial seaweed confined to the inter-tidal zones of the north Atlantic basin and parts of north-western Europe. Commercial formulations of *Ascophyllum nodosum* extracts, when applied to crop plants, have been shown to improve the freezing tolerance in a wide variety of crops. The present study was conducted to investigate the ability of *Ascophyllum nodosum* extract to impart freezing tolerance in *Arabidopsis thaliana*. Our results suggest *A. nodosum* extract imparts significant tolerance towards suboptimal freezing temperatures. In whole plant assays, *viz*; Petri Dish Freezing Assay and Peat Pellet Freezing Assay, treated plants showed significant tolerance to freezing temperatures as compared to water controls. The control plants exhibited severe chlorosis, tissue damage and failed to recover from freezing treatment while the extract treated plants showed no signs of significant tissue damage until -7.5°C in Petri Dish Freezing Assay and -5.5°C in Peat Pellet Freezing Assay. The extend of tissue damage due to freezing treatments were compared using electrolyte leakage assay and histochemical staining using trypan blue. In the electrolyte leakage assay, the LT<sub>50</sub> was lowered by 2°C while the trypan blue staining displayed 30-

40% lesser area of damaged tissue in extract treated plants. Molecular analysis of the major cold responsive genes showed a differential expression in *A. nodosum* extract treated plants.

Keywords: Freezing tolerance, *Ascophyllum nodosum*, *Arabidopsis thaliana*.

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**ICAR7043****ACTIVATION TAGGED ARABIDOPSIS LINES WITH ENHANCED FREEZING TOLERANCE IN THE ABSENCE OF COLD ACCLIMATION**

Category: Abiotic Interactions

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Many temperate plants acquire an increase in their freezing tolerance through cold acclimation, an adaptive process initiated by exposure to low non-freezing temperatures. To genetically dissect this complex adaptation, fifty thousand Arabidopsis activation tagged lines were screened to identify individuals that in the absence of cold acclimation exhibited an enhanced ability to withstand freezing temperatures. These mutant lines named Frigus lines were selected by screening over three successive generations by assessing whole plant survival of two week old Arabidopsis lines after exposure to -5°C. This resulted in the identification of 17 lines that in comparison to wild type displayed a constitutively freezing tolerant phenotype. These lines are presently being analyzed and the sequences flanking the majority of the T-DNA insertion sites have been resolved. Homozygous insertion lines are being generated for each of these 17 lines to enable the precise level of freezing tolerance to be assayed by electrolyte leakage. Here we present the characterization of the Frigus lines to date.

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**ICAR7044****ROLE OF BOR5 IN HIGH BORON TOLERANCE OF *ARABIDOPSIS THALIANA***

Category: Abiotic Interactions

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Boron is an essential micronutrient for the plants and its deficiency as well as toxicity reduces plant growth. BOR1 has been identified as a boron transporter for xylem loading under B limitation in *Arabidopsis thaliana* (Takano et al., 2002). There are six homologous genes (*BOR 2-BOR7*) in the *A. thaliana* genome. This study reports the tissue specificity of the expression and roles of BOR5 in high boron tolerance. In the transgenic lines carrying a promoterBOR5-GUS construct, GUS staining was observed in roots near the root tip, leaf axils, leaf trichomes, siliques, and floret base. It behaves as an efflux type of boron transporter when expressed in yeast. We established four mutant lines having T-DNA insertion. Growth of these lines were examined under normal and high boron conditions. Two lines exhibited improved shoot and root growth compared to the respective wild type, whereas the growth of the other two lines was decreased. Under the normal boron condition, they did not show any significant difference in growth. These apparently contradictory results suggest possible roles of BOR5 in boron homeostasis in *A. thaliana* under high boron condition. We are in the process of detailed characterization of the mutants and the updates will be presented.

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**ICAR7045****DIFFERENTIAL ROLE OF *ARABIDOPSIS* PIP2 AQUAPORINS IN SALT STRESS TOLERANCE**

Category: Abiotic Interactions

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Plants harbor about 30 genes encoding major intrinsic proteins (MIP), which were named aquaporins because of the frequently observed water channel activity, and therefore are supposed to be involved in water relations. In *Arabidopsis thaliana* the clade of plasma membrane intrinsic proteins (PIP) is subdivided into five PIP1 and eight PIP2 members. PIP2 genes are highly homologous to each other. Nevertheless, several lines of evidence such as tissue expression patterns suggested gene-specific and non-redundant functions (see Da Ines et al., 18th ICAR). Principal component analysis applied to publicly available stress-related expression data revealed differential responsiveness of PIP2 transcripts. In addition, several laboratories have studied the response of PIP genes to various water stress conditions (drought, cold, salt stress) and found differential deregulations. Here, we compiled a collection of *pip2* knockout lines which did not show visible phenotype under normal growth conditions. Yet, one isoform knockout displayed an enhanced salt-sensitivity, but was not affected by iso-osmotic sorbitol concentrations. This suggested its specific involvement in salt tolerance. Since tissue expression patterns of other isoforms overlap at least partially, we speculate that the water channel activity per se might not be responsible for this role. Furthermore, promoter analyses using software at [www.genomatix.de](http://www.genomatix.de) revealed differential organization of elements, but no clear clue for any distinct responsiveness related to salt or water stress. Instead, modeling using the known structural template of spinach PIP2;1 [Törnroth-Horsefield et al. (2006) Nature 439, 688] indicated that several amino acid changes with respect to its highly related isoforms clustered in one domain and therefore might alter the channel properties or protein interactions.

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**ICAR7046**

**ARABIDOPSIS TRANSCRIPTIONAL RESPONSES DIFFERENTIATING CLOSELY RELATED CHEMICALS (HERBICIDES) AND CROSS-SPECIES EXTRAPOLATION TO BRASSICA**

Category: Abiotic Interactions

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Using whole genome Affymetrix ATH1 GeneChips we characterized the transcriptional response of *Arabidopsis thaliana* Columbia 24 hours after treatment with five different herbicides. Four of them (chlorsulam, imazapyr, primisulfuron, sulfometuron) inhibit acetolactate synthase (ALS), while one (glyphosate) inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The ALS-inhibiting herbicides affected the expression of numerous genes, particularly those related to protein metabolism and amino acid biosynthesis; they also led to altered transcription of genes involved in C-metabolism, secondary metabolism, abiotic stress response, cell wall modification and growth. In contrast, glyphosate affected the expression of much fewer genes. Interestingly, a set of 101 markers could be compiled which provided a composite diagnostic signature that reliably differentiated the transcriptional responses of *Arabidopsis* to the five herbicides tested, although the ALS-inhibitors encompassed two highly similar chemical structures (sulfonylurea compounds). These herbicide-specific expression patterns were also distinct from responses to major biotic and abiotic stressors and to series of reference chemicals for which data had been selected from quality controlled public ATH1 microarray experiments. Orthologs of selected markers were predicted for *Brassicaceae*, and several such candidates were tested in *B. napus* for responses to the sulfonylurea compounds sulfometuron methyl and primisulfuron methyl. Similar expression patterns were observed as in *Arabidopsis*, indicating that true orthologous genes had been identified.

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**ICAR7047**

**EARL1 TYPE HYPRP GENES PLAY DISTINCT AND OVERLAPPING ROLES IN STRESS PROTECTION PROCESSES**

Category: Abiotic Interactions

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Plants have large families of proteins sharing a conserved eight-cysteine-motif (8CM) domain. EARL1 type proteins in *Arabidopsis* are hybrid proline-rich protein (HyPRP) with a three-domain architecture: a putative signal peptide at the N-terminus, a proline-rich domain (PRD) in the middle, and an 8CM domain at the C-terminus. The biological functions of these proteins are largely unknown. We used transgenic plants and yeast cells to show that different *EARL1* genes have distinct but somewhat overlapping functions. We had previously reported that *EARL1* and two of its paralogs improved freeze survival of yeast cells (Zhang and Schläppi, 2007) and present here confirming evidence for transgenic plants. We further show that both the paralog without a positive effect on freeze survival and to a lower degree *EARL1* improve salt tolerance of transgenic plants. Lastly, we show that the paralogs improving salt tolerance inhibit growth of fungal cells. Taken together, these data suggest that HyPRP genes of the *EARL1* family play different and overlapping roles in protecting plants against abiotic and biotic stresses.

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**ICAR7048**

**OVEREXPRESSION OF ARABIDOPSIS DAMAGED DNA BINDING PROTEIN 1A (DDB1A) ENHANCES DNA REPAIR**

Category: Abiotic Interactions

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DDB1 is a component of multiple complexes involved in genome stability, cell cycle regulation, histone modification, DNA replication and repair. *Arabidopsis* has two homologues of DDB1: DDB1A and DDB1B. In this study we examine the role of DDB1A in *Arabidopsis* DNA repair using a *DDB1A* null mutant (*ddb1a*) and overexpression lines generated using the CaMV 35S promoter. While UV tolerance assays showed no significant difference between wildtype plants and *ddb1a* mutants, a slight delay in repair of (6-4) photoproducts was detected in *ddb1a* mutants. *DDB1A* overexpression lines however exhibited higher levels of UV-resistance as well as faster DNA repair than wildtype. Following UV exposure *DDB1A* mRNA levels increase significantly in wildtype and overexpression lines. *DDB1B* and *DDB2* mRNA levels also increased after UV exposure in wildtype, but induction was not observed in the *DDB1A* loss of function background. In conclusion, these results indicate that DDB1A plays an important role in *Arabidopsis* damaged DNA repair and UV response.

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**ICAR7049**

**THE ROLE OF PHOSPHORYLATION IN THE POST-TRANSLATIONAL REGULATION OF TOC1 IN THE CIRCADIAN CLOCK**

Category: Abiotic Interactions

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The circadian clock controls the appropriate phasing and amplitude of rhythmic processes. Our view of the central oscillator responsible for period maintenance has become more complex as new factors and relationships have been uncovered. Additionally, it has become apparent that post-translational processes, such as phosphorylation, are essential to normal clock function. A core group of clock components in *Arabidopsis* is the five PRR (pseudo response regulator) proteins. Peak expression of TOC1/PRR1, PRR3, PRR5, PRR7 and PRR9 are sequentially phased over the circadian

cycle, and loss of any PRR protein alters period. The F-box protein ZEITLUPE (ZTL) targets TOC1 and PRR5 for proteasome-dependent degradation and the blue-light-dependent post-translational control of ZTL cycling is essential for maintaining the amplitude of TOC1 cycles. Here we identify a second post-translational mechanism controlling TOC1 levels. We demonstrate that each of four PRR proteins examined is nuclear-localized and all are differentially phosphorylated over the circadian cycle. PRR3 and ZTL both interact with TOC1 *in vivo* via the TOC1 N-terminus and all three proteins show peak expression early in the night. Phosphorylation of both TOC1 and PRR3 optimizes their binding to each other, whereas the TOC1/ZTL interaction is only mildly enhanced by TOC1 phosphorylation. Competition assays show that PRR3 can diminish the formation of a TOC1/ZTL complex, which would normally lead to TOC1 degradation. These data are consistent with previous findings showing that TOC1 is stabilized by PRR3, and suggest that the TOC1/PRR3 phosphorylation-dependent interaction may shield TOC1 from ZTL-mediated degradation resulting in enhanced amplitude of TOC1 cycling.

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**ICAR7050**

## REGULATION OF ARABIDOPSIS HFR1 STABILITY AND FUNCTION BY REVERSIBLE PHOSPHORYLATION

Category: Abiotic Interactions

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Plants use an array of photoreceptors to sense the ambient light environment and initiate a cascade of signal transduction events, leading to altered gene expression and adaptive growth and development. Arabidopsis HFR1 encodes a bHLH transcription factor that promotes seedling photomorphogenesis under far-red and blue light conditions. In addition, HFR1 plays a critical role in balancing the shade avoidance response under canopy shade conditions. We previously showed that HFR1 protein is targeted for degradation in darkness by the COP1-SPA1 E3 ubiquitin ligase complex through the ubiquitin-26S proteasome pathway, and is rapidly stabilized under light conditions to promote light signaling. However, the molecular mechanisms by which light regulates HFR1 stability and functionality remain to be fully elucidated. Phosphorylation and dephosphorylation often serve as an "on-and-off" switch for rapid regulation of transcription factor activity through modulating their DNA binding activity, cellular localization, stability and interaction with other proteins. Here, we show that Arabidopsis HFR1 can be phosphorylated by recombinant casein kinase II (CKII) and plant extract *in vitro* and that phosphorylation of HFR1 can be effectively reduced by treatments with two CKII specific inhibitors, 5, 6-dichloro-1-&beta;-D-ribofuranosyl-benzimidazole (DRB) and heparin. We demonstrate that HFR1 physically interacts with the CKB1 and CKB2 regulatory subunits of CKII. Mutagenesis studies indicate that HFR1 is phosphorylated at multiple serine (S) residues in the N-terminal regulatory domain of HFR1 and that a predicted CKII site, S122, represents a major phosphorylation site of HFR1. We also show that phosphorylation of HFR1 is promoted by light. While phosphorylation does not seem to affect nuclear targeting of HFR1 in plant cells, comparison of wild-type, a phosphorylation-deficient and a hyperphosphorylated mutant protein suggests that phosphorylation acts to reduce the degradation rate of HFR1. Together, our results suggest that CKII-mediated phosphorylation represents an important posttranslational modification influencing the stability and signaling activity of Arabidopsis HFR1.

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**ICAR7051**

## FUNCTIONAL CHARACTERIZATION OF A CALCIUM-DEPENDENT CYSTEINE PROTEASE METACASPASE-2D (MCP2D) IN ARABIDOPSIS

Category: Abiotic Interactions

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Programmed cell death (PCD) is a genetically controlled process that regulates development, homeostasis and stress responses in eukaryotes. In spite of the apparent importance of PCD in plants, molecular identity of the key regulators and their control pathways remains largely unknown. Canonical caspases, cysteine proteases that act as key mediators of animal PCD, are absent in plant, fungi and protozoa. Instead, an ancient cysteine protease family called metacaspase exists in these genomes. However, recent studies demonstrated that these proteases efficiently cleave trypsin-type substrates and are unable to cleave caspase-specific substrates *in vitro*. Furthermore, plant and protozoan metacaspases have been shown to be able to replace yeast metacaspase-1 during oxidative stress-induced and/or ageing-related PCD of yeast cells. These characteristics of metacaspases imply that metacaspases are members of a family of cysteine proteases with a role in PCD that is conserved in evolution, in spite of possible differences in their biochemical properties. In this meeting, we report the functional characterization of Arabidopsis metacaspase-2d (AtMCP2d), which is a predominantly expressed member of type-2 metacaspase subfamily (AtMCP2a-2f) in Arabidopsis plants. Our findings from genetic and biochemical studies strongly suggest that this metacaspase plays important roles as a mediator in the activation of PCD induced by mycotoxin and oxidative stress in Arabidopsis.

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**ICAR7052**

## ARABIDOPSIS INFLORESCENCE DEVELOPMENT UNDER LOW SOIL MOISTURE CONDITIONS.

Category: Abiotic Interactions

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Drought stress is known to influence flowering and seed development in plants. For instance, drought can hinder proper pollen development in rice and drought after fertilization can cause kernel abortion in maize. Hence, drought stress can have great consequences on grain yield. Inflorescence development is also controlled by various flowering pathways, such as the autonomous pathway, vernalization and the photoperiod pathway. We are interested finding the mechanisms by which low soil moisture can cause this loss in grain yield.

In our Arabidopsis drought studies we determine soil moisture content using a gravimetric method, which enables us to control exact soil moisture content during inflorescence development. Our results show that under moderately low soil moisture conditions the plant produces small flowers and

short siliques. Under more stringent low moisture conditions the inflorescence meristem activity is terminated prematurely. There are differences found in response to low soil moisture between the ecotypes Landsberg (Ler), Columbia (Col) and Cape Verde Islands (Cvi).

Our analysis of gene expression arrays of Columbia inflorescences during high and low soil moisture conditions show that flower development related genes are down regulated during drought conditions, while sets of hormone related genes are up regulated. In addition, some known drought stress related genes show a response in the inflorescence tissue.

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**ICAR7053****MULTIPLE ROLES OF WRKY22 IN *ARABIDOPSIS THALIANA***

Category: Abiotic Interactions

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WRKY family represents plant specific transcription factors that functions in many aspects of plant development as well as abiotic and biotic stresses adaptation. We report here that *Arabidopsis* WRKY22 plays very important roles in response to salt and pathogen stresses. Both transient and stable fusion GFP proteins showed nuclear localization, consistent with WRKY22 role as a transcription factor. WRKY22 expressed in all the organs with several fold higher expression level in leaves. Low temperature induced its expression level, while high temperature suppressed it. WRKY22 mutant showed better adaptation to lower temperature (4°C) and higher sensitivity to high temperature (37°C), suggesting positive roles of this gene in response to temperature fluctuation. Although the mutant was more sensitive to salt, it was not sensitive to osmotic stress, suggesting it also responded specifically to salinity stress. In addition, hypersensitivity of WRKY22 mutant to salicylic acid and pathogen revealed its positive role in biotic stress.

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**ICAR7054****NATURAL VARIATION IN FREEZING TOLERANCE, CBF GENE SEQUENCE AND EXPRESSION IN THE VERSAILLES CORE COLLECTION OF *ARABIDOPSIS THALIANA***

Category: Abiotic Interactions

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Different plant species vary dramatically in their frost tolerance and most plants from temperate regions are able to increase their freezing tolerance in response to low, non-freezing temperatures, in a process known as cold acclimation. This is accompanied by massive changes in gene expression. A central pathway, estimated to regulate about 20% of all cold-regulated genes, depends on the CBF/DREB1 family of AP2 type transcriptional activators. *CBF* genes appear to be ubiquitous in plant species and are almost always present in multiple copies. In *A. thaliana*, the *CBF1*, *2* and *3* genes were found to be induced by cold and their peak expression occurred prior to the expression of their target (*COR*) genes. However, it still remains to be determined how their expression is regulated and what the precise contribution of each individual gene to freezing tolerance and cold acclimation is. We have taken an approach that relies on the natural variation present between different accessions of wide eco-geographic origins. A phenotyping by electrolyte leakage analysis of the Versailles Arabidopsis core collection of 48 accessions maximizing the naturally-occurring genetic diversity and complemented by 7 additional accessions was performed to assess the variability in freezing tolerance before and after cold acclimation. These data allowed us for the first time to judge the extent of natural genetic variability in freezing tolerance in any plant species. Additionally the *CBF* genes and their promoters were sequenced in 50 of these accessions. Extensive polymorphism was found in all three *CBF* genes. The direct effects of sequence polymorphism were investigated by evaluating the kinetics of *CBF* gene expression, as well as that of a subset of the target *COR* genes, in a set of eight accessions with contrasting freezing tolerance.

Our results indicate that the Versailles core collection contains significant natural variation with respect to freezing tolerance, polymorphism in the *CBF* genes and *CBF* and *COR* gene expression.

## EVOLUTION AND DEVELOPMENT

### **ICAR801**

#### COMPARATIVE FLORAL DEVELOPMENT IN *CARDAMINE HIRSUTA* AND *ARABIDOPSIS THALIANA*

Category: Evolution and Development

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Although some of the general mechanisms underpinning floral organ formation have been established, most notably the regulation of cell identity described in the ABC model of floral development, many questions remain to be answered about how organs achieve their final form. For example, little is understood about the regulation of organ size control or organ number, yet such processes are critical to the generation of morphological diversity in the plant kingdom. To address some of these issues we have chosen to study organ number regulation in *Cardamine hirsuta*, a close relative of *Arabidopsis thaliana* in the *Brassicaceae* family. In floral organ development, there are two striking differences between this plant and *Arabidopsis*. *C. hirsuta* has four stamens instead of six, and has a variable petal number of zero to four in contrast with the invariant four petals observed in *Arabidopsis*. High heritability indicates the relative importance of genes over environment in explaining this variation in petal number within *C. hirsuta* populations. The genetic basis of these species differences in floral organ number is currently unknown. Understanding the regulation of organ number can give insight into basic mechanisms of organ formation, such as meristem function, organ boundaries, or signaling centers. Understanding the basis of variability in petal number might illuminate some general principles of the regulation of natural variation in a population. Characterization of *C. hirsuta* floral development will be presented, including comparative studies of *Arabidopsis* development. Scanning electron microscopy and confocal microscopy have been used to analyze petal and stamen development in *C. hirsuta* to determine whether organ loss in this species is determined by differential organ specification or organ abortion. Additional studies will include clonal analysis to determine the cell lineage contributions to petal and stamen development in *C. hirsuta*.

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### **ICAR802**

#### OSCILLATING SUMMER-LIKE TEMPERATURES PARTIALLY OVERRIDE REPRESSION OF FLOWERING BY *FRIGIDA*

Category: Evolution and Development

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The *Arabidopsis thaliana* flowering time gene *FRIGIDA* (*FR*) maintains high FLC levels, repressing flowering, unless the plant experiences an extended cold period. In controlled chamber studies, plants with a functional *FR* allele grown in the absence of vernalization, flower dramatically later than plants lacking functional *FR*; and this locus is therefore thought to confer a winter annual habit. However, recent field studies conducted in the native European range of *Arabidopsis* unexpectedly found that Columbia (Col) plants introgressed with a functional *FR* allele (Col *FR*) flowered only 10–12 days later in summer plantings than the *FR* null Col ecotype. To test whether the fluctuating temperatures experienced by plants in the field could contribute to the accelerated flowering of Col *FR* under summer field conditions, we designed chamber temperature profiles that mimicked rosette-level, summer conditions at both Norwich, UK and Koeln, Germany field sites. The plants experienced 16 hour long days with an average temperature of 22°C. Diurnal temperatures ranged from 12°C just before dawn to 32°C in the late afternoon. Col *FR* plants grown in the variable temperature treatment bolted significantly earlier than the same lines grown in a 22°C constant treatment. Our results indicate that daily summer temperature fluctuations partially reduce the vernalization requirement of *A. thaliana* Col *FR* plants. In contrast, these variable conditions weakly delay Col plants lacking *FR*. Both vernalized and non-vernalized *vernalization insensitive3* (*vin3*) Col *FR* mutants, which are unable to respond to vernalization, were similarly accelerated in the oscillating treatment, suggesting that the developmental acceleration in the variable treatment does not operate through the known *VIN3*-mediated vernalization pathway. Thus, ecologically realistic experimental conditions suggest new insights into the role of *FR* under natural conditions.

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### **ICAR803**

#### EVOLUTIONARY TRANSGENOMICS: A NEW SCREEN TO IDENTIFY GENES IN PLANT DEVELOPMENT AND EVOLUTION

Category: Evolution and Development

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In an evolutionary transgenic screen, genomic inserts from donor species are moved into the genetic background of a recipient species in order to phenotypically screen transgenic lines. Phenotypic effects caused by inserts can reflect increases in gene dosage or the evolutionary divergence of orthologous sequences. Cases of evolutionary divergence may lead to the identification of genes of large effect that explain phenotypic differences between species. Alternatively, phenotypes caused by diverged sequences could reflect developmental system drift (DSD): divergence in genetic interactions such that genes from one species behave as gain-of-function mutant alleles in the other genetic background. Significantly, DSD effects may uncover cryptic developmental functions of even well characterized genes. In a pilot transgenic screen, 1300 genomic clones of 17–25Kb from the gladeless plant *Leavenworthia alabamica* were screened for phenotypic effects in the *Arabidopsis thaliana* Columbia background. Among the initial findings, one *L. alabamica* insert line has been shown to have a repeatable effect on shoot architecture in *Arabidopsis*. We present results on the repeatability of other phenotypes and describe downstream methods for the identification of causal genes.

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### **ICAR804**

#### COMPARATIVE STUDY OF THE FUNCTIONAL PROPERTIES OF AGL6-LIKE MADS-DOMAIN TRANSCRIPTION FACTORS IN ARABIDOPSIS AND PICEA

Category: Evolution and Development

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MADS-domain transcription factors have important roles in reproductive development in *Arabidopsis* as well as in other seed plants. Our interest is in the evolution of function of these transcription factors in seed plants. *AGL6* and *AGL13* in *Arabidopsis* are the most closely related to the *AP1*-like and *SEP*-like genes which have important functions in meristem and floral organ specification. We have identified two orthologs of these genes, *DAL1* and *DAL14*, from the conifer *Picea abies*, and presented data to suggest that *DAL7* acts to mediate the juvenile-to-adult transition in this tree. Here we present a comparative analysis of the functional properties of *AGL6* and *AGL13* from *Arabidopsis* and *DAL1* and *DAL14* from *Picea*. We have examined the capacity of the proteins to interact with 116 MADS-domain transcription factors from both species using the yeast-2-hybrid system and analyzed their transcriptional activation properties in yeast.

We found that *AGL6* and *DAL1*, but not *AGL13* or *DAL14*, could activate transcription of a reporter gene in yeast. *AGL6* specifically interacted with a large number of MIKC-type and Ma-type MADS-domain proteins, unlike *AGL13* which showed a very limited ability to interact with the other MADS-domain proteins. The two spruce proteins were similar in interaction properties, and shared most interaction partners with *AGL6*, but not *AGL13*. Both *AGL6* and the two spruce proteins interacted with *SOC1*, which has a key role in the regulation of flowering time in *Arabidopsis*. This is interesting since an altered flowering time is a common phenotype of transgenic plants expressing *AGL6*-like genes from other species in *Arabidopsis*. Some of the proteins also, like *AP1* and the *SEP*-proteins, interacted with members of the B-class and C-class MIKC-type MADS-domain proteins.

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## ICAR805

STOMATAL PATTERNING: A CONSERVED SIGNALING PATHWAY?

Category: Evolution and Development

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TOO MANY MOUTHS plays a role in the perception of positional cues that coordinate the spacing of stomata in *Arabidopsis*. *TMM*-like genes exist in plants ranging from bryophytes to angiosperms, a finding that is particularly intriguing because these taxa exhibit widely different modes of leaf development and patterns of stomatal installation. In grasses, which produce strap-shaped leaves, stomata form in a linear sequence with no insertion of new stomata between preexisting stomata, in contrast to broad-leaved eudicots. The more basal monocot *Dioscorea bulbifera* has broad leaves and may exhibit hybrid features of stomatal patterning found in eudicots and grasses. Stomata are also found in lower plants such as the moss *Physcomitrella patens*, but they are infrequent and restricted to the sporophyte capsule. In these organisms it is unknown whether a *TMM*-like protein controls the spacing of stomata, but phylogenetic analysis suggests that the *TMM* clade is ancient and includes the moss putative ortholog. As a first step towards understanding the evolution of stomatal patterning mechanisms, we are evaluating the biological function of *TMM* orthologs from species of evolutionary interest by testing their ability to function in *Arabidopsis*. This will reveal whether these proteins are sufficiently conserved to interact with the *TMM* signal transduction pathway to control stomatal patterning. To date, we have demonstrated that the rice *TMM* protein can restore wild-type stomatal patterning in *tmm* mutant plants, and are currently examining the ability of the *Dioscorea* and *Physcomitrella TMM* orthologs to function in *Arabidopsis*. Ultimately, this work will clarify how proteins involved in positional signaling and cellular patterning are deployed during the evolution of new strategies for leaf development and stomatal installation.

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## ICAR806

UFO IS A TRANSCRIPTONAL COREGULATOR

Category: Evolution and Development

\*Risseeuw, Eddy Venglat, Prakash Xiang, Doaquan Komendant, Kristina Babic, Vivijan Datla, Raju risseeuw@nrc.ca

The *Arabidopsis* gene *UNUSUAL FLORAL ORGANS (UFO)* encodes an F-box protein, which define the substrate specificity of the SCF class of ubiquitin E3 ligases. UFO function is essential together with LEAFY to establish the petal and stamen whorls during flower development, but likely also plays a role in the specification of the floral meristem and possibly other primordia. Ubiquination of members of transcriptional complexes is one mechanism to control the activity of the promoter. To test whether UFO interacts with transcription factors at the promoter, we fused UFO with the engrailed (En) repressor domain and the VP16 activator domain. Indeed over-expression of En-UFO resulted in a loss of function phenotype resembling the *ufo* mutant. *Arabidopsis* plants over-expressing UFO-VP16 exhibited severe leaf serration compared to UFO over-expression without VP16. To our greatest surprise, upon flowering these transgenic plants formed flower meristems and bract primordia on the edge of the top rosette leaves and bracts. These ectopic meristems are competent to produce fertile flowers and bracts. Occasionally a complete inflorescence was formed at the edge of the leaf. The peculiar phenotype could be viewed as an example of the partial shoot, a controversial theory first proposed by Goethe and later by Arber that leaves can be viewed as transformed shoots. Further genetic studies established that the phenotype was independent of the KNAT pathway, which is suggested to be involved in the compound leaf development in some plants. However, the leaf phenotype was enhanced by gibberellin treatment and was completely dependent on functional *LFY* and *SEP* genes, but not other ABC MADS box genes. In the *ap1* mutant, flower meristems on the leaves were rather converted into shoots. Our results provide strong evidence that UFO interacts with transcription factors at the target promoter and further the results from our studies are consistent with the recently reported physical interaction between UFO and LFY.

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## ICAR807

EPIDERMAL CELL FATE REGULATORS MEDIATE WOUND-INDUCED ANTIHERBIVORE DEFENSE.

Category: Evolution and Development

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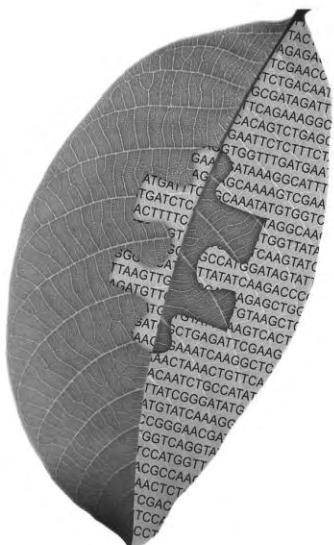
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Trichomes (leaf hairs) of plants have various functions, one of which is a physical barrier against herbivorous insects. Interestingly, various plant species from diverse taxa develop new leaves with increased trichome density in response to the damage to older leaves. Such developmental plasticity is an induced defensive response of plants against herbivores. We aimed to reveal the molecular basis of trichome induction using a model species, *Arabidopsis thaliana*, towards future cross-species comparison to gain insights into the evolutionary origin of this adaptive response.

Jasmonic acid and related compounds, collectively referred to as jasmonates (JA), are rapidly synthesized in response to local stresses, and act as signal-transmitting molecules to induce systemic defensive responses against herbivores and pathogens. We have shown that trichome induction is strictly dependent on both wound-induced biosynthesis, and SCF<sup>COI1</sup> complex-mediated signaling of JA.

To further elucidate the signaling pathway linking JA to trichome density, we next examined the roles of several regulatory proteins which had been implicated in cell-fate specification and differentiation of trichomes. A bHLH transcription factor GLABRA3 (GL3) is a positive regulator of trichome development. We found that GL3 fused with Green Fluorescent Protein (GL3-GFP) strongly accumulate in the nuclei of JA-treated young leaf epidermal cells prior to trichome initiation. We also isolated a novel mutant *unarmed9* (*urm9*) which alters the subcellular localization of GL3-GFP, and almost lacks JA-responsive trichome induction. Finally, genetic analysis of *GL3* loss- and gain-of-function mutants suggested that GL3 bridges JA signaling and trichome induction in a dosage-dependent manner.

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## CELL BIOLOGY

### ICAR901

SERPIN ATSRP4 MAY BE INVOLVED IN ETHYLENE SIGNALING

Category: Cell Biology

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Most serpins are irreversible inhibitors of serine and cysteine proteinases but their regulatory roles in plants are unknown. The *Arabidopsis* genome contains six genes that encode full-length serpins. The amino acid sequence of AtSRP4 suggests it is an inhibitory serpin with specificity for proteinases that cleave after glutamine residues. Expression of *AtSRP4* in seedlings increased gradually from day 2 to day 8 after germination. In adult tissues, *AtSRP4* was ubiquitously expressed, with the highest level in flowers. *AtSRP4* was up-regulated about 30-fold by treatment with AOA, an inhibitor of ACC synthase in ethylene synthesis, but expression did not respond to ACC. Cycloheximide, which disrupts ethylene signaling and is well known as an inhibitor of protein synthesis, induced *AtSRP4* expression more than 300-fold. *AtSRP4* expression increased in response to salt and drought stress, but was slightly reduced by GA treatment and unaffected by ABA and IAA. An AtSRP4-GFP fusion protein was localized to the cytosol. Interestingly, seeds of the homozygous T-DNA insertion mutant, *atsrp4*, gave a low germination rate (~35%) compared to wild-type (nearly 100%). The *atsrp4* mutant displayed a wild-type level of resistance to salinity stress; however, this resistance increased with ACC treatment. Taken together, our results show that AtSRP4 may be associated with ethylene-related signaling, perhaps as occurs in seed development, seed germination or/and stress responses. Future work will identify the target proteinase(s) for AtSRP4 and establish the mechanism by which it may regulate ethylene signaling.

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### ICAR902

EROONAZOLE: A SMALL MOLECULE PERTURBAGEN OF ER STRUCTURE.

Category: Cell Biology

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The endoplasmic reticulum (ER) is a dynamic organelle composed of a polygonal network of tubules and sheets. What determines the morphology of the ER has been of great interest since its discovery in 1945 by Porter, Claude and Fullam (Porter, et al. 1945). In particular how is the tubular structure of the reticulum established and preserved? Recently, biochemical reconstitution of ER tubule formation has led to the discovery that reticulons are necessary for tubule formation *in vitro* (Voeltz, et al. 2006; Hu, et al. 2008).

In an attempt to further dissect the mechanisms that generate and maintain ER morphology, a microscopy based screen of the LATCA collection of bioactive compounds (<http://cutlerlab.blogspot.com/2008/05/latca.html>) was performed using an ER marker line. This effort identified a new small molecule perturbagen of ER structure that we have named eroonazole (**ER balloon**ing **triazole**). Eroonazole treatment causes the ER to degenerate into numerous small vesicles. Observation of other organelles in treated seedlings reveal normal morphology, suggesting the primary target of eroonazole is at the ER. Additionally, time series of eroonazole treated seedlings show that ER streaming occurs rapidly, which suggests that ER-actin interactions are probably intact after treatment. Genetic target identification experiments are underway, using several alleles of a dominant eroonazole resistance locus.

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### ICAR903

IMAGING ACTIN FILAMENTS IN TRANSGENIC ARABIDOPSIS PLANTS EXPRESSING A GREEN FLUORESCENT PROTEIN FUSION TO THE C AND N TERMINI OF THE FIMBRIN ACTIN BINDING DOMAIN 2

Category: Cell Biology

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The role of the actin cytoskeleton in plant development is intimately linked to its dynamic behavior. Therefore, it is essential to continue developing methods for studying actin organization in living plant cells. The discovery of green fluorescent protein (GFP) has popularized the use of translational fusions of GFP with actin side binding proteins to visualize *in vivo* actin filament (F-actin) organization in plants. The most recent of these live cell F-actin reporters are GFP fusions to the actin binding domain 2 (ABD2) of Arabidopsis fimbrin (ABD2-GFP). For reasons not fully understood, a major limitation with the current batch of transgenic plant lines expressing the ABD2-GFP construct is low GFP signal. In an effort to improve ABD2-GFP fluorescence for *in vivo* F-actin imaging, we generated transgenic Arabidopsis plants expressing a construct with GFP fused to both the C and N termini of ABD2 under the control of the CaMV 35S promoter (35S::GFP-ABD2-GFP). The 35S::GFP-ABD2-GFP lines had increased fluorescence compared to the 35S::ABD2-GFP lines and as a result allowed the acquisition of highly resolved images of F-actin in different plant organs and stages of development. Although these new F-actin reporter lines allowed a more comprehensive description of F-actin organization in living Arabidopsis plants, subtle effects on the growth of some cell types were observed. However, the improved resolution of F-actin in living, intact tissues of Arabidopsis provided by this simple modification to the ABD2-GFP construct outweighs the disadvantages and therefore provides another tool for studying actin function during plant development.

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### ICAR904

ARABIDOPSIS HSC70-1 AND WPP-DOMAIN PROTEINS PREVENT AGGREGATION AND PROMOTE NUCLEAR ENVELOPE TARGETING OF ARABIDOPSIS TAIL-ANCHORED PROTEIN WIT1

Category: Cell Biology

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Tail-anchored (TA) proteins are a specialized class of diverse, functionally important, and differently localized transmembrane proteins. TA proteins are characterized by a carboxyl-terminal hydrophobic transmembrane domain, dictating post-translational membrane integration. Molecular chaperones, namely Hsc70 and Hsp40, known for their role in folding and translocation of proteins across membranes, have recently been shown to be involved in targeting of several TA proteins *in vitro*. *Arabidopsis* WPP-domain proteins, WPP1 and WPP2, are plant-unique, nuclear envelope-associated proteins. By tandem affinity purification coupled with mass spectrometry, we have identified *in vivo*-interacting partners of WPP1 and WPP2 - a chaperone HSC70 and a plant-specific putative TA protein, WIT1 (WPP domain-interacting TA protein). WIT1 and GFP-WIT1 are targeted to the nuclear envelope in *Arabidopsis thaliana*. In contrast, GFP-WIT1 forms cytoplasmic aggregates when overexpressed transiently in *Nicotiana benthamiana* leaf epidermis cells. However, when HSC70-1, WPP1 or WPP2 are co-expressed, GFP-WIT1 aggregation is significantly reduced and most GFP-WIT1 is located at the nuclear envelope. HSC70-1, WPP1 and WPP2 were also shown to specifically interact with GFP-WIT1 *in vivo*. A WPP1 mutant with significantly reduced affinity for GFP-WIT1 fails to decrease its aggregation. In addition, we prove the existence of a ternary complex consisting of HSC70-1, WPP1 and WIT1. Based on specific domain requirements, we propose that two independent mechanisms for WIT1 delivery/targeting exist: one that involves WPP domain proteins, which act by binding for the coiled-coil domain and the other mediated by HSC70-1, which is responsible for TMD-dependent TA protein membrane insertion, similar to its mammalian counterpart. WPP1 and WPP2 may work independently or coordinate with HSC70-1 in TA protein delivery. Thus, we propose a novel role for HSC70-1 in targeting of a TA-protein in plants and suggest that WPP-domain proteins are involved in the same pathway.

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### **ICAR905**

PDR9 FUNCTIONS AS A GENETIC SUPPRESSOR OF THE SCF COMPLEX MUTANT TIR1-1

Category: Cell Biology

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The phytohormone auxin is involved in the regulation of virtually all aspects of plant development. Auxin signaling involves complex interactions of auxin biosynthesis, polar transport and localized signaling events. On the cellular level, auxin signal transduction is realized via the TIR1/AFB-mediated signaling pathway. A genetic screen initiated to identify suppressors of *tir1-1* mediated auxin resistance, resulted in the isolation of several independent mutant alleles of the ABC transporter PDR9.

PDR9 has previously been implicated to act as a possible efflux carrier for the synthetic auxin 2,4-D. However, neither loss nor semi-dominant gain-of-function of PDR9 resulted in any phenotype which could be attributed to a function in response to the natural auxin IAA (Ito and Gray 2006). Root growth of our novel *pdr9* mutants is restored to WT sensitivity in response to the synthetic auxin 2,4-D in the *tir1-1* background. Furthermore, the novel *pdr9* alleles confer a restored sensitivity of the auxin responsive reporter construct BA3:GUS in response to 2,4-D treatment as well as in response to IAA application. Thereby, a function for PDR9 in auxin signaling in response to the natural auxin IAA could be demonstrated adding a novel component to the complex regulation of auxin dynamics.

Reference: Ito H, Gray WM (2006) A Gain-of-Function Mutation in the *Arabidopsis* Pleiotropic Drug Resistance Transporter PDR9 Confers Resistance to Auxinic Herbicides. *Plant Physiol.* 142: 63-74

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### **ICAR906**

ARABIDOPSIS SAG13 GENE NEGATIVELY REGULATES DEFENSE AGAINST BACTERIAL PATHOGENS

Category: Cell Biology

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*SENECENCE ASSOCIATED GENE 13 (SAG13)* gene of *Arabidopsis* encodes for a short-chain alcohol dehydrogenase and has been associated with senescence. Here we report that this gene is also involved in regulating defense against bacterial pathogens. *SAG13* gene is strongly induced in response to virulent and avirulent bacterial pathogens. Consistent with the expression profile of a typical defense gene, induction in response to avirulent pathogens is stronger and earlier compared with the virulent pathogens. In addition, *SAG13* transcript accumulates to high levels in several lesion mimic mutants. Interestingly, T-DNA knockout lines of *SAG13* are more resistant to the virulent *Pseudomonas syringae* pv. *tomato* DC3000 compared to the wild-type parent line, suggesting that *SAG13* negatively regulates defense against virulent bacterial pathogens. Pathogen-mediated accumulation of *SAG13* transcript is not affected in several *Arabidopsis* mutants defective in biosynthesis of salicylic acid (SA) and mutants defective in SA-mediated defense signaling. These results suggest that expression of *SAG13* is regulated by SA-independent defense signaling pathway(s). Together with previous reports of involvement of *SAG13* in senescence, our results suggest that *SAG13* is associated with several types of programmed cell death processes in plants.

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### **ICAR907**

CAN CHLOROPLAST-PRODUCED REACTIVE OXYGEN SPECIES REGULATE PLANT PROGRAMMED CELL DEATH?

Category: Cell Biology

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Programmed cell death (PCD) is a gene-controlled, tightly regulated and vital process in plant development and function. PCD induction involves cascades of cellular signals such as reactive oxygen species (ROS). We use *Arabidopsis thaliana* cell suspension cultures as model systems in which cell death may easily be induced by heat treatment.

Heat treatment induces significantly more PCD in dark-grown than in light-grown cultures and suppression of chloroplast biosynthesis in light cultures raises PCD induction levels to those found in dark cultures, suggesting that chloroplasts may be involved in PCD regulation. We have revealed the presence of simple chloroplasts in light culture cells with transmission electron microscopy and have found evidence suggesting that some photosynthetic pathways operate in these cells.

Antioxidant treatment of light cultures also raises PCD induction levels to those found in dark cultures, suggesting the involvement of chloroplast-produced ROS in PCD regulation. The concentration of hydrogen peroxide ( $H_2O_2$ ), a type of ROS, in cultures decreases with increasing light intensity, suggesting  $H_2O_2$  concentration may affect PCD induction. Suppression of chloroplast biosynthesis in light cultures significantly decreases the  $H_2O_2$  concentration in the cultures.

Our results indicate that chloroplast-produced ROS may be involved in the regulation of plant PCD.

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**ICAR908****RESEARCH ON THE DYNAMICS OF ROOT-HAIR SPECIFIC Qa-SNARE AND SEARCHING OF ITS INTERACTING MOLECULES**

Category: Cell Biology

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In higher plants, root hair elongation at trichoblast is a model of tip growth, which couples with polarized membrane trafficking within a cell. We previously reported that SYP123, one of the Qa-SNAREs preferentially localized on plasma membrane (PM), was involved in the elongation of root hairs in *Arabidopsis* and that GFP-SYP123 predominantly accumulates on the tip region of the developing root hair. To elucidate the role of SYP123 molecules on the specific polarized transport pathway to root hair tip, the observation of molecular dynamics of SYP123 was performed by using the transgenic *Arabidopsis* expressing GFP-SYP123 under control of the SYP123 promoter. We analyzed the transgenic plants by FRAP analysis coupled with the treatment of several inhibitors, including actin depolymerization drug, latrunculin B, and membrane traffic inhibitors, brefeldin A. The results indicated that SYP123 recycled dynamically between root hair PM and endomembrane compartments via specific pathway, and this recycling specifically occurred in GFP-SYP123, but not in GFP-SYP132, which was constitutively expressed throughout all tissues of *Arabidopsis*. Furthermore, to identify other SNARE molecules which interact with SYP123, we performed high-throughput screening by using split luciferase complementation assay. New findings obtained by these experiments will be discussed.

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**ICAR909****RNA ELEMENTS THAT INFLUENCE FCA AUTOREGULATION**

Category: Cell Biology

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The RNA binding protein FCA interacts with FY, a homolog of a yeast RNA 3' end processing factor, to promote flowering in *Arabidopsis* by down-regulating the strong floral repressor *FLC*. FCA has been linked to RNA-mediated chromatin silencing at a number of loci in *Arabidopsis* and it has also been demonstrated that FCA is localised to *FLC* chromatin, where it influences the RNAi machinery and epigenetic silencing of *FLC*. FCA/FY also promotes polyadenylation within intron 3 of *FCA* transcripts to negatively autoregulate *FCA*. Mutations to either *fca* or  increase the use of a distal polyA site within *FCA*, whereas overexpression of FCA leads to exclusive use of a poly(A) site within intron 3. Recent evidence supports specialised polyadenylation machinery being recruited in a complex with FY, to promote poly(A) site usage within *FCA* intron 3. It is likely that these *trans*-acting factors bind to *cis*-elements around the poly(A) site to control site choice in *FCA*. We are investigating regions of *FCA* intron 3 that influence poly(A) site choice using a tobacco transient-assay system, transgenic *Arabidopsis*, and Q-PCR to monitor *FCA* processing. Results defining regions of *FCA* which may be involved in poly(A) site choice will be presented.

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**ICAR9010****SEED STORAGE PROTEIN TRAFFICKING AND LOCALIZATION IN LEAVES OF ARABIDOPSIS MUTANTS**

Category: Cell Biology

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Seed storage proteins (SSPs) are typically synthesized exclusively in seeds and are then stored within protein storage vacuoles (PSVs). However, in some recently-developed *Arabidopsis thaliana* mutants, SSP mRNA is expressed in leaves. We have initiated a study of SSP trafficking and localization in these mutants. Our objective is to characterize the pattern of SSP production and accumulation in order to determine whether, and to what extent, mutant *Arabidopsis* lines are synthesizing and storing SSPs in leaves. We will detect SSP expression by immunoblotting and the abundance and morphology of PSVs will be identified using histochemical staining. To localize SSPs and PSVs at a subcellular level, mutant *Arabidopsis* lines will be transformed with translational gene fusions: fluorescent reporter proteins linked to either a SSP or to a PSV marker, and driven by a constitutive promoter, a leaf-specific promoter, or by seed storage protein promoters. Using confocal microscopy, the subcellular localization of SSPs in mutant leaves will be detected and accumulation of these proteins will be co-localized with PSVs. Finally, the developmental pattern of SSP expression in the mutant lines will be studied. This research will further our understanding of protein trafficking and storage in plants and may lead to methods for the production of higher levels of recombinant proteins in leaves.

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**ICAR9011****DYNAMICS OF NON-GREEN PLASTIDS IN SEED INTEGUMENTS OF ARABIDOPSIS**

Category: Cell Biology

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To investigate dynamic aspects of non-green (non-photosynthetic) plastids in live plant cells, we constructed transgenic *Arabidopsis* lines in which an N-terminal transit peptide derived from the plastid division factor AtFtsZ1-1 was stably expressed as a fusion product with yellow fluorescent protein (YFP). The resulting fluorescent protein labeled the plastid stroma, but few remnant cytosolic signals were evident in most non-photosynthetic tissues. This facilitated the detection and observation of non-green plastids under a fluorescent microscope. Comprehensive microscopic observations subsequently revealed that plastids in the outer ovule integuments will adopt highly filamentous forms with the proliferation of stromules at an early phase of amyloplast development during seed coat formation. With the accumulation of starch grains in the stroma, the filamentous plastids became amorphous in shape, reducing the rate of stromule production. Time-lapse imaging also revealed that these amorphous-shaped plastids have fluid envelope membranes and exhibit highly active envelope remodeling and motility in a manner similar to free-living amoeba.

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### **ICAR9012**

ECTOPIC EXPRESSION OF THE N TERMINUS OF FIN219/JAR1 IN ARABIDOPSIS CONFERS A DOMINANT-NEGATIVE PHENOTYPE AND REGULATES THE LEVELS OF COP1 AND HY5 SPECIFICALLY UNDER FAR-RED LIGHT

Category: Cell Biology

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Previous studies indicated that *far-red insensitive 219* (*FIN219*) was involved in phytochrome A-mediated far-red light signaling. *JAR1* was later shown to be the same locus as *FIN219*. To further understand *FIN219/JAR1* function in light signaling, we employed a transgenic approach expressing the N terminus fused with β-glucuronidase in wild-type Columbia. The resulting transgenic seedlings exhibited a dominant-negative long-hypocotyl phenotype specifically under continuous far-red light (cFR), which shared common defects with *fin219/jar1-1* seedlings. Moreover, the long-hypocotyl phenotype did not alter the response to methyl jasmonate in cFR. Intriguingly, various *fin219/jar1* alleles and ectopic expression of the *FIN219/JAR1* N-terminal region influenced the levels of COP1 and HY5 specifically in cFR, but not white light. In addition, the *fin219-null* allele selectively abolished the light-active unphosphorylated form of HY5. Dark-FR light transition studies revealed that under cFR, *FIN219*, COP1 and HY5 were regulated in an antagonistic manner, which involves protein degradation mediated by 26S proteasome. However, ectopic expression of *FIN219* gave an opposite effect in cFR. Overexpression of the N terminus of *FIN219/JAR1* leading to a dominant-negative phenotype under cFR is probably due to the disruption of the correct formation of the endogenous *FIN219* dimer, which thereby affects the expression of COP1 and HY5. Our findings reveal that *FIN219/JAR1* plays a vital role to regulate the levels of COP1 and HY5 to commit to a photomorphogenic development of seedlings under cFR.

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### **ICAR9013**

THE TSIP1, DNAJ TYPE ZN FINGER PROTEIN, CONTROLS TOBACCO PLANT SUSCEPTIBILITY TO THE CUCUMBER MOSAIC VIRUS INFECTION

Category: Cell Biology

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*Cucumber mosaic virus* (CMV) has a broad host range and infects almost 1,000 plants species. The CMV genome consists of three individual single-stranded positive sense RNA. RNA1 and RNA2 code for the 1a and 2a protein, respectively. Both 1a and 2a protein are core elements of the virus replicase complex. To identify plant host factors which interact with CMV 1a protein, a yeast two-hybrid system was used. One of the candidate genes was encoding Tsip1-interacting protein1 (Tsip1), a DnaJ-type Zn finger protein. Additionally, Tsip1 interacted with the CMV 2a protein in yeast. When tobacco plants were inoculated with purified CMV-Kor, disease symptom development was delayed in the Tsip1-overexpression plants compared with the wild type and Tsip1 RNAi line plants. In addition, the accumulation of CMV CP was delayed in Tsip1-overexpression plants, and viral RNA accumulation was decreased both in the infected local leaves and uninfected upper leaves as compared to wild type and Tsip1 RNA line plants. These results indicate that Tsip1-CMV 1a/Tsip1-CMV 2a binding may control virus replication and systemic movement in tobacco plants.

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### **ICAR9014**

IDENTIFICATION OF SUF4 PROTEIN COMPLEX IN *ARABIDOPSIS*

Category: Cell Biology

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We previously reported that *SUPPRESSOR OF FRIGIDA4*, encoding a C2H2-type zinc finger protein, represses flowering by transcriptional activation of *FLOWERING LOCUS C* in *Arabidopsis* (2006, *Plant Cell*, 18, 2985-2998). A MADS box transcription factor, FLC is a strong floral repressor, and is positively regulated by the FRIGIDA (FRI) encoding a coiled-coil protein in late-flowering winter-annual strain. Our results showed that SUF4 interacts with FRI and FRIGIDA-LIKE1 (FRL1) and binds to the *FLC* promoter *in vivo*. Interestingly, LUMINIDEPENDENS (LD), a FLC repressor, interacts with SUF4. LD encodes a homeodomain protein and functions as a floral activator in autonomous pathway. In addition, our transient gene expression results demonstrated that direct physical interactions of SUF4 with FRI, FRL1, and LD. In this study, to verify the *FLC* regulatory complex containing SUF4 in *Arabidopsis*, we first predicted approximate size of these complexes in diverse mutant backgrounds by gel filtration. Also, using epitope-tagged transgenic plants, we are identifying SUF4-MYC complexes in the nuclear fraction using a specific MYC antibody.

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**ICAR9015**

ANALYSIS OF SNARE LOCALIZATION IN POLLEN GRAINS AND ELONGATING POLLEN TUBES

Category: Cell Biology

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The elongation of pollen tube is a highly coordinated process involving polarized secretion of the cell wall materials and membranes to the tip region of the pollen tube. To study the mechanism of pollen tube elongation, we have focused on the SNARE molecules which possibly function in the pollen tube elongation. We generated transgenic Arabidopsis expressing pollen specific Qa-SNAREs (SYP124, SYP125 and SYP131) with GFP-tag under control of their own promoters, and observed the localizations and dynamics of these molecules. In mature pollen grains, the fluorescence of GFP-SYP124, 125 and 131 dispersed inside of the cells, suggesting that those are accumulated in some vesicular structure, but not specific organelles. In contrast, SYP132, which is constitutively expressed through out all tissues, was predominantly localized to the plasma membrane. During pollen tube elongation, SYP124 and SYP125 were highly concentrated on the tip region or proximal region of the pollen tube. However, the localizations of SYP131 and SYP132 were uniformly observed in the plasma membrane of elongating pollen tube. Thus, SYP132 and 131 are constitutive expressed and showed uniformly localization on the plasma membrane, and SYP124 and 125 showed specific localization of the tip-region of the pollen tube. These results suggest that different SNARE sets are separately used in the at least two distinct membrane transport pathways in the same cell during pollen maturation and elongation.

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**ICAR9016**

REGULATION OF PLANT CELL ELONGATION BY KINESIN-4 MOTORS

Category: Cell Biology

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Plant cells achieve cell elongation by depositing cellulose microfibrils transversely to the elongation axis. While cortical microtubules (MTs) are known to play a critical role in cell elongation, the linkage between MTs and cellulose biosynthesis is still unclear. Cotton fibers are single cells undergoing rapid elongation following fertilization, and have allowed us to discover particular microtubule-based motor kinesins that are highly expressed during cell elongation. Among them, members of the Kinesin-4 subfamily are of special interest to us because the *Arabidopsis fra1* mutation at such a gene caused disorganization of cellulose microfibrils while the cortical MTs were not affected. We hypothesized that Kinesin-4 members play a redundant role in the distribution of cellulose synthase along cortical MTs. Three members of Kinesin-4 subfamily, Kinesin-4A, Kinesin-4B, and Kinesin-4C are encoded by the *Arabidopsis* genome. Using promoter-GUS fusion, expression patterns of these genes were analyzed in transgenic *Arabidopsis*. Kinesin-4A and Kinesin-4C were highly expressed in the vascular tissue of expanding leaves, and in the root elongation zone. Multiple alleles of T-DNA insertional mutations at the three kinesin loci have been isolated. While the *kinesin-4a* mutation significantly impaired shoot elongation, mutations of *kinesin-4b* and *kinesin-4c* did not exhibit a noticeable growth defect. Possible redundant functions have been analyzed in homozygous double mutants. The most severe phenotype was detected in the *kinesin-4a / kinesin-4c* double mutant which exhibited an enhanced inhibition of cell elongation compared to the *kinesin-4a* single mutant. Ongoing studies are focused on determining intracellular localization of the three kinesins using mono-specific antibodies and epitope-tagging. We will examine whether intracellular distribution of cellulose synthase complex is dependent on activities of Kinesin-4. This work was supported by the Energy Biosciences Program of the Department of Energy.

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**ICAR9017**

FUNCTIONAL CHARACTERIZATION OF AN F-BOX PROTEIN GENE INVOLVED IN MODULATION OF AUXIN LEVELS IN *ARABIDOPSIS*

Category: Cell Biology

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Auxin performs the pivotal role in leaf initiation, axial polarity patterning and marginal serration during leaf development. The temporal and spatial allocations of auxin are thus important. To tightly control the optimal auxin levels in a specific tissue at a specific time, plants have evolved multiple mechanisms, including auxin biosynthesis, conjugation and degradation. Here, we report the characterization of an *Arabidopsis thaliana* mutant, upward-curled leaf1-Dominant (uc1-D), which exhibited hyponastic leaf phenotypes. UCL1 encodes an F-box protein classified into C5 type-1 with no intron. UCL1 overexpression lines displayed pleiotropic phenotypes including a simple vascular pattern, branched inflorescence, less lateral roots and reduced fertility. Likewise, 35S-UCL1 plants showed decreased DR5-GUS signals in a whole plant; however, the response was recovered by exogenously applied auxin. T-DNA insertion alleles of UCL1 showed reverse phenotypes to those of the 35S-UCL1 plants. The UCL1 gene was spatially expressed in the auxin maxima and its expression was repressed by auxin. The UCL1:GFP protein was localized in the nucleus, raising a possibility that UCL1 controls protein stability of a transcription factor involved in the auxin response. These data suggest that UCL1 play a role in modulating auxin levels.

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**ICAR9018**

*PUCH1* REGULATES FLORAL MERISTEM IDENTITY AND BRACT SUPPRESSION IN *ARABIDOPSIS*

Category: Cell Biology

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In *Arabidopsis*, the inflorescence bears a few number of basal branches and numerous flowers on top. Branches show indeterminate growth and are subtended by cauline leaves, whereas flowers are determinate shoots that lack subtending leaves. To investigate how the inflorescence architecture is determined, we analyzed the function of the *PUCHI* gene encoding a putative AP2/EREBP transcription factor. Mutations in *PUCHI* cause a slight but significant increase in the number of lateral branches produced from the primary inflorescence compared with the wild type, suggesting that very early arising flowers are converted to branches in *puchi*. Moreover, flowers of *puchi* are subtended by rudimentary bracts. In the inflorescence apex, *PUCHI* mRNA is expressed transiently in the adaxial portion of stage 1 to early stage 2 floral meristems (FMs). In addition, a functional GFP-*PUCHI* fusion protein expressed under the control of the native cis-regulatory sequence is localized to the adaxial portion of early FMs similar to *PUCHI* mRNA, indicating that the expression of *PUCHI* protein in the adaxial side is sufficient to suppress bract outgrowth on the abaxial side. To further explore the role of *PUCHI*, we examined the interaction with *BLADE ON PETIOLE1/2 (BOP1/2)* genes that redundantly control flower development and bract suppression. Interestingly, *puchi* enhanced the *bop1 bop2* phenotypes in such that in the *bop1 bop2 puchi* triple mutant almost all flowers are converted to branch-like shoots. The expression of *BOP1/2* in *puchi* and *PUCHI* expression in *bop1 bop2* remain unchanged. Furthermore, expression of *LFY* is reduced in the *bop1 bop2 puchi* triple mutant background, indicating that *LFY* acts downstream of *PUCHI* and *BOP* genes. These observations suggest that *PUCHI* and *BOP* genes are redundantly required to specify the floral meristem identity.

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### ICAR9019

ANALYSIS OF PROPYZAMIDE HYPERSENSITIVE2 (*PHS2*) MUTANT, RELATED WITH MICROTUBULE REGULATION IN *ARABIDOPSIS*

Category: Cell Biology

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In growing plant cell, cortical microtubules play an important role in regulating the direction of cell expansion. Many regulators of microtubule array were already isolated, but it is difficult to describe the mechanism only by their factors. To isolate a new factor related to construction or maintenance of cortical microtubule arrays, we screened mutants which root show abnormal growth response to low concentration of propyzamide, a microtubule disrupting drug. By the weak microtubules disruption, it is expected that the disruption of microtubule regulators is detected sensitively. The *propyzamide hypersensitive 2 (phs2)* is recessive mutant, which shows slightly left-skewing of root growth under a usual MS condition. But in the presence of 3μM propyzamide, the root growth shows right-skewing and is reduced. The aerial parts are almost normal except to twisted siliques, which phenotype is also observed in some tubulin mutants. The *phs2* mutant was mapped on the center of chromosome 4 and had a frame-shift mutation in a novel gene. The genomic fragments including the loci rescued the *phs2* drug responses, resulting that the loci was identified as the *PHS2* gene. The *PHS2* is encoded a Tetrastric Peptide Repeats motif protein. The four homologous genes exist in *Arabidopsis*, and also in rice. But there are no genes homologous to *PHS2* in animal and yeast. To investigate the cellular localization of *PHS2* proteins, we constructed *PHS2-GFP* and *GFP-PHS2* transgenic plants using genomic *PHS2* clone. Their plants rescued the *phs2* phenotype, showing that the GFP-fused *PHS2* protein is functional. The fluorescent pattern showed cortical fibers in the root, hypocotyl and leaf epidermal cells. Moreover these fibers were disrupted by the treatment of microtubule disrupting drug oryzalin and new fibers were generated after washing out of the drug. These results were suggested that *PHS2* is a novel microtubule associate protein, which regulates cortical microtubule array in plant.

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### ICAR9020

ACTIN DEPOLYMERIZING FACTOR 9 IS BOTH NECESSARY AND SUFFICIENT FOR CONTROL OF EXPRESSION OF CONSTANS-LIKE 1 AND CONSTANS-LIKE 2

Category: Cell Biology

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The Actin Depolymerizing Factor (ADF)/Cofilin family of proteins are essential actin-binding proteins found throughout eukaryotes. ADF's classical function is to bind actin filaments at the cellular cortex and modulate filament dynamics by severing monomers from the pointed end of the filaments, thus providing more monomers for growth at the barbed end of the filament. ADF is therefore essential to such vital cellular functions as motility, membrane dynamics, cytokinesis, cytoplasmic streaming, and other functions where actin dynamics play an important role. The *Arabidopsis thaliana* genome encodes eleven diverse ADF proteins.

In our lab, we have shown that Actin Depolymerizing Factor 9 (ADF9) is expressed the weakest of all the ADF proteins in *Arabidopsis*, but is expressed most strongly in young seedlings and meristem tissues. We have also shown that a T-DNA mutant in ADF9, *adf9-1*, flowers earlier than wild-type under long day conditions (16 hours of light, 8 hours of dark). Here we show that the expression of *CONSTANS (CO)*, a central activator of flowering in the photoperiod dependent flowering time pathway, as well as two closely related genes, *CONSTANS-LIKE 1 (COL1)* and *CONSTANS-LIKE 2 (COL2)*, are all up-regulated in *adf9-1*. Interestingly, however, the expression of *COL1* and *COL2* are down-regulated in *ADF9* overexpression plants, indicating that *ADF9* is both necessary and sufficient to control the expression of these genes. ADF9 may, therefore, play an additional role to its classical cytoskeletal role in the development of *Arabidopsis*.

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### ICAR9021

PIN AUXIN EFFLUX CARRIER DEGRADATION BY SNX1-DEPENDENT VACUOLAR TARGETING

Category: Cell Biology

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Plasma membrane proteins and their turnover determine the cell surface identity and contribute to rapid responses to intrinsic and extrinsic cues in all eukaryotic cells including plants. Here we present a simple method for the *in vivo* visualization of lytic vacuole-dependent protein degradation. We molecularly characterize this pathway and identify several plant cargos including plasma membrane-based PIN efflux carriers for the phytohormone auxin (Petrasek et al., 2006). PIN proteins mediate directional auxin fluxes during many developmental processes and are regulated at multiple levels including their polar targeting (Wisniewska et al., 2006) and turnover at the plasma membrane (Abas et al., 2005). We show that in contrast to basal PIN delivery to the plasma membrane, which depends on vesicle trafficking regulator ARF-GEF GNOM (Geldner et al., 2003; Kleine-Vehn et al., 2008), PIN sorting to the lytic vacuole pathway depends on another Brefeldin A-sensitive ARF-GEF. Furthermore, we identify the SORTING NEXIN1 (SNX1) as an important component of this pathway having vacuole gating function at the level of a late endosome/prevacuolar compartment. The *in vivo* visualization of PIN2 turnover revealed a differential PIN2 degradation in different cell types and in response to environmental signals such as light and gravity. Our data suggest that ARF-GEF- and SNX1-dependent PIN sorting to the lytic vacuole pathway is instrumental for fine tuning auxin fluxes during plant development.

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## ICAR9022

### FUNCTIONAL ANALYSIS OF ARABIDOPSIS EB1 PROTEIN FAMILY

Category: Cell Biology

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In plant cells, microtubules (MTs) regulate many cellular functions, such as maintenance of cell shape and cell polarity, intracellular transport, and cell division. A group of MT-associated proteins, called plus end-tracking proteins (+Tips), associate specifically or preferentially with growing MT plus ends, and regulate plus end dynamics and spatial arrangement of MTs. EB1 is a highly conserved member of the +TIP family that plays a central role in forming the +TIP complex. Typical EB1 protein consists of three functional regions, a conserved calponin homology (CH) domain at the N-terminus, an  $\alpha$ -helical C-terminal domain, and a highly acidic tail. *Arabidopsis thaliana* has three EB1 proteins (AtEB1a, AtEB1b, and AtEB1c), but how they differ in cellular functions are still unclear.

When the  $\beta$ -glucuronidase (GUS) reporter gene was expressed under the 2-kb AtEB1 promoters, strong GUS activity was driven in pollen and vascular tissues by AtEB1a and AtEB1b promoters. On the other hand, AtEB1c promoter showed preferential expression in meristematic cells. The *eb1a eb1b* roots exhibited abnormal skewing growth in response to propyzamide whereas the *eb1c* roots showed increased sensitivity to oryzalin at the cell division zone. In interphase cells, GFP-tagged AtEB1a and AtEB1b decorated plus ends of cortical MTs in transgenic *Arabidopsis* plants, but GFP-tagged AtEB1c was associated with the plus ends of mitotic MTs. For AtEB1b and AtEB1c, the CH domain bound MTs *in vitro* and the  $\alpha$ -helical domain promoted dimerization between EB1 members. The acidic tail of AtEB1b inhibited MT-polymerization activity of EB1, but such inhibitory function was not observed for the basic tail of AtEB1c. Interestingly, the AtEB1c tail region contained a bipartite nuclear localization signal, which was shown to be important for its function. These results suggest that EB1c has evolved in the plant lineage to specifically regulate MT functions at mitosis.

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## ICAR9023

### MOLECULAR CHAPERONES IN INTRACELLULAR PROTEIN TARGETING

Category: Cell Biology

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The majority of proteins are synthesised by cytosolic ribosomes and then targeted to a specific compartment within the cell. The information specifying the destination is contained within the amino acid sequence of the protein, which is generally decoded by targeting factors present in the cytosol, and subsequently recognised at the target compartment.

In a class of proteins termed tail-anchored (TA) proteins a hydrophobic tail sequence tethers the protein to a specific membrane. TA proteins are found in many different intracellular membranes, where they play important roles in diverse cell processes, including biochemical pathways, protein targeting and the control of cell death. A key characteristic of TA proteins is that they are inserted into membranes by a C-terminal hydrophobic domain after they have been fully synthesised and released from the ribosome and are therefore used as a model system for posttranslational protein targeting.

Most targeting pathways begin after precursor synthesis, and are dependent on cytosolic molecular chaperones. Current models suggest that chaperones simply prevent precursor aggregation, but there is growing evidence supporting the notion that they also determine the specificity of targeting. Molecular chaperones can adapt to perform a variety of specialised functions by combining with different cochaperones, suggesting a mechanism by which targeting specificity may be generated from complexes of generic chaperones and cochaperones. The recent discovery of chaperone receptors, including TOM70 at the mitochondrial membrane and Toc64 at the chloroplast membrane, suggests a more complex role for molecular chaperones.

We are investigating the possibility that chaperones bind signal sequences and form specific complexes, which are then recognised by membrane receptors at the destination organelle. Analysis and functional testing of chaperone-mediated mechanisms are performed using TA proteins and chaperones from *Arabidopsis thaliana* in organelle-competitive targeting assays.

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**ICAR9024****γH2AX AND E2FS FOCI: HOW TRANSCRIPTIONAL REGULATION MIGHT BE INTEGRATED IN CHROMATIN REPAIR**

Category: Cell Biology

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At the molecular level the kernel of responses elicited by DNA double-strand breaks (DSBs) is commonly assumed to rely upon the dynamic of the "repair foci" which are large proteic structures assembling at the site of DNA lesions. According to this view several repair signalling pathways have been characterized for the last decade, even if a convincing unified model is still lacking, mainly due to an insufficient knowledge about the functions and cooperation of some of the foci components.

Therefore, using the plant *Arabidopsis thaliana* system, our first goal was to investigate the relevance of one early DSBs marker: the phosphorylated form of the histone variant H2AX ( $\gamma$ H2AX). We generated, by the means of an amiRNA construct, a transgenic line with a deficiency in  $\gamma$ H2AX.

Unexpectedly this one does not display a higher genomic instability or sensitivity to genotoxic stress, but dampens the upregulation of several genes involved in repair, suggesting that  $\gamma$ H2AX is instrumental in the transcriptional program following genotoxic exposure.

Proceeding further we unveiled the specific behaviour, in *Arabidopsis* root tip cells, of some plant transcription factors E2Fs fused to GFP which relocalize after a DSBs-inducer treatment and in a strictly ATM-dependent way, into some discrete foci-like structures. Unfortunately till now, we were not able to state if these latter colocalize with  $\gamma$ H2AX foci or if they partake of other subnuclear domains like Cajal bodies, in which transcriptional machinery accumulate.

Taken together these data bring new information about the way transcriptional responses are coupled with chromatin remodelling in the vicinity of DNA damage. They may as well foster an analysis of the DNA repair process, not only at a cellular level, but also in a developmentally-controlled context.

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**ICAR9025****A HOMOLOG OF HUMAN AND YEAST *TDP1* GENES IS ESSENTIAL FOR DNA REPAIR AND AERIAL DEVELOPMENT IN ARABIDOPSIS**

Category: Cell Biology

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Plant growth is controlled by the various developmental genetic programs. Among them, DNA repair is essential genetic program for DNA replication, cell cycle, cell division, embryogenesis, and organ growth. We identify the *Arabidopsis AMI*(*aerial miniature*), that affects plant growth and development. Homozygous *ami-1* plant shows the reduced size in cotyledons, rosette leaves, stems, and flowers under normal condition, whereas it shows a normal root size. Besides, the *ami-1* plant shows largely reduced epidermal cell numbers in all organs and the reduced number of vascular bundle in stem. Furthermore, homozygous *ami-1* has defective in apical dominance, seed setting, flowering, and senescence. *AMI* shares conserved homologous sequences with *TDP1*(Tyrosyl-DNA phosphodiesterase 1) gene of the human and yeast. In human and yeast, *TDP1* is known as a unique DNA repair enzyme that hydrolyzes the phosphodiester bond between the tyrosine residue of topoisomerase 1 and the terminal 3'-phosphate of the DNA. *AMI* protein shows the enzyme activity that remove tyrosine (Y) residue from 3' terminus of artificial substrate, oligonucleotide-tyrosine (18-Y), suggesting that *AMI* functions in a topoisomerase 1-DNA complex lesion and mediates DNA repair mechanism. Moreover, loss of *AMI* function caused marked hypersensitivity to the DNA damage agents, camptothecin and mitomycin C, and increased cell death. Taken together, we suggest that the *AMI* regulates DNA repair mechanism by repairing the defect of topoisomerase 1-DNA complex in *Arabidopsis*.

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**ICAR9026****IDENTIFICATION OF A BIOLOGICALLY ACTIVE SMALL SECRETED PEPTIDE IN *ARABIDOPSIS* BY *IN SILICO* GENE SCREENING FOLLOWED BY LC-MS-BASED STRUCTURE ANALYSIS.**

Category: Cell Biology

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Peptidomics is a challenging field to create a link between genomic information and biological function through biochemical analysis of expressed peptides, including precise identification of post-translational modifications and proteolytic processing. We found that secreted peptides in *Arabidopsis* plants diffuse into the medium of whole-plant submerged culture and can be effectively identified by *o*-chlorophenol extraction followed by LC-MS analysis. Using this system, we first confirmed that 12-amino-acid mature CLE44 peptide accumulated at a considerable level in the culture medium of transgenic plants overexpressing *CLE44*. Next, using an *in silico* approach, we identified a novel gene family encoding small secreted peptides that exhibit significant sequence similarity within the C-terminal short conserved domain. We determined that the mature peptide encoded by At1g47485, a member of this gene family, is a 15-amino-acid peptide containing two hydroxyproline residues derived from the C-terminal conserved domain. This peptide, which we have named CEP1 (C-terminally Encoded Peptide 1), is mainly expressed in the lateral root primordia and, when overexpressed or externally applied, significantly arrests root growth. CEP1 is a candidate for a novel peptide plant hormone.

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**ICAR9027****PROCESSING OF THE RALF PEPTIDE HORMONE IN PLANTS**

Category: Cell Biology

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RALF is a 5 kDa peptide hormone originating from a preproprotein that causes medium alkalinisation when added to a cell suspension culture. RALF can inhibit root growth by blocking cell division and control root hair elongation by modulating the activity of an H<sup>+</sup>-ATPase at their tip. RALF signaling pathway is unknown but involves an increase in intracellular Ca<sup>2+</sup> and ROS, as well as a membrane receptor. How is RALF processing regulated? To assess this, 35S::ScRALF1/3:GFP was inserted in *Solanum chacoense*. By western and immunoblotting with anti-GFP antibody, a strong accumulation of the core GFP was observed. However, when a GFP:HDEL marker protein was used, it was possible to visualize and stabilize the processed peptide, although only weak accumulation of the processed peptide could be observed. This suggests that RALF processing occurs in the Golgi and that its processing could be regulated by developmental cues or stress responses. To better understand this processing, an AtRALFL::AtRALFL:GFP/GFPHDEL construction was inserted in *Arabidopsis* with some motif deletions to identify possible cleavage sites. Some important amino acids were characterized suggesting the involvement of a subtilase. These amino acids are the target of the S1P subtilase in animals and plants. To assess the function of this subtilase in RALF processing, all the constructs were also inserted in the T-DNA knockout line s1p. RALF is a gene family with a conserved C-terminal domain, but not all of the members have the prodomain, questioning the function of this domain? To assess this question, an AtRALFL with or without a prodomain was overexpressed. Only plants expressing a construct without a prodomain showed an obvious phenotype, suggesting a negative role of the prodomain in peptide activity.

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### **ICAR9028**

A GPI-ANCHOR PLASMODESMAL NECK PROTEIN WITH CALLOSE-BINDING ACTIVITY AND POTENTIAL TO REGULATE CELL-TO-CELL TRAFFICKING

Category: Cell Biology

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Plasmodesmata (Pds) traverse the cell wall to establish a symplastic continuum through most of the plant. Despite central roles in plant growth development and defence very little is known about their constituent parts or mechanisms of regulation. Rapid and reversible deposition of callose in the cell wall surrounding the Pd apertures is proposed to provide a regulatory process through physical constriction of the symplastic channel. From a proteomic analysis of *Arabidopsis* suspension cell walls, we identified a family of Pd callose-binding proteins (PDCBs) which target Pds in newly-divided and in differentiated cells. PDCB1 showed specific binding to 1,3-β-glucans in vitro. These proteins contain signal sequences for a glycosylphosphoinositol (GPI)-linkage. GPI-anchor proteins localise to the extracellular face of the plasma membrane. In this position, PDCB proteins can interact with callose in the Pd neck region of the cell wall providing a potential structural anchor between the plasma membrane component of Pds and the cell wall. The location of PDCBs in the neck region of Pds was confirmed by electron microscopy using immunogold-labelling. Although insertional mutants for the respective genes showed no phenotype, increased expression of the protein unexpectedly led to increased callose accumulation and reduced cell-to-cell trafficking, further supporting the association between PDCB-mediated callose deposition and plant cell-to-cell communication.

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### **ICAR9029**

THE SEED COAT OF *ARABIDOPSIS THALIANA* AS A MODEL SYSTEM TO STUDY PLANT PLASMA MEMBRANE TO CELL WALL ADHESION.

Category: Cell Biology

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Traditionally, plant cells are thought of as static and constrained because of their rigid cell walls. However, it is becoming increasingly clear that the cell wall is a dynamic structure that reacts to developmental and environmental cues. As a result, there is considerable interest in determining the key proteins that act to link the plasma membrane and the cell wall. While there are many hypotheses as to which gene families are involved in adhesion, few proteins have been directly implicated in this process. To address this, we have initiated a reverse genetics screen utilizing the mucilage secretory cells of the *Arabidopsis thaliana* seed coat. These particular cells make an excellent model system to study plasma membrane to cell wall association as they undergo developmentally regulated changes in plasma membrane to cell wall adhesion. Therefore, sites of adhesion may be compared with sites of non-adhesion within the same cell.

Gene families with the capacity to interact with both the cell wall and the plasma membrane were chosen as potential adhesion proteins. Twenty-seven candidate genes were identified using publicly available gene expression profiles and data from seed-specific microarrays that were recently performed in the lab. Homozygous mutants were isolated and confirmed for all of these genes. Mutant plants were screened for defects in plasma membrane to cell wall adhesion via plasmolysis, and for changes in seed coat morphology that may result from defects in adhesion. Preliminary results indicate that an arabinogalactan protein (AGP) and several fasciclin-like AGPs may be involved in this process. Currently, further phenotypic analysis is being performed on these potential plasma membrane to cell wall adhesion mutants.

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### **ICAR9030**

EFFECTS OF CELLULOSE SYNTHESIS INHIBITORS THAXTOMIN A AND ISOXABEN ON *ARABIDOPSIS THALIANA* SEEDLINGS.

Category: Cell Biology

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Thaxtomin A and isoxaben are two cellulose synthesis inhibitors. The first one is the main phytotoxin produced by *Streptomyces scabiei*, the causative agent of common scab disease of potato, while the second is a synthetic herbicide. In a previous study, our laboratory has demonstrated that these two substances induce in *Arabidopsis thaliana* suspension-cultured cells a programmed cell death (PCD) (Duval et al., 2005). The cellular mechanism of this induction is still unknown but we show that a similar process occurs in planta. We have also performed a microscopic analysis of roots, the more spectacularly affected organs of treated seedlings. Our observations show a modification in the pattern of differentiation in the root.

These results led us to hypothesise that the modifications induced by these inhibitors interfere with auxin signals or transport. Here we discuss the impact of auxin on this particular type of PCD.

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**ICAR9031**

## ANALYSIS OF CHLOROPLAST RIBOSOME ASSOCIATED PROTEINS

Category: Cell Biology

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The *apg4* mutant was isolated from *Ac/Ds* transposon-tagged lines and was shown to have a disrupted gene for a ribosome binding factor RBFA homologue. The phenotypes of the *apg4* mutant had white cotyledons and yellow or green variegated true leaves. In *Escherichia coli*, the RBFA was shown to be involved in processing of the pre-rRNA to form the mature 16S rRNA. We found that the APG4 is involved in processing of the pre-rRNA to form the mature 23S and 4.5S rRNA and related accumulation of *rrn16* transcripts. The effect on the *apg4* mutation gradually decreased as to plant grew, which may be due to high expression of the *APG4* gene in cotyledon but not in true leaves. We make a prediction that some proteins for the ability to complement the phenotype. RimM(21-kDa protein formerly called 21K)and Era(*E. coli* Ras-like)have similar functions of RBFA in *E. coli*. To analyze the relation between *APG4* and these genes, we identified their homologous genes in *Arabidopsis thaliana*. We report the function and expression of these genes closely related to *APG4*.

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**ICAR9032**

## ER-LOCALIZED PIN5 AUXIN TRANSPORTER MEDIATES CELLULAR AUXIN HOMEOSTASIS

Category: Cell Biology

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Transport-mediated distribution of the phytohormone auxin provides positional cues for multiple plant developmental processes. PIN auxin exporters on behalf of their polar, plasma membrane localization determine direction and rate of intercellular auxin flow. We show that PIN5 - an atypical, and evolutionary ancient member of the PIN family encodes a broadly expressed functional auxin transporter that regulates growth and patterning in *Arabidopsis*. Loss- and gain-of-function phenotypes, changes in free auxin levels and the PIN5 localization at the endoplasmic reticulum suggest a role of PIN5 in transport of auxin from the cytosol into the lumen of the endoplasmic reticulum. Our data identify unexpected mechanism of regulating cellular auxin homeostasis that involves transport-based subcellular compartmentalization of the signaling molecule.

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**ICAR9033**

## ARABIDOPSIS BAX INHIBITOR-1 REGULATES CELL DEATH THROUGH THE SPHINGOLIPID PATHWAY

Category: Cell Biology

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In organisms, including animals and plants, programmed cell death (PCD) is crucial for the maintenance of life and is highly regulated by various factors. The cell-death suppressor Bax Inhibitor-1 (BI-1), an endoplasmic reticulum (ER) membrane protein, is widely conserved in animals and plants; and overexpression of BI-1 suppresses cell-death that is induced by various stimuli in animals, plants and yeast cells.

Recently, we identified *Arabidopsis* cytochrome *b*<sub>5</sub> (AtCb5) as an interactor of *Arabidopsis* BI-1 (AtBI-1) by screening the *Arabidopsis* cDNA library with the split-ubiquitin yeast two-hybrid (suY2H) system. Cb5 is an electron transfer protein mainly localized in the ER membrane. In addition, a Bimolecular Fluorescence Complementation (BiFC) assay and Fluorescence Resonance Energy Transfer (FRET) analysis confirmed that AtBI-1 interacted with AtCb5 in plants.

On the other hand, we disclosed that the AtBI-1-mediated cell death suppression in yeast required *Saccharomyces cerevisiae* fatty acid hydroxylase 1 (ScFAH1), which was a Cb5-containing fusion protein and interacted with AtBI-1. ScFAH1 is a 2-hydroxylase of sphingolipid fatty acid localized in the ER membrane. In contrast, two functional ScFAH1 homologues in *Arabidopsis* (AtFAH1 and AtFAH2) have no Cb5-like domain, and instead interact with AtCb5 in plants. These results suggest that AtBI-1 interacts with AtFAHs via AtCb5 in plant cells.

Furthermore, overexpression of AtBI-1 in *Arabidopsis* increased the amount of 2-hydroxy-very-long-chain fatty acids, which are predominantly included in sphingolipids. A close association between sphingolipids and the control of PCD has been well established in both animals and plants. Thus, AtBI-1 regulates cell survival through the 2-hydroxylation of sphingolipids.

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**ICAR9034**

## MICROTUBULE-NUCLEATION COMPLEXES CONTRIBUTE CORTICAL MICROTUBULE ARRAY ORGANIZATION IN ARABIDOPSIS CELLS

Category: Cell Biology

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Plant cells lack a centrosome-like microtubule organizer. To analyze plant microtubule organizing centers, we characterized *Arabidopsis thaliana* (*At*) GCP2, a component of gamma-tubulin complex (gamma-TuC). AtGCP2 localized as dots along cortical microtubules and at filament branching

points. Analysis of null mutants suggested that *AtGCP2* is essential for cell viability and gametophyte development. We also obtained a weak *gcp2* allele, *spiral3* (*spr3*), which has left-handed helical arrays of cortical microtubules and exhibits right-handed helical growth in axial organs. An amino-acid exchange mutation in *spr3* impairs an interaction with AtGCP2 and another component of gamma-TuC, AtGCP3. In higher plant cells, cortical microtubules are nucleated as branches on the pre-existing microtubule at angles of ~40°. The angles of branching in *spr3* cells were shifted to center around at 50°. Interestingly, additive cellular and morphological phenotypes were observed in a double mutant between *spr3* and *katanin* mutant in which minus ends of newly formed cortical microtubules were not released from the nucleation sites.

Our results indicate that gamma-TuCs containing AtGCP2 are responsible for microtubule nucleation in plant interphase cells and that the microtubule nucleation angle is an important determinant for generating transverse cortical arrays.

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### ICAR9035

A POSSIBLE ROLE OF A NOVEL RING FINGER PROTEIN IN ACTIN REMODELING WITHIN STATOCYTES IN SHOOT GRAVITROPISM

Category: Cell Biology

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Plants can sense the direction of gravity and change the growth orientation of their organs. Amyloplasts sedimentation in the statocyte is thought to be important for gravity perception. The observation of amyloplasts surrounded by actin microfilaments (MFs) implies possible regulatory roles of MFs in amyloplast dynamics in statocytes. However, it has been reported that inhibition of actin polymerization dose not have a detrimental effect on gravitropism of roots and shoots. Hence, it remains unclear how MFs participate in gravity perception within statocyte. Our previous studies have demonstrated that the endodermis is essential for shoot gravitropism in Arabidopsis, and that amyloplasts sedimentation in the endodermal cells is important for gravity sensing. The *sgr9*(*shoot gravitropism 9*) mutant exhibited weak gravitropic response in the inflorescence stem. In addition, its amyloplasts did not sediment to the direction of gravity in endodermal cells. The *SGR9* gene encodes a RING finger protein localized to amyloplasts within the endodermal cells. These results indicate that *SGR9* is involved in amyloplasts sedimentation. Here we demonstrate that actin depolymerization can restore gravitropic response of *sgr9* with pharmacological and genetic approaches. Gravitropic ability of stem segments of *sgr9* was restored by treatment with LatB. The *fiz1*(*frizzy shoot 1*) mutation, a dominant mutation in *ACT8* gene, partially inhibits actin polymerization. Interestingly, the *sgr9 fiz1* heterozygous mutant exhibited almost normal gravitropic response. Simultaneously, amyloplasts sedimented to the direction of gravity. These results suggest that abnormal amyloplasts sedimentation in *sgr9* is due to reduced depolymerization of MFs, resulting in weak shoot gravitropism. These results prompt us to postulate that a certain mechanism to regulate MF depolymerization is required for amyloplasts sedimentation to enable full gravity perception in shoot gravitropism, and that *SGR9* is involved in the mechanism.

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### ICAR9036

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF A NEW *BRI1* ALLELE IN ARABIDOPSIS

Category: Cell Biology

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Brassinosteroids-Insensitive 1 (*BRI1*) has been known to be a master regulator for brassinosteroids (BRs) signaling in many plant species. *BRI1* is a plasma membrane-localized 24 leucine rich-repeats (LRRs) containing receptor-like serine/threonine kinase, and more than 30 mutant alleles of *BRI1* have been reported. All of them displayed dwarfism with downward-curled and rounder aerial rosette part that was not recovered by the treatment of exogenous brassinolide (BL), the most active brassinosteroid. Severity of the growth defect depends on the mutational sites on each mutant allele. Mutational sites identified from the known *bri1* alleles revealed that both the extracellular and the cytoplasmic kinase domains are important for functional *BRI1*. However, to date, no known *bri1* allele defective in LRR-region in extracellular domain has been reported. We tested BR-sensitivity to dwarf mutant collection exhibiting similar characteristics of known *bri1* from the ABRC stocks to obtain more BR-signaling-related mutants, and found one semi-dwarf mutant, previously known as *compacta 3* (*cp3*) displayed reduced sensitivity to brassinolide in root inhibition assay. Compared with its wild type background *Ler-1*, growth of *cp3* was retarded in every part of the plant, such as leaf and petiole length, peduncle and silique length as well as total stature. As the molecular nature of *cp3* has not been known so far, we sequenced full-length region of *BRI1* of the *cp3* and found one nucleotide was change, resulting that serine in 399<sup>th</sup> amino acid of *BRI1* was changed into phenylalanine in the LRR 13 of *BRI1*. To confirm this could be a new *bri1* allele, first, we crossed this to *bri1-301*, another weak *bri1* allele, and observed F1 plants uncovered similar phenotypes to *bri1*. Secondly, we generated and analyzed *cp3* transformant containing wild type *BRI1*. *Cp3* transformed with *BRI1* showed recovered growth patterns and normal BR-sensitivity in transcriptional inhibition of *CPD* expression. Therefore, we renamed *cp3* as *bri1-13*. This is the first new allele of *bri1* whose mutation occurs in LRR region of *BRI1*.

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### ICAR9037

SUB-CELLULAR LOCALIZATION OF THE ARABIDOPSIS G $\beta$  $\gamma$  DIMER DURING ER STRESS IN TOBACCO BY2 CELLS

Category: Cell Biology

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Heterotrimeric G proteins consist of G $\alpha$ , G $\beta$  and G $\gamma$  subunits. However, the G $\beta$  and the G $\gamma$  subunits form a stable complex and function as a single entity, hence are often referred to as the G $\beta$  $\gamma$  dimer. The Arabidopsis genome encodes a single canonical heterotrimeric G protein  $\alpha$ (GPA) and G $\beta$ (AGB) subunit, and two  $\gamma$ (AGG) subunits. We have previously reported that the G $\alpha$  and G $\beta$  $\gamma$  subunits serve both separable and synergistic functions in signaling by reactive oxygen species (ROS) during the oxidative stress response. We have also shown that plants homozygous for a null mutation of the G $\beta$  subunit are substantially more resistant to ER stress-induced cell damage and death than either wildtype plants or plants homozygous for a null

**mutation of the G $\alpha$  subunit. ER stress occurs when the protein-folding machinery is compromised and unfolded proteins accumulate in the ER lumen.** The molecular response that relieves ER stress is termed the unfolded protein response (UPR). We found that the UPR is reduced and delayed in the **G $\beta$  null mutants compared** to that of wildtype plants and the large protein aggregates characteristic of ER stress response were not observed in mutant plants. These observations suggest that signaling through the G $\beta$  subunit of heterotrimeric G protein triggers the cell death response in the UPR.

**Here we investigate the subcellular localization of the Arabidopsis G $\beta$  complex in normal and ER-stressed plant cells.** We used a construct that expresses YFP-AGB and AGG1 from constitutive promoters [35S-YFP-AGB-NOS - 35S-AGG1-NOS]. This construct rescues the G $\beta$  null mutant phenotype of Arabidopsis and hence is functional in plant cells. We also used a fusion construct of RFP and N-acetylglucosaminyl transferase 1 (NAGAT-RFP), an enzyme that participates in glycosylation of secretory proteins, as a marker for Golgi bodies. Previous reports have indicated that overexpression required for the detection of fluorescent fusion proteins may interfere with the identification of functionally important compartments in which the proteins are present at relatively low concentrations. Our biochemical data showed that the abundance of G $\beta$  was greater in the endomembranes than in the plasma membrane. To avoid studying localization patterns from grossly overexpressing cells, we studied the expression patterns only in those cells that did not show exclusively plasma membrane localization of G $\beta$ . Our results from transient co-transformations of tobacco BY2 cells with the above constructs clearly show that G $\beta$  co-localizes with NAGAT-RFP in ER membranes and Golgi bodies, as well as at the plasma membrane. Moreover, co-localization of G $\beta$  and Golgi signals is observed in newly formed cell plates, which are enriched in Golgi-derived materials.

Our results from Tm-treated BY2 cells indicate that the cytoskeleton is destabilized during ER stress. Other laboratories have reported that the cytoskeleton is important for Golgi travel within plant cells. Preliminary data suggest that the localization of G $\beta$  is dependent on the secretory activity of the Golgi apparatus. Localization of G $\beta$  after treatment with tunicamycin, a potent inducer of ER stress, will also be discussed.

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### ICAR9038

PASTICCINO2 IS ESSENTIAL FOR SHOOT EPIDERMIS FORMATION

Category: Cell Biology

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*Arabidopsis PASTICCINO2 (PAS2)* is known as a negative regulator of the cell cycle by inhibiting A-type cyclin-dependent kinase (CDKA). *pas2* mutants had shown ectopic cell divisions and hypersensitivity to cytokinin. Here we report that *PAS2* is mainly expressed in the epidermis of shoots, and the mutants have severe defects in morphogenesis and cuticular formation in the epidermis. Recent *in silico* analysis suggested a possibility that PAS2 functions as a very long chain fatty acid (VLCFA) elongase, enoyl-CoA dehydratase. We found that expressions of some of the genes related to VLCFA biosynthesis were altered in the *pas2* mutants. Also, wax load to the epidermis was defected in the mutants. Taken together, our data indicate that PAS2 is involved not only in regulating cell division but also in VLCFA biosynthesis. We propose that VLCFAs produced in the epidermis play an essential role in organ formation during continuous development of plants.

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### ICAR9039

THE CHLOROPLASTIC LIPOCALIN ATCHL PROTECTS ARABIDOPSIS AGAINST DEHYDRATION STRESS

Category: Cell Biology

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Lipocalins are small ligand-binding proteins found in bacteria and in invertebrate and vertebrate animals. They have an ability to bind small, generally hydrophobic molecules. Animal lipocalins play an important role in the regulation of developmental processes, and they are involved in the responses of organisms to various stress factors and in signal transduction pathways. Recent reports suggested that lipocalins possess oxidant-scavenging properties. Plants also possess two genuine classes of lipocalins, the temperature-induced lipocalins (TILs) and the chloroplastic lipocalins (CHLs). We recently showed that AtTIL is involved in modulating tolerance to oxidative stress in *Arabidopsis thaliana*. The current work aims to elucidate the function of the AtCHL protein. Immunoblot analyses performed with an anti-AtCHL antibody showed that the protein accumulates specifically in the lumen of the chloroplast, and to a higher level after dehydration stress or paraquat treatment. AtCHLprom:GUS lines were generated and histochemical staining revealed that the promoter is active in the tips of young leaves, in mature leaves, in the stem nodes and in root vascular tissues. These data prompted us to assay for the possible involvement of hormones in the activity of the promoter. Only IAA showed a detectable effect, demonstrated by a significant increase in expression in the petiole of mature leaf. For functional characterization, we are performing gain- and loss-of-function analyses on overexpressing (OEX), knock-out (KO), and complementation lines (COMP). The accumulation of the protein upon dehydration stress and paraquat treatment, and the accumulation in the chloroplast lumen are consistent with a possible function of AtCHL in the protection of the photosynthetic machinery during abiotic stress.

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### ICAR9040

MEMBRANE-BOUND TRANSCRIPTION FACTORS PLAY A CRITICAL ROLE IN REGULATING STRESS RESPONSES IN ARABIDOPSIS

Category: Cell Biology

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Controlled release of membrane-tethered, dormant precursors is an intriguing activation mechanism that regulates diverse cellular functions in eukaryotes. An exquisite example is the proteolytic activation of membrane-bound transcription factors (MTFs). Regulated activation of preexisting

dormant MTFs is considered to be an adaptive strategy that ensures prompt responses to environmental changes.

The MTFs are activated by controlled proteolytic cleavage through either one of two distinct, but biochemically related pathways. In regulated ubiquitin (Ub)/proteasome-dependent processing (RUP), they are ubiquitinated and partially degraded by the 26S proteasome. In regulated intramembrane proteolysis (RIP), active forms are released by specific membrane-associated proteases.

Genome-wide analysis revealed that at least thirteen members of the -helical $\alpha$ -Arabidopsis NAC transcription factor family contain strong transmembrane motifs (TMs) in their C-terminal regions and thus are membrane-associated. Interestingly, most of the NAC MTF genes are up-regulated under stress conditions. Membrane release of the NAC MTFs is also activated by diverse environmental factors, suggesting that they are involved in stress responses. Transgenic plants overexpressing partial-size NAC constructs devoid of the TMs, but not those overexpressing full-size constructs, showed distinct phenotypic changes, including dwarfed growth, delayed flowering and seed germination, and alterations in leaf senescence. Furthermore, a number of plant transcription factors are predicted to be anchored to the intracellular membranes, thus indicating that proteolytic activation of MTFs is a regulatory scheme that occurs widely in plant genomes.

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**ICAR9041****CHARACTERIZATION OF THE PLANT ORTHOLOGUE OF TPX2**

Category: Cell Biology

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Higher plant cells are characterized by dispersed microtubule organizing centers. During interphase, they were identified at the nuclear surface, close to the cortex and along pre-existing microtubules. However, the mechanisms of spindle microtubule assembly remain largely unknown. In acentrosomal animal cells like Xenopus oocytes, the Targeting Protein for Xklp2 (TPX2) was characterized as an essential player in perichromosomal spindle assembly, suggesting that it may be a central regulator of spindle formation in the absence of centrosomes. Our aim was first to identify and then to functionally characterize the Arabidopsis orthologue of TPX2.

The best candidate corresponded to a single gene referred as AT1G03780. Stable transformants of Arabidopsis plants and tobacco BY-2 cells expressing GFP-AtTPX2 fusions were obtained.

The fusion protein was targeted within the nucleus in interphase and actively exported shortly before nuclear envelope breakdown (NEB), probably participating in prospindle formation around the prophase nucleus. This behaviour differs from animal cells in which TPX2 nucleates microtubules only after NEB. In prometaphase, AtTPX2 colocalizes with spindle fibers and is rapidly degraded in telophase, like in vertebrates, suggesting that the protein is involved in early steps of mitosis.

We characterized two nuclear localization signals, one nuclear export signal and two microtubule binding domains specific for the Arabidopsis protein, arguing in favor of its intracellular targeting and dynamics we followed.

Furthermore, AtTPX2 was shown to rescue microtubule nucleation in a TPX2-depleted Xenopus extract, indicating that this function is conserved in the plant protein. In addition, the injection of anti-TPX2 antibodies in Tradescantia stamen hair cells inhibited cell division just before NEB.

All together, our data provide new insights into plant cell division, suggesting that throughout evolution, TPX2 has conserved essential functions in spindle assembly.

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**ICAR9042****FUNCTIONAL CHARACTERIZATION OF A NOVEL GENE, EFC, REGULATING LEAF DEVELOPMENT AND FLOWERING TIME**

Category: Cell Biology

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Proper plant development is fine-tuned by multiple regulatory mechanisms. It is well known that chromatin remodeling is a major regulatory mechanism to control flowering time and leaf organogenesis. We isolated an activation tagging dominant mutant, *early flowering and upward curly leaves-D*(*efc-D*), which showed leaf incurvature and early flowering. The phenotype was caused by transcriptional activation of a novel protein, EFC, which was localized in the nucleus. Semi-quantitative RT-PCR showed that expression level of *FLOWERING LOCUS T* (*FT*) was increased in its gain of function mutants. Genetic interaction studies revealed that loss of *FT* function was epistatic to gain of *EFC* function in terms of flowering time but not in leaf morphogenesis, as similarly seen in *INCURVATA2* (*ICU2*), and *CURLY LEAF* (*CLF*). The expression levels of putative target genes of Polycomb group (PcG) proteins were increased in the gain of function mutants of *EFC*, suggesting that *EFC* plays a role in maintaining these target genes at the derepressed state. Taken together, our results suggest that *EFC* may play a role in the regulation of chromatin structure of flowering time genes and leaf morphogenesis genes by de-repressing the target genes of PcG proteins.

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**ICAR9043****AN ANALYSIS OF THE FUNCTION OF ING1 AND ING2, PHD-FINGER DOMAIN PROTEINS, IN ARABIDOPSIS USING BIOINFORMATICS, FUNCTIONAL GENOMICS, AND BIOCHEMISTRY**

Category: Cell Biology

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The Plant HomeoDomain (PHD) zinc finger was first identified in plants, though its presence is nearly ubiquitous in eukaryotes. It consists of a conserved zinc-binding Cys4-His-Cys3 motif. Recent reports have determined that conserved residues within some PHD fingers allow binding to trimethylated lysine 4 of histone H3 (H3K4me3), an important chromatin modification associated with transcriptional activity. In addition, some PHD

fingers have been shown to bind to specific phosphatidylinositol phosphates (PtdInsPs), which may be important for nuclear membrane localization. We have undertaken a bioinformatic analysis of the PHD finger in plants. In *Arabidopsis*, 73 separate PHD finger domains were identified in 60 different proteins. Most of these proteins also contained a domain annotated to function in chromatin remodeling, suggesting a major role for PHD containing proteins in this process. From this analysis we predict that only a small group of PHD fingers from plants bind to H3K4me3. While we predict that many plant PHD fingers have the ability to bind PtdInsPs. To begin to understand the function of the plant PHD domain, we have identified and characterized an *Arabidopsis* loss-of-function mutant and a gain-of-function transgenic in the plant orthologs of the human tumor suppressor, INhibitor of Growth (ING). From these data we hypothesize that ING1 and ING2 serves a critical role in *Arabidopsis* growth and development.

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**ICAR9044****FUNCTION OF ARABIDOPSIS EXL4 AND EXL6 TO FORM POLLEN COATS**

Category: Cell Biology

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Pollen coat is a surface structure of *Arabidopsis* pollen grains, which consists mainly of proteins and lipids. These components are synthesized in tapetal cells, released into locules when the tapetal cells are ruptured, and then deposited on the exine cavities of pollen surfaces. Pollen coats are significant in fertilization, because they are involved in the pollen recognition by stigma and the water absorption by pollen grains. The EXTRACELLULAR LIPASE4 (EXL4) and EXL6, which belong to the GDSL lipases, are most abundant proteins in *Arabidopsis* pollen coat. Both *EXL4* and *EXL6* were strongly expressed in tapetal cells at the flower developmental stage 10 to middle 12, where the components of pollen coat were synthesized actively. The EXL4-GFP and EXL6-GFP were localized in small granules in the tapetal cells. The plants carrying both a T-DNA insertion in *EXL6* and an RNAi construct for *EXL4* (*exl4-RNAi/exl6-1*) showed remarkably reduced fertility. By the TEM and SEM observations, it was appeared that the pollen coat formed only partly on the *exl4-RNAi/exl6-1* pollen grains, and that the pollen grains showed the difficulty in water absorption and germination. Taken together, it is suggested that the EXL4 and EXL6, or possibly their products generated by their lipase activity, are required for the formation of pollen coats. These components might be involved in the pollen-stigma interaction and water absorption.

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**ICAR9045****MTV2 AND MTV4 ENCODE COMPONENTS OF ARABIDOPSIS VACUOLAR TRAFFICKING**

Category: Cell Biology

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Plants possess at least two functionally divergent types of vacuoles, lytic and storage vacuoles with potentially different trafficking routes. The molecular mechanisms behind this distinction are still poorly understood, despite the fact that both lytic and storage vacuoles are indispensable for plant development and the eventual destination of proteins also of great biotechnological interest. We have developed an assay to detect *Arabidopsis* mutants *modified in transport to the vacuole* (*mtv* mutants). The *mtv* mutants abnormally secrete a vacuolar marker, VAC2, which consists of the CLAVATA3 protein fused to the putative storage vacuolar-sorting signal from barley lectin. In the apoplasm, VAC2 causes early meristem termination, a phenotype easily detectable by visual inspection of the plants. We will present the current status of the genetic screen and the characterization of two of the mutations identified, *mtv2* and *mtv4*. The vacuolar sorting receptor encoded by *MTV2* is a limiting factor for trafficking to storage vacuoles in vegetative tissues. In seeds, other sorting receptors can compensate for *MTV2* loss, consistent with the transport system being more robust in specialized storage tissues. *MTV4* encodes an ARF-GAP protein, which may regulate vesicle formation at the Golgi apparatus.

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**ICAR9046****PLETHORA PROTEIN COMPLEXES IN ROOT DEVELOPMENT**

Category: Cell Biology

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Maintenance of the stem cell population located at the apical meristem is essential for repetitive organ initiation during the development of higher plants. *Arabidopsis PLETHORA (PLT)* genes encode plant specific two-AP2 domain transcription factors required for stem cell specification. We have already shown that a clade of several *PLT* homologues is essential for root formation and that ectopically expressed *PLT* genes induce root-like structures. However, the molecular mechanisms by which PLT proteins modulate root formation and maintain stem cell activity remain to be understood.

Through characterization of proteins interacting with multiple PLT proteins and bimolecular fluorescence complementation (BiFC) assays in planta, we found that PLTs differentially interact with another set of plant-specific transcription factors. Some of these interacting proteins have been connected to development and cell cycle regulation, our data strongly suggest that PLT proteins specify root patterning and control cell division through distinct complexes. We shall describe recent progress in understanding how these regulatory partners control root development.

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**ICAR9047****ISOLATION OF NOVEL MUTANTS SHOWING ABNORMAL EXINE STRUCTURE IN ARABIDOPSIS THALIANA**

Category: Cell Biology

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The exine composing the outer wall of pollen grains shows highly diverged structures among higher plants, suggesting it plays important roles for successful pollination. However, little is known either about its biosynthetic process or genes involved in its construction. We screened mutagenized

*Arabidopsis thaliana* by scanning electron microscopy for plants that have pollen grains with abnormal exine structures, which were different from a regular mesh pattern in wild type. We obtained 12 mutants, named *kaonashi* (*kns*), and classified them into four types by exine structures as follows, pretty collapsed structure (2 mutants), very thin layer (1 mutant), incomplete tectum formation (5 mutants), and finer mesh size (4 mutants). Since most of these phenotypes have never been reported, this screening seems very effective to identify many novel genes involved in exine formation. All heterozygotes between wild type and each *kns* mutant had pollen grains with normal exine structure, suggesting that these genes function sporophytically. So we guess that a lot of genes involved in the exine formation are expressed in diploid cells, namely pollen mother cells and/or tapetal cells. Interestingly, many *kns* mutants did not show remarkable sterility, indicating that the precise structure of exine might not be necessary for the pollination. Map-based cloning of several mutants is in progress.

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**ICAR9048**

RABA4D IS REQUIRED FOR THE REGULATION OF POLLEN TUBE TIP GROWTH IN ARABIDOPSIS THALIANA

Category: Cell Biology

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During reproduction in flowering plants, pollen grains form a tube that grows through floral organs during fertilization. These highly polarized cells have a rapid rate of growth which is supported by a tip-focused delivery of membrane and cell wall components and is balanced by a retrieval of excess membrane by endocytosis. Both of these processes are regulated by the Rab family of GTPase proteins. The RabA4 subfamily consists of RabA4b and four other closely-related genes. We previously showed that RabA4b is tip-localized in growing root hair cells and mislocalized in root hair mutants defective in tip growth. To determine whether any other RabA4 subfamily members were expressed in pollen, the expression patterns each subfamily member was tested in roots, stems, leaves, flowers, and pollen. Of the RabA4 subfamily only RabA4d was expressed in pollen. Additionally, RabA4d was expressed only in mature flowers and pollen and not in other plant tissues. Subcellular localization of EYFP-RabA4d in pollen tubes revealed that, like RabA4b in root hairs, the pollen-specific RabA4d was tip-localized and this localization was correlated with pollen tube elongation in these cells. Furthermore, when RabA4d activity was disrupted in RabA4d T-DNA insertion mutants, *in vitro* germinated pollen displayed a bulged pollen tube phenotype and a reduced rate of elongation. Expression of EYFP-RabA4d restored a wild-type phenotype to these cells, demonstrating that this fusion protein is able to functionally replace the endogenous RabA4d protein. *In vivo*, disruption of RABA4D resulted in a male-specific transmission defect in which mutant raba4d pollen is outcompeted by wild-type pollen for fertilization of ovules leading to a decrease in the percentage of raba4d mutant progeny. Analysis of siliques from crosses using pollen from a heterozygous mutant plant and a wild-type female showed that most of the seeds at the base of the siliques are wild-type, which indicated that mutant raba4d pollen tubes have defective growth and/or guidance *in vivo*. Observation of pollen tubes *in vivo* showed that mutant raba4d pollen tubes displaying aberrant growth in the ovary and reduced guidance at the micropyle. These results indicate that RabA4d function is necessary for the regulation of pollen tube growth, and that this Rab GTPase may play important roles in perception of guidance cues during fertilization.

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**ICAR9049**

ANALYSIS OF SUMO GENE FAMILY IN ARABIDOPSIS THALIANA

Category: Cell Biology

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Small ubiquitin-related modifier (SUMO) modification is a reversible post-translational modification, which is essential for normal cell growth in most organisms. SUMO is expressed as a precursor and is proteolytically processed to expose the C-terminal Gly-Gly motif. SUMOylation regulates localization and stability of target proteins, as well as protein-protein interactions, and is involved in multiple cellular processes including cell cycle progression, DNA repair, and transcription. Occasionally, covalently attached SUMO can inhibit or facilitate a protein-protein interaction, as well as inducing the protein conformational change through non-covalent binding with the SUMO interacting motif (SIM) in a target protein. In *Arabidopsis* SUMOs are encoded by a gene family of eight members, *AtSUMO1-8*. It has been reported that *AtSUMO1* and 2 are essential for cell viability, nevertheless, the role of individual SUMO isoforms is still unclear. In order to reveal the possible functional specificity of each *SUMO* gene, we analyzed their gene expression patterns, the C-terminal processing patterns and the ability of SUMOylation. The result of RT-PCR and the histochemical GUS assay revealed tissue specific expression pattern of each *SUMO* gene, except for *AtSUMO8*, whose expression was not detected. For the C-terminal processing test, *AtSUMO1-7* precursors overexpressed and purified from *E. coli* were incubated with plant protein extracts from various organs. The processing of *AtSUMO1*, 2, and 3 was observed, whereas the other SUMOs were not processed in this system. Furthermore, all the analyzed mature SUMOs, *AtSUMO1*, 2, 3, 5, 6, and 7, were shown to be able to sumoylate a substrate protein by *in vivo* SUMOylation system in *E. coli*, despite the lack of the C-terminal Gly-Gly motif in *AtSUMO4*, 6, and 7. These results suggest that individual SUMO isoforms might acquire their own specific function by the distinctive tissue distribution and the specific processing manner, respectively.

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**ICAR9050**

ENDOCYTIC DEGRADATION AND POLARIZED LOCALIZATION OF BORATE TRANSPORTERS DEPENDENT ON SORTING MOTIFS

Category: Cell Biology

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Boron (B) is essential for plant growth but toxic when present in excess. *Arabidopsis thaliana* BOR1 is a plasma membrane borate exporter and is essential for efficient B translocation from roots to shoots under B limitation (Takano et al. 2002). The root-to-shoot translocation of B was enhanced

under B limitation in wild type but not in bor1-1 mutant plants (Takano et al. 2005). The enhanced translocation was suppressed upon resupply of high levels of B within several hours. Using transgenic plants expressing BOR1-GFP under control of the CaMV 35S RNA promoter, we demonstrated that supply of high levels of B induces degradation of BOR1 via endocytic pathway, presumably to avoid accumulation of toxic levels of B in shoots (Takano et al. 2005). In contrast, BOR4, a BOR1 homolog suggested to be involved in B exclusion from root cells, was accumulated stably in high B conditions when expressed as a GFP fusion under control of the 35S promoter (Miwa et al. 2007). Here we show BOR1 is localized preferentially in the plasma membrane of proximal side of various root cells. It is in contrast to the case of BOR4, which is localized in the plasma membrane of distal side in epidermal cells (Miwa et al. 2007). The polarized localization of borate exporters suggests the directional transcellular transport of B. Analysis of BOR1/BOR4 chimera and mutant proteins fused to GFP revealed that sorting motifs embedded in a probable cytoplasmic-loop region of BOR1 but not of BOR4 are involved in the endocytic and polarized trafficking. Our data are consistent with the model that the sorting motifs of BOR1 are required for selective trafficking from the early-endosome/TGN to the multivesicular-body/prevacuolar-compartment under high B conditions and to the proximal domain of plasma membrane under low B conditions.

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**ICAR9051**CHARACTERIZATION OF THE PUF GENE FAMILY IN *ARABIDOPSIS THALIANA*

Category: Cell Biology

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Regulation of gene expression in eukaryotic cells occurs at a number of different levels. Post-transcriptional mechanisms of gene regulation include mRNA stability, mRNA silencing, mRNA localization, and translational control. In some systems, these mechanisms function together to regulate protein synthesis and targeting. For example, in subcellular mRNA localization, mRNAs are typically translationally repressed while being transported to their final destination. Once localized, repression is lifted and translation occurs. One protein that has an important role in mRNA stability and translational repression is Pumilio, a protein first identified in Drosophila. Pumilio proteins, members of the Puf family of RNA-binding proteins, possess a highly conserved C-terminal region made up of 8 – 9 tandem repeats. These conserved domains have been shown to bind directly to transcripts, thereby affecting transcript stability or translation. The identification and characterization of Puf genes has been extensive in animal and fungal cells, but no work has been conducted on plant Pumilio homologs. Genome database searches have identified 26 putative *Arabidopsis* Puf genes. Sequence alignment, phylogenetic tree analysis, and homology modeling of this protein family demonstrate a breadth of variability in this protein in plants, particularly within the amino terminal region of the protein. Structural predictions of the plant Puf proteins suggests that the conserved carboxyl-terminus forms the typical rainbow-like structure that allows for a direct interaction with specific RNA targets. The subcellular localization of these proteins as determined by expression as fluorescent protein fusions will also be discussed.

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**ICAR9052**

## CHARACTERIZATION OF A NOVEL ROOT CELL ELONGATION REGULATOR, UP BEAT1.

Category: Cell Biology

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In plants, stem cell centers known as meristems are located at the tip of the root and shoot. The root meristem generates cells that divide several times before entering a zone of rapid elongation without division (elongation zone) after which they go on to differentiate. The process of cell expansion dramatically increases the volume of the cell, often as much as 100 fold.

Both root meristem size and root growth are regulated systematically as the rates of cell division and elongation are synchronized. However the molecular details of the transcription network regulating rapid root cell elongation are poorly understood. To identify the transcriptional networks regulating rapid root cell elongation, we used our RootMap, which comprises high-resolution gene expression profiles obtained from fine sections along the developmental axis of the *Arabidopsis* root (Brady et al., Science 2007). We hypothesized that transcription factors regulating root meristem size are likely to be expressed at the boundary between the root meristem zone and elongation zone. We chose several transcription factors show a peak of gene expression at this boundary. We then screened *Arabidopsis* T-DNA insertion mutants for these transcription factors. Preliminary characterization of one of these, *UPBEAT1* has shown that downregulation results in a larger plant while upregulation results in a smaller plant. Thus, this gene appears to act like a rheostat in controlling the transition from rapid cell division to cell expansion and consequently the size of the plant. The molecular and cytological characterization of the *upbeat1* mutant should provide new insights into the molecular interactions controlling rapid cell expansion.

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**ICAR9053**

## UNKNOWN RELATIONSHIP BETWEEN LEAF-CELL SIZE AND PLOIDY LEVEL

Category: Cell Biology

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Cell size, and consequently organ size, are generally correlated with the ploidy level. However, no one knows why do such a correlation exists. Here genetic approach in *Arabidopsis* has been adopted to elucidate this enigma.

There are two different ways for polyploidization. One way is the duplication of chromosome number that can be artificially induced by colchicine treatment. In *Arabidopsis*, tetraploidization by such a treatment results in karyotype of  $2n = 4C = 20$ , and tetraploidized cells/organs are larger in size. On the other hand, it has been shown recently that increase of ploidy level by endoreduplication also positively affects cell size. Unlike colchicine-

mediated polyploidization, endoreduplication does not increase the number of chromosomes, but instead, polytene chromosomes are formed. This type of polyploidization keeps the karyotype as  $2n = 10$ , even for 32C trichome cells. Recently, we found that these two ways of polyploidization have the similar effect on cell- and organ-size, as deduced from analysis of polyploidized topo VI mutants (Breuer *et al.* 2007, Plant Cell **19**: 3655-3668).

In the course of this analysis, we found that the impact of tetraploidization on cell and organ size was stronger in the topo VI mutants than in the wild type. This fact contradicts a popular hypothesis which assumes that increases in ploidy level automatically increase cell components in proportion with ploidy level, resulting in larger cells. If such a hypothesis is correct, the increase in cell volume after tetraploidization should be constant and equals 2, being independent of any mutation that affects cell size. To know the relationship between cell size and ploidy level, I polyploidized a series of small-celled mutants including *xs* mutants (Fujikura *et al.*, 2007, Plant Cell Physiol. **48**: 278-286) and examined the effect of the mutations on the impact of ploidy level on cell size. The results were curious: only some *xs* mutations increased the impact of tetraploidization on cell size increase. A possible explanation for this unexpected data will be discussed.

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**ICAR9054**

## THE ROLE OF THE CYTOSKELETON IN PEROXISOMAL MATRIX PROTEIN TRANSLATION AND IMPORT IN PLANT CELLS

Category: Cell Biology

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Microtubules (MTs) play an essential role in the growth and development of eukaryotic cells. A large-scale study aimed at identifying MT binding proteins using tubulin affinity chromatography identified 100 proteins, greater than 40% of which were related to proteins previously reported to have an association with MTs. Interestingly, six of the proteins identified were peroxisomal matrix proteins. Peroxisomal matrix protein import is unique in that the proteins are imported fully folded and oligomerized, which indicates a potential for cytosolic activities. Our research objectives are to explore the role of MTs on the translation and import of peroxisomal matrix proteins. We have shown that two of these proteins, the peroxisomal multifunctional protein (MFP) and malate dehydrogenase (MDH), have MT binding activity in vitro and in vivo, and RNA binding activity in vitro. These proteins have a high affinity for MTs ( $\sim 1 \mu\text{M}$ ), and selective specificity for RNA sequences in vitro. We have also demonstrated that peroxisomes show frequent, apparent associations with MTs while stationary or during actin-mediated transport. Based on these findings, and the findings of others, we propose a model that links the MT and RNA binding activity of MFP and MDH to the regulation of their translation and import into the peroxisome. We are currently performing experiments aimed at determining the effect of MT disruption on the synthesis of MDH and MFP and on their import into peroxisomes. In conclusion, we have evidence indicating that MTs serve to provide a scaffold that assists in the efficient, autoregulated synthesis and import of peroxisomal matrix proteins in plant cells.

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**ICAR9055**

## CHARACTERISATION OF A PUTATIVE PROTEIN RECEPTOR AT THE CHLOROPLAST ENVELOPE

Category: Cell Biology

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The correct targeting of proteins is crucial for the organisation and viability of a cell. Beside the cotranslational targeting of proteins to the ER, there exist multiple pathways for proteins to be targeted to organelles after translation. In this case a cytosolic preprotein-complex is recognized by receptors, which are localized at the outer membrane of the organelle. A search for proteins with domains common to known receptors resulted in an uncharacterised protein from *Arabidopsis thaliana*. Its sequence includes a predicted protein-protein interaction domain and a membrane anchor. According to quantitative real time RNA analysis and western blotting this protein is expressed in all tissues. Evidence from confocal microscopy and targeting assays supports localisation in the chloroplast. We propose this protein to be a novel receptor for preproteins at the chloroplast envelope.

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**ICAR9056**

## THE ROLE OF PROPYZAMIDE HYPERSENSITIVE 1 (PHS1) SIGNALLING IN REGULATION OF PLANT MICROTUBULE FUNCTION

Category: Cell Biology

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The plant cytoskeleton comprises a network of microtubules (MTs) and actin microfilaments that organizes the structures and activities of the cell. Substantial evidence in mammalian systems indicates a potential role of Mitogen-Activated Protein Kinases (MPKs) in mediating MT function and dynamics. In contrast, the mechanisms by which MPKs regulate MT function and dynamics in plants are not well characterized. To examine whether disruption of the polymer status of microtubules would induce changes in the expression of any members of the MPK signaling cascades, we took advantage of the temperature-inducible microtubule disruption phenotype of the *mor1-1* mutant to carry out a full genome microarray analysis at three early time-points (2h, 4h and 8h) during the course of microtubule disruption at the 30 degree restrictive temperature. This analysis revealed that expression of one of the five MPK phosphatases, *Propyzamide Hypersensitive 1 (PHS1)*, was >5-fold up-regulated in the *mor1-1* mutant at the 4-h time-point, indicating that *PHS1* gene expression may be involved in the process by which the cell monitors microtubule polymer status. Histochemical analysis of *PromoterPHS1::GUS* reporter plants showed that the *PHS1* gene was generally expressed through development and in most tissues, including roots, hypocotyl, leaf vascular tissues and guard cells. Transient expression of GFP-PHS1 in tobacco leaf epidermal cells revealed cytoplasmic labeling by both N- and C-terminal GFP fusions, with no apparent labeling on the microtubules. To further understand the biological significance of PHS1 protein in the regulation of MT functions, we generated transgenic plants in which expression of PHS1 is depleted through inducible RNAi

suppression. Depletion of PHS1 leads to phenotypic defects in cell elongation and MT organization, further supporting a role for a PHS1-signaling cascade in mediating microtubule function.

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**ICAR9057****CHARACTERIZATION OF ARABIDOPSIS DELLA PROTEIN DEGRADATION USING A CELL-FREE ASSAY SYSTEM**

Category: Cell Biology

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The phytohormone gibberellin (GA) regulates diverse aspects of plant growth and development, including seed germination, internode elongation and floral induction. GA responses are triggered by inducing the degradation of DELLA proteins, which function as repressors in GA signaling pathway. Genetic studies in Arabidopsis and rice have implied that the degradation of DELLA protein occurs via the ubiquitin-proteasome system. To investigate the specific roles of factors or the biochemical activities involved in this degradation process, we developed an Arabidopsis cell-free system to recapitulate DELLA protein degradation *in vitro*. Using this cell-free system, we were able to show that lysine 29 of ubiquitin is the major site for ubiquitin chain formation to mediate DELLA protein degradation. We also verified the specific roles of GA receptor and multi-subunit E3 ligase components in DELLA protein degradation process. In addition, we showed that the degradation is blocked by the inhibitor of PP1/PP2A phosphatase, suggesting that DELLA protein degradation requires protein serine/threonine dephosphorylation activity.

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**ICAR9058****THE ARABIDOPSIS THALIANA UBC13/UEV1D COMPLEX PROMOTES LYS63-LINKED POLYUBIQUITINATION CHAIN AND IS INVOLVED IN DNA DAMAGE TOLERANCE**

Category: Cell Biology

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Ubiquitination is an important biochemical reaction found in all eukaryotic organisms and is involved in a wide range of cellular processes. While polyubiquitin chains linked through Lys48 of ubiquitin targets specific proteins for degradation, recently described polyubiquitination through the Lys63 chain is thought to regulate the target protein activity instead of its degradation. To date, Ubc13 is the only known ubiquitin-conjugating enzyme (Ubc) capable of catalyzing the Lys63-linked poly-Ub reaction and this function requires physical interaction with a Ubc variant, Uev1 or Mms2. In yeast, the Ubc13-Mms2 complex is required for an error-free DNA damage tolerance (DDT), whereas in mammals, two distinct complexes Ubc13-Mms2 and Ubc13-Uev1 functions in DDT and NF- $\kappa$ B signalling, respectively. Neither Lys63-linked ubiquitination nor DDT has been systematically investigated in plants prior to this study. We isolated two *UBC13* and four *UEV1* genes from *Arabidopsis thaliana*. All *AtUBC13* and *AtUEV1* genes are able to functionally replace the corresponding yeast *UBC13* or *MMS2* genes for the DDT function. All four AtUev1 proteins can form stable complexes with AtUbc13s or with Ubc13s from yeast or human and can promote Ubc13-mediated Lys63-linked poly-Ub chain assembly *in vitro*, suggesting that both DDT and Lys63 poly-Ub are conserved in plants. Although all *AtUBC13* and *AtUEV1* genes are ubiquitously expressed in most tissues, *AtUEV1D* appears to be expressed at a much higher level in germinating seeds and in pollen. We analyzed two independent *Atuev1d* mutant lines and found that seeds from these *Atuev1d* null plants displayed enhanced sensitivity to a DNA damaging agent methyl methanesulfonate (MMS). To our knowledge, this is the first report of Ubc13-Uev complexes that function in tolerating DNA damage in a multicellular organism. Currently, we are investigating roles of Ubc13-Uev1 in plant development and stress responses.

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**ICAR9059****ETC3 FUNCTION IN TRICHOME DEVELOPMENT**

Category: Cell Biology

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**ETC3 function in trichome development**

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Trichome patterning in *Arabidopsis thaliana* is governed by a conserved cassette of bHLH- and MYB-factors, the WD40 protein TTG1 and single repeat MYB R3 factors. The single repeat MYB R3 factors inhibit trichome formation whereas the other factors promote the formation on leaves. It is puzzling that triple mutants of the negative regulators so far generated do not result in an all-trichome phenotype as expected in their complete absence. These mutants still have substantial leaf areas without trichomes. This indicates that further trichome regulators must exist. We show by genetic analysis that ETC3 (Enhancer of TRIPTYCHON and CAPRICE 3) has a redundant role in trichome formation like CPC and TRY. The *etc3* mutant shows a higher trichome density on leaves compared to wildtype. ETC3 is expressed similar to TRY and CPC and it interacts with GL3 in yeast two-hybrid assays. Triple and quadruple mutants show a considerable enhancement of trichomes on leaves.

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**ICAR9060****A PLASTID POLYRIBONUCLEOTIDE PHOSPHORYLASE SUPPRESSES VARIEGATION IN THE ARABIDOPSIS VAR2 MUTANT**

Category: Cell Biology

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The Arabidopsis *var2* variegation mutant defines a nuclear gene for a chloroplast FtsH metalloprotease. Leaf variegation is expressed in homozygous recessive individuals of the mutant. The cells in the green leaf sectors of *var2* contain morphologically normal chloroplasts, whereas cells in the white sectors contain abnormal plastids lacking organized lamellar structures. *var2* mutants are hypersusceptible to photoinhibition, and consistent with this phenotype, VAR2 has been shown to be involved in the D1 repair cycle of photosystem II, likely by affecting turnover of the photodamaged D1 polypeptide. A second-site suppressor screen of *var2* yielded several lines in which the variegation phenotype of *var2* is significantly modified. Some of these lines have a "central yellow" (CY) phenotype, in which the younger leaves on the rosette are pale-green or yellow, then turn fully-green as they develop and expand. One suppressor line with a CY phenotype, 2484, was chosen for further analysis. Map-based cloning revealed that the suppressor gene in 2484 codes for a plastid polyribonucleotide phosphorylase, which involved in rRNA processing and chloroplast protein translation. We designated this gene CY1. Isolation of the *cy1* single mutant showed a phenotype similar to the double mutant, which demonstrates that *cy1* is epistatic to *var2*. Our results suggest that VAR2 and CY1 act antagonistically in chloroplast biogenesis, and that downregulation of CY1 lowers the requirement for VAR2 in plastid development. The isolation of the *cy1* mutant represents an important advance in the generation of tools to understand variegation mechanisms and photoprotection in plants.

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### ICAR9061

POST-TRANSCRIPTIONAL AND POST-TRANSLATIONAL REGULATION OF CCA1 AND LHY IN THE CIRCADIAN OSCILLATOR OF ARABIDOPSIS THALIANA

Category: Cell Biology

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The circadian clock is an endogenous mechanism that generates rhythms with approximately 24-hour periods and enables organisms to predict and adapt to daily and seasonal changes in their environment. These rhythms are generated by transcription/translation feedback loops called oscillators. The main oscillator loop of the model plant Arabidopsis has been shown to consist of three elements: CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYLS (LHY) and TIMING OF CAB EXPRESSION 1 (TOC1). CCA1 and LHY are transcription factors that are expressed early in the morning, bind to the TOC1 promoter and repress its expression. Later in the day, when CCA1 and LHY levels drop, TOC1 is expressed and activates CCA1 and LHY expression by an unknown mechanism. However, numerous reports in other model organisms have shown that the oscillator mechanism requires not only transcription and translation but also post-transcription and post-translation regulations. We are interested in understanding the post-transcriptional and post-translational regulation of CCA1 and LHY and how this affects the Arabidopsis clock. We have shown that CCA1 is regulated post-transcriptionally by light: CCA1 mRNA is stable in the dark and in far-red light but is destabilized by red and blue light. Furthermore, the instability determinants in CCA1 transcripts are probably located in the coding region. This change in transcript stability is likely to be an important entrainment point and contribute to the correct setting of the oscillator with respect to the environment. To examine CCA1 and LHY in vivo at the protein level, we are setting up a system of tagged CCA1 and LHY expressed under the control of the endogenous CCA1 or LHY promoters in transgenic plants. These tagged proteins are been used to check if, where and when CCA1 and LHY interact, how entrainment conditions affect their subcellular localization and how the oscillator operates in different tissues and in different cells in Arabidopsis.

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### ICAR9062

SPL7 IS A KEY FACTOR TO ADAPT COPPER DEFICIENCY IN ARABIDOPSIS

Category: Cell Biology

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Copper is one of essential micronutrients for most living organisms. In higher plants, copper is mainly utilized as a cofactor of proteins involved in photosynthesis, scavenging of reactive oxygen species and ethylene sensing. Most abundant copper protein is plastocyanin (PC), localized to thylakoid lumen of chloroplasts, and is essential for photosynthesis in higher plants. Another major copper protein, copper/zinc superoxide dismutase (CSD), localized to cytosol (CSD1) and chloroplast stroma (CSD2), is involved in the scavenging of reactive oxygen species. In copper deficient conditions, the expression of CSD1 and CSD2 is down-regulated and their function is compensated by iron superoxide dismutase (FSD) specifically expressed in low copper conditions. Previously we demonstrated that a microRNA, *miR398* was involved in this down-regulation of CSD1 and CSD2. *miR398* is expressed in low copper conditions and involved in the degradation of *CSD1* and *CSD2* mRNA directly. Consequently, limited copper is preferentially transferred to indispensable copper protein like PC in copper deficient environments.

In this study, we identified SPL7 (SQUA promoter-binding protein-like 7) as a transcription activator for *miR398*. SPL7 recognized and directly bound to a GTAC core motif, an essential element for copper response in Chlamydomonas, on the promoter region of *miR398* and activated the transcription of *miR398* in copper deficient conditions. In addition SPL7 also up-regulated multiple microRNAs involved in the degradation of copper proteins, FSD, some copper transporters resulting proper copper distribution and copper chaperone leading effective copper mobilization in copper deficient conditions. Taken together, we propose that SPL7 is a master regulatory factor involved in copper homeostasis.

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### ICAR9063

PLANT SPECIFIC CHLOROPLAST PROTEIN, PTAC3 IS REQUIRED FOR TRANSCRIPTION OF BACTERIAL TYPE RNA POLYMERASE IN CHLOROPLAST.

Category: Cell Biology

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Plastids of higher plants are descended from cyanobacterial symbiosis and have their own genome and complete gene expression machinery. However, the plastid gene expression machinery has several unique features. Plastids contain two types of transcriptional machineries (PEP; bacterial type RNA polymerase and NEP; phage type RNA polymerase). Localization of the plastid nucleoids is strictly regulated during chloroplast differentiation. Interestingly, no homologue of cyanobacterial transcription factors nor nucleoid proteins such as Hu has been found in higher plants, suggesting that plastid nucleoids may have unique functions. Recent chloroplast proteome analysis suggested that chloroplast contains not only prokaryotic proteins derived from the ancestors, but also several eukaryotic proteins acquired from host cells during plant evolution. We took notice of pTAC3 that is originally identified as a component of chloroplast transcriptional active chromosome, and has a sap domain and some putative PPR motives. Sap domain is involved in DNA binding in eukaryotic matrix attachment region binding proteins. The transient expression of the GFP fusion protein in *Arabidopsis* protoplasts clearly demonstrated that pTAC3-GFP is localized to plastid nucleoids. T-DNA insertion mutant of pTAC3 showed albino phenotype. Expression of all PEP-dependent genes including photosynthesis and rRNA genes was reduced significantly in the pTAC3 knock-out mutants. To determine the binding sites for pTAC3 in *Arabidopsis* chloroplast genome, we performed chloroplast ChIP assay. pTAC3 mainly binds to promoter regions of the strongly transcribed PEP-dependent genes such as *psbA*. Gel shift analysis revealed that a GST fused sap domain fragment has DNA binding activity, however the sequence specificity is weak. These results suggest that plastid nucleoid localized pTAC3 might function as regulatory factor of PEP.

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**ICAR9064**

SOV1 REVEALS NATURAL VARIATION IN mRNA DECAY PATHWAY IN ARABIDOPSIS

Category: Cell Biology

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The main goal of our research is to identify the mechanisms controlling leaf vein pattern in *Arabidopsis*. We previously characterized mutants in the *VARILOSE (VCS)* gene: *vcs-1* to *vcs-5* showed a similar phenotype, including normal cotyledons and narrow, pointed leaves. These *vcs* alleles were isolated in Landsberg *erecta* (*L.er*) genetic background. In contrast, *vcs* alleles in the Columbia (Col-0) background have a more severe phenotype. These mutants have small chlorotic cotyledons, fail to produce leaf primordia, and show cotyledon vein pattern defects. To investigate how the genetic background influenced the phenotype, we generated *vcs-1/vcs-7* transheterozygotes. The phenotype of transheterozygotes looks like *vcs-1*, indicating a dominant suppressor of *VCS* in *L.er* (we call *SOV1<sup>Ler</sup>*). To gain mechanistic insight, we mapped *SOV1<sup>Ler</sup>*. The predicted protein sequence of SOV1 suggests that it interacts with RNA. We are testing whether SOV1 directly affects RNA decay rates.

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## METABOLISM

### ICAR1001

*GSD1-1D (GIBBERELLIN SENSITIVE DWARF1-1D)* IS A NOVEL MUTATION THAT AFFECTS GIBBERELLIN METABOLISM

Category: Metabolism

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*gsd1-1D [gibberellin(GA)-sensitive dwarf1-1D]* is a new dominant GA sensitive semi-dwarf mutant that has recently been isolated by genetic screening from the pools of *Arabidopsis* transposon-tagged mutants. Genetic analysis, however, revealed that the semi-dwarf phenotype did not cosegregate with the transposon insertion. Phenotypes of this mutant are similar to those observed in other GA deficient mutants (dwarf with dark green leaves and delayed flowering time), and can be restored by treatment with exogenous GAs. Consistent to the mutant phenotype, the *GSD1* gene has been mapped to a region that does not contain any known GA-related gene, suggesting that the *gsd1-1D* mutation affects GA metabolism via a novel mechanism. Progresses in identifying the *GSD1* locus and elucidating its possible role in GA metabolism will be presented.

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### ICAR1002

AAE13 (AT3G16170) ENCODES A MOLONYL-COA LIGASE IN ARABIDOPSIS

Category: Metabolism

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Malonyl CoA is the precursor for fatty acid synthesis and elongation. It is also essential for the biosynthesis of phytoalexins, flavonoids and malonylated compounds. It has been widely accepted that in plants acetyl-CoA carboxylase (EC 6.4.1.2) is the sole source for malonyl-CoA biosynthesis. However, previous studies have suggested that malonyl-CoA might also be made directly from malonic acid by malonyl-CoA ligase (malonyl-CoA synthetase) (EC 6.2.1.14). Here, we report the cloning of the first malonyl-CoA ligase gene, *AAE13* in *Arabidopsis*, from plant kingdom. When expressed in *E. coli*, *AAE13* protein shows very high activity on malonic acid ( $K_m = 631.4 \pm 90.2 \mu\text{M}$ ,  $V_m = 25.4 \pm 1.8 \text{ nmol}/\mu\text{g}/\text{min}$ ). *AAE13* is very specific toward malonic acid. It has only ca. 10% activity on 2-methylmalonic acid and has no activity on other dicarboxylic acids, such as 2-hydroxymalonic acid, oxalic acid, succinic acid, glutaric acid, adipic acid, and pimelic acid. Also it has no activity on short-, medium- and long-chain carboxylic acids ranging from acetic acid to octadecanoic acid. We also measured *AAE13* activity in extracts from different *Arabidopsis* tissues and found the highest activity in the flowers, which is consistent with the highest expression of *AAE13* gene in this tissue. Using GC/MS, we identified low level of malonic acid in flowers of wild-type *Arabidopsis*. However, in the flowers of transgenic *Arabidopsis* overexpressing *AAE13* gene under the driven of 35S promoter, malonic acid is undetectable. When *Arabidopsis* was germinated and grown on agar plates supplemented with different concentrations of malonic acid, the growth of seedlings was significantly inhibited by malonic acid at higher concentrations. GC/MS analysis of the leaf tissue of these plants showed simultaneously accumulation malonic acid and succinic acid, in proportion to exogenous malonic acid in the agar. These results suggested that malonic acid is a competitive inhibitor of succinic acid dehydrogenase and this may explain the toxicity of malonic acid to seedlings. Taking together, our *in vitro* and *in vivo* experiments indicate that *AAE13* is a malonyl-CoA ligase in *Arabidopsis*. This research is supported by NSF *Arabidopsis* 2010 project grant No. 0420199.

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### ICAR1003

STUDY OF ARABIDOPSIS MUTANTS WITH MODIFIED SPHINGOLIPID LONG CHAIN BASE CONTENT AND COMPOSITION

Category: Metabolism

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Sphingolipids have been intensively studied in mammals and yeast because of the important roles they play in basic cellular activities such as endocytosis, protein trafficking, and programmed cell death. The study of sphingolipids in plants, although still lagging behind the progress achieved in yeast and mammals, has made considerable recent advances. This has been facilitated by the availability or generation of T-DNA mutants and RNAi suppression lines for most of the genes involved in sphingolipid metabolism, coupled with recently developed mass spectrometry methods for the comprehensive analysis of plant sphingolipids. We have used these resources to study genes involved in synthesis and modification of sphingolipid long chain bases (LCBs). As part of these studies T-DNA mutants for both AtLCB1 and AtLCB2, the two subunits of *Arabidopsis* serine palmitoyltransferase (SPT), have been characterized, and RNAi lines for AtLCB1 and inducible RNAi lines for AtLCB2 have been generated. Results from the study of these lines conclusively show that sphingolipids are essential for both gametophytic and sporophytic cell viability in *Arabidopsis*. Mutants defective in C-4 hydroxylation of sphingolipid LCBs have also been generated. Detailed analyses of these mutants has shown that complete loss of C-4 LCB hydroxylation results in suppressed growth and increased sphingolipid content, resulting primarily from the accumulation of sphingolipids with C16 fatty acids rather than the more typical very long-chain fatty acids. These results reveal the critical role of LCB hydroxylation in mediating sphingolipid synthesis and plant growth. Such findings increase our understanding of plant sphingolipid function and metabolism that may be applicable to other eukaryotic organisms.

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### ICAR1004

MATERNAL LEU CATABOLISM IS REQUIRED FOR NORMAL SEED DEVELOPMENT

Category: Metabolism

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3-Methylcrotonyl-CoA carboxylase (MCCase) is a nuclear-encoded, mitochondrial-localized biotin-containing enzyme that is required for leucine (Leu) catabolism. In Arabidopsis, single copy genes code for the two MCCase subunits that constitute the holoenzyme. To dissect the physiological role of MCCase in plant metabolism and development, T-DNA and transposon tagged alleles were isolated at both loci, and characterized. Metabolite profiling studies of these mutants are consistent with MCCase participating in a non-redundant system for the catabolism of Leu in mitochondria. The biochemical block in mitochondrial Leu catabolism is associated with impaired reproductive growth phenotypes, e.g. aberrant flower and silique development, larger seeds, and decrease seed germination. Most significantly, the decreased seed germination phenotype is only expressed when homozygous mutant seeds develop on a parent plant that is itself homozygous, but not when these seeds develop on a parent plant that is heterozygous. These observations indicate that maternal mitochondrial Leu catabolism is required for the normal development of viable seeds. These characterizations establish an experimental system for investigating the role of catabolic processes (branched-chain amino acids catabolism) in tissues that are expected to be net anabolic in nature, that is seed development.

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### **ICAR1005**

#### DUAL PROMOTERS AND TRANSLATIONAL REGULATION OF APT1: THE COMPLEXITY OF A HOUSEKEEPING GENE

Category: Metabolism

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APT1 is the predominant enzyme for the salvage of adenine to AMP. Arabidopsis *APT1* ESTs and cDNAs can be divided into 2 classes, which are distinguished by their 5'UTR and first exon sequences. This gene structure became of interest with the identification of an *APT1* insertion mutant designated *oxf1*, which expresses only the shorter of the two *APT1* transcripts. Although having reduced APT activity relative to WT, *oxf1* is completely fertile unlike previously reported *apt1* mutants (e.g. *apt1-3*) that are male sterile. Sequence analysis of the male sterile *apt* genes indicates they contain splicing mutations that interfere with the expression of both transcripts. We suspect that expression of the two *APT1* transcripts is highly regulated in WT and that the *oxf1* mutation disrupts this regulation. Thus we are investigating the possibility that *APT1* transcripts arise from alternative promoters, and are subject to differential regulation.

Semi-quantitative RT-PCR data showed an equal presence of both *APT1* transcripts in leaves and inflorescences of WT. Conversely, immunoblots revealed that the smaller APT1 isoform is much more abundant than the larger APT1, in both tissues. Interestingly, *oxf1* APT abundance was greatly reduced although *APT1* transcript abundance was equivalent to that in WT.

Future research will investigate the translational inhibition occurring at this locus using artificial microRNA (amiRNA) lines and gene expression assays involving reporter genes. The amiRNA lines target the specific *APT1* transcript classes and will provide insight into physiological relevance to the corresponding products. Gene expression assays will locate promoter activity and the role of the 5'UTR as the target of translational regulation. We further propose that the regulation at this locus is metabolically responsive.

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### **ICAR1006**

#### THE ROLE OF TRYPTOPHAN SYNTHESIS IN TRYPTOPHAN SECONDARY METABOLISM

Category: Metabolism

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In Arabidopsis, tryptophan not only functions in protein synthesis but is also a precursor for indole-3-acetic acid and the two classes of defense compounds, indole glucosinolates (IGs) and camalexin. To better understand 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, in the *TSA1* and *TSB1* genes. *trp2-1* and *trp3-1* mutants grown in the restrictive high light condition (without a Trp supplement) have normal IG levels compared to wild-type Col. Thus the *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.

To test this hypothesis we constructed triple mutants between *trp3-1* and the *cyp79B2 cyp79B3* double mutant, which cannot make IGs. We have found that the triple mutant is no longer a Trp auxotroph. Thus, blocking IG production suppresses the high light Trp auxotrophy of *trp3-1*. To test further this model, we measured IG levels in the *atr1D trp2-1* or *atr1D trp3-1* double mutants. The *ATR1* Myb transcription factor positively regulates IG biosynthetic genes. In comparison to wild type, the gain-of-function *atr1D* mutation results in increased transcription of these genes and results in approximately ten-fold higher IG levels when grown under high light. Under these same conditions, *atr1D trp2-1* or *atr1D trp3-1* both synthesize IGs ~six-fold higher than Col and ~three-fold higher than *trp2-1* or *trp3-1* alone and display a more severe Trp auxotrophy. We currently are testing models to explain these findings.

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### **ICAR1007**

#### IDENTIFICATION OF A PROTEIN KINASE AND A RING-FINGER PROTEIN INVOLVED IN PLANT SUGAR RESPONSE

Category: Metabolism

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In plants, metabolism and development are tightly integrated. For example, the levels of soluble sugars, such as glucose and sucrose, regulate diverse metabolic and developmental pathways. To elucidate the role of soluble sugars in plant metabolism and development, our lab has identified and characterized several *sugar insensitive* (*sis*) mutants of Arabidopsis. These *sis* mutants are resistant to the inhibitory effects of high concentrations of sucrose and glucose on early seedling development. Recently the genes corresponding to two *sis* mutants, *sis3* and *sis8*, were identified using a map-based cloning approach. The *SIS3* gene encodes a protein that is predicted to be a member of the RING-finger family and contains transmembrane domains. The *SIS8* gene is predicted to encode a protein kinase. Current efforts are focused on characterizing *SIS3* and *SIS8*. *In vitro* E3 ligase assays have confirmed the polyubiquitination function of *SIS3*. The subcellular locations of both *SIS3* and *SIS8* have been determined via transient expression assays. Possible interacting partners of *SIS8* were identified through a yeast two-hybrid screen. *35S-SIS3* and *35S-SIS8* plant were generated and their seed germination and early seedling developmental responses to high sugar levels and to abscisic acid and gibberellins were analyzed. The expression of sugar-regulated genes in *sis3* and *sis8* knockdown and overexpressor lines has been studied by Q-PCR. Possible signaling mechanisms of *SIS3* and *SIS8* are discussed.

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**ICAR1008**ANALYSIS OF GABA-SHUNT METABOLITES IN *ARABIDOPSIS THALIANA*

Category: Metabolism

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Gamma-amino butyric acid (GABA), a four carbon non-protein amino acid, is the main inhibitory neurotransmitter in mammalia. It is found in all species and has a signaling function in organisms from mammalia to bacteria. In plants, GABA accumulates under various stress conditions, but a function remains unclear. Besides GABA, several substances with a discussed signaling function - gamma-hydroxy butyric acid (GHB) and succinic semialdehyde (SSA) - occur during GABA degradation.

Knock out plants in the GABA-shunt were isolated to figure out if there is an effect on growth, development or metabolite content. Mutants of both catabolic genes (*GABA transaminase* and *succinic semialdehyde dehydrogenase* (*ssadh*)) display phenotypic deviations to wild type: the former show a normal vegetative growth with reduced fertility, whereas the latter are severely affected in growth and development. The *ssadh* knock out plants can be rescued by interrupting the GABA-shunt upstream of the *ssadh* function, thereby preventing accumulation of a substance possibly being toxic or having a signaling function. To test whether the GABA-shunt metabolites may be signaling molecules in planta, wild type and mutant plants were grown on media supplemented with GABA, SSA or GHB, respectively. Each of the three substances has a specific influence on plant growth. External GABA had dose dependent effects, small amounts increase plant growth, higher concentrations decrease plant size. The *ssadh* phenotype could be mimicked by addition of SSA to the growth media, whereas feeding of GHB results in altered root architecture. These findings suggest a signaling function for GABA-shunt intermediates in plants. To figure out which substance is causing the *ssadh* phenotype, the metabolite content of plants grown on MS supplemented with either GHB or SSA was determined.

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**ICAR1009**IDENTIFICATION OF ARABIDOPSIS MUTANTS THAT SUPPRESS THE *CYP83B1* AUXIN OVERPRODUCTION PHENOTYPE

Category: Metabolism

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The plant growth regulator indole-3-acetic acid (IAA) is essential for plant growth and development as well as for a number of environmental responses. The biosynthetic pathways for IAA are not well defined, but it is clear that a great deal of redundancy exists. IAA can be synthesized from the amino acid tryptophan, (tryptophan dependent pathways), or from a precursor of tryptophan (tryptophan independent pathways). Other tryptophan derived plant secondary metabolites include the defense compounds camalexin and indole glucosinolates. The current understanding of IAA biosynthesis mainly comes from Arabidopsis mutants with altered IAA levels. One such mutant is *cyp83B1*, which affects a tryptophan dependent pathway of IAA biosynthesis. *CYP83B1* converts indole-3-acetaldoxime (IAOx) into its aci-nitro compound. IAOx is an intermediate in the pathways for both IAA and indole glucosinolates, as well as camalexin. In the *cyp83B1* mutant, the flow of IAOx is redirected into the IAA biosynthetic pathway, leading to IAA overproduction. The associated phenotypes include retarded growth, a short primary root, increased lateral roots and adventitious rooting from the hypocotyls. Our work focuses on identifying EMS-induced mutants that suppress the IAA overproduction phenotype of *cyp83B1*. To date, we have identified several homozygous suppressor lines that have wild type root phenotypes and restored shoot growth. The characterization of these mutants is described here.

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**ICAR10010**

## GENETIC, BIOCHEMICAL AND PHYSIOLOGICAL STUDIES ACETYL-COA METABOLISM VIA CONDENSATION

Category: Metabolism

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Acetyl-CoA is metabolized via one of three mechanisms, carboxylation, acetylation and condensation. Acetoacetyl-CoA thiolase (AACT) catalyzes the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. The fate of acetoacetyl-CoA depends on the biological context in which it is generated. In the cytosol of plant cells, it is the precursor of mevalonate-derived isoprenoids. In microbes, such as *Rhodospirillum rubrum*, acetoacetyl-CoA is the precursor of the storage polymer polyhydroxybutyrate (PHB). BLASTP analyses have identified two *AACT* genes in the *Arabidopsis* genome, At5g47720 (*AACT1*) and At5g48230 (*AACT2*). These two genes code for proteins that share 75% sequence identity. Two T-DNA insertion alleles at each *AACT* gene have been characterized. These characterizations indicate that although both genes are expressed (as evidenced by RT-PCR analysis), mutations in *AACT2* are embryo lethal whereas null alleles of *AACT1* are viable and show no apparent growth phenotypes. Furthermore, reciprocal crosses show that mutations in *AACT2* affected male transmission.

In *R. rubrum*, the AACT enzyme is encoded within the *phaABC* operon, which is responsible for PHB biosynthesis. Furthermore, *R. rubrum* contains two additional AACT-like genes, called *phaC2* and *phaC3*. To characterize the roles of these genes in acetyl-CoA metabolism, we have generated antibodies to each gene product. In addition, we have developed an inducible-expression system for individually over-expressing each *pha* gene. The results indicated that *phaB* is the key enzyme in synthesizing PHA and over-expressing each PHA biosynthetic gene can increase PHA production. In addition, the data suggested that *phaB* is subject to post transcriptional regulation. In combination, these studies will elucidate the role of AACT in the acetyl-CoA metabolic network.

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**ICAR10011**MULTI-PATHWAY TO GENERATE CASTASTERONE IN *ARABIDOPSIS THALIANA*

Category: Metabolism

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It has been demonstrated that castasterone is biosynthesized from campesterol which carry the same carbon skeleton as that of castasterone. To examine whether castasterone can be biosynthesized by alternative pathways, *in vitro* conversions of 28-norcastasterone, 28-homocastasterone and 6-deoxodolichosterone were tested by the use of a crude enzyme solution prepared from *Arabidopsis thaliana*. When 28-norcastasterone was used as substrate in the presence of *S*-adenosyl-methionine, the enzyme product was identified to be castasterone by GC-MS analysis, indicating that castasterone can be biosynthesized by C-24 methylation of 28-norcastasterone. When 28-homocastasterone was added, GC-MS analysis revealed that the product is castasterone, which provides that castasterone can be generated by C-28 demethylation of 28-homocastasterone. When 6-deoxodolichosterone was used, two products, dolichosterone and castasterone, was identified, indicating that 6-deoxocastasterone was converted into castasterone via dolichosterone by 6-oxidation followed by reduction of C-24 exomethylene. In respect that 28-norcastasterone, dolichosterone and 28-homocastasterone is the end product of biosynthetic pathway for C<sub>27</sub>-BRs, 24-exomethylene C<sub>28</sub>-BRs and C<sub>29</sub>-BRs, respectively, biosynthetic pathways to generate 28-norcastasterone, dolichosterone and 29-homodolichosterone from cholesterol, 24-methylene-cholesterol and sitosterol, respectively, are thought to be alternative pathways to produce the active BR in *Arabidopsis*, castasterone. In conclusion, diverse biosynthetic pathways to produce C<sub>27</sub>-, C<sub>28</sub>- and C<sub>29</sub>-BRs are likely to collect to castasterone for maintaining homeostatic level of an active BR in *Arabidopsis*.

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**ICAR10012**

## NUCLEAR TARGETING OF METHYL RECYCLING ENZYMES IS MEDIATED BY A SPECIFIC PROTEIN-PROTEIN INTERACTION

Category: Metabolism

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Both *S*-adenosyl-L-homocysteine hydrolase (SAHH; EC 3.3.1.1) and adenosine kinase (ADK; EC 2.7.1.20), are essential to sustain the hundreds of the methyltransferase (MT) activities required for plant growth. Both enzymes have generally been regarded as cytosolic enzymes despite the fact that SAM-dependent methylation reactions occur in all cellular compartments to maintain the MT activities throughout the cell. There are, however, no other reports of SAHH movement and no information on ADK being targeted to other compartments. To investigate this, green fluorescent protein (GFP) fusion proteins of ADK or SAHH were expressed in *Arabidopsis* and tobacco plants. To verify if the multiple location of both enzymes is mediated by the interactions or trafficking of other proteins, several protein-protein interaction assays were performed including bimolecular fluorescence complementation (BiFC), yeast 2-hybrid, and pull-down assays.

GFP fusions of ADK and SAHH were localized to the cytosol as previously reported by other research groups; in addition both fusion proteins were present in the nucleus, even though the primary amino acid sequence of neither protein contains a detectable nuclear localization signal (NLS). Deletion analysis of SAHH revealed that an internal stretch of 40 amino acids which is present only in plant and photosynthetic bacteria SAHH sequences is essential for nuclear targeting of SAHH. In addition, computational modeling with ZDOCK predicted the best interaction between ADK and SAHH via region of SAHH. The protein interaction assays suggest that both molecules assemble into protein complexes in the cytosol and nucleus, which means that they might function in transmethylation as an ADK-SAHH complex to maintain functional stoichiometry of the two enzymes with changes in SAH production. In addition, we propose that the complex might be essential for the nucleo-cytoplasmic trafficking of ADK and SAHH via methyltransferases (e.g. Guanine-7-Methyltransferase; At3g20650). This is the first report to evaluate the interaction between ADK and SAHH in plants.

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**ICAR10013**

## THE PROANTHOCYANIDIN-RELATED ARABIDOPSIS TT19 PROTEIN IS A PHENOLIC ACID-GST-BINDING PROTEIN THAT AFFECTS ANTHOCYANIN ACCUMULATION THROUGH A 3' AMINO ACID SUBSTITUTION

Category: Metabolism

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A novel *transparent testa* (*tt*) phenotype showing reduced seed coat color has been recovered from a new activation-tagged population of *Arabidopsis*. Positional cloning and sequencing showed that *tt19-4* was a spontaneous allele to *tt19-7* and contained a G-to-T mutation in a conserved 3'-domain of the *TT19* glutathione-S-transferase gene. This missense mutation converts Trp (amino acid 205) into Leu. Transformation of *tt19-7* with a *TT19-4* cDNA partially complements *tt19-1* by producing wild-type levels of vegetative anthocyanin. *In vitro* enzyme assays indicated that the recombinant wild-type TT19 enzyme can conjugate glutathione to an artificial substrate but not to flavonoids. However, TT19 could bind phenolic

acids, including sinapic acid or gallic acid. This result led us to hypothesize that TT19 recognizes coumaric acid derivatives connected to anthocyanin or gallic acid derivatives connected to epicatechin and potentially transports them into the vacuoles. A 26K *Arabidopsis* cDNA microarray indicated that transcript levels for 326 genes were up-regulated two-fold and 41 genes were down-regulated more than two-fold in *tt19-4* over *tt19-1*. Phytochemical analysis showed similar seed flavonoid composition, but distinct differences in seed proanthocyanin content and seedling anthocyanin content, between wild type, *tt19-1* and *tt19-4* lines. The higher content of unextractable PAs in both mutants than in Columbia suggests the relaxation of a negative control on PA synthesis by the wild-type TT19 protein and confirms a relationship with seed coat color. The data suggests the possibility of two distinct mechanisms for extractable and unextractable PA biosynthesis.

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**ICAR10014**

## IDENTIFICATION AND CHARACTERIZATION OF A TWO-MEMBER GENE FAMILY ENCODING A NOVEL PURINE TRANSPORTER IN ARABIDOPSIS

Category: Metabolism

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In addition to serving as building blocks for DNA and RNA, nucleobases and their metabolites act as enzyme co-factors, contribute to carbohydrate biosynthesis, and serve as energy molecules. Constant synthesis and recycling involves both intra- and inter-cellular shuttling of nucleobases. This necessitates a highly regulated and selective nucleobase membrane transport mechanism. The Arabidopsis genome contains 43 loci among six different families encoding for amino acids sequence homology to fungal and bacterial transporters. One family, consisting of loci At3g10960 and At5g50300, was identified by amino sequence homology to the AzaA adenine-guanine-hypoxanthine transporter of *Aspergillus nidulans*. Our goal is to identify the substrate/solute specificity of these two Arabidopsis putative transporters. A reverse genetics approach was undertaken in which knock-out T-DNA insertion mutants were isolated from Arabidopsis populations in At3g10960 and At5g50300. Segregating populations from T-DNA insertion lines of At3g10960 and At5g50300 were screened by germinating in a medium containing a wild-type lethal concentration of 8-azaguanine (Azg), a toxic analog of guanine. Homozygous insertion mutants, confirmed by PCR analysis, displayed enhanced resistance to Azg. Double homozygous insertion mutants were generated. Growth assays of the single and double homozygous knock-out/insertion mutant lines using a panel of toxic nucleobase and nucleoside analogs revealed that both genes are involved in guanine and adenine transport. The wild type coding regions of At3g10960 and At5g50300 were cloned into yeast expression vectors then transformed into and expressed in yeast deficient in adenine-cytosine-guanine transporter *FCY2* and uracil transporter *FUR4*. Heterologous complementation in yeast confirmed the substrate specificity of At3g10960 and At5g50300 *ex vivo*. Finally, uptake of [<sup>3</sup>H]guanine alone and in competition with other cold nucleobases and nucleosides by the single and double homozygous insertion mutant seedlings provided an *in planta* confirmation of the substrate/solute specificity of this new Arabidopsis family of membrane transporters.

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**ICAR10015**

## CELLULAR LOCALIZATION OF INOSITOL MONOPHOSPHATASE PROTEINS IN ARABIDOPSIS

Category: Metabolism

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*Myo*-inositol synthesis and catabolism are crucial in many multicellular eukaryotes for production of phosphatidylinositol and inositol phosphate signaling molecules. *Myo*-inositol monophosphatase (IMP) is a major enzyme required for the synthesis of *myo*-inositol and breakdown of inositol (1,4,5)-trisphosphate, a potent second messenger involved in many biological activities. Arabidopsis contains a single canonical IMP gene, which we have shown that encodes an active IMPase and L-Gal 1-phosphatase enzyme. To determine whether the IMP gene impacts *myo*-inositol synthesis in Arabidopsis, we isolated T-DNA knockout lines of IMP that exhibit small perturbations in ABA, salt and cold responses. Analysis of metabolite levels in *Imp* mutants showed that less *myo*-inositol and ascorbate accumulate in these mutants. Two other genes in Arabidopsis encode proteins which we have classified as IMP-like (IMPL), because of their greater homology to the prokaryotic IMPs such as the SuhB, and CysQ proteins. Prokaryotic IMPL enzymes are known to dephosphorylate D-Ins 1-P and other substrates *in vitro*, however their *in vivo* substrates are not characterized. We have examined the plant IMPL enzymes, and have found that AtIMPL2 is bifunctional, whereas the AtIMPL1 enzyme only hydrolyzes inositol phosphate substrates. To determine if IMP and IMPL proteins function in the same subcellular compartment, IMP and IMPL green fluorescent protein gene fusions have been constructed and transformed into plants. Our results indicate that IMP is a cytoplasmic enzyme, whereas the IMPL enzymes are likely targeted to a specific organelar location within the plant cell. Together, these data indicate that inositol phosphate hydrolysis by the IMP and IMPL enzymes takes place in different subcellular locations, which may be critical in maintaining inositol homeostasis in the plant cell.

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**ICAR10016**

## ENGINEERING ARABIDOPSIS AND TOBACCO FOR THE PRODUCTION OF PTEROSTILBENE, A HIGH-POTENCY RESVERATROL ANALOGUE

Category: Metabolism

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Stilbenoid phytoalexins, such as resveratrol, play an important defense role in several plant species against pathogens such as *Botrytis cinerea*. In addition to their antimicrobial properties, resveratrol and related stilbenes have also generated considerable interest as nutraceuticals, due to their potential role in promoting cardiovascular health, inhibiting tumor formation, and increasing cell longevity. Several studies have demonstrated that pterostilbene, a methyether derivative of resveratrol, possesses similar potent antioxidant and anticancer properties, and in addition could provide host plants with more broad-spectrum resistance to fungal pathogens. A dual-transgene cassette approach was therefore employed using a resveratrol synthase sequence from peanut in conjunction with an O-methyltransferase from *Sorghum bicolor* capable of catalyzing the conversion of resveratrol

to pterostilbene, both expressed behind the CaMV 35S promoter in transgenic *Arabidopsis* as well as tobacco. Significant levels of pterostilbene accumulated in transformants of both species, demonstrating the potential utility of this strategy for the development of novel crop germplasm with enhanced nutraceutical value and disease resistance.

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**ICAR10017****CROSSTALK BETWEEN THE BIOSYNTHETIC PATHWAYS FOR INDOLE GLUCOSINOLATES AND IAA**

Category: Metabolism

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In *Arabidopsis*, tryptophan (Trp) is a precursor for the auxin, IAA, and for two defense compounds: the anti-microbial compound camalexin and the anti-herbivory class compounds called indole glucosinolates (IGs). Two *Arabidopsis* cytochrome P450s, CYP79B2 and CYP79B3, convert Trp to indole-3-acetaldoxime (IAOx). The cloning of and subsequent genetic analysis of the CYP79B2 and CYP79B3 genes has pointed to IAOx being a key metabolite in the production of IAA, IGs and camalexin. CYP79B2-overexpressing plants (CYP79B2-OEX) have elevated levels of free IAA and IGs while the cyp79B2 cyp79B3 double mutant has decreased free IAA and make no IGs or camalexin.

As revealed by the superroot mutants, a block in the IG branch of the pathway leads to an accumulation of IAA manifested by a high-auxin phenotype. This high-auxin phenotype is suppressed in the cyp79B2 cyp79B3 sur1 and cyp79B2 cyp79B3 sur2 triple mutants suggesting that in superroot mutants production of IAA from IAOx is dysregulated. To understand more about how these pathways are regulated, we are creating conditional cyp79B2 and sur1 strains that will allow inducible control of IAOx or IG production. We are currently characterizing the effects of manipulating these pathways at the chemical and morphological levels.

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**ICAR10018****THE OVER-EXPRESSION OF GDU-LIKE GENES LEADS TO MODIFICATION IN AMINO ACID CONTENT AND TRANSPORT**

Category: Metabolism

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GDU1 is a small membrane protein of unknown function that bears no similarities with known proteins. The over-expression of *GDU1* in plants induces a remarkable phenotype first identified in the *gdu1-1D* mutant: the plants over-accumulate free amino acids and secrete pure glutamine at the hydathodes. GDU1 belongs to a novel family of proteins found only in higher plants and mosses. All these proteins present a single membrane domain and a highly conserved 25 amino acid motif present in the cytosolic C-terminus. Apart from GDU1, *Arabidopsis* genome encodes six GDU1-similar proteins, none of which has been characterized so far.

Clues about the localization of the expression of these six *GDU* genes were obtained by RT-PCR and promoter-GUS fusions. These genes all appeared to be expressed at different levels in the various organs of the plant, but all of them were present in the vascular tissues. Major overlaps of the expression patterns were also observed. GFP fusions showed that these proteins are all present at the membrane. Plants expressing the coding sequences of the *GDU* genes under the control of the 35S promoter displayed phenotypes reminiscent of the one of *gdu1-1D*: free amino acid levels were increased and several plants secreted glutamine. Analysis of the expression of all members of the family in the various transgenic lines revealed also a complex cross-regulation of *GDU* genes. It was noticed that all the over-expressors were tolerant to external concentrations of amino acids known to be lethal to wild type plants. This can in part be explained by the observation that all over-expressors take up less amino acids than the wild type. These data suggest that the GDU proteins are involved in the same process as GDU1, the identity of which is not clear at present. It can either link metabolism to membrane, for instance involving amino acid sensing, or directly affect and regulate amino acid transport activity.

## References:

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Pratelli, R., and Pilot, G. (2006). FEBS lett. 580, 6961-6966.

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**ICAR10019****FUNCTIONAL ANALYSIS OF A PECTATE LYASE-LIKE GENE (PMR6) IN ARABIDOPSIS THALIANA**

Category: Metabolism

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Functional Analysis of a pectate lyase-like gene (PMR6) in *Arabidopsis thaliana*  
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During a screen for powdery mildew resistant *Arabidopsis* mutants, the powdery mildew resistant mutant, pmr6, was identified; the wild type is susceptible to this fungal pathogen. PMR6 encodes a pectate lyase-like protein, with a GPI anchor in its C-terminal, which does not exist in the other 26 pectate lyase-like proteins in *Arabidopsis*. Our research aims at three main goals: (1) to identify the specific changes in the cell walls of the mutant plants; (2) to measure the biochemical activities of the gene product; (3) to develop an understanding of why this mutant is powdery mildew resistant. PMR6 binds to an unidentified component of a pectic extract from *Arabidopsis* (designated as PMR6 ligand), but does not bind to homogalacturonan, xylogalacturonan, xyloglucan, rhamnogalacturonan I, rhamnogalacturonan II, or arabinoxylan. Filtration studies suggest PMR6 binds to a negatively charged high MW component of pectin.

Transgenic plants expressing PMR6:GUS indicate that PMR6 is most highly expressed in rapidly expanding tissues, such as hypocotyls and expansion zone of roots. A translational fusion to GFP indicates the protein is mainly expressed in inner cell walls and plasma membranes.

In order to further study the functions of PMR6 protein, mutations in residues predicted to be important for pectate lyase function were made. These mutated plasmids were transformed into pmr6-1 mutant plants to assay for phenotypic rescue. All the transgenic plants have similar lengths of hypocotyls as pmr6-1, which is about 70% of that of wild type plants. In disease resistance tests of plants to powdery mildew fungi, only D217L mutation, restored the transgenic plants to susceptibility to pathogen; all other mutations, including mutations in the critical residues of pectate lyase active domain, remained resistant to powdery mildew. This suggests that active site residues, but not the D217 amino acid implicated in  $\text{Ca}^{2+}$  binding, are required for both normal stature and disease susceptibility in wild-type plants.

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#### ICAR10020

##### ETHYLENE SIGNALLING IS REQUIRED FOR THE ACCELERATION OF CELL DEATH INDUCED BY ACTIVATION OF ATMEK5 IN ARABIDOPSIS

Category: Metabolism

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Mitogen-activated protein kinases (MAPKs) are involved in regulation of plant growth, development, and responses to a wide variety of stimuli. In a conditional gain-of-function transgenic system, constitutively activation of AtMEK5, a MAPK kinase in *Arabidopsis*, was proved to activate endogenous AtMAPK3 and AtMAPK6, which led to a dramatic increase in ethylene production and induced a HR-like cell death. However, the role of the accelerated ethylene production in regulating this HR-like cell death is still unknown. Using the *Arabidopsis* transgenic plants which express AtMEK5<sup>DD</sup>, an active mutant of AtMEK5 under the control of steroid-inducible promoter, we tested the contribution of ethylene to the cell death. We found that the ethylene biosynthesis is an earlier event comparing with the cell death. Inhibiting AtMEK5-induced ethylene production by using inhibitors of ACC-synthases, or ACC-oxidases, or ethylene receptors delayed the cell death. In mutants *AtMEK5*<sup>DD</sup>/*etr1-1* and *AtMEK5*<sup>DD</sup>/*ein2-1*, which shown ethylene insensitivity, the AtMEK5<sup>DD</sup> protein expression, activity of AtMAPK3 and AtMAPK6, and ethylene production shown normally induction as in *AtMEK5*<sup>DD</sup> transgenic plant, but the cell death was also delayed. These data suggest that ethylene perception is required to accelerate the cell death induced by AtMEK5 activation.

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#### ICAR10021

##### IDENTIFICATION AND CHARACTERIZATION OF ARABIDOPSIS ECERIFERUM8 (CER8), A GENE IMPORTANT FOR CUTICULAR WAX BIOSYNTHESIS

Category: Metabolism

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As one of the major components of the plant cuticle, waxes play essential roles in many ecophysiological functions, such as resistance to drought and pathogens. *Arabidopsis thaliana* mutants with abnormal wax load or composition are named *eceriferum* (*cer*), which can typically be identified by the naked eye as being glossy. So far, more than 30 *cer* loci have been identified, but the exact roles of most of these *CER* genes still remain unknown.

By using a combination of forward and reverse genetic approaches, we have identified *CER8* gene as *Long-Chain Acyl-CoA Synthetase 1* (*LACS1*, *At2g47240*). The allelism of *cer8* and *lacs1* was confirmed by complementation crosses and reverse-transcription PCR. Scanning electron microscopy (SEM) of *cer8* mutants showed that these mutants have more wax "plates" and "small trunks", instead of the predominant tube-shape wax crystals found on wild-type plants. Gas chromatography analysis of *cer8* mutant lines and corresponding wild-type plants showed that the main wax components including C29 alkane, C29 ketone and C29 secondary alcohol as well as the total wax load of *cer8* mutants were decreased to approximately half that of the wild-type levels, while the C30 free fatty acids increased by 17-19 fold. This is consistent with a defect in very-long-chain acyl CoA synthetase activity. On the molecular level, the promoter region (~4,000bp upstream from the ATG starting site) of *CER8/LACS1* was fused with the GUS reporter gene, which was subsequently transformed into Col-WT to characterize the gene expression profile of *CER8*. Moreover, *CER8* cDNA was cloned into plant binary vectors, under the 35S promoter and the strong epidermal-specific *CER6* promoter respectively, and the effects of over-expression will be presented. Based on these results and previously published studies on plant lipid metabolism, a functional model of the role of *CER8/LACS1* in wax biosynthesis is proposed.

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#### ICAR10022

##### STUDY OF THE GENE EXPRESSION PATTERNS AND SUBSTRATE SPECIFICITIES OF ALCOHOL-FORMING FATTY ACYL-COA REDUCTASES (FARS) FROM ARABIDOPSIS THALIANA

Category: Metabolism

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Fatty Acyl-CoA reductases (FARs) catalyze the formation of primary fatty alcohols from fatty acyl-CoAs. An eight-member family of alcohol-forming FARs has been identified in *Arabidopsis thaliana*. One of these genes, *CER4* (*At4g33790*), is responsible for the production of very-long-chain fatty alcohols present in the waxy cuticle of the aerial plant surface (1). *CER4* is also found to express in all of the cell layers of the elongation zone of young roots. Two other genes of the Arabidopsis FAR family, *FAR4* (*At3g44540*) and *FAR5* (*At3g44550*), were found to be highly expressed in root tissues by inspection of DNA microarray data. Using promoter-reporter gene fusions, we found that these two genes are specifically expressed in the cell layer surrounding the central vasculature of the root. Suberin and associated waxes (e.g. fatty alcohols) are deposited in the wound periderm, casparyan strips and the endodermal cell walls of the primary root, which forms a protective hydrophobic barrier that limits water and solute loss from the central stele of roots. Thus, these enzymes are likely responsible for the production of fatty alcohols associated with suberin in roots. Analysis of DNA microarray data indicated that these two genes are expressed in the seed and it is likely that they are expressed in the seed coat as this layer is the site of suberin deposition. These genes were also found to be expressed in response to mechanical wounding in the seed coat and aerial tissues of the plant. The substrate specificities of the FAR enzymes were investigated by measuring the accumulation of fatty alcohols in yeast heterologously expressing Arabidopsis FAR enzymes, wherein, FAR4 resulted in accumulation of C20 fatty alcohols and FAR5 resulted in accumulation of C16, C18 and C20 fatty alcohols with preference to C18 alcohols. We are studying the chemical phenotypes in the roots and seed coat of T-DNA knockout mutant lines of each of these genes, which may reveal important roles of fatty alcohols in these specific cell types. We expect that this study will provide valuable information for producing drought-tolerant plants and for using the plant seed coat as a platform for the synthesis of high-value bio-products.

(1) Rowland, O., Zheng, H., Hepworth, S.R., Lam, P., Jetter, R. and Kunst, L. (2006). *CER4 Encodes an Alcohol-Forming Fatty Acyl-CoA Reductase Involved in Cuticular Wax Production in Arabidopsis*. *Plant Physiol.* 142, 866-877.

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### **ICAR10023**

ABIOTIC STRESS-INDUCIBLE *ATCPT5* ENCODES A NOVEL *CIS*-PRENYLTRANSFERASE CATALYZING THE FORMATION OF *Z,E*-HEPTAPRENYL DIPHOSPHATE

Category: Metabolism

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In contrast to other biological species, higher plants biosynthesize various *Z,E*-mixed polyisoprenoids, including dolichol and polyprenol, showing a broad distribution of the carbon chain length from  $C_{30}$  to more than  $C_{130}$ . While dolichol is well characterized as a glycosyl carrier lipid for the biosynthesis of glycoproteins in eukaryotes, a physiological significance of polyprenols in higher plants is hardly understood. In addition, neither the chain length-physiological function relationship nor corresponding enzymes for each of *Z,E*-mixed polyisoprenoids have been elucidated. The structural backbone of *Z,E*-mixed polyisoprenoid is formed by *cis*-prenyltransferase (CPT), catalyzing the sequential *cis* (*Z*)-condensation of isopentenyl diphosphate with *all-E*-allylic diphosphate. In this meeting, we report identification and characterization of AtCPT5, a *cis*-prenyltransferase in *Arabidopsis thaliana*, shown to be induced in response to various abiotic stimuli.

Histochemical analyses of the transgenic Arabidopsis plants carrying the AtCPT5 promoter:: *GUS* revealed that *AtCPT5* expressed predominantly in root. Furthermore, induction of *AtCPT5* by abiotic stresses, such as high salinity, low temperature and ABA treatment, was observed only in roots. Transgenic Arabidopsis overexpressing *AtCPT5* showed decreased elongation of root in the standard growth condition. Root elongation assay revealed that *AtCPT5*-overexpressing seedlings were more sensitive to cold stress than wild type. The CPT assay with crude protein from the AtCPT5-overexpressing *Escherichia coli*, *Saccharomyces cerevisiae* and T87 Arabidopsis cultured cells showed the increased formation of  $C_{30-35}$  polyprenyl products. The partially purified recombinant AtCPT5 expressed in *E. coli* indicated certain CPT activity catalyzing  $C_{35}$  polyisoprenoid as a major product. Interestingly, ultimate product chain-length was not affected by the chain-length of *all-E*-allylic diphosphate, ranging from  $C_{10}$  to  $C_{20}$ , indicating a strict product chain-length recognition mechanism of AtCPT5. These results indicate that AtCPT5 is a novel *cis*-prenyltransferase catalyzing the formation of *Z,E*-heptaprenyl diphosphate, which is considered to function in abiotic stress response in root.

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### **ICAR10024**

5'-METHYLTIOADENOSINE NUCLEOSIDASE-DEFICIENT PLANTS EXHIBIT DEVELOPMENTAL ABNORMALITIES

Category: Metabolism

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Plants generate 5'-methylthioadenosine (MTA) as a by-product of both ethylene and polyamine biosynthesis, and re-cycle it via the Yang cycle to methionine. In this cycle 5'-methylthioadenosine nucleosidase (MTN) irreversibly hydrolyses MTA to adenine and methylthioribose. The importance of MTA metabolism to plant development remains to be elucidated.

The *Arabidopsis thaliana* genome has two genes annotated as encoding MTN activity, the coding regions of which share 73% nucleotide identity and 60% predicted amino acid identity. Both recombinant MTNs hydrolyze MTA with similar kinetics, although MTN2 shows modest affinity for S-adenosylhomocysteine as well. Single knockout lines of *mtn1* and *mtn2* are phenotypically indistinguishable from the wild type when grown on Murashige-Skoog solid media, or in soil at normal growth conditions. When solid media is supplemented with 500  $\mu$ M MTA there is a distinct reduction

in the root length growth of *mtn1* seedlings; wild type and *mtn2* seedling roots are unaffected. Consistent with this phenotype enzyme assays indicate that MTN1 provides approximately 80% of the total MTN activity detected in 4-day-old seedlings or rosette leaves of 3-week-old plants.

*mtn1-1 mtn2-1* double mutants have both developmental and morphological defects. These mutants take 5.5 weeks to bolt as compared to 3.5 weeks for the wild type. Double mutants are also sterile as a result of defects in both male and female gametes. Pleiotropic phenotypes including fasciation and thick veins are visible in all organs studied (stems, leaves, inflorescence and siliques). Future research will be focused on determining whether this phenotype is due to one or multiple interrupted pathways.

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**ICAR10025****METABOLOMIC AND GENETIC ANALYSES OF FLAVONOL SYNTHESIS IN *ARABIDOPSIS THALIANA***

Category: Metabolism

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Flavonol synthase, the enzyme that catalyses the conversion of flavonols into dihydroflavonols, is part of the flavonoid biosynthesis pathway. In *Arabidopsis thaliana*, this activity is thought to be encoded by several loci. In addition to the *FLAVONOL SYNTHASE1* (*FLS1*) locus that has been confirmed by enzyme activity assays, five other loci, designated *FLAVONOL SYNTHASE-LIKE1-5* (*FSL1-5*), display similarity of the deduced amino acid sequences to the *FLS1* protein. We studied the putative *A. thaliana* *FLS* gene family using a combination of genetic and metabolite analyses approaches.

An *A. thaliana* *fls1* null mutant (*fls1-2*) was identified and characterized. *fls1-2* seedlings show a drastic reduction in flavonol glycoside content and concomitant accumulation of glycosylated forms of dihydroflavonols, the substrate of the FLS reaction. The level of anthocyanin accumulation in the *fls1-2* mutant is greatly higher than in the wildtype, indicating that precursors usually channelled to flavonols are used to build anthocyanins.

Additionally, with the help of a *LEUCOANTHOCYANIDIN DIOXYGENASE* (*LDOX*) *fls1-2* double mutant, we present evidence that the remaining flavonol glycosides found in the *fls1-2* mutant are synthesized in planta by the FLS-like side activity of the *LDOX* enzyme.

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**ICAR10026****ACYL-COA SYNTHETASES INVOLVED IN CUTIN BIOSYNTHESIS IN *ARABIDOPSIS***

Category: Metabolism

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Cutin is the structural polymer of the cuticle, the interface between epidermis and the environment, which covers aerial parts of terrestrial plants and prevents excessive non-stomatal water loss. The major cutin monomers are derivatives of long chain fatty acids. In *Arabidopsis*, the most highly abundant ones are  $\alpha$ ,  $\omega$ -dicarboxylic acids and  $\omega$ -hydroxy fatty acids. *LACS2*, a member of the Long-chain Acyl-CoA Synthetase gene family is essential for normal cuticle development in *Arabidopsis*. A *lacs2* null mutant has a defect in cuticular barrier as indicated by a thinner leaf cuticular membrane than that of wild type plant. And *lacs2* leaves can support pollens germination, while wild type leaves cannot. Here, we show *LACS1* (At2g47240) is also involved in cuticle development. *LACS1* and *LACS2* have 59% similarity at protein level. Transcriptome analysis shows that *LACS1* is highly expressed in epidermal cells where the cutin monomers and cuticular wax are synthesized. Unlike *lacs2*, a *lacs1* null mutant does not have any visible phenotype under normal growth conditions. The *lacs1/lacs2* double mutant has more severe cuticle defect. Organ fusion, a phenotype found in several cuticle-defect mutants, is observed from both the flower buds and rosette leaves of the double mutant. The permeability of leaves of the double mutant to toluidine blue is greater than each of the single mutant. The amount of several cutin monomers of *lacs1/lacs2* is significantly lower than that of *lacs2*. We conclude that *LACS1* and *LACS2* have overlapping functions in the biosynthesis of the major cutin monomers.

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**ICAR10027****FUNCTIONAL CHARACTERIZATION OF ATAZG2, A HIGH AFFINITY PURINE TRANSPORTER IN *ARABIDOPSIS THALIANA***

Category: Metabolism

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Nucleobases and derivatives play essential roles in all living organisms. Since the site of their synthesis frequently differs from the site of use, transport is required in multicellular organisms. Several gene families encoding for putative nucleobase transporters have been identified in plants. In this work, a functional characterization of *AtAzg2* (*A. thaliana* aza-guanine resistant 2) is presented. This gene belongs to a novel family of purine transporters recently identified in *Aspergillus nidulans* (*azgA*: aza-guanine resistant Aspergillus).

*AtAzg2* was functionally expressed in a yeast mutant deficient in adenine uptake (*fcy2*) to study its transport features. It was able to transport purines, including adenine, hypoxanthine and guanine with high affinity, but not their nucleosides. Interestingly, *AtAzg2* transported purines within a broad pH range (pH4 to pH8) with similar velocities. *In planta* studies were conducted on T-DNA insertion mutants and overexpressor lines. Compared with the controls, *AtAzg2* overexpressor lines showed higher adenine uptake rates, indicating that this protein transports purines *in vivo*. Expression results, using GUS as a reporter for promoter activity, indicated the expression of *AtAzg2* at a very low level in different organs.

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**ICAR10028**

PREPARATION AND SPECIFICITY OF ANTIBODY AGAINST AtPLC4, A PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C IN ARABIDOPSIS

Category: Metabolism

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Phosphoinositide-specific phospholipase Cs (PI-PLCs) are important enzymes in eukaryotes which catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into the two second messengers inositol 1,4,5-trisphosphate and diacylglycerol. Arabidopsis genome contains 9 PI-PLC genes. The overall structure of the predicted Arabidopsis AtPLCs protein is similar to those of plant PI-PLCs and animal PI-PLC zeta. *AtPLC4*, an abiotic stress induced gene, has been reported to encode an active PI-PLC isoform. However, the exact roles of *AtPLC4* in plant remain to be elicited. N-terminal of AtPLC4, referred to as AtPLC4N which includes the first 108 amino acid residues of the, was expressed as a recombinant protein in E.coli. After purification, AtPLC4N was used as antigen to generate antibody. Purified recombinant proteins including AtPLC1 to AtPLC5, AtPLC8, AtPLC9 and AtPLC4N were transferred onto the same blot to test specificity of the prepared antibody. Western blot result shows that only AtPLC4 and AtPLC4N can be recognized by the antibody. Flag-tagged AtPLCs were also expressed in tobacco leaves transient expression system and IP with anti-Flag M2 antibody. Western blot result shown that AtPLC4 antibody only recognized Flag-AtPLC4 protein. In addition, the AtPLC4 antibody recognized a protein of approximately 68kD in the plasma membrane fraction and cytosolic fractions prepared from Arabidopsis plants. These results demonstrate that we have generated an AtPLC4 specific antibody.

This work was supported by National Natural Science Foundation of China (30771124 to H Yang and 30770203 to D Ren).

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**ICAR10029**

MULTIPLE ROLES OF CATIONIC AMINO ACID TRANSPORTER IN ARABIDOPSIS THALIANA : DIFFERENT LOCALIZATION AND FUNCTION

Category: Metabolism

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Amino acids (AAs) are the basic blocks of proteins, but also serve other versatile roles in plants, such as signaling roles for growth and development. Amino acids are translocated from source tissue to sink organs, but also can serve as a rich nitrogen source for seedlings. On the cellular scale, AAs are transported between different intercellular compartments. The amount of this intracellular amino acid shuttling is presently unclear, but it must be of critical importance during storage protein degradation and subsequent release of amino acids to the primary metabolism. Furthermore, amino acids are transiently stored in the vacuole during diurnal cycle and several amino acid transport activities have been classically identified on the tonoplast membrane. However, the molecular identity of internal amino acid transporters for amino acid storage and remobilization is much less clear. The Arabidopsis genome encodes more than 50 putative amino acid transporters. Most of these belong to the ATP super family (AAPs, LHTs, etc.). A second group of structurally unrelated transporters has 14 members, of which 9 are called CATs (for cationic amino acid transporters). We have identified several members of this transporter class on various cellular membranes and investigate their contribution to subcellular amino acid storage and compartmentation using knock-out lines and over-expressors. While AtCAT1 is a plasma membrane transporter that imports cationic amino acids such as lysine and arginine, AtCAT2 and AtCAT4 are localized on the tonoplast and AtCAT3 is localized to the ER membrane. As CAT1 expression level (knock out vs. over expressor) determines the sensitivity of seedlings to micromole concentrations of lysine, AtCAT2 knock-outs and over-expressors are growing fine under a number of conditions. AtCAT2 is strongly expressed in seedlings, predominantly in roots and the results suggest that other transporters can compensate the loss of AtCAT2. Molecular sequence motifs were identified in these highly similar transporters that participate in the localization to the various compartments.

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**ICAR10030**

TRANSCRIPTOME COEXPRESSION ANALYSIS LEADING TO DECODING GENE-METABOLITE CORRELATIONS IN ARABIDOPSIS FLAVONOID

METABOLISM

Category: Metabolism

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The flavonoids comprise one of the major secondary metabolite groups, with over 7,000 known compounds. Formation of the basic flavonoid skeleton has been well studied in terms of molecular biology and natural product chemistry. However, the pathways for sequential modification, such as glycosylation, acylation and methylation, are still relatively unexplored, even though modification produces a huge chemical diversity and is essential for the stable accumulation. Candidate genes presumably involved in the flavonoid pathway were delimited by transcriptome coexpression network analysis using public databases, leading to the detailed analysis of two flavonoid pathway genes, *UGT5* and *RHM1*. The levels of flavonol 3-*O*-pentosides were reduced in *ugt5* knockdown mutants, suggesting that UGT5 is a flavonol pentosyltransferase. Recombinant UGT5 protein could convert quercetin to quercetin 3-*O*-arabinose. The strict substrate specificity of UGT5 for flavonol aglycones and UDP-arabinose strongly suggest that UGT5 is the first flavonol arabinosyltransferase characterized. Flavonol 3-*O*-rhamnoside levels were lower in *rhm1* knockdown mutants, but levels of flavonol 3-*O*-glucosides and 3-*O*-arabinosides were higher. These results suggest that the rate of flavonol 3-*O*-glycosylation is affected by the supply of UDP-rhamnose produced by RHM1. The precise identification of flavonoids in conjunction with transcriptomics thus led to the identification of a novel gene function and a more complete understanding of a plant metabolic network.

## GENOMICS, PROTEOMICS AND METABOLOMICS

### **ICAR1101**

#### THE PLEIOTROPIC EFFECTS OF THE BAR GENE AND GLUFOSINATE ON THE ARABIDOPSIS

Category: Genomics, Proteomics and Metabolomics

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The Arabidopsis transcriptome was studied using the Affymetrix Arabidopsis ATH1 GeneChip in wild-type plants and glufosinate-tolerant transgenic plants expressing the bar gene. Pleiotropic effects were specifically generated in the transcriptomes of transgenic plants by both the bar gene and glufosinate treatments. In the absence of glufosinate, 4 genes were differentially expressed in the transgenic lines and another 80 genes were differentially expressed in the presence of glufosinate, of which 29 were specific to transgenic plants. In contrast, the number of differentially-expressed genes specific to wild-type plants was 194 genes during the early response at 6h of glufosinate treatment and rose to 3712 genes during the late response at 48h. Whereas the wild-type plants undergo extensive transcriptional reprofiling in response to herbicide-induced stress and finally plant death the transgenic plants appear to activate other detoxification processes to offset the toxic effects of the residual herbicide or their derivatives. The study provides the first description of the pleiotropic effects of the bar gene and glufosinate on the plant transcriptome. The results indicate that care must be taken in the functional assessment of transgenic plants where bar is used as the selectable marker gene as it is associated with pleiotropic effects that alter the transcriptome.

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### **ICAR1102**

#### THE GLK1 'REGULON' ENCODES DISEASE DEFENSE RELATED PROTEINS AND CONFERS RESISTANCE TO FUSARIUM GRAMINEARUM IN ARABIDOPSIS

Category: Genomics, Proteomics and Metabolomics

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The maize Golden2-like or GLK genes encode proteins belonging to a class of GARP domain transcriptional activators (Rossini et al, 2001, Plant Cell 13:1231). GARP domain transcriptional activators are involved in plant specific processes and GLK has been observed to be involved in the regulation of chloroplast development (Yasumura et al, 2005, Plant Cell 17:1894) and adaptation to cold stress (Savitch et al, 2005, Plant Cell Physiol 46:1525). *In planta* targets of GLK regulated transcription, however, have not been identified. We use GLK1 overexpression (OE) to study reprogramming of gene expression networks in Arabidopsis and to identify an associated phenotype. Affymetrix Gene Chip and RT-PCR analyses indicated that GLK1 OE in Arabidopsis reprogrammed gene expression networks to enhance a high constitutive expression of genes encoding disease defense related proteins. These include PR10, isochorismate synthase, antimicrobial peptides, glycosyl hydrolases, MATE efflux and other genes associated with pathogen response and detoxification. However, PR1, an indicator of systemic acquired resistance (SAR), was downregulated in GLK1 OE. GLK1 OE in Arabidopsis confers resistance to *Fusarium graminearum*, a broad host pathogen responsible for major losses in cereal crops. This is the first identification of the GLK1 'regulon' and a novel role for GLK1 in plant defense. Comparisons of transcriptional regulation by overexpression of heterologous GLK1(s) from other plant species in Arabidopsis is also described

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### **ICAR1103**

#### METABOLITE PROFILING OF ARABIDOPSIS REVEALS COMPOUNDS THAT AFFECT INFECTION BY THE NECROTROPHIC FUNGAL PATHOGEN *ALTERNARIA BRASSICICOLA*.

Category: Genomics, Proteomics and Metabolomics

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In order to ward off invading microbial pathogens, plants must activate a battery of defense responses in a timely manner. A functional genomics platform was used to gain insights into the metabolic changes that occur following the infection of Arabidopsis with the necrotrophic fungal pathogen *Alternaria brassicicola*. We analyzed Arabidopsis mutants with known defects in defense signaling (dde2-2, ein2-1, sid2-2) along with wild-type Col-0. Of the 394 metabolites evaluated, 16 showed significant genotype interactions while 252 were significantly altered in response to pathogen infection. A co-response analysis of transcriptional and metabolic responses revealed tight transcript-metabolite correlations for some of the metabolites evaluated. Exogenous foliar applications of some of the metabolites showing significant treatment effects showed that sugar alcohols and GABA promote susceptibility to *A. brassicicola*, while trehalose limits pathogen growth. Our data showed that metabolite profiling provides a robust approach to identifying compounds that limit pathogen growth and enhance plant resistance.

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### **ICAR1104**

#### PREDICTION OF SMALL RNA TARGET SITES AND GENES IN THE *ARABIDOPSIS THALIANA* GENOME

Category: Genomics, Proteomics and Metabolomics

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The AthaMap database generates a genome-wide map of predicted transcription factor binding sites (TFBS) for *Arabidopsis thaliana*. AthaMap includes various online tools for the analysis of transcriptional gene regulation like a basic search function to display TFBS in a specific genomic region, a Colocalization function for the identification of combinatorial *cis*-acting elements, and a Gene Analysis function to determine the occurrences of TFBS in a set of genes for comparative studies. To also cover post-transcriptional gene regulation, predicted target sites from an *A. thaliana* small RNA transcriptome screening were annotated to AthaMap. mRNA transcripts were associated to these sites and putative post-transcriptionally regulated genes were identified. These genes are tagged when using the Gene Analysis function and can be excluded from the analysis to enhance prediction accuracy by exclusively considering transcriptionally regulated genes. With the recent annotation of small RNAs, AthaMap now offers a genome-wide online resource to the scientific community for transcriptional as well as post-transcriptional gene regulation in *Arabidopsis thaliana*. AthaMap is available online at <http://www.athamap.de/>

## UNCOVERING THE ARABIDOPSIS THALIANA NECTARY TRANSCRIPTOME

Category: Genomics, Proteomics and Metabolomics

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Many flowering plants attract pollinators by offering a reward of floral nectar. Remarkably, the molecular events involved in the development of nectaries (the organs that produce nectar), as well as the synthesis and secretion of nectar, are poorly understood. Indeed, no genes have been shown to directly affect the *de novo* production or quality of floral nectar. To address this gap in knowledge, we have used Affymetrix microarrays to follow global changes in gene expression in *Arabidopsis thaliana* nectaries at two developmental time points (pre-secretory and secretory nectaries), and we have also produced ~6,600 ESTs from *Brassica rapa* (oilseed rape) nectary cDNA libraries. From this data we have identified over 70 genes that are expressed >10-fold higher in nectaries than in any other tissues examined, with a significant subset being upregulated at specific floral developmental stages. Several non-nectary-specific pathways, such as those involved in auxin metabolism and signaling, are also highly upregulated in nectaries. It is hypothesized that these highly expressed, nectary-specific genes and pathways are required for nectary development and/or function. Putative functions of these genes range from transcription factors to sugar transport and modifying enzymes. Preliminary characterization of candidate gene mutants will be presented.

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**ICAR1106**

## FUNCTIONAL CHARACTERIZATION OF AN ARABIDOPSIS NUCLEAR GENE ENCODING A VARIANT OF THE GENERAL TRANSCRIPTIONAL FACTOR TFIIB

Category: Genomics, Proteomics and Metabolomics

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Initiation of mRNA transcription is a key step in the regulation of gene expression. In eukaryotes, nuclear gene expression is accomplished by three evolutionary conserved RNA polymerases (RNAP), namely RNAPI, II, and III. Despite their complexity, the nuclear RNAPs are not capable of selective promoter recognition and employ a set of auxiliary factors, known as General Transcription Factors (GTFs), for transcription initiation to be achieved. Among those factors, two evolutionarily conserved GTFs, the TATA box binding protein (TBP) and the general transcription factor B (TFIIB or Brf) play a central role in preinitiation complex assembly by bridging core promoter sequence and RNAP. Although the GTFs have been initially thought to be ubiquitous, it is now well documented that animals have evolved variants of TBP and B-type factors to regulate their complex patterns of gene expression. However, with the exception of the plant-specific TFIIB-related protein 1 (pBrp1), there is so far little information regarding the existence of GTF variants in plants. In the present work, we report the identification of *PRBP2*, a *Arabidopsis* gene coding for a novel TFIIB variant, hereafter refer to as plant-specific TFIIB-related protein 2 (pBrp2). In contrast to *PRBP1* that is widely distributed among plant species, *PRBP2* presents a more restricted phylogenetic distribution, being detected to date only in the Brassicaceae family, an observation that suggests a recent emergence of this gene. Moreover, our data indicate that *PRBP2* is specifically expressed in the reproductive organs of the plant, in particular in the pollen and embryo. To assess the function of pBrp2 in plants, we have obtained an *Arabidopsis* KO line containing the T-DNA inserted in the coding region of pBrp2 and preliminary results concerning the function of the gene will be presented.

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**ICAR1107**

## THE ARABIDOPSIS BRAHMA CHROMATIN REMODELLING ATPASE IS INVOLVED IN REPRESSION OF SEED MATURATION GENES IN LEAVES

Category: Genomics, Proteomics and Metabolomics

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Synthesis and accumulation of seed storage proteins (SSPs) is an important aspect of the seed maturation program. Genes encoding SSPs are specifically and highly expressed in the seed during maturation. However, the mechanisms that repress the expression of these genes in leaf tissue are not well understood. To gain insight into the repression mechanisms, we performed a genetic screen for mutants that express SSPs in leaves. Here we show that mutations affecting BRAHMA (BRM), a SNF2 chromatin remodelling ATPase, cause ectopic expression of a subset of SSPs and other embryogenesis-related genes in leaf tissue. Consistent with the notion that such SNF2-like ATPases form protein complexes *in vivo*, we observed similar phenotypes for mutations of AtSWI3C, a BRM interacting partner, and BSH, a SNF5 homolog and essential SWI/SNF subunit. Chromatin immunoprecipitation experiments show that BRM is recruited to the promoters of a number of embryogenesis genes in wild-type leaves, including the 2S genes, expressed in brm leaves. Consistent with its role in nucleosome remodelling, BRM appears to affect the chromatin structure of the At2S2 promoter. Thus, the BRM-containing chromatin remodelling ATPase complex involved in many aspects of plant development mediates the repression of SSPs in leaf tissue.

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**ICAR1108**

## METABOLITE CHANGES IN TRANSGENIC ARABIDOPSIS OVEREXPRESSING CHALCONE SYNTHASE

Category: Genomics, Proteomics and Metabolomics

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Chalcone synthase (CHS) is a key enzyme of the phenylpropanoid pathway catalyzing the first step in flavonoid biosynthesis. Elucidation of gene

functions can be pursued through the systematic perturbation of gene expression followed by quantitative and qualitative analyses of gene expression products including mRNA, protein, and metabolite levels. To understand more about the functions of *chs* in plant we analyzed *chs* transgenic *Arabidopsis* at transcriptomic, proteomic and metabolomic level. Results of RT-PCR and q-PCR showed that mRNA expression is higher in *chs* transgenic T3, T5 lines and lower in T1 and T6 but western blots showed that *chs* protein is higher in T2 and T5 and lower in T1 and T6. Using <sup>1</sup>D nuclear magnetic resonance (NMR) and principal component analysis (PCA) to study changes in metabolism we found that kaemferol glycoside, quercetin, feruloyl malate, amino acid and malic acid are higher and fumaric acid, adenosine, sugar, fatty acids, synapoyl, coumaroyl malate are lower in transgenic plants. These results suggest that changes in the level of enzyme gene expression do not always affect the intracellular levels of the related metabolites. Metabolome analysis and transcriptome analysis show different aspects of metabolism both are necessary to understand metabolism and in fact supplement each other. Metabolic profiling clearly has the potential to provide not only specific information on the function of a gene product, but can also give more general insight into the behavior of complex metabolic and physiological networks.

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**ICAR1109**

## TOWARDS IN PLANTA-VISUALISATION OF A TRANSCRIPTION FACTOR COMPLEX

Category: Genomics, Proteomics and Metabolomics

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In the model plant *Arabidopsis thaliana* the anthocyanin biosynthesis is regulated by the combinatorial interaction of PRODUCTION OF ANTHOCYANIN PIGMENT (PAP)-group MYB proteins and BHLH proteins of subgroup IIIf. A regulatory complex is build from a MYB factor, a BHLH factor, and the more generally acting WDR factor TRANSPARENT TESTA GLABRA1 (TTG1) [1]. We investigated the complex formed by PAP1/MYB75 and TT8/BHLH42 with the DFR promoter DNA. Binding of tagged purified proteins to the DFR promoter DNA was detected through changes in diffusion constants measured by fluorescence correlation spectroscopy (FCS) [2]. Simultaneous binding of both transcription factors to specific binding sites on double stranded DNA was shown, while binding of single transcription factors has not been observed. Furthermore, attachment sites of PAP1/MYB75 and TT8/BHLH42 at the DFR promoter DNA have been identified. Tobacco protoplast transfections using fusions with different fluorescent proteins (eGFP, mCherry) showed accumulation of PAP1/MYB75 and TT8/BHLH42 in the nucleus. The biological functionality of the fusion proteins was confirmed in vivo in *arabidopsis* protoplast co-transfection assays showing activation of the DFR promoter and in planta by complementation of transcription factor mutant plants. Stable complementation lines have been used for in planta localization studies using fluorescence imaging. Parallel localization studies were performed in seedlings of double complementation lines to visualize the transcription factor complex in planta in single cells. Fluorescence resonance energy transfer (FRET) allows observation between the fluorophores and was used to investigate individual transcription complexes in vivo [3].

References:

- [1] A. Baudry et al., *The Plant Journal* 39, 366-380 (2004).
  - [2] S.T. Hess et al., *Biochemistry* 41, 697-705 (2002).
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**ICAR11010**

## PROTEOME ANALYSIS OF 2,4,6-TRINITROTOLUENE STRESS RESPONSE IN ARABIDOPSIS THALIANA USING DIGE

Category: Genomics, Proteomics and Metabolomics

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2,4,6-trinitrotoluene (TNT) is one of the most commonly

The aim of this study was to investigate the phytodegradation of 2,4,6-trinitrotoluene by *Arabidopsis thaliana* at the proteome level. *Arabidopsis thaliana* (Columbia ecotype) plants were grown for 5 weeks in the growth chamber (23°C, 16 h light), and then treated with 50 mg.l<sup>-1</sup> TNT. The plants were harvested after 2 days. Differential protein expression was analyzed by two-dimensional difference gel electrophoresis, which enables the separation of up to three separate protein samples in the same 2-D gel and reduces the effects of gel to gel variation due to the incorporation of an internal standard.

Results showed total of approximately 700 major protein spots. TNT caused only small changes in the *Arabidopsis thaliana* proteome. Pixel densities for 12 protein spots differed significantly between TNT treatments and control plants. Differential expression of these proteins will be validated by Western blotting and proteins will be identified by MS techniques.

Key words: Phytoremediation, stress, 2,4,6-trinitrotoluene, DIGE, Arabidopsis, differential in-gel electrophoresis, proteome analysis.

Acknowledgement: This study was supported by projects FT-TA3/118 and 2B06187.

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**ICAR11011**

## TRANSCRIPTOME ANALYSIS OF LASER MICRODISSECTED PROCAMBIAL CELLS FROM A DEVELOPING LEAF.

Category: Genomics, Proteomics and Metabolomics

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We have used a reverse genetics approach using laser capture microdissection in conjunction with DNA microarrays to profile the procambial cells from a developing *Arabidopsis* leaf. The procambial set after filtering out genes expressed in the mature leaf cell types contained most of the genes previously identified by forward genetic mutant screens like CVP1, MP, SCF. We also verified the expression of a number of new genes in this set by making transgenic promoter gus lines and show their procambial/vascular cell specific expression. TDNA (SALK) insertion lines for several previously uncharacterized genes in the PC set exhibit aberrant venation, including an unusual RING E3 ligase/kinase that shows differentiation defects in the cotyledonary leaf and appears to be a seedling lethal.

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### ICAR11012

#### THIOREDOXIN INTERACTIONS IN THE CHLOROPLAST LUMEN

Category: Genomics, Proteomics and Metabolomics

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Thioredoxins, originally discovered as a link between photosynthesis and metabolism, are a family of small disulfide-reductase proteins found in all organisms. *Arabidopsis thaliana* has at least 44 known genes coding for thioredoxins or thioredoxin-like proteins, of which 21 are predicted or experimentally shown to be located in plastids [1]. The recent identification of many putative thioredoxin targets implies that redox signaling mediated via thioredoxins plays an important role, not only in photosynthesis but also in a wide range of processes in the chloroplast. Within the thylakoid membrane, the focal point of oxygenic photosynthesis, lies the thylakoid lumen, a small sub-organelle compartment containing a distinct protein population. The proteome of the thylakoid lumen of *Arabidopsis thaliana* is comprised of approximately 80 proteins [2]. While many of the proteins have unknown functions, a number of them are important enzymes regulating oxygen-evolution and the xanthophyll cycle. Although no thioredoxin has so far been identified in the chloroplast lumen, several observations strongly indicate the presence of a thio-transduction pathway in the lumen: an x-ray structure study of the luminal immunophilin FKBP13 by Gopalan et al. [3] showed that this protein had a pair of disulfide bonds which could be reduced by *E.coli* thioredoxin and Marchand et al. [4 and 5] found that the extrinsic Photosystem II proteins PsbO1 and PsbO2, and also the luminal pentapeptide protein TL17 could be reduced by thioredoxin h3.

In this study we have identified putative thioredoxin targets in the chloroplast lumen of *Arabidopsis thaliana* using two complementary proteomic methods, fluorescence two-dimensional gel-electrophoresis and two-dimensional gel-electrophoresis coupled with differential alkylation of reduced cysteine residues. Besides the previously identified luminal thioredoxin targets PsbO1, PsbO2, TL17 and FKBP13, several novel thioredoxin targets were identified. Among the proposed targets are the xanthophyll cycle enzyme violaxanthin de-epoxidase and proteins of unknown function.

#### References

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- [2] Schubert et al. (2002) J. Biol. Chem. 277:8354-8365
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### ICAR11013

#### FUNCTIONAL CHARACTERIZATION OF HUA2 PROTEIN

Category: Genomics, Proteomics and Metabolomics

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Coordinate control of processes that occur at the transition from vegetative to reproductive phase in plants are 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 implications for the coordinated control of induction and maintenance of floral state. Our lab has previously demonstrated that the natural variant *HUA2-Sy-O* is unique, uncoupling the effects on *FLC* and *AG*. This suggests that *HUA2* affects *FLC* and *AG* responses via different mechanisms. To gain more insight into *HUA2* function, potential *HUA2* interacting proteins were identified by performing yeast two-hybrid screens. *UBP1*, *RBP45* and *AtPrp40* were identified to interact with *HUA2*. These interacting proteins are known to participate in eukaryotic splicing, suggesting that *HUA2* functions inside the nucleus as a pre-mRNA processing factor. We have demonstrated that *HUA2* affects splicing and 3' end processing of the *AG* primary transcript.

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### ICAR11014

#### FUNCTIONAL CHARACTERISATION OF PLANT SERINE HYDROLASES BY ACTIVITY-BASED PROTEIN PROFILING AND MUDPIT-ANALYSIS.

Category: Genomics, Proteomics and Metabolomics

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The *Arabidopsis* genome encodes for hundreds of serine hydrolases, like esterases, amidases, peptidases, glycosidases. The large size of this group of enzymes has so far hampered a concerted analysis and functional characterisation. Enzyme activities can be monitored using 'activity-based protein

**profiling' (ABPP), a method that is based on the** use of tagged, mechanism-based inhibitors (probes) that react with whole classes of enzymes in an activity-dependent manner. We previously introduced this technique in plant science by monitoring activities of papain-like cysteine proteases. In order to gain more insight into the activity of serine hydrolases in plants, we profiled their activity with the biotinylated irreversible serine hydrolase inhibitor FP (a fluorophosphonate). The labelled proteins were purified by affinity chromatography and identified by MuDPIT-analysis (Multi Dimensional Protein Identification Technology). Several FP targets were cloned into expression vectors and confirmed using the agroinfiltration-based transient overexpression in *Nicotiana benthamiana*. The combination of ABPP with the low sensitivity levels of the MuDPIT-technology allowed us to identify more than 70 targets of FP from a single Arabidopsis leaf extract, which includes numerous subtilase-like proteases, serine carboxypeptidases, SGNH-lipases, prolyl-oligopeptidases and pectinacetyl esterases. For most of these FP targets this is the first evidence that these enzymes are active in leaves. We believe that the presented method will allow scientists to monitor the activity of serine hydrolases during different developmental stages as well as during biotic and abiotic stresses.

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**ICAR11015**

## HOMOLOGOUS RECOMBINATION INDUCED BY SITE-SPECIFIC DOUBLE STRAND BREAK AGENTS IN ARABIDOPSIS

Category: Genomics, Proteomics and Metabolomics

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DNA Double Strand Breaks (DSBs) can be repaired by two major pathways: Homologous Recombination (HR), or Non-Homologous End Joining (NHEJ). In yeast, the HR pathway is predominant and homology-based gene targeting is very efficient. In plants, NHEJ is predominant and gene targeting is rare. However, recent studies have shown that DSBs induced by two site-specific DSB agents, transposon excision or meganucleases, can stimulate HR in higher plants.

To determine how DSB repair pathways coordinate and compete with each other, a Homologous Recombination System (HRS) consisting of three different constructs (HRS1, HRS2 and HRS3) has been designed and introduced into Arabidopsis. The constructs employ both a visual marker (GUS) and a selective marker (NPTII which confers Kanamycin resistance) to detect various repair and recombination events. A duplication as recombination donor sequence is present in different orientations nearby a DSB site, induced by either cutting with the meganuclease I-SceI or excision of the transposon Ds. This will enable comparisons of the pathway of DSB repair for the two mechanistically different DSB inducers at the same locus. As designed, the three constructs are able to efficiently report both somatic and/or germinal recombination frequencies. The results will help to establish repair pathway frequencies in wild-type plants. Ultimately, these data will be used as base lines for comparison with plants that under- or over-express genes involved in DSB repair and chromatin structure to determine their impact on homologous recombination. Data collections are in progress, and lines exhibiting rather high somatic and germinal recombination frequencies have been identified.

The ability to efficiently measure HR frequencies and to obtain germinal recombinants suggests that the HRS may provide new insights into the biology of homologous recombination that will facilitate the development of genome modification tools for plants.

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**ICAR11016**

## TAIR8 GENOME RELEASE AND NEW LAGE-SCALE DATASETS AT TAIR

Category: Genomics, Proteomics and Metabolomics

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A new *A. thaliana* genome release, TAIR8, is now publicly available at TAIR. The TAIR8 release contains 27,235 protein coding genes, 4759 pseudogenes or transposable elements and 1288 ncRNAs. A total of 1291 new genes and 2009 new gene models were added. Overall 23% of all existing TAIR7 genes (7380 genes) were updated for TAIR8.

In addition to providing detailed information on Arabidopsis gene number statistics, this poster will focus on new types of datasets that have recently been made available at TAIR including genome-wide methylation, phosphorylation and promoter data, aligned Brassica sequences, the VISTA genome alignment plots, orthologous genes, polymorphic sequences, transcriptome data, community annotations and more. These data types are visible in our new genome browser Gbrowse and the corresponding files can be downloaded from our ftp site.

We will also briefly discuss upcoming projects for TAIR9 and beyond.

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**ICAR11017**

## EL25, A NOVEL PROTEIN, MAY PARTICIPATE IN EMBRYOGENESIS IN ARABIDOPSIS THALIANA

Category: Genomics, Proteomics and Metabolomics

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Plant embryogenesis refers to the process of development of plant embryos, being either a sexual or asexual reproductive process that forms new plants. Although there are about 4000 genes required for normal embryo development, the mechanisms to date are still unclear. Embryo related T-DNA insertional mutagenesis represents a promising approach to the molecular isolation of genes involved in the embryo development with essential functions.

In our present study, we have obtained 13 T-DNA mutants of *Arabidopsis thaliana* from salk which may defective in embryo development. One of the mutants named *EL25*(embryo lethals 25) was studied in detail. The mutation located in the exon of the gene on chr5. The gene contains a single open reading frame of 273 amino acids and encodes a putative protein with calculated molecular mass of 31.1kDa. It contains about 78 amino acids of glycogen binding domain (GBD) in beta subunit of AMP-activated protein kinase (AMPK) in C-terminal ends. AMPK is a metabolic stress sensing protein that senses AMP/ATP and has recently been found to act as a glycogen sensor as well. But in higher plants, whether it plays any specific role in plant development is still unknown.

To analyze the expression patterns of *EL25* in *Arabidopsis thaliana*, semi-quantitative RT-PCR analysis was performed. The results showed that the *EL25* transcripts were constitutively detected in roots, stems, leaves, flowers and with little stronger in siliques. The germination test showed that the germination ability of *el25* seeds were lower than that of wild type, only 8% seeds of homozygous were bud as well as 96% seeds of wild types were. The *el25* have fewer lateral roots and shorter axial root than those of wild type. The preliminary analysis indicated that the gene tagged by T-DNA encode a novel protein which may play an important role in embryogenesis. To confirm whether the embryo lethality observed in the *el25* is caused by the insertional mutation in *EL25* or not, this gene was amplified through PCR according to its genomic sequence, and the sense binary vector was constructed for *Arabidopsis* transformation. The further study of *el25* mutant, and the function of this gene were still undergoing.

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**ICAR11018****THE ARABIDOPSIS PROLIFERATIVE ENDOSPERM TRANSCRIPTOME**

Category: Genomics, Proteomics and Metabolomics

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During the early stages of seed development, arabidopsis endosperm is syncytial and proliferates rapidly through repeated rounds of mitosis without cytokinesis. This stage of endosperm development is important in determining final seed size and is a model for studying various aspects of cellular and molecular biology, such as the cell cycle and genomic imprinting. However, the small size of arabidopsis seed, the syncytial nature of the proliferative endosperm, and the surrounding maternal tissues, make high-throughput molecular analysis of the early endosperm technically difficult. Laser capture microdissection has enabled high-resolution transcript analysis of the proliferative stage of arabidopsis endosperm development at 4 days after pollination, when seeds are at the early to mid-heart stage of embryo development. Comparative analysis of gene ontology representation of the proliferating endosperm transcriptome (11,676 genes) revealed a developmental programme dominated by the expression of genes associated with cell-cycle, DNA processing, chromatin assembly, protein synthesis, cytoskeletal and microtubule related processes, and cell/organelle biogenesis and organisation. Analysis of core cell-cycle genes implicates specific gene-family members as playing important roles in controlling syncytial cell division. Hormone marker analysis indicated the importance of cytokinin in the proliferating endosperm and suggests specific roles for particular cytokinin signalling genes during endosperm development. Comparisons with publicly available microarray data revealed ~800 early seed-specific genes that were preferentially expressed in the endosperm, including 71 transcription factors that might have key roles in the endosperm. The list of endosperm-expressed genes also appears to be enriched for imprinted loci and we are currently screening this list to identify novel imprinted genes. In summary, this work should provide a useful resource for obtaining novel insight into early seed development and identifies targets for further characterisation.

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**ICAR11019****BIOMASS AND HETEROSES IN ARABIDOPSIS THALIANA: QTL MAPPING, METABOLITE PROFILING AND ASSOCIATION MAPPING**

Category: Genomics, Proteomics and Metabolomics

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The term „heterosis“ was coined by G.H. Shull in 1914 to describe the „increased vigor manifested by crossbred organisms as compared with the corresponding inbreds as the specific result of unlikeness in the constitution of the uniting parental gametes“. Heterosis is extensively used in plant breeding, e.g. in maize, but the underlying mechanisms are still largely unknown. We study growth and heterosis in the model plant *Arabidopsis thaliana* with the aim to identify and characterise at the molecular level loci underlying this effect. To this end, growth related parameters such as biomass, leaf area and relative growth rate are recorded in collections of natural accessions, recombinant inbred line (RIL) populations, and complete series of introgression lines (ILs).

Using large RIL and IL populations, six QTL for biomass per se, up to eleven QTL for heterosis for biomass, and 157 metabolic QTL for 84 metabolites could be identified. The complementarities of the RIL and IL approaches led to the detection of additional QTL. The genetic analyses were extended to include association testing on populations composed of accessions or complex crosses thereof that have been genotyped using single nucleotide polymorphism (SNP), single feature polymorphism (SFP) and simple sequence length polymorphism (SSLP) markers. A preliminary analysis using a panel of 99 accessions genotyped with 499 SNP markers revealed 28 marker associations for the trait biomass, explaining ca. 50% of the total genetic variance.

Metabolic profiling by GC-MS was used to study the potential link between biomass and metabolic composition. Pearson correlation analyses showed only weak relations between biomass and levels of individual metabolites, but a highly significant canonical correlation was observed revealing a close link between biomass and a specific combination of metabolites. Meta-QTL analyses of the measured and the predicted biomass (canonical variate calculated from metabolic profiles) were consistent. The demonstrated predictive power of metabolic composition for biomass features this composite measure as an excellent biomarker and opens new opportunities to enhance plant breeding specifically in the context of renewable resources.

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**ICAR11020****ANNO-J: INTERACTIVE WEB-BASED GENOME BROWSING IN ARABIDOPSIS FOR LARGE DATASETS IN FUNCTIONAL GENOMICS**

Category: Genomics, Proteomics and Metabolomics

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Next-generation deep sequencing and high-throughput proteomics are generating larger amounts of data than ever before, leading to data handling and visualization difficulties. Anno-J is a new web-based genome annotation browser that has been designed to display these data in parallel by making the most of Web 2.0 technologies. The program provides CSS-based style control, data syndication, a dynamic AJAX-powered interface, and independence from server architecture. Tracks are discrete plugins that may be developed and hosted by remote providers, freeing each to implement ad hoc functionality. Data may be hosted directly by data creators, ensuring that Anno-J users are always looking at up-to-date information. An example of Anno-J in use may be viewed at <http://neomorph.salk.edu/epigenome/> where it has been used by the JR Ecker Laboratory at the Salk Institute for a study of the epigenome in *Arabidopsis thaliana* (Lister et al 2008). This implementation shows multiple tracks from Illumina GA sequencing-by-synthesis data, including strand-specific sequencing of the complete cytosine methylome and the smRNA and mRNA components of the transcriptome. Anno-J is an open-source project that is free for personal and academic use. Documentation, source code and related resources are available from <http://www.annoj.org>. Plugin tracks for deep sequencing data, microarray data, and peptide mass spectra have been generated and are currently being used.

Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, Ecker JR. (Apr 17, 2008) Highly Integrated Single-Base Resolution Maps of the Epigenome in *Arabidopsis*. *Cell.*, doi:10.1016/j.cell.2008.03.029

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### **ICAR11021**

#### THE *ARABIDOPSIS* SEEDGENES PROJECT: APPROACHING SATURATION FOR ESSENTIAL GENES

Category: Genomics, Proteomics and Metabolomics

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The purpose of the SeedGenes Project ([www.seedgenes.org](http://www.seedgenes.org)) is to present detailed information on essential genes in *Arabidopsis*. Emphasis is placed on genes required for seed development. The December 2007 database release includes more than 350 total genes and 600 mutant alleles. When pending additions found through the SeedGenes Access page are included, over 350 *EMB* genes alone have been identified to date. Approximately 65% of these gene identities have been confirmed through molecular complementation or the analysis of duplicate mutant alleles. Another 150 to 200 genes required for gametophyte development have been reported in the literature, although many of these identities remain to be confirmed. At least 50 genes required for seedling survival have also been described.

Lethals represent a critical and informative part of the *Arabidopsis* mutant collection. A robust dataset of essential genes is needed to define the minimal gene set required for plant growth and development and to complement ongoing research with other model organisms. We estimate that 30-50% of the *EMB* genes in *Arabidopsis* have been identified to date. Because forward genetic screens for lethals become less efficient with progress towards saturation, most of the remaining essential genes will likely be identified through reverse genetics. We describe here several different strategies that we have pursued to identify additional *EMB* genes by focusing on candidates that: (A) represent orthologs of essential genes in other organisms; (B) function in the same metabolic pathway or cellular process as known *EMBs*; (C) share protein interactors with a known *EMB* gene product; and (D) are associated with insertion mutants that fail to generate knockout homozygotes.

Research supported by the NSF 2010 Program. Individuals working on mutant alleles of genes first described in SeedGenes are requested to include the original *EMB* gene symbol in future publications.

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### **ICAR11022**

#### WHOLE-GENOME TRANSCRIPTOME ANALYSIS IN ARABIDOPSIS SEEDS USING TILING ARRAY

Category: Genomics, Proteomics and Metabolomics

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The phytohormone abscisic acid (ABA) plays an important role for seed dormancy. Indeed, ABA-deficient mutant, *aba2*, shows reduced seed dormancy, whereas ABA-over-accumulation mutant, *cyp707a1 cyp707a2 cyp707a3* triple mutant, exhibits strong seed dormancy. Thus, ABA regulates a large number of genes that is involved in seed dormancy. To explore genome-wide expression patterns and regulatory mechanisms in *Arabidopsis* seeds, comprehensive expression analysis was performed using whole-genome tiling array. Although there was large difference in the endogenous ABA levels between *aba2* and *cyp707a* triple mutant dry seeds, a large number of genes was not drastically changed in these mutants, indicating that the difference of endogenous ABA levels in dry seeds does not influence global gene expression. In contrast to dry seeds, the transcript levels of many genes in both mutants were drastically changed compared to those of wild type. Transcriptome analysis identified 343 ABA-upregulated and 545 ABA-downregulated AGI code genes at 24 h after imbibition. In addition, the promoters of ABA-upregulated genes were enriched for CACGTG sequence that consistent with one of the most typical ABA-responsive elements (ABREs). Moreover, whole-genome transcriptome analysis identified 4884 non-redundant novel genes in *Arabidopsis* seeds. Among novel genes, 4559 novel genes probably encoded hypothetical non-coding RNAs (ncRNAs). We identified 127 ABA-upregulated and 13 ABA-downregulated novel genes at 24 h after imbibition. Furthermore, 72 ncRNAs were conserved in several plant species. At present, we're proceeding analysis of functional ncRNA.

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### **ICAR11023**

#### ACTIVITY-BASED PROTEIN PROFILING IN *ARABIDOPSIS*

Category: Genomics, Proteomics and Metabolomics

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In this postgenomic era, plant scientists face the daunting task of assigning functions to the more than 30,000 proteins that are encoded by the *Arabidopsis* genome. Several genome-wide technologies have been developed that have generated a tremendous wealth of information about genomes, transcriptomes and proteomes of *Arabidopsis*, yielding insights into diverse biological processes. Yet a crucial piece of information is missing between the proteome and the processes in which proteins participate, namely: **activity**. The actual activity of a protein is difficult to predict from its presence since activity is predominantly regulated by various post-translational processes, such as phosphorylation, translocation and processing. Information on protein activity is essential to the annotation and understanding of protein functions since it is the activity of a protein, rather than its abundance, that ultimately dictates its role in a living organism. We aim to uncover the proteome activity layer of *Arabidopsis* through a novel, genome-wide technology called activity-based proteome profiling (ABPP). ABPP can be applied on living tissues or on protein extracts and is based on labeled small molecules (probes) that react with active site residues of a broad range of proteins in an activity-dependent manner. The aim of this work is to generate activity information by cross-proteome and cross-condition comparisons of *Arabidopsis* proteomes. We separate and visualize fluorescent labeled proteins on protein gels and identify them subsequently by mass spectrometry (MS) with the intention to annotate 1001 different proteins from various mechanistic classes. Finally, inhibitory signatures will be generated to sub-classify a large redundant protein family into functional units.

#### **ICAR11024**

#### GENOMIC ANALYSES OF NOVEL CODING SHORT OPEN READING FRAMES IN ARABIDOPSIS: INVOLVEMENT IN FLOWER DEVELOPMENT

Category: Genomics, Proteomics and Metabolomics

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Transcriptome sequencing and whole genome tiling array studies have revealed significant levels of expression from numerous intergenic regions in animals and plants, suggesting the presence of genic sequences in un-annotated "intergenic" regions. In *Arabidopsis*, computational studies have identified many coding short ORFs (sORFs) with the potential to constitute novel coding genes (for example, Hanada et al. 2007, Genome Res. 17, 632). We have used custom-designed DNA microarrays to study the expression of ~6,000 sORFs in *Arabidopsis* floral tissues and during the process of flower development, using either the floral organ identity mutants (*ap1*, *ap2*, *ap3*, *ag*), or an AP1-GR-based floral induction system (described in Wellmer et al. 2006, PLoS Genetics 2, e117). Many of the sORFs are expressed in floral tissues, and ~400 show either predominant expression in a given tissue or differential expression throughout development. Further computational studies indicate that many of the expressed sORFs may indeed correspond to novel genes.

#### **ICAR11025**

#### QUANTITATIVE PROTEOMICS OF A CHLOROPLAST SRP54 SORTING MUTANT AND ITS GENETIC INTERACTIONS WITH CLPC1 AND TIG IN ARABIDOPSIS THALIANA

Category: Genomics, Proteomics and Metabolomics

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Protein homeostasis within the chloroplast is critical for plant development and function. cpSRP54 is involved in co- and post-translational sorting of thylakoid proteins. To better understand the developmental impact of a deletion in cpSRP54, we employed a quantitative comparative proteomics approach of young *Arabidopsis thaliana* wt (Col-0) seedlings and young seedlings of a cpSRP54 mutant (*ffc1-2*) using isobaric stable isotope labeling (iTRAQ). Complementary western blot analysis of individual leaves showed that all four SRP sorting components (except SecYE), were strongly down-regulated with progressive leaf development in both wt and *ffc1-2*. cpSRP54 deletion led to a differential reduction of LHC proteins, an increase in PsbS, and decreased ratio between Photosystems I and II. Chloroplast chaperones Cpn60, HSP70 and ClpC1, FtsH proteases, and other proteins involved chloroplast gene expression increased in *ffc1-2*. Peroxisomal and mitochondrial photorespiratory enzymes, Calvin cycle enzymes, as well as the envelope triosphosphate translocator were up-regulated. Isolated thylakoid proteomes of mature *ffc1-2* and wt rosettes were also compared using iTRAQ, while isotope coded affinity tags (ICAT) and 2DE gels were used to compare mature chloroplast stroma of wt and *ffc1-2* plants. The significance of ClpC1 up-regulation was confirmed through the generation of an *ffc1-2* × *clpc1* double mutant. To further investigate the role of cpSRP54 in co-translational targeting, and because of the dual role of SRP54 and TIG in *E. coli* in co-translational binding of nascent hydrophobic domains at the ribosome, we have generated a double mutant in cpSRP54 and AtTIG. It is possible that cpSRP54 and AtTIG may interact in the same targeting pathway, or functionally interact with each other at the chloroplast ribosome. Our findings are integrated in a testable model for chloroplast protein biogenesis.

#### **ICAR11026**

#### METABOLIC SYSTEMS OF METHIONINE-DERIVED GLUCOSINOLATE IN ARABIDOPSIS

Category: Genomics, Proteomics and Metabolomics

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Using the public co-expression database based on the *Arabidopsis* transcriptomics, we can predict the co-regulated gene group in the multiple conditions, biotic/abiotic stress, growth stages and organ specificity. We have previously identified three R2R3-MYB genes which are co-expressed with known glucosinolate biosynthesis enzyme genes in the co-expression database (ATTEDII). The omics approaches, transcriptomics and metabolomics, of the MYB genes knockout *Arabidopsis* lines, confirming that the MYB genes are positive regulators acting the genes expression of the major methionine-derived glucosinolate (MET-GSL) biosynthesis steps, and we named the genes: production of methionine-derived glucosinolates

(*PMG1/MYB28*, *PMG2/MYB29* and *PMG3/MYB76*) [1]. Here we report that further analysis using the double knockout lines of the *PMG* genes. In double knockout lines of *pmg1/pmg2* and *pmg1/pmg3*, MET-GSLs decreased than the single mutant lines. Especially, the absence of every MET-GSL was observed in the *pmg1/pmg2*, and the expressions of MET-GSL biosynthetic genes were significantly repressed in the transcriptomic data. To follow the metabolic changes associated with the absence of MET-GSL in *pmg1/pmg2*, primary metabolites in leaves and seeds were analyzed using capillary electrophoresis mass spectrometry (CE-MS). Principal component analysis (PCA) applied to the metabolomic data of leaves and seeds showed that only seeds had significant changes, suggesting that there is a different regulatory system between leaves and seeds. Further experiments on the mutants are being conducted to confirm the metabolic systems.

1. M. Y. Hirai, K. Sugiyama, Y. Sawada et al., PNAS (2007).

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### ICAR11027

FUNCTIONAL CHARACTERIZATION OF A GENE HOMOLOGOUS TO AT1G74730 UPREGULATED IN EMBRYONIC BRASSICA NAPUS MICROSPORES

Category: Genomics, Proteomics and Metabolomics

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Cultured canola microspores undergo embryogenesis instead of forming pollen when exposed to a mild heat stress. The microspore culture system is useful for studying embryogenesis because microspore-derived embryos are very similar to the zygotic embryos with respect to their morphology, chemical composition and physiology. Therefore, it provides an easy way to access and study all of the different stages of embryogenesis, starting from the initiation phase. Moreover, the similarities make it possible to use the system to identify a gene that is involved in zygotic embryogenesis. A previous study comparing the transcriptomes of three-day-old sorted embryogenic and pollen-like, (nonembryogenic), cells of *B.napus* microspore cultures identified 100 transcripts that were upregulated in embryogenic cells, including a fragment of a gene of unknown function homologous to AT1G73740. This objective of the current study is to characterize the homologous gene in *B. napus* and to determine its function by over-expression and RNA silencing in *B. napus* and *Arabidopsis*. The complete gene was isolated and sequenced from *B. napus* by PCR and RT-PCR using primers designed from the AT1G73740 sequence and named as *BnMicEmUP* (*B. napus* microspore embryogenesis up regulated gene). Two forms of *BnMicEmUP* mRNA (597bp and 588 bp) and two forms of genomic DNA (711bp and 702bp) were identified. These *BnMicEmUPs* share high nucleotide similarity with AT1G73740 *Arabidopsis* (about 85%) within the coding region and have a similar genomic organization to the *Arabidopsis* gene. To investigate the function of *BnMicEmUP* two silencing constructs were made with the Pgk5941 vector and two different *BnMicEmUP* fragments. One fragment is highly gene-specific and the other fragment is ~90% identical for the two forms of the gene. Vectors with a 35s promoter (pB1121) and an inducible promoter (pER8) were constructed for over-expression of the *BnMicEmUP<sub>597</sub>* gene. The constructs were delivered by *Agrobacterium*-mediated transformation of *A. thaliana* using the floral dip method and *Brassica* using hypocotyl explants. The effects of gene over-expression and suppression on embryo formation and plant development will be investigated.

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### ICAR11028

TRANSCRIPTOME ANALYSIS OF PLASTID GENE EXPRESSION IN ARABIDOPSIS

Category: Genomics, Proteomics and Metabolomics

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Chloroplasts are the site of photosynthesis and contain their own genome encoding ~50 photosynthesis related proteins and ~80 housekeeping proteins and RNAs. Photosynthesis genes are mainly transcribed by bacterial type RNA polymerase PEP that was derived from a cyanobacterial ancestor. On the other hand, housekeeping genes that are involved in transcription and translation events are transcribed by other phage-type RNA polymerase, NEP. As an organelle specialized for carrying out photosynthesis, chloroplast genes are actively transcribed in chloroplasts in green tissues, but their expression is largely silent in proplastids of meristematic cells and root plastids. However, little is known about broad patterns of variation of chloroplast gene expression in various tissues and under stress conditions. In this study, we analyzed tissue-specific and stress-induced gene expression in plastids, by using a chloroplast tiling macroarray. (1) PEP-dependent photosynthesis gene expression is activated during chloroplast development. AtSIG2 and AtSIG6 play crucial roles in the regulation of PEP during chloroplast development. (2) NEP-dependent *accD* gene is constitutively expressed in both leaves and roots, while expression of other PEP- and NEP-dependent genes is largely suppressed in roots, suggesting that *accD* transcription is differentially regulated by a specific factor. (3) Accumulation of several intron-containing genes was specifically reduced in the dark. (4) The *psbD* light-responsive promoter (*psbD* LRP) is specifically activated by various abiotic stresses, including high salinity, high osmolarity, cold and high light, but not by elicitors and H<sub>2</sub>O<sub>2</sub>. However, biotic and abiotic stresses have no effect on expression of other chloroplast-encoded genes. It is assumed that stress-induced activation of *psbD* LRP is mediated by a stress-specific sigma factor, AtSIG5.

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### ICAR11029

GLOBAL ANALYSIS OF THE LEAFY TRANSCRIPTIONAL NETWORK: TRANSITIONING TO REPRODUCTIVE DEVELOPMENT IN *ARABIDOPSIS THALIANA*

Category: Genomics, Proteomics and Metabolomics

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The transition from vegetative to reproductive development in *Arabidopsis thaliana* involves the integration of multiple environmental and endogenous cues and the translation of these cues into the developmental program of flower-morphogenesis. The LEAFY (LFY) transcription factor has been identified as the key integrator and master regulator of this transition. In addition to its role in the initiation of floral development during the meristem identity (MI) transition, LFY also has a later role in the induction of homeotic genes that specify floral organ identity (i.e. floral patterning). Using a post-translationally activatable form of LFY, a ChIP-on-chip approach was taken to identify targets of LFY during the initiation of flower

development. Preliminary analyses suggest that LFY binds to over 1000 targets, suggesting it plays a large role in the transition to reproductive development in Arabidopsis. All known MI regulators were identified as LFY targets in this dataset. In addition, new potential LFY targets include genes involved in leaf development, hormone signaling, chromatin remodeling and flowering time, suggesting a possible link between meristem identity and these pathways.

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**ICAR11030****DISSECTING ARABIDOPSIS HOST AND NONHOST INTERACTIONS WITH PSEUDOMONAS SYRINGAE TAKING ADVANTAGE OF COMPARATIVE EVOLUTIONARY GENOMICS**

Category: Genomics, Proteomics and Metabolomics

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The bacterial plant pathogen *Pseudomonas syringae* is a wide spread species that causes damage to agricultural crops globally. We have selected a group of closely related isolates within the *P. syringae* species for investigating host range and virulence evolution and plant non-host resistance. The selected group of strains includes the completely sequenced *A. thaliana* and tomato pathogen *PtoDC3000* and additional strains that are potent pathogens on *A. thaliana*. Other members of this group, such as *PtoT1* and *PtoJL1065*, are not pathogenic on *A. thaliana*. Despite of distinctive host range and virulence, these isolates have been confirmed to share over 99 DNA percent identity with DC3000 in housekeeping genes. Relatively high recombination frequency among these isolates has been detected. Preliminary genome sequencing of T1 and molecular analysis of additional T1-like isolates have revealed diversity in a relatively small number of genomic regions, which are believed to be the primary contributors to the observed virulence and host range differences between isolates. Thus, these isolates represent a perfect combination of closeness in phylogeny and divergence in host range and virulence. We are currently comparing the defense responses of *A. thaliana* to DC3000 and T1-like isolates. We have found that T1-like isolates are unable to cause disease nor grow to the level of DC3000 on *A. thaliana* Col-1, several single and double mutants defective in gene-for-gene resistance, in SA mediated signaling, and in Pathogen Associated Molecular Patterns (PAMPs)-triggered immunity. Some evidence suggests that unknown PAMPs of T1 are eliciting yet unidentified basal defenses. On the other hand, some of T1's Type III effectors seem to promote disease symptoms or suppress defense responses. These preliminary results reveal the complexity of the T1 - *A. thaliana* interaction. Using this system, we expect to uncover novel signaling pathways and defense mechanisms in *A. thaliana* and PAMPs, Type III effectors, and other genetic elements with novel functions during pathogenesis in *P. syringae*.

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**ICAR11031****TRANSCRIPTIONAL CASCADE IN CELL FATE SPECIFICATION**

Category: Genomics, Proteomics and Metabolomics

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Cell fate specification is one of the most interesting questions in developmental biology. From the view of genomics, a particular cell identity can be recognized as the characteristic transcription profile of that cell type. From the simplest perspective, cells receiving the master signal• of a particular cell fate will express some cell-type-specific transcriptional factor(s) which will then turn on more downstream cell-type-specific events and eventually lead to the cell-type-specific transcription profile and make cells of that particular identity. Important questions in cell fate specification are: What is the “master signal”• of a particular cell identity? How is the downstream cell-type-specific transcriptional cascade turned on? What are the components of the cascade?

It has been found that plant hormone auxin plays an important role in cell fate specification in the root apical meristem. Exogenous auxin treatment of the Arabidopsis root can induce ectopic quiescent center and columella identity in the root apical meristem. We aim to study how auxin signaling can cause the identity change and to find the components of transcription cascade downstream of auxin to specify quiescent center and columella fates. While the cells are changing their identity on auxin, we can sort out these competent cells by taking advantage of the GFP marker specifically expressed in that cell type. Then we can examine their cell-type-specific transcription profile by microarray experiment using RNA of the cells that are changing their identity. If we sample along the cell identity transition, we will be able to find the genes turned on upon auxin signaling and put them in order. And these genes represent at least part of the transcription cascade that builds the quiescent center or columella cells.

**ICAR1201**

A SYSTEMS BIOLOGY APPROACH TO IDENTIFY TRANSCRIPTION NETWORKS IN ARABIDOPSIS LEAF SENESCENCE

Category: Bioinformatics, Modeling, and Systems Biology

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The leaf is one of the fundamental organs of higher plants and its primary function is carbon and energy assimilation through photosynthesis. Senescence is the process by which the plant dismantles the leaf structure and mobilises the valuable nutrient reserves that have been stored in the leaf during development, for use in further growth. The leaf is also the key organ by which plants detect and respond to environmental changes. Environmental stress leads to premature senescence and there are many complex cross-linking signalling pathways that are involved with both senescence and responses to stress.

Several highly replicated and detailed time course experiments have been carried out to develop and test network modelling methodology. The first of these experiments was aimed at a detailed analysis of gene expression changes in a developing leaf from just before full expansion to senescence in plants grown in long days. A highly replicated microarray experiment was carried out and these data have been analysed to identify genes whose expression is significantly modulated over time. In another microarray experiment, gene expression during senescence in plants grown in short days has been measured. In both cases significantly changing genes were clustered using SplineCluster (Heard et al. 2006 JASA 101:18-29) and groups have been fit for state space modelling (SSM) using the approach of Beal et al. (2005 Bioinformatics 21:349-356) to infer regulatory network models. These models have suggested a number of novel regulatory hub genes and a selection of these have been tested using knockout and/or overexpression mutants. Comparisons made between the models from the two experiments will help to reveal common regulatory interactions that are involved in controlling leaf senescence under two conditions. Further timecourse experiments are underway using leaf tissues subjected to biotic or abiotic stress and analysis of these will reveal the extent of cross talk between stress responses and senescence.

**ICAR1202**

GREENPHYLDB: A PLANT COMPARATIVE GENOMICS PLATFORM

Category: Bioinformatics, Modeling, and Systems Biology

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**GreenPhyIDB** (<http://greenphyl.cines.fr>) is a comprehensive platform designed to facilitate comparative functional genomics in *Arabidopsis thaliana* and *Oryza sativa* genes. GreenPhyIDB integrates phylogenetic concepts for high accuracy prediction of ortholog relationships and putative intra-species neo-functionalization and/or genetic redundancy. The main function of GreenPhyIDB allows to assign *A. thaliana* and *O. sativa* sequences to gene families using a semi-automatic clustering procedure and to create orthologous groups using a phylogenomic approach. GreenPhyIDB nowadays comprises the most complete list of plant gene families (6,423 families) which have been manually curated. GreenPhyIDB also contains all the phylogenomic relationships computed for 4,406 families and is the only plant phylogenomics database that provides an indicator of phylogenetic prediction through bootstrap procedure.

Moreover, GreenPhyIDB platform includes a specific analysis tool named **GOST** (GreenPhy Orthologous Search Tool) developed to predict *O. sativa/A. thaliana* ortholog using a protein sequence from any other plant species.

**ICAR1203**

GLOBAL DISCOVERY AND FUNCTIONAL ANNOTATION OF ARABIDOPSIS CIS-REGULATORY ELEMENTS

Category: Bioinformatics, Modeling, and Systems Biology

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A basic unit of a gene regulatory network is the binding of a protein (Transcription Factor or TF) to specific patterns of nucleotides in the promoters of other genes called a cis-regulatory element (CRE). Our high throughput computer program tests the biological significance of CREs by first identifying putative CREs around every *Arabidopsis* gene, including 5', 3' UTR, and introns, and correlating presence/absence, position and orientation of the element with gene expression on 1300 different DNA microarrays. This is designed as a distributed high performance computing system with web server modules, statistics and computing module driven by a cluster machine and SQL management module to store and update biological datasets. Our goal is to use this algorithm to test every known CRE and systematically discover all *Arabidopsis* CREs, thus building a comprehensive component library of the promoter. Additionally, our program functionally annotates CREs by calculating the P-value (based on statistical test) for the involvement of that CRE in each experimental condition, mutant gene, treatment and tissue/organ, and by correlating the position, orientation, and distance from the transcription start site that are required for the element to function based on analysis of naturally occurring variation in the genome. For example, genes with the ABRE element respond to over 60 different elicitors, including ABA, cold, heat, pathogens, GA, sucrose, and circadian rhythm in a tissue specific manner, but only if the element is located in within 1200bp upstream of the transcription start site or in the 3'UTR. While both orientations are active, no response is observed if ABRE is located in the 5'UTR, introns, further upstream, or in the 3' downstream region. This regulatory fingerprint and the determination of functional range, active orientation and dependency on other elements forms the basis for our improved annotation, as well as eliminating many false positives from current databases.

**ICAR1204**

NOVEL GENE DISCOVERY IN THE BIOSYNTHESIS OF SUBERIN, A BIOPOLYMER

Category: Bioinformatics, Modeling, and Systems Biology

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Suberin, durable and water-insoluble, has become a green polymer candidate to replace plastic. Suberin composition in *Arabidopsis thaliana* is identical to that in potato, and thus the study in former could be applied to economically important species. Suberin is localized in the root endodermis, and synthesized in potato periderm when subjected to biotic and abiotic stresses. We describe our bioinformatics approach to identify new genes in the suberin biosynthesis, including a novel approach for expression clustering using random matrix theory and high performance computing (HPC). We identified co-expressed genes in response to salt and cold stress, and in the root endodermis from our manually curated candidate list of 1107 genes. One cluster of genes (module 6) included many known suberin biosynthetic enzymes and was a good candidate suberin transcriptomic module. Consequently, we identified 1104 additional genes based co-expression with module 6 genes. Our tentative gene list was further refined by identifying cis-regulatory elements in promoters of putative suberin synthesis enzymes and regulators. We found 2880 genes shared at least 2 of 4 cis-regulatory elements identified in module 6 gene promoters. *Arabidopsis* predicted protein interactions were employed to discover 827 new gene-encoding proteins interacting with the proteins encoded by our curated genes. A final list of 429 tentative genes was identified by two out of three methods; while 6 novel candidate genes were identified by all three criteria. In addition, we started testing our candidate genes experimentally and found interesting results in root development.

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#### **ICAR1205**

TOOLS FOR DATABASE-ASSISTED IDENTIFICATION OF *CIS*-REGULATORY ELEMENTS IN PATHOGEN-RESPONSIVE GENES

Category: Bioinformatics, Modeling, and Systems Biology

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Plant defense mechanisms are based on effective pathogen recognition and signal transduction during plant pathogenesis. PathoPlant® has been developed as a relational database to display relevant components and reactions involved in signal transduction related to plant-pathogen interactions. Expression of specific proteins directed towards infecting pathogens involves transcriptional gene activation upon pathogen attack. Therefore, PathoPlant has been complemented by 66 Affymetrix and cDNA microarray gene expression experiments with *Arabidopsis thaliana* subjected to pathogen infection and elicitor treatment. Web tools enable identification of plant genes regulated by a specific stimulus and of gene sets co-regulated by up to three stimuli. This results in a table listing the genes and the respective stimuli. The search can be restricted to certain induction factors to identify, e.g. strongly up- or down-regulated genes. To identify common *cis*-regulatory elements in co-regulated genes, a resulting gene list can directly be exported to the AthaMap database of transcription factor binding sites. Sets of co-regulated genes can further be used for motif sampling to identify new *cis*-regulatory elements in pathogen-responsive promoters. A new feature within the gene expression analysis of PathoPlant is the possibility to highlight 5323 genes that have been tagged as potential targets of small RNAs. These genes can be subtracted from the analysis in order to enhance prediction accuracy by considering exclusively transcriptionally regulated genes. PathoPlant combines different tools to establish predictions and to learn more about the complex interactions between plants and pathogens. PathoPlant is available online at <http://www.pathoplant.de/>

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#### **ICAR1206**

VIRTUALPLANT: A SOFTWARE PLATFORM TO SUPPORT SYSTEMS BIOLOGY RESEARCH IN THE POST-GENOMIC ERA.

Category: Bioinformatics, Modeling, and Systems Biology

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In the post-genomic era, data generation is no longer the limiting factor for advancing biological research. Instead, data integration, analysis and interpretation are the bottlenecks and challenges that biologists face everyday in genomic research. We wish to aid biologists to take advantage of the burgeoning amount of genomic data, by developing a software platform that enables scientists to visualize, integrate and analyze genomic data from a **systems biology perspective**. We term this software platform "VirtualPlant".

The VirtualPlant platform integrates genome-wide data concerning the known and predicted relationships among genes, proteins and molecules, as well as genome-scale experimental measurements. VirtualPlant automates the use of mathematical and statistical methods to help summarize and quantify the data. VirtualPlant implements and combines these quantitative and qualitative (e.g. visual) approaches to data integration and analysis using a web-accessible interface. **VirtualPlant can be used to help biologists mine genomic data to address questions such as: "What are the molecular mechanisms by which internal and external perturbations affect processes and gene networks controlling growth and development?"**

Whereas the VirtualPlant project was developed specifically for *Arabidopsis*, the data structures, algorithms, and visualization tools are designed in a species-independent way. Our future plans include providing support for other plant species. The VirtualPlant system is available from

<http://www.virtualplant.org>

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#### **ICAR1207**

DEVELOPMENT OF A NEW VARIETY OF RICE FOR EFFECTIVE PREVENTION OF PEOPLE AND THEIR ENVIRONMENT FROM ARSENIC CONTAMINATION  
Category: Bioinformatics, Modeling, and Systems Biology

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The final goal of our research is to eliminate arsenic contamination of rice, the staple food in many countries e. g., Bangladesh, China or Japan. For instance, in Bangladesh every year more than 30 millions people are affected from rice-derived arsenic contamination leading to severe damage of kidney, liver, lungs, bladder and many other neurological and vascular disorders. To solve this problem we plan to generate genetically modified varieties of rice either by modifying native gene(s) responsible for arsenic uptake or by insertion of foreign genes responsible for arsenic metabolism "in planta". We will also attempt to modify molecular mechanisms involved in localization of arsenic to non-edible parts of the plant. For identification of these genes we have employed data mining, an *in silico* analysis based on searching of the existing genomic databases. Data mining resulted in identification of four candidate genes that are involved either in uptake, transport or cellular localization of arsenic in plants. However, we found only one gene that might be involved in arsenic metabolism in rice. We are now studying the 3-D structures of these genes for further understanding of their function. As an alternative to *in silico* analysis we have also screened available T-DNA insertion mutants for identification of the candidate genes. Results obtained in both *in silico* analyses and screening of T-DNA insertion mutants will be then utilized for designing gene cloning experiments e. g., cloning of target genes by PCR, inverse PCR, RT-PCR or plasmid rescue. The cloned target genes will be studied in heterologous systems such as the yeast or *E. coli*. Vectors containing the target genes will be constructed for transformation of rice. For validation of the transgenic results we will also include yeast and *Arabidopsis thaliana* as models in our experiments. For investigation of potential genes/gene products we constructed a kinetic model to outline strategies for developing genetically modified plants exhibiting a significant reduction in arsenic concentration in the edible parts (straw and grain). This model contains equations for uptake, metabolism and sequestration of different types of arsenic (AsV, AsIII, MMAA and DMAA). The model has been implemented in the software XPP and validated against data existing in the literature.

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### ICAR1208

#### PREPERATION AND USAGE OF GENE COEXPRESSION DATA

Category: Bioinformatics, Modeling, and Systems Biology

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Gene coexpression provides key information to understand living systems because coexpressed genes are often involved in the same or related biological pathways. Coexpression data are now used for a wide variety of experimental designs, such as gene targeting, regulatory investigations and/or identification of potential partners in protein-protein interactions. We constructed two databases for *Arabidopsis* (ATTED-II, <http://www.atted.bio.titech.ac.jp>) and mammals (COXPRESdb, <http://cpxpresdb.hgc.jp>). Based on pairwise gene coexpression, coexpressed gene networks were prepared in these databases. To support gene coexpression, known protein-protein interactions, common metabolic pathways and conserved coexpression were also represented on the networks. We used Google Maps API to visualize large networks interactively. The relationships of the coexpression database with other large-scale data will be discussed, in addition to data construction procedures and typical usages of coexpression data.

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### ICAR1209

#### COMBINING 'OMICs DATA FOR A SYSTEMS LEVEL PICTURE OF ORGANELLE METABOLISM IN PLANTS

Category: Bioinformatics, Modeling, and Systems Biology

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Energy metabolism in plants is conducted by three organelles: the mitochondrion, chloroplast and peroxisome. The proteomes of these organelles have recently been elucidated by experimental proteomics data in combination with other non-high throughput methods, such as green fluorescent protein (GFP) tagging. Such data in the model plant *Arabidopsis* have been collated in our database of SUbcellular Localization in *Arabidopsis* (SUBA). Organelle proteomes were combined with public data on metabolic pathways in the Aracyc database, as well as manually-curated pathway data, to construct metabolic networks for these organelles. Networks were visualized and analyzed with the Cytoscape software. We present and analyze various network topology parameters. Some of these suggested refinements to the metabolic networks during the course of their construction. For example, terminal metabolite nodes, which represent species that are imported or exported from the organelle in question, were examined in the context of expectations based on known behavior. Well-characterized biochemical pathways, such as the beta-oxidation pathway in plant peroxisomes, are present in the constructed metabolic networks, and we also find possibly novel biochemical pathways that can be investigated further experimentally. Our networks represent a starting point for quantitative modeling of organelle metabolism in plants.

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### ICAR12010

#### COMBINING THE *S. CEREVISIAE* PROTEIN-PROTEIN INTERACTION NETWORK WITH AN *A. THALIANA* GENE EXPRESSION NETWORK

Category: Bioinformatics, Modeling, and Systems Biology

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For each gene in *A. thaliana* that has an ortholog in *S. cerevisiae*, we have identified which genes it is co-regulated with based on over 300 microarray chips. The *S. cerevisiae* protein-protein interaction network, as extracted from the BIND database, was converted into the corresponding *A. thaliana* orthologs. The two resulting networks (i.e. the gene expression network and the orthologous protein-protein interaction database) were then compared. This analysis found that genes that are likely to interact based on the *S. cerevisiae* protein-protein based interaction network are statistically likely to be co-regulated. This demonstrates that using genome-wide scale data from *S. cerevisiae* can provide useful information with respect to *A. thaliana*. With the redundancy in the *A. thaliana* genome, this allows us to identify likely interacting partners based on co-regulated partners.

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**ICAR12011**

GROUND TISSUE NETWORKS OF THE *ARABIDOPSIS* ROOT

Category: Bioinformatics, Modeling, and Systems Biology

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The successful function of tissues is dictated by the ability of cells to acquire identity and differentiate appropriately in response to spatial, temporal, and environmental signals. To understand how cells integrate these inputs into complex tissue processes, informational pathways that specify and instruct cells will need to be studied. Recent technological advances combining fluorescence activated cell sorting methodologies (FACS) of cell-type-specific fluorescent reporter lines and genomic approaches have led to cell-specific gene expression patterns in the *Arabidopsis* root, called the 'root map' on the developmental time scale with unprecedented resolution (Birnbaum *et al.*, 2003; Brady *et al.*, 2007). Describing all of the relationships between molecular components and signals governing cellular identity and behavior in the *Arabidopsis* root (networks and network states) will require systems biology approaches. As a first step, data from the root map has been mined and different experimental techniques are being used to unveil the transcriptional circuitry that underlies the successful function of ground tissue networks in the *Arabidopsis* root.

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**ICAR12012**

IDENTIFICATION OF TRANSCRIPTIONAL TARGETS OF THE TRANSCRIPTION FACTOR, ATMYB61

Category: Bioinformatics, Modeling, and Systems Biology

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AtMYB61, a member of the R2R3-MYB family of transcription factors in *Arabidopsis thaliana*, modulates gene expression in response to diurnal cues so as to appropriately modify the aperture of stomata. AtMYB61 also alters gene expression in response to sugars, resulting in modification of plant architecture and cell wall structure. Recently, three putative downstream target genes of AtMYB61 were identified. The three putative targets of AtMYB61 were predicted on the basis of comparative transcriptome analyses between microarrays that examined gene expression changes that were modulated by difference in AtMYB61 activity and sugar and those that examined the co-expression of AtMYB61 across development and in different organs (Pearson correlation coefficient >.75). These gene targets encode the following gene products: a KNOTTED1-like transcription factor (KNAT7, At1g62990); a caffeoyl-CoA 3-O-methyltransferase (CCoAOMT7, At4g26220); and a pectin-methylesterase (PME, At2g45220). Statistically over-represented motifs were identified in the 5' non-coding regions of the three putative target genes, and these correspond to previously characterized AC element motifs that function as R2R3-MYB targets. The consensus motif functions as a bona fide target for AtMYB61 binding as determined by an electrophoretic mobility shift assay. Binding between the gene regulatory sequences of the putative target genes, which contain multiples of these motifs, was confirmed via electrophoretic mobility shift assays. Altogether these experiments provide assessment of the ability of AtMYB61 to bind to gene regulatory sequences present in the 5' non-coding sequences of the three putative downstream targets: KNAT7, CCoAOMT7 and a PME, substantiating its role as a potential regulator of the transcription of these genes.

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**ICAR12013**

A COMPUTATIONAL PIPELINE FOR THE PREDICTION OF TRANSCRIPTION FACTOR BINDING SITES IN A. THALIANA APPLIED TO TISSUE-SPECIFIC EXPRESSION AND ABIOTIC STRESS RESPONSE.

Category: Bioinformatics, Modeling, and Systems Biology

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The *de novo* prediction of transcription factor binding sites (TFBS) from promoter sequences has received considerable attention in the literature of late. While many methods have been proposed, none have emerged as a definitive means for accurately predicting cis-regulatory motifs. Moreover, as would be expected, the definition of a valid biological context is critical to the successful identification of biologically relevant motifs. In the case of methods that search the promoter sequences of coregulated gene clusters, success requires both of: 1) a high-degree of confidence in the existence of a common regulatory mechanism among genes in the cluster and, 2) biological accuracy in the promoter sequence search space chosen for each gene. Indeed, many methods will return significant motifs even for randomly chosen promoter clusters and are often subject to "over-fitting" issues in their predictions. As a means of assessing predictions from various methods within a common statistical framework, we have developed a sampling procedure, Bootmer2, that will return a non-parametrically determined enrichment score for any putative binding site as compared to a carefully chosen promoter background. In addition, we apply an algorithm, Promomer2, that will exhaustively permute all TFBS possibilities for subsequent enrichment assessment using Bootmer2. These tools, in combination with several popular prediction programs, have been applied to predicting TFBS in highly coregulated gene clusters from *A. thaliana*. Gene clusters are obtained using a novel coregulation technique and show expression profiles that demonstrate exclusive responses to certain abiotic stress conditions or tissue-specificity. Results include putative TFBS in abscisic acid responsive genes belonging to both the bZIP and MYB regulated components of the pathway and elements potentially regulating the seed specific expression of many genes.

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**ICAR12014**

PLANT GENOME CENTRAL A PORTAL TO GENOMIC RESOURCE AT NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION

Category: Bioinformatics, Modeling, and Systems Biology

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National Center for Biotechnology Information (NCBI) provides integrated systems for the data storage, retrieval, and analysis. Entrez information systems enables text search and retrieval across various databases at NCBI. Main NCBI genome browser, Map Viewer, allows for the aligned display of different types of maps like genetic, physical, and sequence based as well as many different objects like Genes, STSs, markers, and probes. There are currently forty one higher plants, two algae and the nucleomorph genomes of *Guillardia theta* and *Hemiselmis andersenii* displayed in the Map Viewer. Genome Project database is a collection of all large scale sequencing and mapping projects that allows for the display of project specific data and provides for the status of the various sequencing projects. In addition there are plant specific tools – PlantBLAST for BLAST against accessions associated with mapped loci and plant-EST BLAST that BLASTs against ESTs from those plants with more than 50,000 ESTs. Plant Genome Central is a web portal to all the above tools and plant specific resources at NCBI providing a framework for comparative genomics and potential gene discoveries

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**ICAR12015**

UNDERSTANDING THE FUNCTION OF UNKNOWN GENES IN ARABIDOPSIS BY QUANTITATIVE REAL TIME PCR EXPRESSION PROFILING AND PROMOTER-REPORTER EXPRESSION ANALYSIS IN TRANSGENIC PLANTS

Category: Bioinformatics, Modeling, and Systems Biology

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A variety of platforms, including cDNA microarray, Affymetrix GeneChip, whole genome tiling array, massively parallel signature sequencing, have been used extensively to study gene expression in *Arabidopsis thaliana*. However, there are still many genes in the Arabidopsis genome that could not be profiled effectively by any of these methods, generally due to the low abundance of their transcripts in mRNA populations. We are therefore generating expression profiles by quantitative real time PCR (qRT-PCR) which is about 2-3 orders of magnitude more sensitive than hybridization-based technologies for many of the function of unknown genes in Arabidopsis currently lacking reliable expression data. To date, we have performed qRT-PCR on about 2,000 genes using mRNAs from leaf, root, bud, siliques and T87 cell culture and seedlings treated with IAA, SA and salt. Over 90% of the genes were expressed in at least one of our current cDNA populations and ~ 40% of them showed differential expression in at least 2 out of 8 conditions. We are using co-expression analysis to associate these low-expressing genes with functionally annotated genes and pathways. In addition, we have developed a high throughput pipeline to generate promoter-reporter constructs and transgenic Arabidopsis plants for a subset of these genes of unknown function. So far, promoters from 587 genes have been cloned into a GFP reporter construct and 495 have been transformed into Arabidopsis. All the GFP expression patterns detected in these transgenic plants are localized to small regions of tissues and cell types. Both the qPCR and reporter construct data can be found at [www.tigr.org/tdb/e2k1/ath1/qpcr/index.shtml](http://www.tigr.org/tdb/e2k1/ath1/qpcr/index.shtml). Supported by NSF 2010.

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## NON-ARABIDOPSIS SYSTEMS

### ICAR1301

IDENTIFICATION OF *ARABIDOPSIS* HOMOLOGOUS STRESS RESPONSE GENES IN TWO ECONOMICALLY IMPORTANT FRUIT CROPS: *CITRUS SP.* AND *VITIS SP.*

Category: Non-Arabidopsis systems  
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Environmental stresses like water deficit and nutrient excess or deficit in the soil affect productivity in economically important crops. The study of diverse plant stresses in the model plant *Arabidopsis thaliana* facilitates the elucidation of the physiologic mechanisms underlying adaptation to extreme conditions and the identification of genes responsible of stress tolerance. Nevertheless these genes need to be identified and studied in unrelated economically important plant species.

We are interested in the identification of stress response genes in two fruit crops: *Citrus sp.* and *Vitis sp.* (grapevine). This will allow in the future to genetically improve these crops. Using different public database we identified in *Vitis vinifera* a gene homologous to *MYB60*, a transcription factor which regulates stomatal aperture. At $MYB60$  mutants show drought tolerance. In grapevine, *VvMYB60* gene is mostly expressed in seeds and leaves. Its sequence analysis shows the presence of several ABA responsive and water stress responsive elements, the same as its *Arabidopsis* putative orthologue. Gene fusions of *VvMYB60* to *GFP* indicated protein localization in the cell nucleus. Another gene of interest, a boron transporter, was found in *Citrus macrophylla*. Boron is a nutrient differentially distributed in soils in our country generating important losses due its excess or deficiency. *CmBor1* is homologous to *AtBor1*, both with ten trans-membrane domains. *CmBor1* is mostly expressed in roots and stems but also in fruit and floral tissues. Gene fusions of *CmBor1* to *GFP* indicated protein localization in the plasma membrane. Complementation studies in yeasts are now in progress to functionally characterize this boron transporter.

Acknowledgements: CORFO-07Genoma 01, Innova-Chile 204-4037, Chilean Wine Consortium and Millennium Nucleus for Plant Functional Genomics (P06-009-F)

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### ICAR1302

FUNCTIONAL ANALYSIS OF SELECTED PROMOTER SEQUENCES OF DEFENCE-RELATED GENES IN POPLAR AND ARABIDOPSIS.

Category: Non-Arabidopsis systems  
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The recent completion of the *Populus trichocarpa* (poplar) genome sequence enables comparative genomics studies between this woody perennial and the two previously sequenced angiosperms, Arabidopsis and rice. We performed data mining on a select set of genes related to the stress response (wounding and plant-microbe interactions) by combining large data-sets available from Arabidopsis research and our own results on poplar. An *in silico* analysis of the promoter sequences for the selected set of genes was conducted looking for the presence of W-boxes, a *cis*-element recognised and bound to by the defence-related transcription factor WRKY. Our analysis confirmed that the defence-related promoters, from both Arabidopsis and poplar, contained more W-boxes than were found in randomly chosen non-defence-related promoters. We took advantage of using a transient transformation system using Agrobacterium to perform rapid functional studies of several promoters simultaneously. This approach allowed us to measure promoter strength, in response to stress treatments, using GUS as a reporter gene. Further delineation of important promoter elements will be possible by deletion analysis. Identified promoter elements will then be used to discover genes encoding the proteins binding to these regions using the yeast one-hybrid approach.

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### ICAR1303

SECRETORY VESICLE STREAMING AND TARGETING IN POLLEN TUBE GROWTH

Category: Non-Arabidopsis systems  
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Changes in cell shape associated with plant cell growth and morphogenesis require the concerted action of two mechanical processes: the deformation (stretching) of the existing cell wall and the local secretion of cell wall precursors. The precise targeting of the secreted material is therefore pivotal in order to obtain a particular change in cell shape. Tip growing cells such as pollen tubes and root hairs are ideal model systems to study the processes involved in plant cell morphogenesis. In these cells the surface expansion and secretion events are confined to a very small area at the very tip of the cell resulting in unidirectional growth that is easily quantified.

Our goal is to generate a mechanical model of the growth process in the rapidly growing pollen tube. An important quantitative parameter required for this model is the precise location and quantity of the delivered wall material. These components (polysaccharides and proteins) are exported by exocytotic events. The fusion sites of the secretory vesicles with the plasma membrane are expected to correspond to the zones where the new material is incorporated in the cell wall.

We monitored the intense vesicle trafficking at the apex of lily pollen tubes by labeling the vesicles with the phosphomembrane fluorescent dyes FM 1-43. Using space-time image correlation spectroscopy (STICS) and fluorescent recovery after photobleaching (FRAP), we characterized the vesicle dynamics in the apical and subapical regions, and specified the vesicle targeting at the apex.

Plants have numerous genes encoding potential trafficking proteins but the ones involved in exocytosis events at the pollen tube tip, are still unknown. We therefore undertook the identification of *Arabidopsis* proteins involved in vesicle targeting and fusion to the tip plasma membrane, using GFP-fusions and reverse genetic strategies.

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**ICAR1304**

CONTROL OF SHOOT BRANCHING IN SOLANACEAE

Category: Non-*Arabidopsis* systems

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The morphological diversity of plant architecture greatly depends on the control of axillary meristem development. These meristems may remain dormant as axillary buds or grow out to give new shoots in response to endogenous and environmental signals that allow the plant to adapt its growing habits to changing conditions. Understanding the pathways by which external and developmental signals are translated into growth arrest/stimulation at axillary positions, is a biological problem of great agronomical interest. The *Arabidopsis* gene BRANCHED1 (BRC1) coding for a TCP transcription factor, plays a central role in this process: it integrates external and endogenous signals and act as local switches of axillary bud growth: its activity causes axillary bud arrest and its down-regulation is necessary for branch elongation.

We are now investigating the conservation of this function in Solanaceae. We have isolated the orthologs of BRC1 in tomato and potato and we are currently investigating their function in these species during axillary meristem development and axillary bud outgrowth. This process affects the formation of branches in both species but also stolon development and tuber sprouting in potato.

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**ICAR1305**

RSG, A BZIP TRANSCRIPTIONAL FACTOR, IS INVOLVED IN THE FEEDBACK REGULATION OF THE GA 20-OXIDASE GENE

Category: Non-*Arabidopsis* systems

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RSG(for REPRESSION OF SHOOT GROWTH) is a tobacco transcriptional activator with a basic leucine zipper domain that is involved in the regulation of endogenous amounts of gibberellins (GAs). The dominant negative form of RSG repressed the expression of the *ent*-kaurene oxidase gene of the GA biosynthetic pathway in transformed tobacco plants. This down regulation reduced endogenous amounts of GAs and severely inhibited the process of cell elongation of stems, resulting in a dwarf phenotype. We found that GA levels regulate the intracellular localization of RSG. RSG translocated into the nucleus in response to a reduction in GA levels. GA treatment could reverse this nuclear accumulation. This observation suggested that RSG plays a role in the feedback regulation of GA biosynthetic genes. The genes of *GA 20-oxidase* and *GA 3-oxidase* are regulated by feedback mechanisms in several plants, including *Arabidopsis* and tobacco. We found that the dominant negative form of RSG inhibited the feedback regulation of the *GA 20-oxidase* gene but not of the *GA 3-oxidase* gene.

To explore the molecular mechanisms of the transcriptional regulation of the *GA 20-oxidase* gene by RSG, we identified a *cis*-acting sequence responsible for the GA-negative feedback using transgenic plants. Furthermore, we found that RSG directly binds to the *cis*-acting sequence in the gel retardation assay. The mutation in the *cis*-acting sequence abolished both GA-negative feedback and RSG binding. Chromatin immunoprecipitation analysis showed that RSG binds to the *GA 20-oxidase* promoter in vivo in response to a reduction in GA levels. The RSG orthologue in *Arabidopsis* promoted the transcription of a gene encoding *Arabidopsis GA 20-oxidase* in the transient assay. Our results suggest that RSG play a role in the homeostasis of GAs through binding to the *cis*-acting sequence of the GA-negative feedback of the *GA 20-oxidase* gene.

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**ICAR1306**

CHARACTERIZATION OF THE INTERACTION BETWEEN TWO POPLAR STRESS RESPONSIVE MAPKS AND AN EAR-REPRESSOR THAT BELONGS TO THE CYS2/HIS2-TYPE ZINC FINGER PROTEIN FAMILY.

Category: Non-*Arabidopsis* systems

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MAPK cascades contribute to the establishment of plant disease resistance by modulating numerous downstream signalling components including transcription factors. Such DNA binding proteins in turn regulate expression of specific gene sets that will insure plant adaptation and survival. In order to shed some light on molecular events involved in poplar rust resistance, we have isolated several stress responsive MAPK interacting partners. Among these putative interactors, we discovered a Cys2/His2-type zinc finger protein that was named PtZFP1 (*Populus trichocarpa* Zinc Finger Protein 1). This putative transcription factor belongs to a large family of transcriptional repressors that depend on an EAR motif for their repression activity. Interestingly, this motif was also found to be essential for MAPK binding in our experiments. Close examination of PtZFP1 primary protein sequence revealed a functional bipartite MAPK docking site that partially overlaps with the EAR motif. Since features of MAPK docking surface were conserved among other classes of EAR-repressors, these results suggest a novel and exciting mode of defense mechanisms regulation involving stress responsive MAPKs and EAR-repressors.

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**ICAR1307**

## IDENTIFICATION OF PHOTOSYNTHESIS-RELATED GENES USING FOX HUNTING SYSTEM

Category: Non-Arabidopsis systems

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Photosynthesis is one of the most important determinants of plant productivity. To identify photosynthesis-related genes, we generated Arabidopsis FOX (Full-length cDNA Overexpressor) lines that Arabidopsis and Rice full-length cDNAs were over-expressed in Arabidopsis. We have used imaging of chlorophyll fluorescence to screen photosynthesis-related mutants. To confirm over-expression of transformed cDNAs cause the observed phenotype, we generated Arabidopsis transformants overexpressing each cDNA isolated from candidates, resulting that 13 (Arabidopsis) and 12 (rice) re-transformed lines showed the same phenotype as the original lines. Overexpression of chloroplast ribosomal protein and chloroplast Clp protease subunit arising from both Rice and Arabidopsis cDNAs lead to similar photosynthetic phenotype, suggesting that Rice cDNAs can serve the same function as Arabidopsis. For further photosynthetic characterization, we measured steady state chlorophyll fluorescence using PAM fluorometry. Isolated candidates could be classified into five categories depending on the chlorophyll fluorescence parameters. Moreover, we analyzed gene expression profile of three re-transformed lines showing similar photosynthetic characteristics using DNA microarray. We found commonly regulated genes among three candidates, suggesting that candidates have shared influences on gene expression. Some of rice transformants overexpressing rice cDNAs isolated from candidates exhibited the same photosynthetic phenotype as the original lines, indicating that FOX hunting system can be used for identification of useful genes derived from different organisms.

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### ICAR1308

#### GENETIC ANALYSIS OF SELF-COMPATIBLE MUTANTS IN *BRASSICA RAPA*

Category: Non-Arabidopsis systems

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Most flowering plants produce hermaphrodite flowers, which can lead to inbreeding and loss of genetic diversity. To prevent inbreeding, *Brassica* species have self-incompatibility (SI) system for rejecting the self pollen. It has been revealed that SI in *Brassica* is primarily controlled by a multi-allelic *S* locus which includes two tightly linked genes, *SRK* (the female determinant) and *SP11* (the male determinant). However, little is known about downstream factors of signal transduction and rejection mechanism of self pollen. The objective of this study is to identify factors related to SI, by genetic analysis of self-compatibility (SC) mutant in *Brassica rapa*.

We isolated five SC mutant lines, LVC28, LVC17, K28, K4 and K2. In five SC mutants, *SRK* and three known downstream factors, *MLPK*, *ARC1* and *THL*, expressed normally, and *SP11* also expressed in all lines, except for LVC17, indicating that all known SI factors are functional in four SC mutants lines, at least. In genetic analysis of *F<sub>2</sub>* population between LVC28 (SC) and S<sup>8</sup> rapid cycling (SI), the segregation ratio of SI to SC was 5 to 1. In this population, genotype of *S* locus did not link to segregation pattern of SC phenotype. These results suggest that more than one SI-related gene has been mutated and are independent from *S* locus in LVC28. To identify new SI factors leading to SC phenotype in the mutant lines, we are currently investigating the linkage DNA markers for SC phenotype in *F<sub>2</sub>* population of mutant lines.

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### ICAR1309

#### INVESTIGATION OF A PUTATIVE MOBILE, LONG-DISTANCE SIGNAL THAT PROMOTES FLOWERING IN MAIZE

Category: Non-Arabidopsis systems

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#### Investigation of a Putative Mobile, Long-Distance Signal that Promotes Flowering in Maize

Chloé Lazakis and Joseph Colasanti

The transition to floral development is a crucial stage in the life of plants. Higher plants use a multitude of perceptive measures to regulate the floral transition, which involves the careful coordination of both environmental and endogenous cues. Recent molecular and genetic analyses in Long-Day (LD) *Arabidopsis* suggest that a long-distance florigenic signal may be the protein encoded by *FLOWERING LOCUS T* (*FT*). *FT* is activated in mature leaves by the CONSTANS (CO) regulatory protein and travels through the phloem to the shoot apex where it interacts with the transcription factor *FLOWERING LOCUS D* (FD). The FT/FD complex then activates a cascade of floral identity genes to promote the transition to flowering. In contrast to *Arabidopsis*, temperate maize is a Day-Neutral (DN) plant, while teosinte, its ancient progenitor, has an obligate requirement for Short-Days (SD) to flower. Recent discoveries of *FD* and *FT* homologs (*DLF* and *ZCN*, respectively) in maize support the notion of a universal, long-distance florigenic compound that induces floral transition. We are attempting to verify if *FT* and *FD* functional orthologs exist in maize and if the *FT/FD* regulatory module functions to promote the transition to flowering in this monocot species. Furthermore, we are comparing the expression of these genes in wildtype vs. *indeterminate1* mutant (severe late flowering) maize. *INDETERMINATE1* (*ID1*), which encodes a zinc finger regulatory protein, is one of only two genes known to specifically affect the transition to flowering time in maize. In addition, expression of the putative maize *FT* and *FD* orthologs will be examined in florally induced vs. un-induced teosinte.

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**ICAR13010**

ORGAN-SPECIFIC AND PATHOGEN INDUCIBLE EXPRESSION PROFILES OF POPLAR MAP KINASE KINASES (PTMKKS) AND MAP KINASE (PTMPKs).

Category: Non-Arabidopsis systems

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MAPK signal transduction modules play crucial roles in regulating many biological processes in plants, and their components are encoded by highly conserved multi-gene families. While extensive work has been conducted on the post-translational regulation of specific *MKKs* and *MPKs* in various plant species, there has been no systematic investigation of the transcriptional regulation of these genes. Expression profiles of all members of the *PTMKK* and *PTMPK* gene families were analyzed in various hybrid poplar organs (*Populus trichocarpa* X *P. deltoides*) using real-time quantitative PCR (RTqPCR). In parallel, we also investigated the expression profiles of the poplar *PTMKKs* and *PTMPKs*, in *P. nigra* X *P. maximowiczii* (*NM*) following leaf infections with the biotrophic rust fungi *Melampsora medudae* f. sp *deltoides* (*Mmd*) and *M. larici populina* (*Mlp*) which give rise to complete susceptibility and quantitative resistance respectively. This study highlights the technical challenges with primer design for RTqPCR analyses when hybrid poplars are used. Our results showed contrasting expression levels of the *PTMKK* and *PTMPK* genes in different poplar organs. Moreover, as in other plant systems, we clearly demonstrate that a specific set of poplar *PTMKKs* and *PTMPKs* are differentially regulated following pathogen challenge.

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**ICAR13011**

COMPARATIVE GENOMICS BETWEEN *ARABIDOPSIS* AND *BRASSICA*

Category: Non-Arabidopsis systems

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The cultivated *Brassica* species represent the group of crops most closely related to *Arabidopsis thaliana*, the model plant for dicots. Recent studies in this species have yielded new insights into plant genetics and genomics. With a completion of the full-genome sequencing of *A. thaliana*, a pattern of segmental chromosomal collinearity has been identified between *Arabidopsis* and *Brassica* crops. The level of genomic synteny between them provides a good opportunity to study how genetic and morphological variation has developed during the evolution of the genome, including the endurance of certain genetic structures in *Arabidopsis* and related *Brassica* species. The international research community has launched a Multinational *Brassica* Genome Project, one aim of which is to sequence the genome of a *Brassica* species. The genome chosen, on the basis that it is the smallest *Brassica* genome, is that of Chinese cabbage, *B. rapa*. To take a full advantage of such efforts, we aim to integrate genetic and genomic resources across the Brassicaceae and focus on genetic map integration of *Brassica* A genome within the most important *Brassica* crop especially in North America and Europe, oilseed rape (*B. napus*), the species adopted for genome sequencing, *B. rapa*, and the model species, *A. thaliana*. This will contribute to an understanding of the genetic and chromosomal relationship between them and open new avenues for improvement of *Brassica* crops. It will also yield interesting insights into various researches such as an evolution of plant genome including polyploidy, genomic rearrangement, and synteny.

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**ICAR13012**

MYCOVITALITY VS. MYCOHETEROTROPHY IN PLANTS WITH MINUTE SEEDS

Category: Non-Arabidopsis systems

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Phylogenetically-young Orchidaceae are among the most diversified and adaptable plant groups on earth. Terrestrial orchids grow in the ground, as opposed to orchids that grow on trees (epiphytes) or rocks (lithophytes). More than 10,000 dust-like seeds are produced in a single capsule and, usually and surprisingly, with no embryo or food reserves for germination. Dr. Vujanovic's research team newly discovered Mycovitalism\* - a phenomenon describing a relationship between endophytic fungi and seeds, maintaining their vitality and leading to germination. Mycoheterotrophism is considered as a later mycotrophic stage between two eukaryotic organisms - plant and compatible fungus - leading to further improvement of plant nutrition and stress tolerance. An innovative fungal bioassay to address the question of orchid seed viability is described in which viability is evaluated using co-culture with compatible Fusarium strain. In viable seeds, this leads to seed coloration and germination. The Fusarium phylogenetical status was defined based on sequences of the EF-1 alpha gene. Culture-independent PCR-DGGE fingerprinting method was optimized to select promising strains for orchid seed viability testing in orchid production using in vitro biotechnology. The Orchidaceae were selected as a model system for future studies on plant seed dormancy, vitality and embryogenesis under environmental stress conditions.

\* 2007. Mycovitality and mycoheterotrophy: Where lies dormancy in terrestrial orchid and plants with minute seeds? SYMBIOSIS 44: 93-100

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## NOVEL TOOLS, TECHNIQUES AND COMMUNITY RESOURCES

### ICAR1401

DEVELOPING A NOVEL TISSUE-SPECIFIC SYSTEM IN ARABIDOPSIS

Category: Novel Tools, Techniques, and Community Resources

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There are few effective ways of dealing with essential genes in plants. Tools like allelic series and weak knockdowns are only sometimes effective and not always feasible. Thus, developing a tissue-specific knock down system for the analysis of essential genes, similar in principle to what now is being used in mice and nematodes, could become a valuable tool. Petals are good targets for tissue-specific RNAi because they are large and visible organs, yet not required for growth or reproduction. *Arabidopsis APETALA3 (AP3)* and *PISTILLATA (P)* are floral homeotic genes required for petal development. It has been shown that a portion of the *AP3* or *P* promoters confers petal-specific expression during the cell division phase of growth. The portion of the *AP3* or *P* promoters was used to drive the expression of the GUS RNAi transgene in plants expressing *GUS*. Tissue-specific knockdown of GUS expression was observed for both *AP3* and *P* petal-specific RNAi (psRNAi) transgenes. Petal specificity was further confirmed by fusion the portions of the *AP3* or *P* promoters to *GUS* cDNA. We are currently testing this novel psRNAi system to knockdown the expression of *CENH3* and *MIS12*, both essential genes required for chromosome movement and cell division in plants. The development of an efficient tissue-specific RNAi technique would in principle be useful for many fields of plant biology where essential genes are being studied.

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### ICAR1402

OPTIMIZATION OF GROWTH CONDITIONS FOR FROZEN STORED ARABIDOPSIS THALIANA POLLEN

Category: Novel Tools, Techniques, and Community Resources

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One of the rare weak points of the model plant *Arabidopsis* is the technical problem associated with the germination of its male gametophyte and the generation of the pollen tube *in vitro*. *Arabidopsis* pollen being tricellular it has a notoriously low *in vitro* germination rate compared to species with bicellular pollen. This drawback strongly affects the reproducibility of experiments based on this cellular system. Together with the fact that pollen collection from this species is tedious, these are obstacles for the standard use of *Arabidopsis* pollen for experiments that require high numbers of pollen tubes and for which the germination rates under control conditions need to be highly reproducible. The possibility of freeze storing pollen after bulk collection is a potential way to solve these problems but necessitates methods that ensure continued viability and reproducible ability to germinate.

Our objective was the optimization of germination conditions for *Arabidopsis* pollen that had been freeze stored. We optimized the concentrations of various media components conventionally used for *in vitro* pollen germination. In addition to these components, medium pH and growth temperatures were tested.

Here we summarize the optimized conditions for pollen germination and growth in different media and under different experimental setups. We suggest how to optimally use these methods for different practical experiments ranging from morphological observations of pollen tubes in optical and electron microscopy to their bulk use for molecular and biochemical analyses or for setups for which a specific medium stiffness is critical.

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### ICAR1403

LATCA 3.6K: A LIBRARY OF BIOLOGICALLY ACTIVE SMALL MOLECULES FOR PLANT CHEMICAL GENOMICS.

Category: Novel Tools, Techniques, and Community Resources

Tszufung F. Chow, \*Andrew Defries, Simon E. Alfred, Jignasha Patel, Yang Zhao, Pauline Fung, Xiaofei Li, Peter McCourt and Sean R. Cutler

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Bioactive compound libraries are valuable tools in chemical genomics that can be used to validate new assays and identify known agents that perturb a process of interest. Given their utility, we have assembled a 3680-member screening library named LATCA (for Library of Active Compounds on *Arabidopsis*). Approximately 450 of the LATCA compounds are known, from the literature, to be active in plants, yeast, animals or bacteria. The remaining compounds are mostly uncharacterized and were identified as bioactive in growth-based screens of novel-structure compound libraries. We have systematically characterized LATCA by classifying the phenotypes induced by compounds in *Arabidopsis* etiolated hypocotyls using a binary phenotypic annotation scheme. Hierarchical clustering of this data generated phenoclusters, which were combined with compound-similarity clustering data. These comparisons uncovered both expected and new core structures that phenotype known bioactive compounds such as 2,4-D, isoxaben, oryzalin, uniconazole and brassinazole. To explore the utility of LATCA, we used it in a microscopy-based screen for modulators of ER morphology. This uncovered eroonazole, a new compound with plant-specific activity that causes cortical ER tubules to degenerate into balloon-like structures, suggesting it targets a factor required for ER-tubule maintenance. Thus, LATCA can be used to identify new classes of bioactive molecules, validate assays and identify well-characterized compounds that perturb a process of interest.

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### ICAR1404

ARACYC AND THE PLANT METABOLIC NETWORK: CONNECTING ARABIDOPSIS RESEARCH TO A NEW MULTI-SPECIES METABOLIC RESOURCE

Category: Novel Tools, Techniques, and Community Resources

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AraCyc is a metabolic pathway database containing experimentally verified and computationally predicted pathways for *Arabidopsis thaliana*. Curated information about the genes, enzymes, reactions, and compounds associated with these biochemical pathways is publicly available through the AraCyc website ([www.arabidopsis.org/biocyc/](http://www.arabidopsis.org/biocyc/)). The results of new research on *Arabidopsis* metabolism are continuously added to the database, leading to the release of AraCyc 4.5 in June 2008.

The Plant Metabolic Network (PMN) ([www.plantcyc.org](http://www.plantcyc.org)) is a new project designed to extend the current metabolic resources beyond *Arabidopsis* to all

plants. Its central database, PlantCyc 1.0 includes data from over 250 species of plants in the context of over 500 pathways. The extensively curated information in AraCyc, as well as additional plant pathway data in MetaCyc, are important constituents of PlantCyc. Following its debut, in June 2008, PlantCyc will be used to generate novel metabolic databases for plants with newly sequenced genomes, such as poplar. In this process, putative orthologs are identified by the PMN and related biochemical pathways are predicted by the Pathologic software. These pathways can be displayed together on a metabolic overview map for each species and researchers can use them for the analysis of gene expression, metabolomic, and proteomic data. Over time, as the data content and predictive power of PlantCyc increases, the metabolic overview maps and associated pathway, gene, enzyme, compound and reaction data for individual plant species databases, including AraCyc, will be revised and expanded. Together, AraCyc and the PMN can promote, facilitate, and help connect basic and applied aspects of metabolic research in *Arabidopsis* and other plant species.

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**ICAR1405****NEW REPORTER CONSTRUCTS TO STUDY SITE-SPECIFIC RECOMBINATION IN PATHOGEN INFECTED PLANTS**

Category: Novel Tools, Techniques, and Community Resources

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Molecular aspects of incompatible plant-pathogen interactions have been studied more extensively as compared to compatible one. Analysis of the compatible interaction between Tobacco Mosaic Virus (TMV) and *Nicotiana tabacum* (tobacco) have revealed new details of the plant response. A novel signal has been identified that travels faster than the pathogen in systemic tissues of plants. The signal results in the increase of the frequency of homologous recombination in non-infected systemic tissues of the infected plants. Moreover, the signal leads to various transgenerational changes. The changes include elevated frequency of recombination, instability at certain resistance gene loci, global genome DNA hypermethylation and locus-specific hypomethylation. The hypomethylated loci encode genes with the homology to the gene of resistance to TMV, the *N* gene. Specific changes were noticed in Leucine rich repeats (LRR) regions of these genes. This finding suggests that plant resistance genes could have evolved by recombination mediated duplication and unequal crossing-over events in the LRR regions. Since all the previous studies were done on the transgene, and no data on the rearrangements within LRR regions had been obtained, we attempted to generate the recombination reporter based on the various regions of homology of several resistance genes. In these constructs expression of a reporter gene depends on a recombination event in an upstream sequence under study. In our study, LRR regions from *Arabidopsis* RPS5 and RPP5, and *N* gene from tobacco were selected for construction of the reporter system. Transient expression analyses have confirmed the functionality of the constructs. This new technique opens the opportunity for analysis of sequence-specific recombination rate, which was previously difficult to get done.

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**ICAR1406****SPECIFIC LABELLING OF CELL TYPES, STRUCTURES AND DEVELOPMENTAL STAGES IN ARABIDOPSIS LEAVES**

Category: Novel Tools, Techniques, and Community Resources

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Although our lives are dependent on plants, our understanding of how they grow and how different levels of organisation (*i.e.* whole plant, organ, cell, molecular module and molecule) are linked is still not understood. Therefore, the AGRON-OMICS consortium is using existing and novel tools to collect data that enable us to model the growth of the *Arabidopsis* leaf under non-limiting and limiting environmental conditions (*e.g.* drought). After initiation of the leaf primordial, biomass accumulation is controlled mainly by cell proliferation and expansion in the leaves. However, the *Arabidopsis* leaf is a complex organ made up of at least 18 individual cell types (10 epidermal, 3 mesophyll and 5 vascular) and 11 structures. At the same time, the growing leaf contains cells at different stages of development with the cells furthest from the petiole being the first to stop expanding and subsequently undergo senescence. Sampling entire leaves can therefore give a distorted view of what is going on in only a subset of the cells. Recently, sectioning and GFP lines, expressing GFP in a cell type specific manner, was used to demonstrate this effect in root tips. It was shown that the cell identity and distance from the root tip had a significant effect on the expression profiles of a large number of genes (Birnbaum *et al.*, 2003; Brady *et al.*, 2007). Also, lines containing a cell-type specific GAL4 trans-activation system were used to show that xylem-pole pericycle cells are necessary for lateral root development (Laplace *et al.*, 2005).

The two examples mentioned above show the power of such tools. We are therefore using the LhG4 trans-activation system to develop lines that will allow us to label all the specific cell types, structures and developmental stages in the *Arabidopsis* leaf. This will allow us to create a high-resolution expression map of the leaf and to specifically over-express or repress transcription of genes in a spatial and temporal manner.

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**ICAR1407****USING MATLAB TO PROCESS IMAGES FOR THE ANALYSIS OF PLANT ORGAN GROWTH AND CURVATURE**

Category: Novel Tools, Techniques, and Community Resources

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Image analyses of plant growth phenomena can be achieved by a number of commercially available software programs. However, these programs require considerable user manipulation when collecting the measurements, making it time-consuming to examine large sets of images. Additionally, the software programs that process images do not necessarily offer the ability to integrate complex mathematical calculations within the same program. In this report, we present the use of MATLAB mathematical software and its associated Image Processing Toolbox to create a plant image analysis tool for the collection and measurement of the growth and curvature of plant stems and roots. Our objectives were to create a tool that 1) uses a single program to accomplish multiple steps in image processing and analysis, 2) can be modified for a variety of mathematical measurements,

and 3) has the potential to be automated to process sets of images. The plant image analysis tool first reduces the image of the stem or root to an array of x- and y-coordinates that define the center of the organ. These coordinates are used for measurements of overall growth, curvature, angle, and point of maximum curvature (vertex). Using this tool, we have been able to process images collected from the hypocotyls of horizontally-reoriented etiolated *Arabidopsis* mutants associated with the 1-aminocyclopropane carboxylic acid synthase (*At-ACS*) genes. Our analysis revealed that the increased curvature (compared to wild type plants) observed in two ethylene-overproducing mutants (*eto2* and a T-DNA insertion in *At-ACS4*) was not accompanied by increased growth or a change in vertex location. Current studies are using the plant image analysis tool to evaluate gravitropic curvature in pea stems and *Arabidopsis* roots and inflorescence stalks, as well as for basic organ growth studies.

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**ICAR1408**

## GATEWAY ENTRY CLONES AND DESTINATION VECTORS FOR PLANT GENOME ANALYSIS

Category: Novel Tools, Techniques, and Community Resources

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The Gateway™ technology ([www.invitrogen.com](http://www.invitrogen.com)) has been developed to facilitate the transfer of DNA segments between plasmids by site-specific recombinational cloning. We have constructed a large collection of Gateway-compatible destination vectors for a wide range of gene function analyses in transgenic plant cells. Using MultiSite recombination Gateway cassettes, plant binary destination vectors have also been created in which two or three segments can be transferred contiguously or in independent expression unit, in a single LR clonase *in vitro* reaction. Our destination vectors carry one of three plant selectable markers coding for resistance to kanamycin (*nptII*), hygromycin (*hpt*) or glufosinate ammonium (*bar*), and are available in small high copy number plasmids.

To further streamline the construction of recombinant genes, we have built series of reference Gateway entry clones carrying promoters, terminators, and reporter open reading frames most commonly used in plant research. This collection obeys simple engineering rules: the genetic elements (parts) are designed in a standard format: they are interchangeable, fully documented, and can be combined at will according to the desired output. The Gateway entry clones and destination vectors can be obtained on line (<http://www.psb.ugent.be/gateway>). This web site provides recombinational cloning instructions, as well as experimentally verified sequences, maps and Vector NTI files for each plasmid.

Karimi et al. (2002) GATEWAY™ vectors for *Agrobacterium*-mediated plant transformation. Trends Plant Sci. 7:193-195.

Karimi et al. (2005) Modular cloning in plant cells. Trends Plant Sci. 10:103-105.

Karimi et al. (2007) Building blocks for plant gene assembly. Plant Physiol. 145:1183-1191.

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**ICAR1409**

## USE OF ARABIDOPSIS IN AN INQUIRY-BASED UNDERGRADUATE GENETICS LAB

Category: Novel Tools, Techniques, and Community Resources

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Arabidopsis has been used successfully in a one-semester undergraduate genetics project lab at Adelphi University as an experimental model for undergraduate students to learn key concepts and techniques in classical and molecular genetics and develop their research skills. Students begin with guided labs that introduce the analysis of inheritance patterns, characterization of phenotype using a simple physiological assay, and the fundamentals of PCR-based mapping, all using a collection of auxin-related mutants isolated in the author's research. Students then design and carry out their own phenotypic analysis experiment and pursue rough mapping of their assigned mutants independently. At the end of the course they present their results in a poster session. The approach of the course could be easily adapted to use different types of mutants or genes in various stages of characterization. Student responses to the course have been positive. This inquiry-based course helps foster students' development as scientists and supports a strong culture of student-centered research at Adelphi.

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**ICAR14010**NEW TECHNOLOGY FOR INDUCING DELETION MUTANTS IN *ARABIDOPSIS THALIANA*

Category: Novel Tools, Techniques, and Community Resources

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Ion beams consist of ions accelerated in accelerators, such as cyclotrons or synchrotrons. Ion beam irradiation is an excellent technology for producing mutations to improve horticultural and agricultural crops with high efficiency, and we have marketed seven new flower cultivars in Japan, the United States, Canada, and the European Union since 2002. In the case of *Triticum monococcum*, two independent mutants of wheat APETALA1 were isolated using N ion beam irradiation, followed by phenotype observation and polymerase chain reaction (PCR) screening of target genes in the M<sub>2</sub> generation. This technology is useful for both forward and reverse genetics, and can be used to induce deletion mutations in genome science. In this study, we optimized the conditions of the ion beam irradiation used to induce mutations at high frequency using the cyclotrons in the RIKEN RI-beam factory (RIBF). Dry seeds were irradiated with C (1.52 GeV), N (1.76 GeV), Ne (2.46 GeV), Ar (3.18 GeV), and Fe (3.77 GeV) ions at doses of 5 to 500 Gy, with the linear energy transfers (LET) controlled at 22.5-640 keV/μm. As a result, a LET of 30 keV/μm was the most effective for inducing albino mutants. Then, we screened the morphological mutants from the M<sub>2</sub> progenies after C-, Ne-, and Ar-ion irradiation. Around 300 mutants were isolated from 26,000 M<sub>2</sub> plants, including pale green, variegated, and narrow-leaved mutants. We collected *hy* and *gl* mutants and characterized their mutations. With C-ion irradiation, three deletions (1, 2, and 3 bp) were identified from seven *hy* mutants, and one deletion (47 bp) and one base change (G→A) were found from two *gl* mutants. With Ne-ion irradiation, one deletion (440 bp) was identified from one *hy* mutant. With Ar-ion irradiation, one deletion (16 bp) was identified from three isolated *hy* mutants. These findings revealed that the LET is an important factor for the effective mutagenesis, and that the ion beams predominantly cause DNA deletions.

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**ICAR14011**

REPORT ON PLANT RESOURCE PROJECT IN RIKEN BRC

Category: Novel Tools, Techniques, and Community Resources

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RIKEN BioResource Center (BRC) preserves and distributes biological resources through National BioResource Project (NBRP) supported by Japanese government. Experimental Plant Division provides plant materials including genomic resource of Arabidopsis such as RIKEN Arabidopsis full-length cDNA (RAFL) clones and RIKEN Arabidopsis transposon-tagged mutant (RATM) lines to the world research community. Since 2002, we have distributed approx. 23,000 cDNA clones and mutant lines to more than 1,000 laboratories in the world.

In last year, we started distribution of homozygous seeds of RATM lines. By the end of this year, homozygous seeds for 2,000 lines will be ready for distribution. Seeds for FOX hunting lines of Arabidopsis established by RIKEN Plant Science Center (PSC) will be available in this year. Availability of the full-length cDNA clones of model plants such as *Physcomitrella patens* will be also presented.

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**ICAR14012**

RACE DATABASE: A DATABASE ON A LARGE-SCALE COLLECTION AND PHENOTYPE ANALYSIS OF KNOCKOUT MUTANTS FOR NUCLEAR-ENCODED CHLOROPLAST PROTEINS

Category: Novel Tools, Techniques, and Community Resources

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Most of the chloroplast proteins are nuclear-encoded and function in development and environmental responses of chloroplast. For the functional analysis of the nuclear-encoded chloroplast proteins, we systematically collected their knockout mutant lines in *Arabidopsis*. Based on databases of tagged mutant lines, such as RIKEN, JIC, Wisconsin, CSHL and SALK, we collected 3,240 tagged-lines disrupted by *Ds/Spm* transposon or T-DNA that encode 1,368 chloroplast proteins, which share about 66 % of 2,090 predicted chloroplast proteins (Richly and Leister, 2004). From these mutant resources, we observed visible phenotypes systematically at 3-week-old seedling stage grown on agar plates and recorded their phenotype and germination rates of mutants showing abnormal phenotypes, and then collected homozygous lines without clear phenotypes. Mutants with heterozygous but no homozygous knockouts were also identified in our collections, of which genes are essential in embryogenesis. Now we identified 98 mutants showed clear and reproducible visible phenotypes, 82 mutants with no homozygous mutants, and 1,037 of homozygous mutants without clear phenotypes.

The RIKEN Arabidopsis Chloroplast Encyclopedia (RACE) database presents molecular and phenotypic information on our resources, as well as essential, non-redundant genes of *Arabidopsis* that show an abnormal phenotype at seedlings and developing seeds stages when disrupted by mutation. The RACE database provides tools for searching by AGI code, mutant line number and phenotype, and rapid access to detailed information on the resources. Moreover, our homozygous collections can be expected to yield powerful tools to accelerate and enhance the functional genomics of nuclear-encoded chloroplast proteins in *Arabidopsis*. We have constructed the database for opening to the public, and will share the mutant resource and phenotype information.

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**ICAR14013**

FLOW CYTOMETRY TECHNOLOGY FOR ANALYSIS AND SORTING OF ARABIDOPSIS SEEDS

Category: Novel Tools, Techniques, and Community Resources

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The small size of Arabidopsis seeds makes them a challenging subject material for manual manipulation. Many studies of seeds, involving such parameters as seed size or the expression of some transgenic fluorescent protein, are difficult to pursue because of the difficulty of manually handling them. We show that the COPAS flow cytometry instrument facilitates analyzing and sorting Arabidopsis seeds and distinguishing differences in size, optical density, and the transgenic expression of fluorescent proteins.

This instrumentation allows for automated analysis and sorting of Arabidopsis seeds and provides a greatly increased throughput. By automating the current, time consuming manual processes, the time required for experiments is dramatically reduced, human error is eliminated, and new experiments that previously could not be considered are now possible.

This report demonstrates the capability of the COPAS PLUS instrument to detect different optical and fluorescent properties of the various strains of Arabidopsis. We were able to detect and dispense different seed populations based on size and fluorescent characteristics integrated over the length of the seed. Furthermore, data analysis of the acquired samples allows for quantitative comparison of different seed samples or populations.

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**ICAR14014**

PURIFICATION OF RESISTANCE PROTEIN COMPLEXES USING A BIOTINYLATED AFFINITY TAG

Category: Novel Tools, Techniques, and Community Resources

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Arabidopsis genome encodes about 150 NB-LRR resistance (R) genes, at least some of which are known to confer gene-for-gene resistance

against particular pathogens. Many resistance proteins are associated with the plasma membrane and expressed at low levels. Some studies suggest that NB-LRR proteins function in a large protein complex which may change dynamically upon activation. Identification of unknown members of the resistance protein complexes will provide insights into the mechanisms controlling early events in gene-for-gene resistance.

To facilitate purification of resistance protein complexes, we devised a tripartite peptide sequence termed the HPB tag. This tag consists of one copy of the HA epitope tag, a Prescision® protease recognition site, and the biotinylation site of the Arabidopsis 3-methylcrotonyl-CoA carboxylase. The biotinylation site gets biotinylated in vivo and facilitates high-affinity purification using a streptavidin matrix, while the HA tag allows tracking of tagged proteins using a Western Blot. We tagged multiple proteins with the HPB tag and found that all the tagged proteins are functional and highly biotinylated in vivo. Using transgenic Arabidopsis plants carrying the RPS2::HPB gene driven by the RPS2 promoter, we developed a method to purify RPS2-HPB protein complexes. The optimized purification method involves membrane fractionation enrichment, chemical crosslinking, and solubilization followed by streptavidin bead capture. The purified complexes were subjected to LC MS/MS analysis to identify the proteins present in the complexes. Starting with only 30g of whole plant tissue, we successfully identified RIN4, which is known to physically interact with RPS2, as well as many other potential RPS2-complex components. An alternative to the conventional TAP tagging purification method, the HPB tag-based method utilizes much stronger affinity of binding and fewer steps, making it suitable for purification of low abundance protein complexes.

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#### **ICAR14015**

##### ACTIVITIES RELATED TO GENOMICS AND PHENOMICS AT ABRC, 2008

Category: Novel Tools, Techniques, and Community Resources

\*Scholl, Randy Knee, Emma Rivero, Luz Crist, Deborah Mann, James Calhoun, Christopher Case, Natalie Miller, Julie Muthuel, Bhuma Pfingsten, Sarah Posey, Garret Vivian, Pamela Wickramasinghe, Damitha Yan, Hehua Zhang, Zhen Arabidopsis Biological Resource Center, Department of Plant Cellular and Molecular Biology and Plant Biotechnology Center, The Ohio State University

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The Arabidopsis Biological Resource Center (ABRC) maintains stocks relevant to genome exploration and functional genomics. Several flank-tagged insertion collections are distributed, representing more than 25,000 mutated loci. Included are: T-DNA lines from 1) SALK Institute, J. Ecker lab, 2) Syngenta Biotechnology, including the lines originally associated with an MTA, 3) GABI-Kat lines, and 4) Wisconsin Ds-Lox population, plus the transposon lines from Cold Spring Harbor Laboratory, Institute of Molecular Agrobiology (IMA), and John Innes Centre. RNAi lines from the Chromatin Functional Genomics and AGRIKOLA Consortia are also distributed.

Initiatives involving insertion lines include: a) receipt of a total of 17,990 confirmed, purified insertion lines from J. Ecker; b) making these lines available for large phenotypic studies - we have begun distribution of the first installment of a complete single-line set, including a one-allele set of 6,868 lines, and a two-allele set of 8,889 lines; c) fingerprinting of all natural accessions by J. Borevitz; d) receipt of the majority of the SSP ORF/cDNAs in a Gateway™ Entry vector; e) receipt of Gateway™ compatible expression clones from S. P. Dinesh-Kumar; f) receipt of split ubiquitin vectors and clones from W. Frommer; and g) receipt of organelle-targeted, multi-color GFP vectors from A. Nebenfuehr.

The total number of full length entry clones at ABRC is over 29,000, representing approximately 13,500 loci and includes donations from SSP, (J. Ecker, A. Theologis, R. Davis), Salk (J. Ecker), TIGR (C. Town), Peking/Yale (X. W. Deng) and J. Callis. In addition, 5,200 expression constructs from S. P. Dinesh-Kumar, S. Clouse, and J. Doonan are available. Versatile destination vectors (for Gateway™ and other systems) for various expression applications in plants, bacteria and yeast are also distributed.

ABRC is supported by the National Science Foundation.

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#### **ICAR14016**

##### THE ARABIDOPSIS BIOLOGICAL RESOURCE CENTER – 2008 ACTIVITIES AND COLLECTIONS

Category: Novel Tools, Techniques, and Community Resources

\*Scholl, Randy Rivero, Luz Crist, Deborah Knee, Emma Mann, James Calhoun, Christopher Case, Natalie Miller, Julie Muthuel, Bhuma Pfingsten, Sarah Posey, Garret Vivian, Pamela Wickramasinghe, Damitha Yan, Hehua Zhang, Zhen Arabidopsis Biological Resource Center, Department of Plant Cellular and Molecular Biology and Plant Biotechnology Center, The Ohio State University

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The Arabidopsis Biological Resource Center (ABRC) has been collecting, preserving and distributing seed and DNA stocks of Arabidopsis since 1991. ABRC stock information is accessible through TAIR (<http://arabidopsis.org>), maintained by the Carnegie Institution of Washington.

Seed stocks added to our collection bring the present totals to: A) 17,990 confirmed, purified T-DNA lines from J. Ecker; B) 1,080 purified T-DNA insertion lines from other researchers; C) 7,455 T-DNA lines from GABI-Kat; D) 2,543 mutant lines; E) 20 recombinant inbred populations; F) 900 unique natural accessions; H) 7,330 insertion lines from John Innes Centre; and I) 659 AGRIKOLA Consortium lines.

T-DNA lines of the SALK, SAIL, Wisconsin and GABI-Kat collections provide insertions in 25,000 different Arabidopsis genes. It is expected that additional major donations of the SALK confirmed T-DNA lines will be received. The collection of natural accessions has been greatly enhanced with donation of diverse sets of lines from M. Koornneef, M. Pigliucci, B. Schaal and J. Beck. These stocks essentially double the number of genetically distinct natural variants in the collection. All members of this collection have been, or will be, fingerprinted in the J. Borevitz lab.

New DNA stocks added to the collection bring the totals to: A) 10,209 sequence-validated open Reading Frame (ORF) clones in a Gateway™ entry vector from J. Ecker so that the majority of the SSP ORF collection is now available in a Gateway™ system as well as the pUNI system, B) 5,241 Gateway™ Expression clones from S. P. Dinesh-Kumar and others, C) 164 vectors and D) 745 cloned genes and constructs.

During the past year, ABRC distributed approximately 100,000 seed and DNA stocks to researchers worldwide. Distributions of T-DNA lines contribute

to the very high numbers of seed stocks being sent, and the ORF clones represent 60% of the DNA stocks being shipped.

ABRC is supported by the National Science Foundation.

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**ICAR14017**

DEVELOPMENT OF RNAI-IN PROTOPLASTS FOR A RAPID, HIGH-THROUGHPUT PROCEDURE FOR TARGETED GENE-INACTIVATION AND FUNCTIONAL ANALYSES OF ARABIDOPSIS GENES

Category: Novel Tools, Techniques, and Community Resources

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The genome sequence of *Arabidopsis* has been available for more than seven years, but only about 17% of genes have ascribed functions. The impeding step in their functional analyses is the availability of suitable reverse genetic tools. Commonly used approaches, including transposon/T-DNA insertional mutagenesis, and double-stranded (ds)RNA interference (RNAi), are limited by the time and space required to generate, isolate and maintain multiple mutant plant lines, and are not practical for rapid screens. To circumvent these difficulties, we developed a procedure consisting of isolating protoplasts from *Arabidopsis*, and RNAi-based gene inactivation in these protoplasts. This method allows rapid and high-throughput analyses of genes function that are time, space, and cost-efficient.

The success of RNAi in protoplast is contingent on the yield and transformation efficiency. For RNAi, in vitro synthesized dsRNAs against *Atpcs-1* (dsRNA*Atpcs-1*), encoding a phytochelin synthase that is required for heavy metal tolerance, was transfected into protoplasts from wild type *Arabidopsis*. In our method, one gram of tissues from 14-day-old seedlings yields  $5 \times 10^6$ - $10^7$  protoplasts; the transformation efficiency is more than 90%. We also performed RNAi against GFP, the green fluorescence protein from *Aequorea victoria*. In this case, protoplasts were co-transfected with dsRNA*GFP* and the vector expressing GFP. As determined by RT-PCR and fluorescent microscopy, levels of *Atpcs-1* and *GFP* transcripts and GFP-mediated fluorescence decrease by at least 80% after 24 h after transformation; these RNAi effects lasted for 96h. Currently, we are developing rapid screening strategies based on selectable conditional phenotypes resulting from gene-inactivation.

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**ICAR14018**

ARABIDOPSIS THALIANA AS A PLATFORM FOR CROP FUNCTIONAL GENOMICS

Category: Novel Tools, Techniques, and Community Resources

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*Arabidopsis thaliana* as a model plant system is amenable to efficient transformation and has been genetically well-characterized. We have used *Arabidopsis* as a platform to functionally identify and characterize genes from a crop plant. A cDNA library was constructed from tomato fruits within two weeks after fertilization using a modified binary vector pBI121. The library was used to transform *Arabidopsis* ecotype Columbia. About 7000 mutants have been generated and mutations in leaf shape, plant architecture, and flower-related mutations are characterized. The technique can be used for any other plant species as a gene discovery tool.

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**ICAR14019**

INTRODUCING MEDICAGO TRUNCATULA TO THE ARABIDOPSIS COMMUNITY

Category: Novel Tools, Techniques, and Community Resources

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*Medicago truncatula* is emerging as a model legume species. Apart from nodulation, *M. truncatula* is also a good model system for compound-leaf development, flowering and metabolite research. More importantly, the availability of various resources makes it feasible for studies in genetics, genomics, proteomics and metabolomics. Funding from NSF and the Noble Foundation has resulted in a collection of over 226,000 *M. truncatula* ESTs and an ongoing genome sequencing project to be completed by 2008. Due to the limitation of transformation efficiency, large scale generation by T-DNA insertional mutagenesis (as of SALK lines for *Arabidopsis*) is not practical for *M. truncatula*. In the last four years, the Noble Foundation has funded and initiated a project to generate a large scale mutant population by fast-neutron bombardment and Tnt1 retrotransposon mutagenesis. To date, we have collected M2 seeds from more than 100,000 fast-neutron mutated M1 plants. PCR-based reverse genetic screening using DNA pools is available. We have regenerated more than 7500 Tnt1-tagged lines, representing approximately 190,000 insertions. We plan to regenerate ~2000 lines each year for the next three years. An efficient, PCR-based approach to identify Tnt1 insertions in genes of interest has been established. Using this approach, we have identified mutants with Tnt1 insertions in 68 out of 75 target genes. So far, the overall success rate in identifying mutant(s) in desired genes is ~90%. Substantial progress has also been made in generating high quality flanking sequence tags (FSTs) from Tnt1 mutant lines. TAIL-PCR has been used to generate more than 6,000 FST sequences. A user-friendly web-based database has been established, which contains information about mutant lines, photos of different phenotypes, as well as FST sequences mapped to pseudochromosomes and can be viewed using the popular genome browser, GBROWSE.

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**ICAR14020**

SPATIO-TEMPORAL LEAF GROWTH OF ARABIDOPSIS THALIANA AND CHARACTERISATION OF STARCH METABOLISM MUTANTS

Category: Novel Tools, Techniques, and Community Resources

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High resolution of growth responses to environmental changes or inducing/inhibiting substances and also the characterisation of altered growth dynamics in mutants/transgenic plants will give valuable information on plant growth control relevant for breeding and improving plant yield.

Three independent techniques are presented for visualizing and quantifying temporal dynamics of leaf growth of *Arabidopsis thaliana*. A digital image sequence processing (DISP) based non-invasive technique also allows a spatial resolution of leaf growth (Wiese et al. 2007). Rotary resistance transducers (RRTs) are used for the characterisation of length growth dynamics. A third technique detects growth of floating leaf discs with high temporal resolution (Biskup et al., 2008). These techniques combined with *Arabidopsis* enable the characterisation of temporal (and spatial) leaf growth in mutants and transgenic plants in various conditions and to analyse the molecular control underlying and influencing diel growth.

Wild-type *Arabidopsis thaliana* leaves of different ecotypes show highest relative growth rates (RGR) at dawn and lowest RGR at the beginning of the night. Along the lamina, a basipetal gradient of growth rate distribution is shown, similar to other dicotyledonous species. Growth analysis of mutants in starch metabolism, known to be retarded in growth dependent on the day length, revealed altered temporal growth patterns, with reduced nocturnal growth. These mutants show an endogenous change in the diel sugar availability *in vivo*. The diel growth pattern of *Arabidopsis* wild-type and mutant leaves are controlled in the growing leaf tissue, independent of the whole plant context as shown by temporal growth analysis of leaf discs reproducing the detected growth pattern for wild type and starch mutants. This technique also allows adding substances to leaf discs and to analyse diel and short term growth responses influenced by osmotics, sugars, hormones and other substances directly on the leaf.

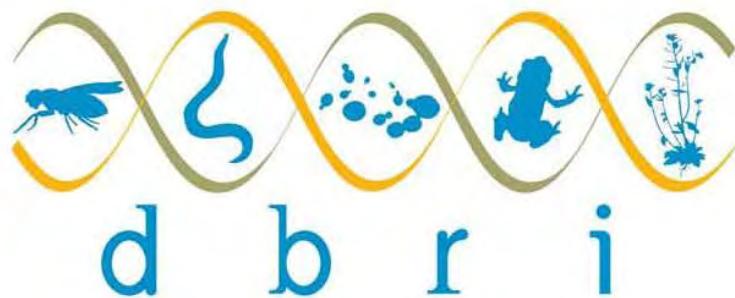
A combined DISP setup for leaves and roots in parallel is currently established, which enables the investigation of interactions between root and shoot growth, a question of high relevance for whole plant productivity.

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The Developmental Biology Research Initiative (DBRI) is dedicated to hypothesis-driven research in non-mammalian model organisms to address fundamental problems in cell and developmental biology.

Its goal is to advance knowledge in a research area that has enormous potential applications for biotechnology and medicine.



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 Rodriguez, Ramiro (ICAR1112)  
 Rodriguez-Franco, Marta (ICAR4062)  
 Roh, Hyungmin (ICAR9042)  
 Rolland, Filip (ICAR4063)  
 Rosin, Faye (C4B02) **SPEAKER**  
 Rowland, Owen (ICAR10022)  
 Rowland, Owen (ICAR10021)  
 Rubio, Vicente (ICAR4064)  
 Runions, John (ICAR1113)  
 Ruppel, Nicholas (ICAR1114)  
 Rutschow, Heidi (ICAR11025)  
 Ryu, Stephen (ICAR4065)  
 Safaei, Natasha (ICAR9043)  
 Saha, Dipnarayan (ICAR1115)  
 Saito, Kazuki (P302) **SPEAKER**  
 sajja, uday (ICAR1116)  
 Salomon, Susanne (C3A03)  
**SPEAKER**  
 Samuels, Lacey (ICAR208)  
 Sandvik, Silje (ICAR6027)  
 Saqib, Muhammad (ICAR7044)  
 Sasaki, Taku (ICAR6028)  
 Sassa, Michika (ICAR9044)  
 SATO, Masanao (ICAR5029)  
 Sauer, Michael (ICAR9045)  
 Sawada, Yuji (ICAR11026)  
 Schaeffner, Tony (ICAR7046)  
 Schaeffner, Tony (ICAR7045)  
 Schlappi, Michael (ICAR7047)  
 Schneider, Katja (ICAR1117)  
 Scholl, Randy (ICAR14016)  
 Scholl, Randy (ICAR14015)  
 Schroeder, Dana (ICAR7048)  
 Schuetz, Mathias (ICAR1118)  
 Schuler, Mary (P301) **SPEAKER**  
 Schultz, Elizabeth (ICAR1119)  
 Schwab, Rebecca (C3B04)  
**SPEAKER**  
 Schwartz, Christopher (ICAR308)  
 Sena, Giovanni (C1A04)  
**SPEAKER**  
 Shah, Jyoti (ICAR4066)  
 Shah, Jyoti (ICAR5030)  
 Shahmir, Fariba (ICAR11027)  
 Shahollari, Bationa (ICAR4067)  
 Sheahan, Brianna (ICAR1120)  
 Shearer, Heather (ICAR5031)  
 Shi, Chunlin (ICAR1121)  
 Shibasaki, Kyohei (ICAR1122)  
 Shiina, Takashi (ICAR11028)  
 Shimotohno, Akie (ICAR9046)  
 Smalle, Jan (ICAR4068)  
 Somers, David (ICAR7049)  
 Somerville, Chris (K01)

**SPEAKER**

Somssich, Imre (ICAR5032)  
Spielau, Claudia (ICAR4069)  
Spitzer, Christoph (ICAR1123)  
Stokes, Michael (ICAR4070)  
Straus, Marco (ICAR5033)  
Sullivan, Julie (ICAR5034)  
Suwabe, Keita (ICAR13011)  
**SUWASTIKA, I** (ICAR4071)  
Suzuki, Toshiya (ICAR9047)  
Szumlanski, Amy (ICAR9048)  
Tabata, Ryo (ICAR1124)  
Tagami, Yuko (ICAR6029)  
Takahashi, Hirotaka (ICAR4072)  
Takahashi, Seiji (ICAR10023)  
Takamura, Yusuke (ICAR9049)  
Takano, Junpei (ICAR9050)  
Takano, Sho (ICAR1125)  
Tam, Patrick (ICAR9051)  
Tameshige, Toshiaki (ICAR1126)  
Tanimoto, Mimi (ICAR1127)  
Tanurdzic, Milos (ICAR6030)  
Tatusova, Tatiana (ICAR12014)  
Taylor, Neil (ICAR209)  
Teotia, Sachin (ICAR1128)  
Tojo, Takuto (ICAR1129)  
Town, Chris (ICAR12015)  
Tsang, Edward (ICAR1130)  
Tsuchida, Yuhei (ICAR1131)  
Tsuchiya, Yuichiro (ICAR1132)  
Tsuda, Kenichi (ICAR5035)  
Tsukagoshi, Hironaka (ICAR9052)  
Tsukaya, Hirokazu (ICAR9053)  
Turgeon, Paul (ICAR1133)  
Turnbull, Colin (ICAR1134)  
Urbanus, Susan (ICAR1135)  
Urquhart, William (ICAR5036)  
Usher, Sarah (ICAR6031)  
Vajinder, Kumar (ICAR1136)  
van der Does, Dieuwertje  
(ICAR5037)  
van Hulten, Marieke (ICAR5038)  
van wijk , klaas (ICAR1137)

**SPEAKER**

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Vatamaniuk, Olena (ICAR14017)  
Vaughn, Laura (ICAR309)  
Vickerman, Lori (ICAR9054)  
Vilhjalmsson, Bjarni (ICAR3010)  
Vittorioso, Paola (ICAR4073)  
Vlachonasios, Konstantinos  
(ICAR6032)  
von Loeffelholz, Clara Ottilie  
(ICAR9055)  
Vujanovic, Vladimir (ICAR13012)  
Wada, Takuji (ICAR1139)  
Waduwara, Ishari (ICAR10024)  
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Wang, Feng (ICAR9057)  
Wang, haiyang (ICAR7050)  
Wang, Haiyang (ICAR4075)  
Wang, Jianying (ICAR5039)  
Wang, Lin (ICAR5040)  
Wang, Yi-Hong (ICAR14018)  
Wang, Yong-Fei (ICAR4076)  
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Wasteneys, Geoffrey (ICAR2010)  
Watanabe, Naohide (ICAR7051)  
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Wen, Rui (ICAR9058)  
Weng, Hua (ICAR10026)  
Wenkel, Stephan (ICAR1142)  
Wester, Katja (ICAR9059)  
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**SPEAKER**

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# **GENERAL MEETING INFORMATION**

## **Location of Events and Exhibits**

All events take place at the Hyatt Regency Hotel in Montreal, except the Conference Banquet, which will be held at McGill Residence Hall Ballroom.

Please refer to the program overview for room locations of specific events.

## **Registration**

The registration desk will be available for questions and registration during the hours below. It will be located in the foyer outside the Grand Salon at the Hyatt Regency.

Wednesday, July 22<sup>nd</sup>  
12:00 pm - 7:00 pm

Saturday, July 26  
8:00 am - 7:00 pm

Thursday, July 24  
7:30 am - 8:00 pm

Sunday, July 27  
8:00 am - 11:00 am

Friday, July 25  
8:00 am - 1:30 pm &  
7:00 pm - 8:00 pm

## **Onsite T-shirt and Banquet Ticket Sales**

You can purchase t-shirts or conference banquet tickets onsite anytime the registration desk is open. We accept checks written in USA dollars on US banks, credit cards (Visa, MasterCard and American Express) and US and Canadian cash. T-shirts and tickets are sold on a first come, first serve basis. T-shirts are \$10 each and banquet tickets are \$50 each.

## **Internet Café**

There will be an Internet Café available in the Grand Salon Foyer. The hours for the Internet Café are the same hours as the registration booth listed above.

## **Speaker Ready Room**

There is a speaker ready room available near the Grand Salon foyer for presentation testing. Speakers should test their presentations at least one hour prior to their presentation time and be at their session at least 10 minutes before they are scheduled to speak. The schedule for the speaker ready room follows:

Wednesday, July 23  
12:00 pm - 7:30 pm

Friday, July 25  
8:00 am - 8:00 pm

Sunday, July 27  
8:00 am - 10:00 am

Thursday, July 24  
7:30 am - 8:00 pm

Saturday, July 26  
8:00 am - 7:00 pm

## **Airport Shuttles**

Free airport shuttles will be available to conference attendees on the closing day of the conference. Buses will run between 6 am and 4 pm to the airport on July 27. More information is available at the registration desk.

## **Cameras/Video/Audio Recording**

Taking photos, video, or audio recording of any kind of the posters and sessions will be **PROHIBITED** unless an author/speaker provides specific permission. This prohibition includes but is not limited to the use of camera phones and any other digital recording devices.

## **Exhibits & Posters**

Exhibits and posters are open during exhibit hours as listed on your schedule. Please refrain from entering exhibits when they are closed. The posters will be organized by category. Exclusive poster session times are listed in the schedule. Please attend your posters during the time slots based on your final poster number. Poster abstracts will be available in the printed abstract book or online. Late submissions are online only.

## **Babysitting Services**

Services are provided by the Hyatt Regency and can be arranged in advance or on-site. Please contact the hotel concierge directly to make arrangements or with any questions.

\*15.00\$ per hour for 1-4 children of the same family (minimum 3 hours) – for 5 and more if not the same family there will be 2 sitters (minimum 3 hours)

\*The guest may cancel, no charge, one day in advance, 3 hours (45\$) will be charged if the cancellation occurs on the day of assignment.

\*The payment may be in cash only.

\*After 23h or 11h PM, a taxi has to be paid to the sitter (minimum 20\$)

\*The payment has to be done at the return of the guest. No check will be accepted, if the guest needs money, he can go to the reception desk and ask for a money advance.

\*The guest has to contact the concierge to organize for babysitting.

## **Conference Smoking Policy**

Smoking is not allowed at any meeting functions or areas.

## **Additional Information/Questions**

The conference Registration Desk will be able to assist you with any additional questions regarding the meeting.

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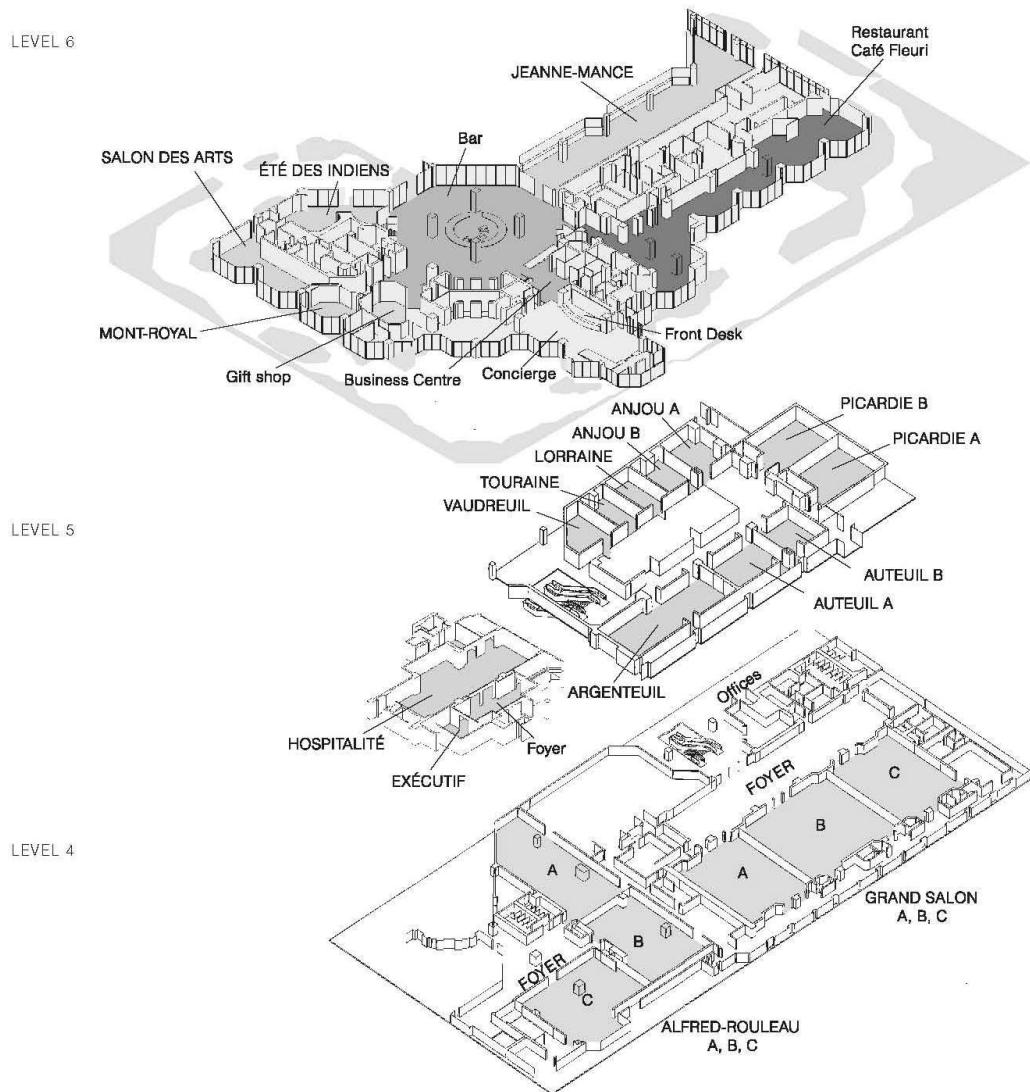
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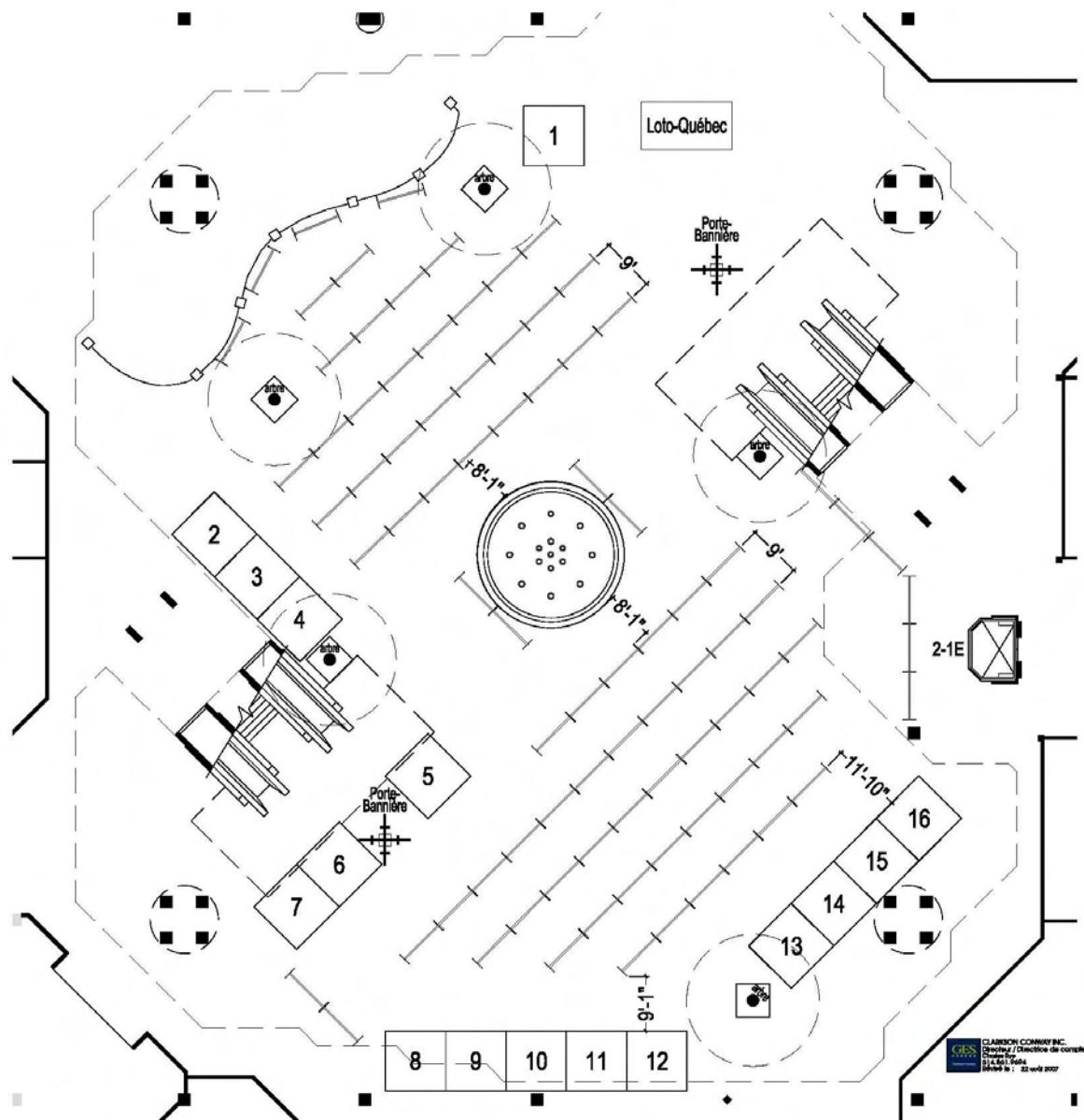
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# **Site-seeing and Tourism in Montreal**

**In the next few pages, there is some information on Montreal and how to get around and suggestions of what to see and do.**

## **(1) Hyatt metro stop**

The Hyatt is near the Place des Arts subway stop. Metro information and maps can be found at:  
<http://www.stm.info/english/metro/a-index.htm>

**(2) Tourism brochures** will be given to each attendee when they check in at conference registration.

## **(3) Montreal Tourism websites:**

<http://www.bonjourquebec.com/us-en/accueil0.html>

<http://www.tourisme-montreal.org/> (click 'My Montreal Planner' to make your own itinerary that you can email to yourself).

## **(4) A few events in the local Montreal area during the Arabidopsis conference**

L'International des Feux Loto-Québec présenté par TELUS

<http://www.internationaldesfeuxloto-quebec.com/en/>

Each year this international fireworks competition draws the biggest pyrotechnics firms to La Ronde (<http://www.laronde.com/larondeen/>), Montréal's largest amusement park. On the agenda: spectacular, 30-minute pyromusical extravaganzas. All of the performances begin at 10:00 p.m. Reserved tickets can be purchased at La Ronde on the day of the event (the entry fee includes unlimited access to rides and attractions on the site throughout the day) or through the Admission network via their website ([www.admission.com](http://www.admission.com)) or by telephone, at 514-790-1245 or 1-800-361-4595 (toll-free). The park (with rides and roller coasters) is about 4 miles from the Hyatt; the nearest metro stop is Jean Drapeau/Ile-Sainte-Helene, then take the 167 La Ronde bus to the park.

Fireworks shows during the Arabidopsis Conference:

July 23- **Canada 'Aurora: Fire in the Sky'**

July 26- **Austria 'Heaven and Hell'**

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Ephemeral Landscape

<http://www.paysagesephemeres.com/home.php>

Ephemeral Landscapes invites the public to take in a series of 10 astonishing but temporary landscape interventions created along Mont-Royal Avenue. Located about 2 miles from the Hyatt and a few blocks from the Sherbrooke metro stop.

Dates: July 02 2008 - August 31 2008

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Theatre in the Parks

<http://www.repercussiontheatre.com/en/>

A classical theatre festival presenting plays for free in parks throughout the Greater Montreal Area.

Directions by metro to each location available on the website.

Shows during the Arabidopsis Conference:

The Tempest (in English): Friday, July 25 at 8:30 PM, Parc Lafontaine;  
Saturday, July 26 at 7:30 PM, Parc Westmount

The Tempest (in French): Sunday, July 27 at 7:30 PM Parc Westmount

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#### Les FrancoFolies de Montréal

[http://www.francofolies.com/Francos2008/accueil\\_en.aspx](http://www.francofolies.com/Francos2008/accueil_en.aspx)

For the 20th consecutive year, the world's second largest French-speaking city hosts the largest musical event in the French-speaking world: The FrancoFolies de Montréal! This one-of-a-kind event, which takes place in the heart of downtown Montréal (immediately adjacent to the Hyatt), offers over 50 indoor concerts, and a wild array of free outdoor shows (to be announced July 9).

Dates: July 24 2008 - August 03 2008

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#### Montreal International Dragon Boat Race Festival

<http://www.montrealdragonboat.com/english/events.php>

If you've never heard of it, Dragon Boat Racing is pretty cool- and free admission and shuttle buses can't be beat. See the FAQ for more info on what this is see:

<http://www.montrealdragonboat.com/english/festivalfaq.php>.

Teams from around the world and from around Montreal compete in 250 meter and 500 meter races. Live entertainment: Folk dancing and music groups from various cultural communities, children's drawing contests, and ethnic delicacies are available throughout the festivities and sponsors exhibit various products and services. Attracting over 200 teams from across North America, the weekend promises to be unforgettable! There are free shuttle buses on the corner of La Gauchetiere Ouest and St-Urbain streets that goes from the Chinatown to the Olympic Basin every 30 minutes starting from 10am to 6pm.

Dates: July 26 2008 - July 27 2008 at the Olympic Basin on Parc Jean-Drapeau.

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#### Rogers Cup

<http://www.rogerscup.com/1/en/home/Default.asp>

The women's edition of the Rogers Cup presented by National Bank will be held at Montréal's Uniprix Stadium. This international event is one of ten Tier I tournaments on the Sony Ericsson WTA Tour (Women's Tennis Association). Located about 4 miles from the Hyatt, the nearest metro stop is De Castelnau station on the blue line. The station is a five minute walk from Uniprix Stadium. The Jarry Metro station on the orange line is also nearby.

Dates: July 26 2008 - August 03 2008

#### **(5) Bring your own wine!**

Many Montreal restaurants have a bring your own wine policy. This lets you pick up the bottle you want at the price you want to spend. See: <http://english.montrealplus.ca/portal/feature/6001/index.jsp#3>

## **(6) Notre Dame Basilica of Montreal**

<http://www.basiliquenddm.org/en/>

Walking distance, about 1 mile, from the Hyatt at 110 Notre-Dame Street West. The church is open daily to visitors, seven days a week. M-F: 8:00 AM to 4:30 PM; Sat.: 8:00 AM to 4:00 PM (note: closes at 12 noon on July 26) ; Sun.: 12:30-4:00 PM

**Special event: 'And then there was light' sound and light show, 35 minutes long.**

Tuesday to Friday : 6:30 p.m. and 8:30 p.m. Saturday: 7:00 p.m. and 8:30 p.m.

## **(7) Mount Royal Park**

<http://www.montreal.com/parks/mtroyal.html>

Designed by Frederick Law Olmsted, who created New York City's Central Park, this park boasts a vast network of walking paths and stairs that will lead to you a variety of wooded areas and landscapes. Magnificent lookouts offer breathtaking views of the city and region. Lastly, Les amis de la montagne (friends) offer numerous education, conservation and recreational activities. Metro: Mount Royal then take the 11 bus.

## **(8) Museum of Contemporary Art**

<http://www.macm.org/en/index.html>

Located right near the Hyatt, the Musée d'art contemporain de Montréal moves to the rhythm of the summer's festivals and cultural events. With its "jazzy" atmosphere, music-filled terrace, sculpture garden in full bloom, the Musée promises many enjoyable, refreshing moments. Step into the world of today's art and discover some amazing, even provocative works.

## **(9) Montreal Science Centre**

<http://www.montrealsciencecentre.com/en/index2.htm>

Located within walking distance (about 1 mile) from the Hyatt in the heart of the Quays of the Old Port, the Montréal Science Centre is a unique complex offering an educational and entertaining outing for the whole family. The Science Centre features scientific exhibition halls, technical innovations, interactive movie games, an IMAX theatre, boutiques and restaurants.

## **(10) Montreal Botanical Gardens**

<http://www2.ville.montreal.qc.ca/jardin/en/menu.htm>

With its collection of 22,000 plant species and cultivars, 10 exhibition greenhouses, some thirty thematic gardens, and teams of researchers and activities staff, the Montréal Botanical Garden ranks as one of the world's largest and most spectacular botanical gardens. No matter what the season, visitors to the Montréal Botanical Garden are sure to be captivated by the colors and fragrances from around the world

as they wander from the delightful Chinese Garden to the heart of the Sonoran desert, from the peaceful oasis of the Japanese Garden to the classically designed French garden or the woodlands of the Laurentians.

Hours: 9 am- 6 pm, Mon-Sun

### **(11) Rue Sainte Catherine shopping district**

[http://www.bonjourquebec.com/us-en/07fev\\_rue\\_stecath0.html](http://www.bonjourquebec.com/us-en/07fev_rue_stecath0.html)

Located adjacent to the Hyatt, Rue Ste-Cathérine, is Montréal's main shopping thoroughfare with department stores and shops of all kinds, as well as numerous restaurants, ranging widely in the type of food and price.



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