



18th International Conference on Arabidopsis Research

Beijing, China
June 20-23, 2007

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Welcome to the 18th International Conference on Arabidopsis Research

Dear Colleagues,

I would like to personally welcome you to the 18th International Conference on Arabidopsis Research. From an initial group of about 25 scientists that participated in the first International Arabidopsis Symposium in Germany in 1965, there are currently more than 16,000 Arabidopsis researchers in over 6,000 laboratories worldwide. While the second and third conferences occurred in roughly 10 year intervals, interest in Arabidopsis research rapidly increased in the 1980's. Beginning in 1995 this led to annual meetings, now called the International Conference on Arabidopsis Research (ICAR); since that year, the Arabidopsis meeting convenes approximately two out of every three years in the United States, with each third meeting held outside the U.S.

The Arabidopsis community is represented by the Multinational Arabidopsis Steering Committee (MASC), a committee made up of representatives of countries and programs around the world involved in Arabidopsis research. When held in the U.S., the International Conference of Arabidopsis Research is traditionally organized by the North American Arabidopsis Steering Committee (NAASC). This year the Conference takes place in Beijing, China, marking the first time the annual meeting will be held in an Asian country. Five local institutions will jointly organize the conference: the National Institute of Biological Sciences, The Institute of Botany and Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences, Peking University, and China Agricultural University.

In addition to presentations of significant and innovative Arabidopsis-based research, the conference will include discussions of other relevant plant systems throughout most of the sessions. This complementary approach, initially allocated one special session during the 2004 Berlin ICAR, will emphasize the importance and usefulness of Arabidopsis as the reference plant and its impact on other systems, including economically important species.

This year more than 1500 delegates will attend the Arabidopsis meeting which will feature 83 oral presentations in Plenary and Concurrent sessions, as well as more than 40 additional oral presentations in 8 Scientific Workshops. In addition, there will be over 700 Scientific posters presented and available for viewing at all times during the conference which will allow fruitful networking and collaboration.

I would like to thank all the members of the local, regional, and international Organizing Committees for their efforts in developing an exciting scientific program and in ensuring the success of the meeting. Additionally, I would like to thank the conference sponsors and exhibitors for their support.



Xing Wang Deng
Chair of the Local Organizing Committee
On behalf of MASC

Organizer

The Multinational Arabidopsis Steering Committee (MASC)

Ian Small (Australia, Chair), Xing Wang Deng (USA, Co-Chair), Thomas Altmann (Germany), Ruth Bastow (UK), Philip Benfey (USA), David Bouchez (France), Jorge Casal (Argentina), Bill Crosby (Canada), Klaus Harter (Germany), Marie-Theres Hauser (Austria), Pierre Hilson (Belgium), Jaakko Kangasjarvi (Nordic Arabidopsis Network), Sean May (UK), Peter McCourt (Canada), Ortrun Mittelsten-Scheid (Austria), Javier Paz-Ares (Spain), Barry Pogson (Australia and New Zealand), Ben Scheres (The Netherlands), Randy Scholl (USA), Kazuo Shinozaki (Japan), Willem Stiekema (The Netherlands), Paola Vittorioso (Italy), Weicai Yang (China)

MASC Coordinator: Joanna Friesner (USA)

The North American Arabidopsis Steering Committee (NAASC)

Judith Bender (USA), Philip Benfey (USA), Caren Chang (USA), Xuemei Chen (USA), Xing Wang Deng (USA, Chair), Joe Kieber (USA), Rob McClung (USA), Julian Schroeder (USA)

Regional Advisory Committee

Zhihong Xu (China, Chair), Sudip Chattopadhyay (India), Xiaoya Chen (China), Zhangliang Chen (China), Jiayang Li (China), Hong Gil Nam (South Korea), Kiyotaka Okada (Japan), Kazuo Shinozaki (Japan), Qifa Zhang (China)

Local Organizing Committee

Kang Chong	The Institute of Botany , Chinese Academy of Sciences	Beijing
Xing Wang Deng (Chair)	National Institute of Biological Sciences	Beijing
Hongya Gu	Peking University	Beijing
Hai Huang	Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences Chinese Academy of Sciences	Shanghai
Chunming Liu	The Institute of Botany , Chinese Academy of Sciences	Beijing
Chentao Lin	The Chinese Academy of Agricultural Sciences	Beijing
Ligeng Ma	National Institute of Biological Sciences	Beijing
Shiping Wang	Huazhong Agricultural University	Wuhan
Ping Wu	Zhejiang University	Hangzhou
Weihua Wu	China Agricultural University	Beijing

Daoxin Xie	Tsinghua University	Beijing
Hongwei Xue	Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences Chinese Academy of Sciences	Shanghai
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Shuhua Yang	China Agricultural University	Beijing
Weicai Yang	Institute of Genetics and Developmental Biology, Chinese Academy of Sciences	Beijing
Jianmin Zhou	National Institute of Biological Sciences	Beijing
Yuxian Zhu	Peking University	Beijing
Jianru Zuo	Institute of Genetics and Developmental Biology, Chinese Academy of Sciences	Beijing

Joanna Friesner (USA), Co-coordinator for 18th International Conference on Arabidopsis Research, MASC Coordinator

Qin Zheng (China), Co-coordinator for 18th International Conference on Arabidopsis Research

Program Overview

Poster Schedule

All posters will remain up for the entire meeting and can be set up at **No. 102, No. 106 and No. 107 meeting halls**, beginning at **2:00 pm June 20th, 2007**. There will be three poster sessions, one Wednesday evening, one Thursday evening, and one Saturday evening. To determine when you should stand next to your poster:

Find your abstract in this book; your poster number is the number it is assigned in this book, NOT the number it was assigned when you originally submitted it.

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

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

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

Wednesday, June 20, 2007

8:00 am – 10:00pm

Registration

1st Floor Lounge

6:00 - 7:30 pm

Welcome Reception

1st Floor Banquet Hall

7:30 - 9:30 pm

Workshops

(1) Linking Stress Signals to Growth

Deshpal Verma and Hong Ma

2nd Floor Room No. 88

(2) Ubiquitination in Plant Development

and Signaling

Qi Xie and Minami Matsui

2nd Floor Room No.76

(3) Web services for Arabidopsis Data Integration and Bioinformatics Tools
for Arabidopsis Microarray Databases

2nd Floor Room No. 87

Chris Town and Anika Joecker

7:30 - 9:30 pm

POSTER SESSION I

3rd Floor Hall No. 102, 106&107

Please present (stand by) your poster if your abstract number in this book is EVEN

Thursday, June 21, 2007

8:00 am – 9:30 pm **Posters open all day** **3rd Floor Hall No. 102, 106 & 107****8:00 am - 6:00 pm** **Registration Continues** **1st Floor Lounge****7:00 - 8:30 am** **Breakfast at Hotels**
For 15th & 16th Dist. Guests: 16th Dist. 1st Floor Banquet Hall
For 10th & 12th Dist. Guest: 10th Dist. Hangzhou Restaurant**8:20 - 8:30 am** **Welcome and Announcements** **3rd Floor Hall No. 103 & 105****8:30 - 10:00 am** **PLENARY SESSION 1:
*Developmental Mechanisms*** **3rd Floor Hall No. 103 & 105****Session Chair:** Elliot Meyerowitz, California Institute of Technology, USA**Session Co-chair:** Hong Ma, Pennsylvania State University, USA**8:30 am** **Elliot Meyerowitz, Chair, California Institute of Technology, USA**
Cellular dynamics in the shoot apical meristem**9:00 am** **Ueli Grossniklaus, University of Zürich, Switzerland**
When partners don't talk: the molecular basis of cell-cell communication during double fertilization**9:20 am** **Wilhelm Gruissem, Institute of Plant Sciences, ETHZ, Switzerland**
Role of the Arabidopsis retinoblastoma-related (RBR) protein in cell specification**9:40 am** **Jiayang Li, Institute of Genetics and Developmental Biology, China**
Rice, a model system for studying plant architecture**10:00 - 10:30 am** **Refreshment Break** **3rd Floor Lobby****11:00 - 12:30 pm** **PLENARY SESSION 2:
*Genomics and Genetics*** **3rd Floor Hall No. 103 & 105**
(Sponsored by: Pioneer HI-Bred International, Inc. A DuPont Company)**Session Chair:** Joe Ecker, The Salk Institute, USA**Session Co-chair:** Xuemei Chen, University of California, Riverside, USA

11:00 am	Joe Ecker, Chair, The Salk Institute, USA Mapping genotype into phenotype: natural, induced and epigenetic variation in <i>Arabidopsis</i> genomes
11:30 am	Maarten Koornneef, Wageningen University, Netherlands The use of <i>Arabidopsis</i> natural variation for gene function analysis
11:50 am	Steve Jacobsen, University of California, Los Angeles, USA Genetics and Genomics of DNA methylation in <i>Arabidopsis</i>
12:10 pm	Qifa Zhang, Huazhong Agricultural University, China Genetic and molecular characterization of inter-subspecific hybrid sterility and wide compatibility in rice

12:00 - 1:30 pm **Lunch** **1st Floor Banquet Hall**

1:30 - 3:00 pm S1 and S3 CONCURRENT SESSIONS:

3rd Floor Hall No. 103

S1 Session: Developmental Mechanisms 1

Session Chair: Weicai Yang, Institute of Genetics and Developmental Biology, China

1:30 pm	Philip Benfey, Duke University, USA Getting to the root of cell identity
1:50 pm	Neelima Sinha, University of California, Davis, USA The development and evolution of leaves
2:10 pm	Hongwei Xue, Shanghai Institutes for Biological Sciences, China Steroid binging protein in photomorphogenesis and BR signaling
2:30 pm	David Smyth, Monash University, Australia Organogenesis of the perianth in <i>Arabidopsis</i> flowers is facilitated by the dual action of PETAL LOSS and AUX1. S-12
2:45 pm	Lynette Brownfield, University of Leicester, UK Regulatory proteins, DUO1 and DUO3, link cell cycle progression and cell specification in male germ line development. S-13

S3 Session: *Genomics and Genetics 1*

3rd Floor Hall No. 101

Session Chair: Xiaofeng Cao, Institute of Genetics and Developmental Biology, China

1:30 pm Christian Hardtke, University of Lausanne, Switzerland
Exploiting the natural genetic variation in recombinant inbred lines:
two accessions, multiple traits

1:50 pm	Ko Shimamoto, Nara Institute of Science and Technology, Japan Florigen in rice
2:10 pm	Xuemei Chen, University of California, Riverside MicroRNA biogenesis and function in Arabidopsis
2:30 pm	Eric Lam, Rutgers University, USA CHROMATIN CHARTING: Global mapping and characterization of epigenetic control mechanisms
2:45 pm	Marco Todesco, Max Planck Institute, Tübingen, Germany QTL analysis of developmental trade-offs: plastochron and pathogen response in <i>Arabidopsis thaliana</i>

3:00 - 3:30 pm	Refreshment Break	3rd Floor Lobby
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3:30 - 5:00 pm	S2 and S4 CONCURRENT SESSIONS:	
	S2 Session: Developmental Mechanisms 2	3rd Floor Hall No. 103
	Session Chair: Chun-ming Liu, The Institute of Botany, Chinese Academy of Sciences, China	
3:30 pm	Kiyotaka Okada, Kyoto University, Japan Axis-dependent gene expression in the lateral organ formation	
3:50 pm	George Coupland, Max Planck Institute, Cologne, Germany Transcriptional and post-transcriptional regulation in the control of flowering by photoperiod	
4:10 pm	Chuck Gasser, University of California, Davis, USA Organ formation and gene expression patterning in ovule development	
4:30 pm	Elena Caro, Centro de Biología Molecular, Madrid, Spain A chromatin link that couples cell division to <i>Arabidopsis</i> root epidermis cell fate through epigenetic changes of patterning genes. S-22	
4:45 pm	Annelie Carlsbecker, Uppsala University, Sweden Genetic analysis of the vascular patterning of the <i>Arabidopsis</i> root. S-23	

S4 Session: Genomics and Genetics 2	3rd Floor Hall No. 101
Session Chair: Zhizhong Gong, China Agricultural University, China	

3:30 pm	Rob Martienssen, Cold Spring Harbor Laboratory, USA Second generation Arabidopsis genomics with second generation sequencing instruments
3:50 pm	Peter Shaw, John Innes Center, Norwich, UK The nucleolus is involved in mRNA surveillance in Arabidopsis
4:10 pm	Bonnie Bartel, Rice University, USA Using Arabidopsis to find new roles for peroxins and peroxisomes
4:30 pm	Joost Keurentjes, Wageningen University, Netherlands Regulatory network construction in Arabidopsis using genome-wide gene expression QTLs. S-27
4:45 pm	Rodrigo Gutierrez, P. Universidad Católica de Chile, Chile Global analysis of the Arabidopsis transcriptome data suggests methylation is a key factor that determines regulation of gene expression. S-28

6:00 - 7:30 pm	Dinner	1st Floor Banquet Hall
7:30 - 9:30 pm	Workshops (1) Chemical Genetics <i>Zhenbiao Yang and Sean Cutler</i> (2) Metabolomics <i>Mike Beale</i> (3) Proteomics <i>Wolfram Weckwerth and Harvey Millar</i> (4) TAIR (The Arabidopsis Information Resource) <i>Eva Huala and Donghui Li</i>	2nd Floor Room No. 78 2nd Floor Room No.92 2nd Floor Room No.87 2nd Floor RoomNo.88

7:30 - 9:30 pm POSTER SESSION 2 3rd Floor Hall No. 102, 106&107

Please present (stand by) your poster if your abstract number in this book is ODD

Friday, June 22, 2007

8:00 am – 9:30pm	Posters open all day	3rd Floor Hall No. 102, 106 & 107
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8:00 am - 6:00 pm	Registration Continues	1st Floor Lounge
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7:00 - 8:30 am	Breakfast at Hotels For 15 th & 16 th Dist. Guests: 16 th Dist. 1 st Floor Banquet Hall For 10 th & 12 th Dist. Guest: 10 th Dist. Hangzhou Restaurant
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8:30 - 10:00 am	PLENARY SESSION 3: <i>Plant Responses to the Environment</i> (Sponsored by: Monsanto)	3rd Floor Hall No. 103 & 105
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Session Chair: Caroline Dean, The Sainsbury Lab, John Innes Center, UK

Session Co-chair: Chentao Lin, University of California, Los Angeles, USA

8:30 am	Caroline Dean, Chair, The Sainsbury Lab, John Innes Center, UK The molecular basis of vernalization
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9:00 am	Kazuo Shinozaki, RIKEN, Japan Regulatory gene network in drought and ABA responses
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9:20 am	Barbara Hohn, Friedrich Miescher Institute, Switzerland Transgenerational memory of stress in plants
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9:40 am	Javier Paz-Ares, Centro Nacional de Biotecnología, Madrid, Spain Phosphate Starvation Signaling in Arabidopsis
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10:00 - 10:30 am	Refreshment Break	3rd Floor Lobby
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11:00 - 12:30 pm	PLENARY SESSION 4: <i>Epigenetics and Plant Defense</i>	3rd Floor Hall No. 103 & 105
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Session Chair: David Baulcombe, The Sainsbury Lab, John Innes Center, UK

Session Co-chair: Shengyang He, Michigan State University, USA

11:00 am	David Baulcombe, Chair, The Sainsbury Lab, John Innes Center, UK Short silencing RNA networks
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11:30 am	Ton Bisseling, Wageningen University, The Netherlands Does Rhizobium root nodule symbiosis involve a legume specific toolbox?
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11:50 am	Brian Staskawicz, University of California, Berkeley, USA Recognition of pathogen effectors and activation of disease resistance signaling pathways in <i>Arabidopsis thaliana</i>
12:10 pm	James Carrington, Oregon State University, USA Diversification of Small RNA Pathways

12:00 - 1:30 pm	Lunch	1st Floor Banquet Hall
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1:30 - 3:00 pm S5 and S7 CONCURRENT SESSIONS:

S5 Session: Responses to the Environment 1 **3rd Floor Hall No. 103**
Session Chair: Jianru Zuo, Institute of Genetics and Developmental Biology, CAS, China

1:30 pm	Clark Lagarias, University of California, Davis, USA Functional analysis of dominant GAF-domain tyrosine mutants of <i>Arabidopsis</i> phytochromes in transgenic plants
1:50 pm	Hong Gil Nam, Pohang University of Science and Technology, South Korea Photoreceptor-interacting proteins as immediate facilities for environmental light information processing
2:10 pm	Sudip Chattopadhyay, National Centre for Plant Genome Research, India ZBF3/CAM7 plays an important role as transcriptional regulator in <i>Arabidopsis</i> seedling development
2:30 pm	Paul Dijkwel, University of Groningen, Netherlands Mutation of an enzyme involved in the de novo synthesis of NAD causes early leaf senescence in <i>Arabidopsis thaliana</i> . S-40
2:45 pm	Haiyang Wang, Cornell University, USA Transposase-derived proteins FHY3 and FAR1 From <i>Arabidopsis</i> modulate phyA signaling homeostasis through direct activation of "FHY1" and "FHL" expression. S-41

S7 Session: Responses to Microbials 1 **3rd Floor Hall No. 101**
Session Chair: Jianmin Zhou, National Institute of Biological Sciences, China

1:30 pm	Ken Shirasu, RIKEN, Japan HSP90, SGT1, and RAR1 form a ternary chaperone complex to regulate resistance-protein dependent plant immunity
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1:50 pm	Roger Innes, Indiana University, USA Molecular mechanisms underlying the activation of NBS-LRR proteins
2:10 pm	Ralph Panstruga, Max Planck Institute, Cologne, Germany Durable broad-spectrum powdery mildew resistance in crops and cereals: What can we learn from <i>Arabidopsis</i> ?
2:30 pm	Xin Li, University of British Columbia, Canada MOS7 is essential for plant innate immunity. S-45
2:45 pm	Sjoerd Van der Ent, Utrecht University, Netherlands Transcriptional regulators in rhizobacteria-induced systemic resistance. S-46

3:00 - 3:30 pm	Refreshment Break	3rd Floor Lobby
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3:30 - 5:00 pm	S6 and S8 CONCURRENT SESSIONS:	
	S6 Session: Responses to the Environment 2 Session Chair: Hongquan Yang, Shanghai Jiao Tong University, China	3rd Floor Hall No. 103
3:30 pm	Klaus Apel, Institute of Plant Sciences, ETHZ, Switzerland The genetic basis of singlet oxygen-mediated signaling of stress responses	
3:50 pm	Masamitsu Wada, National Institute for Basic Biology, Okazaki, Japan Molecular mechanism of chloroplast photorelocation movement	
4:10 pm	Julian Schroeder, University of California, San Diego, USA Identification of CO ₂ and ABA signaling components and novel membrane transduction mechanisms in guard cells	
4:30 pm	David Somers, Ohio State University, USA Blue-light enhanced post-translational control of circadian cycling of the F-box protein ZEITLUPE occurs via the LOV domain. S-50	
4:45 pm	Vicente Rubio, National Center of Biotechnology, Spain Cryptochrome signaling to the plant clock associates with inhibition of COP1-mediated ELF3 ubiquitination. S-51	

S8 Session: Responses to Microbials 2**3rd Floor Hall No. 101****Session Chair:** Daoxin Xie, Tsinghua University, China**3:30 pm****Jane Parker, Max Planck Institute, Cologne, Germany**

Positioning Arabidopsis EDS1 in pathogen and oxidative stress responses

3:50 pm**Jonathan Jones, The Sainsbury Lab, John Innes Center, UK**

Little and large pathogen effectors in plant resistance and susceptibility

4:10 pm**Shauna Somerville, Carnegie Institution, Stanford Univ., USA**

Penetration resistance: the first line of defense against invasive pathogens

4:30 pm**Shunyuan Xiao, University of Maryland Biotechnology Institute, USA**

Dissection of the signaling pathway of RPW8-mediated broad-spectrum disease resistance. S-55

4:45 pm**Tingting Xiang, National Institute of Biological Sciences, China**

Pseudomonas syringae effector AvrPto blocks innate immunity by targeting receptor kinases. S-56

6:00 - 9:30 pm**Conference Dinner
Traditional Chinese Show****1st Floor Banquet Hall**

Saturday, June 23, 2007

8:00 am – 9:30pm	Posters open all day	3rd Floor Hall No. 102, 106&107
8:00 am - 6:00 pm	Registration Continues	1st Floor Lounge
7:00 - 8:30 am	Breakfast at Hotels For 15 th & 16 th Dist. Guests: 16 th Dist. 1 st Floor Banquet Hall For 10 th & 12 th Dist. Guest: 10 th Dist. Hangzhou Restaurant	
8:30 - 10:00 am	PLENARY SESSION 5: <i>Signal Transduction/Cell Biology</i>	3rd Floor Hall No. 103 & 105
Session Chair: Mark Estelle, Indiana University, USA Session Co-chair: Yongbao Xue, Institute of Genetics and Developmental Biology, CAS, China		
8:30 am	Mark Estelle, Chair, Indiana University, USA Genetic and biochemical studies of the auxin receptor reveal a novel mechanism of hormone perception	
9:00 am	Gerd Jürgens, University of Tübingen, Germany Specific and redundant ARF-GEF functions in membrane trafficking	
9:20 am	Weihua Wu, China Agricultural University, China AKT1 regulation and potassium uptake in Arabidopsis	
9:40 am	Yongbiao Xue, Institute of Genetics and Developmental Biology, CAS, China Molecular control of S-RNase-based self-incompatibility	
10:00 - 10:30 am	Refreshment Break	3rd Floor Lobby
10:30 - 11:35 am	PLENARY SESSION 6: <i>Metabolism/Bioenergy</i>	3rd Floor Hall No. 103 & 105
Session Chair: Xinnian Dong, Duke University, USA		
10:30 am	Clint Chapple, Purdue University, USA Looking beyond Arabidopsis and angiosperms for biomass crop improvement genes	
10:55 am	Harro Bouwmeester, Plant Research International, Netherlands Metabolic engineering of terpene biosynthesis in Arabidopsis and consequences for plant-environment communication	

11:15 am	Geoffrey Fincher, University of Adelaide, Australia Recent advances in plant cell wall biology	
12:00 - 1:30 pm	Lunch	1st Floor Banquet Hall
1:30 - 3:00 pm	S9 and S11 CONCURRENT SESSIONS:	
	S9 Session: Signal Transduction Session Chair: Yuxian Zhu, Peking University, China	3rd Floor Hall No. 103
1:30 pm	Makoto Matsuoka, Nagoya University, Japan GIBBERELLIN INSENSITIVE DWARF1 (GID1), a soluble gibberellin receptor in rice	
1:50 pm	Tatsuo Kakimoto, Osaka University, Japan Secretory peptides that regulate stomatal patterning	
2:10 pm	Ligeng Ma, National Institute of Biological Sciences, China Heterotrimeric G proteins and ABA signaling in Arabidopsis	
2:30 pm	Pradeep Kachroo, University of Kentucky, USA Plastidial oleiclevels modulate defense signaling by regulating expression of resistance genes. S-67	
2:45 pm	Wolfgang Droege-Laser, University of Göttingen, Germany The C/S1 network of bZIP transcription factors: combinatorial control of developmentally and stress regulated transcription by bZIP heterodimers. S-68	
	S11 Session: Metabolism/Hormonal Responses Session Chair: Chong Kang, Institute of Botany, CAS, China	3rd Floor Hall No. 101
1:30 pm	Ken Feldmann, Ceres Inc., USA A high throughput over-expression pipeline in Arabidopsis to discover genes important in phytohormone biosynthesis and regulation	
1:50 pm	Mary Lou Guerinot, Dartmouth University, USA Metals, mutants and mayhem	
2:10 pm	Tai-Ping Sun, Duke University, USA Early events in gibberellin signaling	

2:30 pm**Jane Ward, National Centre for Plant & Microbial Metabolomics, UK**

Metabolites in Motion - tracking the dynamic metabolome with 1H NMR. S-72

2:45 pm**Joshua Gendron, The Carnegie Institution, Stanford Univ., USA**Brassinosteroids regulate organ boundary formation and organ separation in *Arabidopsis*. S-73

3:00 - 3:30 pm**Refreshment Break****3rd Floor Lobby**

3:30 - 5:00 pm**S10 and S12 CONCURRENT SESSIONS:****S10 Session: Cell Biology****3rd Floor Hall No. 103****Session Chair: Lijia Qu, Peking University, China****3:30 pm****Bill Lucas, University of California, Davis, USA**

Plasmodesmata: pathways for intercellular trafficking of macromolecules

3:50 pm**David Ehrhardt, The Carnegie Institution, Stanford Univ., USA**

A dynamic scaffold: cytoskeletal organization of cellulose synthase

4:10 pm**Richard Vierstra, University of Madison, Wisconsin, USA**Control of *Arabidopsis* hormone signaling by the ubiquitin/26S proteasome system**4:30 pm****Masaaki Ueda, Nara Institute of Science and Technology, Japan**

B2-type cyclin-dependent kinase is controlled by protein degradation. S-77

4:45 pm**Takashi Ueda, University of Tokyo, Japan**

Plant evolves a unique mechanism of endocytosis. S-78

S12 Session: Developmental Mechanisms 3**3rd Floor Hall No. 101****Session Chair: Hai Huang, Institute of Plant Physiology & Ecology, SIBS, CAS, China****3:30 pm****John Schiefelbein, University of Michigan, USA**Cell patterning in the *Arabidopsis* root epidermis**3:50 pm****Ben Scheres, Utrecht University, Netherlands**Stem cell and meristem action in *Arabidopsis*: a numbers game?

4:10 pm	Judy Callis, University of California, Davis, USA RING-type ubiquitin E3 ligase family in <i>Arabidopsis thaliana</i>
4:30 pm	Mengjuan Guo, Cold Spring Harbor Laboratory, USA Molecular mechanism of KNOX gene repression by the <i>Arabidopsis</i> ASYMMETRIC LEAVES1 complex. S-82
4:45 pm	Stephan Wenkel, Carnegie Institution, Stanford Univ., USA Feedback control of leaf polarity by a family of small leucine zipper proteins. S-83

6:00 - 7:30 pm	Dinner	1st Floor Banquet Hall
7:30 - 9:30 pm	Workshops 3 (1) Peptide Signal Transduction in Plants <i>Chun-Ming Liu and Rüdiger Simon</i>	2nd Floor Room No.87
7:30 -9:30pm	POSTER SESSION 3	3rd Floor Hall No. 102, 106&107

End of Conference

Workshop Descriptions and Schedule

Wednesday, June 20: 7:30-9:30 pm

(1) Linking Stress Signals to Growth- 2nd Floor Room No. 88

Workshop chairs: **Deshpal Verma** (Ohio State Univ., USA) and **Hong Ma** (Penn State Univ., USA)

Overview: All abiotic and biotic stress signals eventually affect plant growth. How these signals converge to alter translation and transcription machineries is not known. A body of evidence is accumulating about various stress pathways and a common thread is evolving which may allow us to understand how plant growth may be regulated under stress. This workshop will shed some light on a number of different signaling systems controlling gene expression and growth of plants.

7:30-8:00 pm	Deshpal Verma (Ohio State Univ., USA), <i>TOR kinase pathway in plants: Linking stress to growth signals and adaptation of the plant</i>
8:00-8:20 pm	Jianhua Zhu (Univ. of California, Riverside, USA) <i>Involvement of Arabidopsis HOS15 in histone deacetylation and cold tolerance</i>
8:20-8:45 pm	Hong Ma (Penn State Univ., USA) <i>Function of DNA repair genes in meiosis</i>
8:45-9:10 pm	Hong-Wei Guo (Peking University, China) <i>EIN3-like1 is a critical signaling component in EBF1/2-mediated ethylene response and plant development in Arabidopsis</i>
9:10-9:30 pm	Pip Wilson (Australian National Univ., Canberra) <i>The drought tolerant Arabidopsis alx8" mutant has altered leaf morphology as well as changes in stress response signalling pathways under normal growth conditions and during abiotic stress</i>

(2) Ubiquitination in Plant Development and Signaling- 2nd Floor Room No. 76

Workshop chairs: **Qi Xie** (Institute of Genetics and Developmental Biology, China) and **Minami Matsui** (RIKEN, Japan)

Overview: Ubiquitination has been demonstrated to play important roles in regulation of broad range of cellular processes, including cell division, differentiation, signal transduction, protein trafficking, and quality control in eukaryotic system. In plants, ubiquitination is involved in hormone signaling, environmental responses and pathogenesis of some diseases. This workshop will inform the community about the recent progress in Arabidopsis ubiquitination research. Presentations will focus on functional genomics and genetics to dissect the roles of SCF complex and RING finger proteins in plant development and signaling.

7:30-7:50 pm	Richard Vierstra (Univ. of Wisconsin-Madison, USA), <i>Introductory remarks about ubiquitination in plants</i>
7:50-8:10 pm	Yiyue Zhang (IGDB, China), <i>A novel ring finger protein in ABA signaling</i>
8:10-8:30 pm	Danny Chamovitz (Tel Aviv Univ., Israel), <i>Arabidopsis eIF3e in COP9 signalosome</i>
8:30-8:50 pm	Hirofumi Kuroda (RIKEN, Japan), <i>An ORFeome-based analysis of F-box protein genes in Arabidopsis</i>
8:50-9:10 pm	Yajie Niu (Washington State Univ., USA), <i>Degradation of HAVOC repressor proteins by SCFCOII during jasmonate signaling</i>
9:10-9:30 pm	Juan Carlos del Pozo (INIA, Spain), <i>Role of SCF-SKP2A in controlling cell division</i>

(3) Web Services for Arabidopsis Data Integration and Bioinformatics Tools for Arabidopsis Microarray Databases- 2nd Floor Room No. 87

Workshop chairs: **Chris Town** (J. Craig Venter Institute, USA) and **Anika Joecker** (Max Planck Institute for Plant Breeding Research, Germany)

Overview: The first part of the workshop will showcase a set of tools developed at the University of Leeds for mining microarray data, and will be followed by a series of presentations on the concepts of web services and their implementation. The ever increasing amount and complexity of Arabidopsis genomic data presents a growing problem that should be of concern to both the user and the bioinformatic community. Exploiting the full potential of these large and diverse datasets is currently hindered by the limited mechanisms of availability and the lack of integration. Web services provide a means whereby data residing at many different locations can be seamlessly integrated to provide the user with richer data sets. Unlike web pages that are idiosyncratic in their layout and content, and must be visited by a researcher one at a time, web services (in this case BioMOBY) provide data in well structured and agreed formats, document their availability through a central registry and can be combined to provide richer views of the data. At the workshop, we will present an overview of what web services are and how they work. This will be followed by a series of short presentations describing specific examples of web services in action and mechanisms to encourage a much more widespread use of web services discussed.

7:30-8:00 pm	Iain Manfield (Univ. of Leeds, UK), <i>Arabidopsis Co-expression Tool (ACT); Web-based tools to identify co-regulated sets of genes and predict gene function</i>
8:00-8:15 pm	Chris Town (J. Craig Venter Institute, USA), <i>Introduction to Web Services</i>
8:15-8:30 pm	Anika Joecker (Max Planck Institute for Plant Breeding Research, Germany), <i>AFAWE: Using web services and the Taverna workflow engine for automated protein function prediction through comprehensive data integration.</i>
8:30-8:45 pm	Andreas Groscurth (Max Planck Institute for Plant Breeding Research, Germany), <i>One stop shops: Aggregators to collect data from multiple source databases and their use in web-based data integration</i>
8:45-8:55 pm	Sean May (Univ. of Nottingham, UK), <i>Web Services at the Nottingham Arabidopsis Stock Centre (NASC)</i>
8:55-9:15 pm	Applications: output from the 2007 Developers' Workshop Manny Katari (New York Univ., USA), <i>Virtual Plant</i>
9:15-9:30 pm	Harvey Millar (Univ. of Western Australia), <i>Subcellular proteomics database (SUBA)</i> Jim Carrington (Oregon State Univ.), <i>Small RNA database</i> Chris Town (J. Craig Venter Institute, USA), <i>Developing workflows using Taverna</i>

Thursday, June 22: 7:30-9:30 pm

(1) Chemical Genetics- 2nd Floor Room No. 78

Workshop chairs: **Zhenbiao Yang** (Univ. of California, Riverside, USA)
and **Sean Cutler** (Univ. of California, Riverside, USA)

Overview: Chemical genetics uses small molecules to dissect biological pathways, analogous to the use of mutations in classical genetics. This workshop is designed to inform the community of the progress in the applications of chemical genetic approaches to *Arabidopsis* research. Presentations from the leading groups in this area will focus on both the discovery and use of new small molecules that perturb a variety of developmental and cell biological processes.

7:30-7:55 pm	Sean Cutler (Univ. of California, Riverside, USA), <i>Chemical genetic interrogation of natural variation</i>
7:55-8:20 pm	Glenn Hicks (Univ. of California, Riverside, USA), <i>Dissecting plasma membrane localization, vesicular trafficking and polar growth via chemical genomics</i>
8:20-8:45 pm	Yunde Zhao (Univ. of California, San Diego, USA), <i>Dissecting auxin pathways with a chemical genomics approach</i>
8:45-9:10 pm	Zhenbiao Yang (Univ. of California, Riverside, USA), <i>Chemical genetic interrogation of protein-protein interaction</i>
9:10-9:30 pm	Li Chuanyou (Institute of Genetics and Developmental Biology, Beijing, China), <i>Bestatin, an inhibitor of aminopeptidases, provides a chemical genetics approach to dissect jasmonate signaling in Arabidopsis</i>

(2) Metabolomics- 2nd Floor Room No. 92

Workshop chair: **Mike Beale** (National Centre for Plant and Microbial Metabolomics, UK)

Overview: Metabolomics has been defined as the quantitative measurement of all low molecular weight metabolites in a given sample, cell or tissue and the integration of the data in the context of gene function analysis. This type of analysis allows a full and global comparison of the differences between cell types, tissues, organs and whole organisms (plants, animals and microbes) to probe unknown aspects of gene function, physiology and metabolism for a plethora of future research goals. Metabolomics involves large scale analytical data collection and a range of spectroscopic methods are available to collect such data. The workshop is designed to inform the community of the progress in the application of metabolomics in *Arabidopsis* research. Presentations from the leading international groups in this area will focus on using this technology in functional genomic and QTL studies.

7:30-7:40 pm	Mike Beale (National Centre for Plant and Microbial Metabolomics, UK), <i>Introduction to Metabolomics</i>
7:40-8:00 pm	Kazuki Saito (RIKEN and Chiba Univ., Japan), <i>Metabolomics-based functional genomics in Arabidopsis thaliana</i>
8:00-8:20 pm	Joost Keurentjes (Wageningen Univ., The Netherlands), <i>Constructing genetic regulatory networks for plant metabolism using untargeted high throughput metabolomics</i>
8:20-8:30 pm	Short Break
8:30-8:45 pm	Nina Sipari (Univ. of Joensuu, Finland), <i>Metabolite profiling of the plant RCD1-SRO gene family: Oxidative stress</i>

8:45-9:00 pm	Jane Ward (National Centre for Plant and Microbial Metabolomics, UK) <i>Application of NMR fingerprinting to successfully predict gene function – Towards a mutant classification resource</i>
9:00-9:20 pm	Basil Nikolau (Iowa State Univ., USA) <i>Developing metabolomics as a functional genomics tool for deciphering metabolic and regulatory functions of Arabidopsis genes</i>
9:20-9:30 pm	Mike Beale <i>Concluding Remarks</i>

(3) Proteomics- 2nd Floor Room No. 87

Workshop chairs: **Wolfram Weckwerth** (Max Planck Institute- Potsdam/Golm, Germany) and **Harvey Millar** (Univ. of Western Australia, Australia)

Overview: Plant researchers are gaining more and more interest in proteomics. This is reflected in the increase in annual numbers of publications from 11 in 2000 to 92 in 2006. *Arabidopsis thaliana* is playing a major role as the plant model system of choice for proteomic studies. Exploiting classical methods like two-dimensional gel electrophoresis or emerging techniques like shotgun proteomics in combination with pattern matching to genome sequences is allowing the connection of whole proteome dynamics with both genome-wide RNA expression and metabolite dynamics. The value of accurate genome-wide protein identification and quantification can not be overestimated given the poor protein:RNA expression correlation typically observed. Measuring the active players - the proteins - is therefore a prerequisite to understand molecular dynamics and biochemical causality in plant systems. In the workshop a general introduction to the recent and most popular techniques will be followed by four short talks selected from abstracts covering a range of topics such as combining transcript and protein analysis, and understanding translational processes, hormonal signalling and drought and cold stresses through protein analysis. The workshop will conclude with the participants having a short formal discussion on trends and needs for the future and an informal discussion accompanied by a small poster session of proteomics related research from the conference.

7:30-7:50 pm	Wolfram Weckwerth (MPI-Potsdam/Golm, Germany), and Harvey Millar (Univ. of Western Australia), <i>Introduction and Overview</i>
7:50-8:03 pm	Adam Caroll (Univ. of Western Australia), <i>Towards understanding translational control: Systematic analysis of the <i>Arabidopsis</i> cytosolic ribosome proteome provides detailed insights into its protein complement and their post-translational modification</i>
8:03-8:16 pm	Zhi-Yong Wang (Carnegie Institution, Stanford, USA), <i>Proteomic and genomic studies of the brassinosteroid signal transduction pathway</i>
8:16-8:29 pm	YQ Zhang (Univ. of Alberta, Canada), <i>Transcriptomic and proteomic profiling reveals new insights into salt stress responses in <i>Arabidopsis</i> root</i>
8:29-8:42 pm	Hans-Peter Mock (Inst. for Plant Genetics and Crop Plant Research, Germany), <i>Proteome analysis of the cold stress response in <i>Arabidopsis thaliana</i></i>
8:42-9:02 pm	General Discussion (led by Workshop Organizers)
9:02-9:30 pm	Poster presentations and individual discussion

(4) TAIR Workshop - 2nd Floor Room No. 88Workshop chairs: **Eva Huala** and **Donghui Li** (Carnegie Institution, Stanford, USA)

Overview: The Arabidopsis Information Resource (TAIR; www.arabidopsis.org) is a community database for *Arabidopsis thaliana*. The workshop is designed for users who wish to more effectively utilize the curated data and software resources provided by TAIR. Specifically we will address curation of three major data types: gene structure, gene function and metabolic pathway annotation. We will describe our progress and give details of the TAIR7 genome release and will outline plans for the TAIR8 release. TAIR curators annotate gene function using terms developed by the Gene Ontology (GO) Consortium that describe the molecular function, biological process, and subcellular location of a gene product. We will use TAIR's GO annotations to demonstrate how controlled vocabularies allow for standardization of annotation and assist in comparative genomics. We will describe the process of GO annotation at TAIR and how the annotations can be used to classify large data sets or to facilitate annotation of other sequenced plant genomes. We will demonstrate how to access TAIR's GO annotations in bulk and to derive a global view of the status of functional annotation of the *Arabidopsis* genome. Plant metabolic databases have been developed to visualize the universe of primary and specialized (secondary) metabolism, to present the flexibility and interconnectivity of biochemical networks and to serve as matrix to predict Pathway/Genome Database's (PGDB's) based on sequenced genomes. We will introduce the contents and curation of the multi-organism database MetaCyc and the *Arabidopsis thaliana* specific database AraCyc. The major functions and applications of MetaCyc and AraCyc will be presented including the visualization of large-scale data on the Omics viewer.

7:30-7:50 pm	Eva Huala <i>Introduction to TAIR</i>
7:50-8:10 pm	Eva Huala <i>Structural annotation of the Arabidopsis genome</i>
8:10-8:40 pm	Donghui Li <i>Functional annotation of the Arabidopsis genome using Gene Ontology and Plant Ontology controlled vocabularies</i>
8:40-9:00 pm	Donghui Li <i>AraCyc metabolic pathway annotation in Arabidopsis</i>
9:00-9:30 pm	Question and answer session

Saturday, June 23: 7:30-9:30 pm**(1) Peptide Signal Transduction in Plants - 2nd Floor Room No. 88**

Workshop chairs: **Chun-ming Liu** (Institute of Botany, Chinese Academy of Sciences, China) and **Rüdiger Simon** (Univ. of Düsseldorf, Germany)

Overview: Peptides represent a very important class of signal molecules in animals, especially in neural and endocrinical systems. Substantial evidence accumulated in the last decades or so suggested that plants also use peptides for intercellular communication, to create position signal and for defense response. The Workshop for Peptide Signaling will outline some recent developments in this area.

7:30-8:00 pm	Yoshikatsu Matsubayashi (Nagoya Univ., Japan), <i>Physiological functions of two structurally distinct tyrosine-sulfated peptides in Arabidopsis growth and development</i>
8:00-8:30 pm	Rüdiger Simon (Univ. of Düsseldorf, Germany) <i>Components of CLE signalling pathways</i>
8:30-9:00 pm	Chun-ming Liu (Institute of Botany, Chinese Academy of Sciences, China), <i>Towards the understanding of the function of CLV3 and CLE19 peptides</i>
9:00-9:30 pm	TBA

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S-1

Cellular Dynamics in the Shoot Apical Meristem. Elliot Meyerowitz¹, Vijay Chickarmane¹, Sean Gordon¹, Marcus Heisler¹, Yuling Jiao¹, Wuxing Li¹, Zack Nimchuk¹, Carolyn Ohno¹, Xiang Qu¹, Adrienne Roeder¹, Kaoru Sugimoto¹. ¹California Institute of Technology, Pasadena, California, USA

The shoot apical meristems (SAMs) of a flowering plant are the sets of stem cells at the tip of each shoot, which ultimately provide the cells that make the stem, leaves, and flowers. The cells of the SAM are highly dynamic, but at the same time there is a highly stereotyped spatial structure, with cells of different types (or perhaps in different states) characteristically in specific and predictable positions. As cells divide their positions change, and therefore the stable pattern of gene expression is maintained despite the fact that the cells themselves do not remain in place. This dynamism calls for new methods of observation, manipulation, and analysis.

To describe and manipulate the cells of the *Arabidopsis* SAM, we have developed a set of live-imaging methods, a set of reporters for gene expression domains, and protein localization domains within cells of the SAM, and also a set of methods for changing the patterns of gene expression in the meristem while we are watching. By combining these methods we have shown that cells in the meristem both differentiate from the stem cell state, and return to this state depending on their positions and on signaling between different cells the stable patterns of gene expression found in unstable populations of cells are maintained by a series of feedbacks mediated by cell-cell signaling. Furthermore, using live imaging and reporter constructs we and others have found that the SAM has a private and highly controlled circulatory system for auxin, which serves as the morphogen that induces new leaf and floral primordia. This circulatory system is based on movement within each SAM cell of a polar auxin transporter, with this movement determined by the auxin gradient itself. This feedback system leads to successive peaks of auxin concentration in the precise pattern that is the phyllotactic spiral the regular positions of new leaf or floral primordia. This example seems to define a new class of developmental models, based on regulated and active transport of morphogens, rather than on diffusion or inhibitory cell-cell interactions.

S-2

When partners don't talk: the molecular basis of cell-cell communication during double fertilization. Juan-Miguel Escobar-Restrepo¹, Norbert Huck¹, Sharon Kessler¹, Valeria Gagliardini¹, Jacqueline Geyselinck¹, Wei-Cai Yang², Ueli Grossniklaus¹. ¹University of Zurich, Zürich, Switzerland, ²Chinese Academy of Sciences, Beijing, China

Research in our laboratory focuses on the developmental genetics of plant reproduction. We have used genetic and molecular approaches to identify genes controlling the underlying developmental processes using *Arabidopsis thaliana* and *Zea mays* as model systems. Our studies have shown that both genetic and epigenetic mechanisms play a key role in plant reproduction. In this presentation I will focus on cell-cell interactions during double fertilization. We have isolated a female gametophytic mutant, *feronia*, which disrupts double fertilization: in *feronia* mutant embryo sacs the pollen tubes, even if wild-type, are unable to release the sperm cells to effect fertilization. This phenotype suggests that the female gametophyte plays a crucial role in pollen tube reception and, thus, controls the behavior of the male gametophyte (Huck et al., 2003, *Development* 130:2149). The *feronia* and *sirene* mutants, which display similar phenotypes (Rotman et al., 2003, *Curr Biol.* 13:432), define novel signaling processes between the male and female gametophytes in the process of double fertilization. I will report on the molecular characterization of *FERONIA*, which is expressed at high levels in the synergid cells through which the pollen tube enters to effect fertilization. The molecular nature and subcellular localization of the *FERONIA* protein is consistent with its proposed function in a signaling process. Interestingly, some interspecific crosses result in phenotypes that are very similar to those observed in the *feronia* and *sirene* mutants. The evolutionary implications of these findings will be discussed.

S-3

Role of the *Arabidopsis* Retinoblastoma-related (RBR) protein in cell specification. Wilhelm Gruisse¹, Lorenzo Borghi¹, Ueli Grossniklaus², Ruben Gutza¹, Amal Johnson², Johannes Füller¹, Yec-han Laizet¹. ¹ETH Zurich, Switzerland, ²University of Zurich, Switzerland

The mammalian Retinoblastoma gene ("RB") is best known for its role in G1/S control and tumor formation, but other functions of the RB protein are still poorly understood. We have previously shown that loss of function of the *Arabidopsis* RB-related (RBR) protein is gametophytically lethal and results in supernumerary nuclei (1). The female gametophyte develops from the megasporangium and consists of six haploid cells and one homo-diploid cell that represent four different cell types, thus making it uniquely suited for dissecting the role of RBR in cell specification. Using markers for the egg cell, synergids, central cell and antipodal cells we could show that all cell types in *rbr* female gametophytes become mis-specified. These results suggest that the maternal RBR contribution to the megasporangium is not sufficient to properly connect the three ensuing mitotic divisions to specification processes, or to direct specification during cellularization. To further test the hypothesis that RBR is required for cell specification, we constructed conditional alleles to perturb the RBR pathway during other developmental processes. Targeting RNAi-induced downregulation of "RBR" expression to the root meristematic region produced supernumerary stem cells, suggesting that specification of cells exiting the stem cell was impaired (2). Loss of a complementing "RBRlox" allele during leaf development does not block cell division, but "rbr/rbr" sectors do not specify cells for stomata and trichome development. Similar results were obtained when RNAi-induced downregulation of "RBR" expression was targeted to the shoot apical region during leaf primordia development. Together, the results support a model that RBR functions at a central node, which connects mitotic activity to cell specification processes.

1. Ebel C, Mariconti L, Gruisse W. (2004) Plant retinoblastoma homologues control nuclear proliferation in the female gametophyte. *Nature* 429: 776-780

2. Wildwater M et al. (2005) The "RETINOBLASTOMA-RELATED" gene regulates stem cell maintenance in *Arabidopsis* roots. *Cell* 123: 1337-1349

S-4

Rice, a Model System for Studying Plant Architecture Jiayang Li¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Rice is an ideal system for studying plant architecture of cereal crops. Rice plant architecture, a collection of the important agronomic traits that determine its grain production, is mainly affected by factors including tillering (tiller number and tiller angle), plant height and panicle morphology. To elucidate the molecular mechanisms that control rice plant architecture, we have identified several related mutants and isolated the corresponding genes. Among them, the rice mutant *lazy1* (*la1*), originally reported as *lazy* (*la*) in 1938, is a classical mutant for studying the rice tiller angle due to its extremely spreading growth phenotype. We have now cloned the *LA1* gene through a map-based approach and show that *LA1* is a novel gene that plays an important role as a negative regulator of polar auxin transport (PAT). Loss-of-function of *LA1* enhances PAT greatly and thus alters the endogenous IAA distribution in shoots, leading to the reduced gravitropism and therefore the tiller-spreading phenotype of rice plants. We also found that the tiller angle of *LA1* RNAi transgenic plants showed a correlation to endogenous *LA1* expression levels, implying a potential application of *LA1* to modify rice tiller angle in molecular breeding. In addition, we have identified and characterized the *DWARF27* (*D27*) gene that controls rice tiller number and the *SMALL PANICLE* (*SP*) gene that regulates the panicle size, respectively. Further functional studies on these essential regulators will provide a molecular basis for developing elite rice and other cereal crops in the future.

S-5

MAPPING GENOTYPE INTO PHENOTYPE: NATURAL, INDUCED AND EPIGENETIC VARIATION IN ARABIDOPSIS GENOMES. Joseph R. Ecker¹. ¹The Salk Institute for Biological Studies, La Jolla, CA USA

A precise and comprehensive understanding of DNA sequence and epigenetic variation in natural populations of a species will be essential for elucidating the basis of phenotypic variation. We have applied oligonucleotide tiling microarrays and next-generation ultra-high throughput DNA sequencing methods to identify DNA sequence and epigenetic variation in wild strains (accessions) of *Arabidopsis thaliana* that were chosen for maximal genetic diversity. The entire Arabidopsis Genome Initiative (AGI) reference genome sequence of *A. thaliana* Col-0 was tiled on high-density tiling microarrays at single base resolution using over a billion 25 base oligonucleotides. The arrays were hybridized with genomic DNA from 19 accessions, isothermally amplified DNA to minimize ascertainment biases. In addition, whole genome sequencing using a Solexa/Illumina Genetic Analyzer allowed more complete sequence coverage of the genomes of several strains (Col-0, Ler-1, Cvi-0, etc.), allowing independent assessment of the quality of the AGI Sanger and Perlegen array-based genome sequences. The degree of polymorphism and types of genes that harbor major effect polymorphisms in natural populations will be described. We are also using next-generation sequencing technology to develop methods for creating large populations of T-DNA insertion mutants in diverse genetic backgrounds as well as for determining the extent of epigenetic diversity in wild accessions. A proposal for cost effective re-sequencing of 1,001 *Arabidopsis* genomes will be outlined. When coupled with community-wide phenotypic screens for traits of interest, this database would allow realization of genome-wide association mapping studies and provide an unprecedented resource for understanding the genetic/epigenetic basis of phenotypic variation in plants.

S-6

The use of *Arabidopsis* natural variation for gene function analysis. Maarten Koornneef¹, Xuexing Huang¹, Wim Soppe¹, Matthieu Reymond¹, Joost Keurentjes², Leonie Bentsink³. ¹ Max Planck Institute for Plant Breeding Research, Cologne, Germany, ² Wageningen University, The Netherlands, ³ Utrecht University, The Netherlands

The functional analysis of genes in *Arabidopsis* has followed the routes of both reverse genetics (from DNA to mutant phenotype) and forward genetics (from variant phenotype to DNA). For the latter approach in addition to using induced mutants, natural variation is a resource of increasing importance. *Arabidopsis thaliana* accessions have been collected in a wide range of habitats in the Northern hemisphere. Because these accessions come from different habitats, it is assumed that selection for adaptation to these local environments has occurred and provides genetic variation of responses to environmental factors.

Natural genetic variation is most effectively studied using immortal mapping populations. QTL and genetic networks involved in the variation of multiple traits can be revealed by studying such populations. Moreover, co-location of QTL for different traits can indicate functions for genes that are not expected. Especially the combination of omics with QTL analysis provides large data sets for such systems biology. Natural variation is also a useful resource for studying traits that are not accessible in mutant screens in the common laboratory strains because the phenotypes of interest cannot be observed using these genetic backgrounds. This is illustrated by the study of flowering time, seed dormancy and plant performance QTL. Furthermore epistatic interactions are common when studying the genetics of complex traits. Such interactions often result in novel phenotypes that occur in the progeny of accession crosses. Mapping populations derived from inter-crossing multiple accessions may detect as much as possible such interactions. Map based cloning approach is commonly used to clone the gene(s) underlying the effect of a single QTL that has been Mendelized by backcrossing. The presence of candidate gene(s) in the region of interest and the use of loss of function mutants helps identifying the relevant gene(s) as has been demonstrated for seed dormancy QTL.

S-7

Genetics and Genomics of DNA methylation in *Arabidopsis*

Xiaoyu Zhang¹, Lianna Johnson¹, Ian Henderson¹, Peng Peng¹, Israel Ausin¹, Magnolia Bostick¹, Julie Law¹, Carey Li¹, Yana Bernatavichute¹, Suhua Feng¹, Steve Jacobsen¹. ¹HHMI/University of California-Los Angeles, Los Angeles, CA, USA

Our laboratory studies epigenetic gene regulation using *Arabidopsis thaliana* as a model genetic system. We utilize epigenetic mutations at the *SUPERMAN* and *FWA* genes, which are caused by heritable changes in DNA methylation and chromatin structure of these loci. These epigenetic mutations allow us to follow the methylation state of the genes by easily scorable morphological phenotypes, and allow for straightforward genetic analysis of the underlying epigenetic silencing. Using suppressor mutant screening approaches, we are characterizing genes that are required for the maintenance of DNA methylation and silencing. These include the DNA methyltransferase CHROMOMETHYLASE3, which appears to be the major enzyme controlling CpNpG methylation in the genome, and KRYPTONITE, a lysine 9 histone H3 protein methyltransferase gene, which is also required for the maintenance of CpNpG methylation. A third gene, ARGONAUTE4, encodes a type of protein normally found in RNA interference pathways, suggesting that small RNAs target chromatin modifications. We are also screening for mutants specifically blocked in the initiation of DNA methylation. This has thus far resulted in the identification of seven genes, including the de novo DNA methyltransferase enzyme DRM2 (the ortholog of mammalian Dnmt3). We also discovered that several RNA silencing genes including DICER-LIKE3, RNA DEPENDENT RNA POLYMERASE2, ARGONAUTE4, NRPD1a, NRPD1b, and DRD1 are required to establish DNA methylation, again showing that small RNAs are at the heart of DNA methylation control. Current work is directed toward understanding the mechanism of action of these proteins in DNA methylation control. We have also begun genomic studies of DNA methylation, as well as the histone modifications and small RNAs, which are important in DNA methylation control. These studies are allowing a much broader study of the function of epigenetic modification in wild type, and a much more detailed analysis of mutants.

S-8

Genetic and molecular characterization of inter-subspecific hybrid sterility and wide compatibility in rice. Qifa Zhang¹, Jiangjiong Chen¹, Jihua Ding¹, Yidan Ouyang¹, Jiangyi Yang¹, Huihui Yu¹, Hongyi Du¹, Yunhe Jiang¹, Shuqing Qiu¹, Xiang Song¹, Kede Liu¹, Xianghua Li¹, Caiguo Xu¹. ¹National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China

The Asian cultivated rice (*Oryza sativa* L.) consists of several ecological groups, among which indica and japonica are the two major ones, often referred to as two subspecies. Indica varieties are widely cultivated in tropical and subtropical areas, and japonica cultivars are grown in temperate regions. Although hybrids between the two subspecies frequently show strong heterosis, reduced fertility occurs in most of the hybrids ranging from partial to complete sterility, which has hindered utilization of the inter-subspecific heterosis. Wide compatibility varieties comprise a special germplasm class that produce hybrids with normal fertility when crossed with both indica and japonica subspecies. We have been conducting a systematic study on the genetic and molecular basis of the I-J hybrid sterility and wide compatibility. The results of the cytological study showed that both male and female gamete abortion, and reduced affinity between the uniting gametes all occur in the hybrids, resulting in greatly reduced fertility. QTL analysis identified and confirmed two major genetic loci, S5 and f5, specifying embryo-sac and pollen abortion, respectively. A tri-allelic system was found at each of the loci; an indica allele Si, a japonica allele Sj and a neutral allele (or wide compatibility gene) Sn, such that an individual heterozygous for Si/Sj would show reduced fertility, and all the other genotypes produce normal fertility. Map-based cloning has been conducted to isolate these genes. The gene for S5 has been characterized as encoding an aspartic protease. Current progresses of this work will be presented in the meeting.

S-9

Getting to the root of cell identity. Philip Benfey¹, Siobhan Brady¹, Hongchang Cui¹, David Orlando¹, Jose Dinneny¹, Terri Long¹, Twigg Richard¹, Daniel Mace¹. ¹Duke University

Central processes in development include creating distinctions between cells and producing coordination among different cells so that they function as units. While signaling and transcription are equally important for development, high throughput techniques for identifying the nodes and links in transcriptional networks have matured more rapidly. For plants the simplifying aspects of development in an organ such as the root, make it highly tractable for the application of these approaches. In an effort to identify the transcriptional networks that regulate cell identity in the root we are generating three datasets: 1) global expression profiles; 2) cellular localization of transcription factors; and 3) transcription factor targets. To understand the role of transcriptional networks in development, each of these datasets needs to be at cell-type specific resolution. To acquire global expression profiles we developed technology that uses sorted marked populations of cells with subsequent hybridization of the labeled RNA to microarrays. Employing this methodology, we now have data that cover most of the cell types in the root. We have also developed methods for identifying expression profiles along the developmental axis of the root. Combining these datasets gives us spatial and temporal expression patterns at high resolution. Analysis of these data provides insight into the regulatory basis of cell identity under standard growth conditions.

To investigate the response to environmental stimuli at the cellular level, we are profiling expression after cell sorting in response to a number of stresses. Many of the genes that are induced or repressed in response to these stresses in a tissue-specific manner have not been previously characterized. We are also developing technology that will allow us to follow the effects of stimuli on hundreds of genes at cell-type specific resolution over time. Initial results suggest that there is a core set of functions for each cell type overlaid with a set of cell-type specific responses to different stimuli.

This work is supported by grants from the NIH, NSF and DARPA.

S-10

The Development and Evolution of Leaves. Neelima Sinha¹. ¹University of California, Davis, USA

The Class I Knotted-like homeobox (KNOX 1) genes are highly expressed in the shoot apical meristem but not expressed in the emerging leaf primordium in tobacco, maize, or Arabidopsis. In tomato, KNOX1 expression (LeT6, TKN1) is seen in the early leaf primordium (Chen et al. 1997; Hareven et al. 1996). It is worth noting that tomato has compound leaves while the other organisms thus far tested have simple leaves. We have analyzed compound leaf producing shoot apices in clades with independently derived compound leaves and shown that with one exception (a derived clade in the Fabaceae) compound leaves always show expression of KNOX genes (Bharathan et al., 2002). In the derived pea clade the LFY/FLO gene regulates this function of generating leaf complexity. While KNOX genes appear to be important for generating leaf complexity (except in a derived clade in the Fabaceae) we find that other genes like PHANTASTICA might play a role in determining the form of the compound leaf generated. Transgenic plants overexpressing antisense PHAN suggest that PHAN, by modulating dorsiventrality, has a role in regulating the number of leaflets and their placement in a compound leaf. The role of other key regulatory genes is also being explored in the context of normal and "unusual" leaf morphologies seen in nature.

S-11

Steroid binding protein in photomorphogenesis and BR signaling. Hong-Wei Xue¹, Li Song¹, Qiu-Ming Shi¹. ¹National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Science, Chinese Academy of Sciences, China

Steroid-binding proteins (SBPs) are essential for growth and development of both animals and higher plants. Most known animal SBPs not only regulate the concentrations of general available steroids and hence control their metabolic clearance rates, but also function in steroid signal transduction through interacting with specific receptors on the plasma membrane of target cells, thus directly involve in regulation of cell growth and differentiation (Rosner et al., 1999; Breuner and Orchinik, 2002). Four SBP coding genes have been annotated in Arabidopsis and MSBP1, which could bind progesterone, 5-dihydrotestosterone, 24-epi-brassinolide (24-eBL), and stigmasterol with different affinities in vitro, and whose expression is regulated by light, serves as a negative regulator of cell elongation in Arabidopsis and plays negative roles in BR signaling. Our further studies indicate that expression of MSBP1 was regulated by key factors HY5/HYH and involve in plant photomorphogenesis. In addition, MSBP1 suppress BR signaling through interactions with BAK1.

S-12

Organogenesis of the perianth in *Arabidopsis* flowers is facilitated by the dual action of PETAL LOSS and AUX1. Edwin Lam-pugnani¹, Aydin Kilinc¹, David Smyth¹. ¹Monash University, Melbourne, Vic., Australia

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 effect 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 mutant (*no petals*, *nop*) lacking petals and nectaries (which arise internal to the petals). We show that the *nop* mutant alone has normal flowers, but that it is incompletely dominant for petal initiation in the *ptl* homozygous background. Inversely, *ptl* shows incomplete dominance for petal initiation in the *nop* homozygous background. This reciprocal co-dominance indicates that the two genes influence a common process in petal initiation.

The *NOP* gene was positionally cloned and identified as *AUX1*, an auxin influx carrier. We propose that auxin functions as a signal to promote the initiation of petals. Loss of *AUX1* function alone apparently does not weaken the signal below an organ-inducing threshold level, but the joint loss of *PTL* function results in distortions of growth in the inter-sepal zone such that auxin levels are now insufficient to promote petal initiation in the adjacent regions.

S-13

Regulatory proteins, DUO1 and DUO3, link cell cycle progression and cell specification in male germ line development.
Lynette Brownfield¹, Said Hafidh¹, Anjusha Durbarry¹, Anna Sidrova¹, Tony Wardle¹, David Twell¹.¹ Department of Biology, University of Leicester, Leicester, UK.

The post-meiotic pathway of male gametophyte development in flowering plants that leads to the production of fertile male gametes or sperm cells is well documented in research that spans a century. However the developmental mechanisms that integrate germ line cell divisions and gamete specification remain largely unknown. We have previously described a class of mutants, duo pollen (duo), that specifically block generative cell cycle progression and provide compelling evidence for gametophytic control of germ line cell cycle progression (Durbarry et al. (2005) *Plant Physiol.* 137, 297-307).

In duo1 pollen the generative cell fails at G2/M phase of the cell cycle while the vegetative cell is unaffected. DUO1 is a member of the R2R3 Myb transcription factor family that is expressed specifically in the male germ line (generative and sperm cells), with the protein first detected shortly after pollen mitosis I. We have used several marker lines to explore the role of DUO1 in pollen development. We found that CyclinB1;1, is down regulated in duo1 pollen highlighting the involvement of DUO1 in the decision of generative cells to enter mitosis. Coupled with cell cycle progression, DUO1 also regulates sperm cell specification as several proteins expressed exclusively in the male germ cells are down regulated in duo1 pollen.

We have also identified DUO3, a novel protein with a single MYB-like domain and acidic rich domains. In contrast to DUO1, DUO3 is widely expressed in shoot and root meristems, vascular tissue and in generative and vegetative cells. Analysis of male germ line markers down regulated in duo1 pollen reveals distinct patterns of male germ line-specific transcription that depend on DUO3. Thus, we conclude that DUO1 and DUO3 have essential and overlapping roles in male germ-line development. These data provide the first molecular evidence for a regulatory link between cell cycle progression and the specification of functional sperm cells.

S-14

Exploiting the natural genetic variation in recombinant inbred lines: two accessions, multiple traits.. Christian Hardtke¹, Richard Sibout¹.¹University of Lausanne, Lausanne, Switzerland

Arabidopsis wild accessions are an increasingly popular resource to identify allelic variants and even novel genes of interest for a given developmental process. In our lab, we exploit this natural genetic variation to identify factors that modulate growth rate and thereby contribute to morphological differences between accessions. Growth is most easily monitored in the root, where growth measurements are essentially one-dimensional. In a survey of primary root growth rate in a sample of 44 accessions, we identified one line, Uk-1, in which root growth is markedly reduced. Molecular genetic analysis of this line revealed that this phenotype is largely due to loss-of-function of one locus, named BREVIS RADIX (BRX). Gene expression analyses and physiological experiments suggest that BRX plays a role in the interaction of the auxin and brassinosteroid pathways. Both hormones have been implicated in vascular differentiation, and therefore it was not surprising that in another survey of natural variation in secondary growth of the hypocotyl vasculature, Uk-1 was among the lines with a high phloem to xylem ratio. This phenotype has also been observed in certain brassinosteroid signaling mutants. Indeed, vascular growth is decreased in Uk-1 plants, both in the hypocotyl and root, when compared to transgenic Uk-1 plants complemented by a 3S:BRX transgene. Surprisingly though, the xylem to phloem ratio in the hypocotyl is not influenced by BRX, a result that is also confirmed by QTL analysis of a recombinant inbred line population derived from a Uk-1 x Sav-0 cross. Rather, this trait mapped to the same large effect QTL that explains the difference in flowering time between Uk-1 and Sav-0. This finding prompted us to revisit our secondary growth survey of natural accessions. Indeed, nearly all natural variation in secondary growth of the vasculature could be explained by natural variation in flowering time. The implications of this result for screening secondary growth mutants and further details will be discussed.

S-15

Florigen in rice. Ko Shimamoto¹, Shojiro Tamaki¹, Shoichi Matsuo¹, Hann Lin Wong¹, Shuji Yokoi¹.¹ Nara Institute of Science and Technology

Rice is a model of short day plants (SDP) for the molecular genetic study of the photoperiodic regulation of flowering. Rice has a highly conserved genetic pathway for flower induction in SD consisting of OsGI, Hd1, and Hd3a genes. In LD, Hd1 suppresses Hd3a expression while in SD Hd1 activates Hd3a expression. Therefore, this dual role of Hd1 in the regulation of Hd3a expression is a molecular basis of the photoperiodic regulation of flowering in rice (Izawa et al., 2002, Hayama et al., 2003). We are examining the possibility that Hd3a encodes florigen, which is considered to be induced in leaf and transported to the shoot apex and causes flowering. The florigen hypothesis was postulated 70 years ago but its nature is not known. Results of the analysis on transgenic rice expressing the Hd3a:GFP gene fused with three different promoters suggest that Hd3a protein is florigen.

S-16

microRNA biogenesis and function in *Arabidopsis*. Bin Yu¹, Zhiyong Yang¹, Julien Curaba¹, YunJu Kim¹, Vanitharani Ramachandran¹, Binglian Zheng¹, Theresa Dinh¹, Lijuan Ji¹, Liu Bi¹, Xuermei Chen¹. ¹University of California, Riverside, CA, USA

We are interested in how microRNAs (miRNAs) are produced, modified, and degraded in plants, and how miRNAs act in developmental processes. We are using a combination of genetic and biochemical approaches to study miRNA metabolism and function. We have previously demonstrated that plant miRNAs are methylated on the 2' OH of the 3' terminal ribose by the methyltransferase protein HEN1. The methyl group protects miRNAs from an uridylation activity and an exonuclease activity that target the 3' OH. From a genetic suppressor screen using the weak *hen1-2* allele, we isolated mutations in the largest and second largest subunits of pol IVa that restore miRNA methylation in the *hen1-2* background. I will present evidence that HEN1 and pol IVa have antagonistic activities in miRNA biogenesis and that one role of methylation is to protect miRNAs from the activities of pol IVa.

S-17

CHROMATIN CHARTING: Global mapping and characterization of epigenetic control mechanisms. Eric Lam¹, Faye Rosin¹, Naohide Watanabe¹, Naohiro Kato¹, Juana-Mari Arroyo², Jean-Luc Cacace¹, Joe Simorowski², Umamaheswari Ramu², Matthew Vaughn², Yuda Fang², Bruce May², Richard McCombie², David Spector², Robert Martienssen^{2,1} Rutgers University, New Brunswick, NJ, USA,²Cold Spring Harbor laboratory, Cold Spring Harbor, NY, USA

In the phenomena known as position effect (PE), integration location is hypothesized to affect the expression of transgenes. To globally characterize PEs in the genome, which is likely mediated by epigenetic mechanisms, we generated 611 Ds-tagged lines of *Arabidopsis*, designated CCT lines. The Ds-tagging cassette contains a CaMV 35S::luciferase gene which allows monitoring of PEs, and a lac operator repeat array that allows the visual tracking of the tagged loci by expressing fusions of fluorescent proteins with LacI within the nuclei of live plants. By inserting the same 35S::Luc cassette into various loci within the genome together with the pNos::NPTII selection marker for kanamycin resistance, we seek to quantify and assess the chromosomal location contribution to gene expression that is superimposed on cis-element-specific controls on the global level (i.e. the "transcription potential" for a particular location within the genome). Our first gene expression potential map with 277 CCT lines dispersed over all five chromosomes revealed that over 80% of the insertions have gene expression levels that are within 2-fold of the average activity. Thus, for the majority of locations within the *arabidopsis* genome, we conclude that the activity of a particular transgene is essentially constant and PE is not a factor. The remaining 15 to 20% of the insertion loci, however, exhibit significant enhancement or suppression of one or both of our marker genes. We designate the latter class as Position Effect Loci (PEL). Focusing on a 100 kb region on the top arm of Chr. 2, we found that insertions into a 50 kb "heterochromatin island" adjacent to the Nucleolar Organizing Region (NOR) are silenced more than 10 fold in their associated gene activities, with root tissues exhibiting much more extensive silencing. Hypermethylation at CpG sites appears to be an important contributor for this PE silencing and is correlated with physical constraint of the silenced locus to the nucleolus.

S-18

QTL analysis of developmental trade-offs: plastochron and pathogen response in *Arabidopsis thaliana*. Marco Todesco¹, Sureshkumar Balasubramanian¹, Sridevi Sureshkumar¹, Chris Schwart², Petra Epple³, Jeff Dangl³, Norman Wirthman¹, Julin Ma-Loof⁴, Justin Borevitz⁴, Joanne Chory^{4,5}, Detlef Weigel^{1,4,1} Max-Planck-Institute for Developmental Biology, Tuebingen, Germany,² University of Wisconsin, Madison, WI, USA,³ University of North Carolina, Chapel Hill, NC, USA,⁴ Salk Institute for Biological Sciences, La Jolla, CA, USA,⁵ Howard Hughes Medical Institute, La Jolla, CA, USA

Plants modulate their growth and development based on environmental cues. The shoot apical meristem (SAM) generates leaves in a particular spatial arrangement (phyllotaxis) at defined time intervals (plastochron length). Environmental cues such as light, temperature and presence of pathogens may affect both traits although underlying mechanisms are currently unknown. Here we report co-localisation of a QTL for plastochron length with a natural variant in a pathogen response gene, suggesting a built-in trade-off between immunity and growth rates.

We screened 24 accessions of *Arabidopsis thaliana* in three different growth conditions to reveal extensive variation in plastochron length. Exploiting this natural variation, we performed QTL analysis in two different temperature conditions using a newly developed Recombinant Inbred Lines (RIL) population derived from Col-0 (Columbia) and Est-1 (Estland). The QTL analysis revealed a major temperature-sensitive QTL for plastochron length in Chromosome 4, and a co-localising major QTL for leaf lesioning, a trait peculiar to Est-1 plants. Using an Heterogenous Inbred Family (HIF48) we fine mapped the lesioning phenotype to a 12 kb region. Expression analysis suggests that the lesioning is associated with constitutive activation of pathogen response pathways; we are in the process of testing whether this response is functional. Sequence analysis of the genes present in the QTL interval reveals substantial natural variation not only between Est-1 and Col-0, but also among other wild strains. Transgenic analysis through complementation and amiRNA-mediated silencing is currently underway to confirm the causality for the QTL. The apparent co-localisation of a plastochron QTL and potential variation in pathogen response has important implications for adaptation to the environment.

S-19

Axis-dependent gene expression in the lateral organ formation. Kiyotaka Okada¹, Koichi Toyokura², Keiro Watanabe², Toshiaki Tarneshige², Yuhei Tsuchida², Ryuji Tsugeki². ¹National Institute for Basic Biology, Myodaiji, Okazaki, Aichi, Japan, ²Graduate School of Science, Kyoto University, Kyoto, Japan

In order to unveil the signaling cascade which lead region-specific gene expression responsible for axis-dependent organ formation, we are examining expression pattern of three genes working in the early stages of lateral organ development. Lateral organs, such as leaves, young floral buds and floral organs develop from primordia formed at the fixed position in the peripheral region of shoot apical meristem, and are considered to follow three axes; basal-apical, central-marginal, and adaxial-abaxial. The direction of each axis could be fixed in relation to the position of the meristem. FILAMENTOUS FLOWER (FIL), a member of YABBY family, is responsible to the formation of abaxial side tissue and is expressed in the abaxial side of lateral organ primordia. Promoter analysis of FIL showed that the region-specific expression is based on two discrete cis elements; one is responsible to promote expression at both abaxial and adaxial sides, and the other is to repress at adaxial side. On the contrary, PHABULOSA (PHB), a member of homeobox family, is required for formation of the adaxial side tissue, and is expressed at the adaxial side. Recent researches propose that the region-specific expression of PHB is controlled by microRNA which is expressed at the abaxial side. These observations suggest that the two sides in lateral organ primordia mutually control specific gene expression. However, double-staining analysis of FIL- and PHB-expressing regions showed the two regions are partially overlapped, not supporting the simple mutual-repression model. The third gene, PRESSES FLOWER (PRS), a member of homeobox/WOX family, is expressed at the marginal region of lateral organ primordia. But the FIL-PHB overlapping region covers the PRS expressing region. Based on these observations and other ongoing experiments, we are trying to present a model of axes-dependent gene expression and lateral organ development.

S-21

Organ formation and gene expression patterning in ovule development. Charles Gasser¹, Ryan Brown¹, Thomas Gallagher¹, Dior Kelley¹, Mona Monfared¹, Christina Passerini¹. ¹University of California, Davis, CA, USA

We are studying the genetic and molecular mechanisms underlying the process of ovule morphogenesis in *Arabidopsis*. We have focused in particular on initiation and laminar growth of integuments, the lateral structures developing on the flanks of an ovule primordium. Classical genetic analysis has identified a number of genes necessary for normal ovule morphogenesis. The majority of such genes identified to date encode putative transcription factors. Several such factors belong to families that are associated with ab/adaxial polarity determination in the primary lateral organs of seed plants. *INNER NO OUTER* (*INO*) plays an essential role in the asymmetric growth of the outer integument and encodes an ovule-specific member of the YABBY family of transcription factors. *ABERRANT TESTA SHAPE* (*ATS*) encodes a member of the KANADI protein family and is required for the separation of the inner and outer integuments and for growth of the inner integument. Other KANADI proteins appear to be required for outer integument development. We propose a model in which the activity of these genes in integuments parallels the action of gene family members in primary lateral organ development. In this model, the spatial confinement of expression of these abaxial factors is essential to their function and results in part from interactions with yet to be identified adaxial factors. Studies on the regulation of *INO* have revealed the presence of specific regulatory elements in the promoter sequence, and have allowed the identification of transcription factors that may be involved in *INO* expression. Identification of ovule regulatory genes in *Arabidopsis* has enabled the isolation of orthologous genes from other species with divergent ovule morphologies, helping to illuminate the developmental basis of this diversification. Supported by grants from U. S. National Science Foundation (IBN-0419531, MCB-0517104).

S-20

Transcriptional and post-transcriptional regulation in the control of flowering by photoperiod. Franziska Turck¹, Fabio Fornera¹, Seonghoe Jang¹, Virginie Marchal¹, Coral Vincent¹, Antonis Giakountis¹, Maria Albani¹, Renhou Wang¹, George Coupland¹. ¹Max Planck Institute for Plant Breeding Research, Cologne, Germany

In many plants the transition to flowering is controlled by seasonal cues such as changing day length and temperature. In *Arabidopsis* a circadian-clock regulated pathway that promotes flowering specifically in response to the longer day lengths of spring and early summer has been described. This pathway includes the *GIGANTEA* (GI), *CONSTANS* (CO) and FT proteins, which act in the vascular tissue of the leaves to promote synthesis or transport of a systemic signal that triggers flower development at the shoot meristem. We have studied how circadian-clock regulation and acute responses to light combine to activate this pathway in response to long days. We have analyzed the mechanisms regulating the precise timing of CO and FT gene expression during the day by studying the chromatin modifications on the FT gene, natural genetic variation for circadian phase in *Arabidopsis* and by identifying critical transcription factors through systematic misexpression, and these areas will be described. The importance in flowering control of acute light signalling in regulating protein stability by influencing the activity of the ubiquitin ligase COP1 will also be discussed.

S-22

A chromatin link that couples cell division to *Arabidopsis* root epidermis cell fate through epigenetic changes of patterning genes. Elena Caro¹, M Mar Castellano¹, Crisanto Gutierrez¹. ¹Centro de Biología Molecular, CSIC-UAM, Cantoblanco, Madrid, Spain

Cell proliferation and cell fate decisions are strictly coupled during plant embryogenesis and organogenesis. However, the molecular links that coordinate them are poorly understood. In the *Arabidopsis* root meristem epidermis, expression of the homeobox GLABRA2 (GL2) gene occurs in the trichoblasts, determining that these cells will differentiate into non-hair cells, whereas GL2 is repressed in trichoblasts, that form root hair cells. This decision requires signaling from the cortical cell layer, transcriptional regulation and a change in chromatin accessibility. We initially observed that CDT1, a protein that controls DNA replication at the G1/S transition, activates GL2 expression, suggesting a link between cell division and cell fate decisions.

To define the molecular mechanism further, we identified a novel GL2-Expression Modulator (GEM) protein as a CDT1-interactor. GEM negatively affects both epidermal cell division and GL2 expression. Consistent with this, the level of GEM directly correlates with root hair density. GEM also affects the spatial pattern of GL2 expression. We found that GEM binds to the GL2 promoter by interacting with TTG1 (TRANSPARENT TESTA GLABRA1), a WD40-repeat protein involved in GL2-mediated cell fate decision. Our data suggest that CDT1 and TTG1 compete in vivo for their binding to GEM. Moreover, GEM also binds to and negatively regulates the expression of CAPRICE (CPC), involved in GL2 repression in trichoblasts. We hypothesized that GEM might regulate GL2 expression by controlling the histone epigenetic marks in its promoter. Indeed, we demonstrated that GEM mediates the acquisition and/or maintenance of a correct histone H3 acetylation and H3K9 methylation at both GL2 and CPC promoters, specifically just upstream of the corresponding ORFs.

Our study provides direct evidence for GEM as a crucial component involved in root architecture, that couples cell division, fate and differentiation during *Arabidopsis* root development (1). Further experiments defining this novel pathway that relies on GEM functions will be presented.

1. Caro et al. *Nature* (2007, in press).

S-23

Genetic analysis of the vascular patterning of the *Arabidopsis* root. Annelie Carlsson¹, Ove Lindgren², Jan Dettmer², Christina Roberts¹, Anne Honkanen², Satu Lehesranta², Siripong Thitamadee², Yk Helariutta². ¹Dept. of Evolution, Genomics and Systematics, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden, ²Institute of Biotechnology, University of Helsinki, Finland

The vasculature of plants is of immense importance as it provides paths for the transport of water, sugars, hormones and other signalling molecules through the phloem and xylem, in addition to providing physical support to the plant through the lignified cell walls of the xylem and fibres associated with the vasculature. Despite its importance, little is known about the regulation of the development of the tissues making up the vasculature. The influence of various hormones have been emphasized by several studies, but few regulatory factors have been identified. In order to identify novel components influencing the vascular patterning of the *Arabidopsis* root, we have performed a genetic screen for mutants miss-expressing a phloem marker gene. This screen resulted in the identification of a set of novel mutants with patterning and/or cell proliferation defects specific to the stele. We named these mutants *distorted root vascular pattern* (*dva*). Collectively, these mutants have short primary roots, display a lack of At-SUC2::GFP expression at the root tip, accompanied by a delayed and distorted phloem development. In *dva1* and several of the other mutants we detected ectopic differentiation of xylem in the pericycle along the xylem axis, a phenotype associated with loss of pericycle markers. The phenotype of *dva1*, thus, indicates that the DVA1 protein is required for normal patterning of the root vasculature. We present a detailed characterisation of the *dva1* mutant.

S-24

Second Generation *Arabidopsis* Genomics with Second Generation Sequencing Instruments. William McCombie¹, Robert Martienssen¹. ¹Cold Spring Harbor Laboratory

The sequence of *Arabidopsis thaliana* (Columbia) was completed in 2000. A major breakthrough in DNA sequencing instrumentation has led to new instruments which are capable of generating significant coverage of the *Arabidopsis* genome in a period of as little as 3 days. These instruments will have significant impact on the next generation of studies of the *Arabidopsis* genome and transcriptome. We are working on a number of applications for these technologies in *Arabidopsis*. One of these is adding to the sequence of the original Columbia accession. While that sequence is among the highest quality sequences of a eukaryotic organism, there remain areas of the genome that are not complete. We will attempt to fill in the gaps in the Columbia accession reference sequence. In addition, the instruments have significant capabilities for sequencing other accessions of *Arabidopsis* and comparing them to Columbia. We have done this with Landsberg erecta. The resulting sequence, while interesting for showing the differences between the accessions is also very important because it generates a high density single nucleotide polymorphism map between them. This demonstrates the ease at which such maps can be generated between any two accessions of *Arabidopsis*. The resulting data should have a significant impact on polymorphism based mapping. In addition, we have sequenced several Landsberg erecta lines with transposon insertions showing that sequencing the genome can be carried out to determine the site of transposon insertions. Lastly, we present data showing that the next generation sequencers coupled with genome-wide bisulphite treatment can be used to determine the methylation state of the genome under varying conditions. The studies in aggregate show the significant impact of the new technologies on studies of the *Arabidopsis* genome, its evolution, correlation between structural and functional elements among different accessions of *Arabidopsis* and the impact of methylation on gene expression.

S-25

The nucleolus is involved in mRNA surveillance in *Arabidopsis*. Peter Shaw¹, Ali Pendle¹, Olga Koroleva¹, Peter McKeown¹, Dominika Lewandowska², Sang Hyun Kim², John Brown². ¹John Innes Centre, Norwich, UK, ²Scottish Crop Research Institute, Dundee, Scotland

The nucleolus is the site of rDNA transcription, rRNA processing and ribosomal subunit assembly, but is also involved in a range of other RNA/RNP functions, cell cycle control and stress responses. To investigate the wider functions of the nucleolus in plants, we carried out a proteomic analysis of *Arabidopsis* nucleoli, in which we identified 217 proteins of which about 70% were also present in the human nucleolus. The proteins include many expected nucleolar proteins involved in ribosome biogenesis, ribosomal proteins, snoRNP core proteins, DEAD box helicases, RNA/DNA/chromatin interacting proteins, splicing and translation factors and RNA transport factors. In addition, proteins with unknown function were identified, some of which were plant-specific proteins and some of which were found in both the *Arabidopsis* and human nucleolar proteomes. Full-length cDNAs for 76 of the identified proteins were expressed as GFP-protein fusions in *Arabidopsis* cells. Nearly 90% of the protein fusions are clearly nucleolar or associated with the nucleolus. Many also show labelling of other nuclear bodies.

Surprisingly, the *Arabidopsis* nucleolar proteome contained components of the exon junction complex (EJC), which links transcription and splicing with mRNA export, surveillance and decay. GFP-fusions for these and a number of other EJC proteins confirmed an association with the nucleolus.

Evidence for a role for the plant nucleolus in NMD was obtained from an examination of nucleolar mRNAs. Poly A+ mRNA libraries were made from purified nucleoli, as well as purified nuclei and whole cell extracts. Full-length sequences were obtained from randomly selected clones from each library. This showed that the nucleolar library was highly enriched in aberrant or mis-spliced mRNAs. Furthermore, GFP fusions to upf3 and upf2, proteins known to be associated with nonsense-mediated decay (NMD), were clearly localised to the nucleolus. Taken together these results show that spliced or aberrant mRNAs move through the nucleolus, either as part of the mRNA export process or in mRNA surveillance and NMD.

S-26

Using Arabidopsis to find new roles for peroxins and peroxisomes. Bonnie Bartel¹. ¹Rice University

Plant peroxisomes carry out diverse functions, including fatty acid beta-oxidation, hydrogen peroxide decomposition, certain steps in photorespiration, and the conversion of indole-3-butyric acid (IBA) to the auxin indole-3-acetic acid. These single membrane bound organelles contain no DNA; all peroxisomal matrix proteins must be post-translationally imported. Proteins required for matrix protein import are known as peroxins. More than 20 yeast peroxins have been identified through genetic screens, and human homologs for many of these peroxins underlie the peroxisome biogenesis disorders. Although many peroxins playing roles in matrix protein import have been identified, the process is still incompletely understood, and not all proteins involved in matrix protein import in yeast and mammals have identified homologs in Arabidopsis. We found that IBA resistance provides a facile screen for peroxisome defects in Arabidopsis, and have positionally cloned 12 genes that when disrupted lead to IBA response defects. We will report on the insights derived from genetic, physiological, and cell biological analyses of these mutants.

S-28

Global analysis of the Arabidopsis transcriptome data suggests methylation is a key factor that determines regulation of gene expression. Felipe Aceituno¹, Nick Moseyko², Seung Rhee², Rodrigo Gutierrez¹. ¹Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile. ²Department of Plant Biology, Carnegie Institution of Washington, 260 Panama St, Stanford, CA 94305, USA.

DNA microarray technology is the most widely used approach for monitoring genome-wide gene expression changes. For Arabidopsis, there are >1,800 microarray hybridizations that correspond to >470 different experimental conditions in ATH1 Affymetrix gene chips alone. The large amount of public data accumulated, offers a unique opportunity to infer general principles that govern regulation of gene expression. The ATH1 hybridizations were normalized and filtered to eliminate low-quality hybridizations. We then classified and compared control and treatment hybridizations. We determined differential gene expression between control and treatment hybridizations using the RankProducts method. As expected, the experimental conditions represented in our data set behave differently in terms of the nature and number of genes affected.

Interestingly, organ was the variable that changes expression of the largest number of genes, on average ten-fold more than any other experimental variable. Our data indicates the Arabidopsis transcriptome is robust to most perturbations and it is established during development.

We defined "gene responsiveness" as the number of comparisons (control vs treatment) in which a gene changed its expression significantly. Genes with the highest and lowest responsiveness defined hypervariable and housekeeping genes. In an effort to identify basic principles that explain regulation of gene expression, we contrasted several structural features in these two groups of genes. Notably, transcript region methylation clearly distinguished housekeeping from hypervariable genes. Moreover, transcript region methylation inversely correlated ($R^2 = 0.8$) with gene responsiveness on a genome-wide scale (based on two available methylome datasets [Zhang et al 2006; Zilberman et al 2007]). This data suggests that methylation in the transcribed region of an Arabidopsis gene is a key determinant of the capacity of this gene to respond to internal or external cues.

S-27

Regulatory network construction in Arabidopsis using genome-wide gene expression QTLs. Joost J. B. Keurentjes^{1,2}, Jingyuan Fu³, Ritsert C. Jansen³, Inez R. Terpstra⁴, Guido van den Ackerveken⁴, L. Basten Snoek⁵, Anton J. M. Peeters⁵, Dick Vreugdenhil², Maarten Koornneef¹. ¹Laboratory of Genetics, Wageningen University, NL-6703 BD Wageningen, The Netherlands, ²Laboratory of Plant Physiology, Wageningen University, NL-6703 BD Wageningen, The Netherlands, ³Groningen Bioinformatics Centre, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, NL-9751 NN Haren, The Netherlands, ⁴Molecular Genetics Group, Department of Biology, Utrecht University, NL-3584 CH Utrecht, The Netherlands, ⁵Laboratory of Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, NL-3584 CA Utrecht, The Netherlands

In analogy to classical traits, quantitative genetic variation is often observed for transcript levels of genes. Jansen and Nap therefore introduced the concept of genetical genomics, where Quantitative Trait Locus (QTL) analysis is applied to levels of transcript abundance, identifying genomic loci controlling the observed variation in expression (eQTLs). A logical next step would be the construction of genetic regulatory networks but the main reason holding back the identification of gene by gene regulation has been the lack of a reliable identification of candidate regulators. Consequently, the molecular dissection of quantitative trait regulation is still in its infancy and would greatly benefit from approaches reducing the number of candidate genes in a QTL support interval. We therefore developed a novel approach for the assignment of maximum likelihood regulators by combining QTL analysis of gene expression profiling and iterative Group A-analysis (iGA) of functionally related genes. To apply the concept of genetical genomics to higher plants we analyzed genome-wide gene expression variation in a large and well-studied Recombinant Inbred Line (RIL) population of *Arabidopsis thaliana*. We show that for many genes the variation in transcript level can be explained by genetic factors. By integrating current knowledge of the genetics of a specific trait, we demonstrate the construction of genetic regulatory networks, which can serve to form hypotheses about as yet unknown regulatory steps.

S-29

The molecular basis of vernalization. Caroline Dean¹. ¹The John Innes Centre, Norwich, UK

At a certain stage in their life-cycle plants flower and undergo the transition from vegetative to reproductive development. The correct timing of flowering is crucial for reproductive success so plants integrate multiple environmental and endogenous signals. The Dean laboratory is studying the importance of prolonged cold or winter for flowering, a process known as vernalization. The need for vernalization ensures plants over winter in a vegetative form and flower in the favourable conditions of spring. Vernalization requirement has been bred into many crop species to extend their geographical range and variation in vernalization is a key parameter in adaptation of plants to different climates.

We have used a molecular genetic analysis in *Arabidopsis thaliana* to identify genes involved in determining both the need for vernalization and the ability to vernalize. The pathways we study share a common downstream target, FLC, a gene encoding a MADS box repressor of flowering. The talk will describe our current understanding of these pathways, how FLC chromatin regulation has been identified as a central mechanism and how the functioning of the pathways has changed as *Arabidopsis* has adapted to different climates.

S-30

REGULATORY GENE NETWORK IN DROUGHT AND ABA RESPONSES. Kazuo Shinozaki¹, Kazuko Yamaguchi-Shinozaki², Motoaki Seki¹. ¹RIKEN Plant Science Center, Yokohama, Japan,²The University of Tokyo, Tokyo & Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

Drought stress induces a variety of genes of which products function in drought stress tolerance and response in plants. Many stress-inducible genes have been used to improve stress tolerance by gene transfer. In this meeting, we present our recent studies on regulatory networks in drought and ABA responses. We have identified complex regulatory systems in stress-responsive gene expression: ABA-dependent and ABA-independent systems. In one of the ABA-independent pathways, a cis-acting element (DRE/CRT) and its binding proteins, DREB2s, are important cis- and trans-acting elements in drought-responsive gene expression, respectively. DREB2 is also involved in heat stress response. In the ABA-dependent pathways, bZIP transcription factors (AREB/ABF) are involved in the major process. Protein phosphorylation is important for the activation of AREB proteins. The MYB/MYC and NAC transcription factors are involved in ABA-responsive gene expression and jasmonic acid responses.

In the ABA-dependent pathway, stress-inducible NCED3 is mainly involved in the ABA biosynthesis during drought stress. We analyzed metabolic profiles regulated by ABA using T-DNA tagged mutant and with GC-MS and LC-MS. We discuss the function of CYP707A3 in the regulation of ABA metabolism during stress responses. We also report the functions of SnRK2 protein kinases in drought and ABA responses using mutants and transgenic overexpressors. In addition, recent progress in transcriptome analysis for novel transcripts including noncoding RNAs, miRNAs, and sense-antisense RNAs will be presented based on tiling array analysis and 454 sequencing of small RNAs.

Umezawa et al. Curr Opin Biotech 17: 113-122 (2006)

Yamaguchi-Shinozaki and Shinozaki. Ann Rev Plant Bio 57: 781-803 (2006)

S-31

Transgenerational memory of stress in plants. Jean Molinier¹, Gerhard Ries², Cyril Zipfel³, Barbara Hohn⁴. ¹Institut de Biologie Moléculaire des Plantes, Strasbourg, France,²BioMedInvestor AG, Basel, Switzerland,³The Sainsbury Laboratory, John Innes Centre, Norwich, UK,⁴Friedrich Miescher Institute, Basel, Switzerland

Plants are constantly exposed to stresses from the environment. Both biotic and abiotic stresses can result in tolerance to conditions such as unbalanced water supply, light stress, extreme temperatures and pathogens. Influences such as these have also been documented to result in elevated rates of genomic changes such as transposition or homologous recombination. However, it has not been analysed whether the impact of these influences could also be inherited. Experiments using *Arabidopsis thaliana* plants transgenic for markers for genomic changes, however, showed that exposure of these plants to UV-B or to the bacterial elicitor flagellin exhibited changes of homologous recombination far exceeding those of unexposed plants. Quite unexpectedly, however, the progeny of these plants still exhibited elevated recombination rates, although they themselves were not influenced by the agents their parents were exposed to. This change in the recombination frequency must be due to an epigenetic modification of an unknown locus or of unknown loci; it was the whole population that reacted to the challenge. If a mutation would have caused the described effect only a very small number of plants would have shown the changed behavior. The change was not located at the genomic position of the transgene used to monitor recombination, since plants lacking the transgene transmitted their experience of being treated to an untreated crossing partner containing the recombination monitoring transgene. This resulted in increased recombination frequencies in the progeny of these plants. These experiments may point to the importance of epigenetic changes of plant chromatin in permitting evolutionarily important flexibilities useful for adaptation.

S-32

PHOSPHATE STARVATION SIGNALLING IN ARABIDOPSIS. Re-gla Bustos¹, Jsose Manuel Franco-Zorrilla¹, Pablo Catarecha¹, Gabriel Castrillo¹, Isabel Mateos¹, Mabel Puga¹, Vicente Rubio¹, Antonio Leyva¹, Javier Paz-Ares¹. ¹Centro Nacional de Biotecnología-CSIC, Madrid, Spain

Plants have evolved adaptive responses to cope with growth under phosphate (Pi) limiting conditions. This rescue system is under the control of a highly elaborated regulatory mechanism, which, besides Pi, is modulated by sugars, cytokinins and other unknown signal(s) mediating long distance systemic repression. We have found that transcription of Pi starvation-induced genes is repressed by arsenate, potentially reflecting the existence of a savage system to protect plants from arsenate, particularly in Pi poor soils. One component of the Pi starvation regulatory system is transcription factor PHR1, which recognises an imperfect palindromic motif (GNATATNC, P1BS) that is over-represented in the promoter region of Pi starvation induced genes. We have shown that PHR1/P1BS acts as key integrator in Pi starvation signalling. Thus, a minimal promoter containing a multimerised P1BS motif is specifically induced by Pi starvation and is responsive to the stimulatory effect of sugars and the inhibitory effects of cytokinins, long distance repression signals and arsenate. Control of Pi starvation responses also involves the participation of Pi starvation responsive miRNAs (miR399) and other noncoding RNAs, namely the IPS1 family members. Functional characterisation of IPS1 has disclosed a novel mechanism of inhibition of miRNA activity, in which a non-coding RNA sequesters a miRNA and reduces its effective levels. We coin the term target mimicry to define this riboregulatory mechanism of miRNA activity

S-33

Short silencing RNA networks. David Baulcombe¹. ¹The Sainsbury Laboratory, John Innes Centre, Norwich, UK

RNA silencing was first implicated as a defense system against plant viruses. However, from genetic analysis and through the use of high throughput sequencing, it is now clear that this process pervades many aspects of regulation at the RNA and DNA level. I will propose that the short silencing RNAs serve roles as negative switches, as mediators of systems robustness through positive and negative feedback and as initiators of epigenetic mechanisms. Current understanding of silencing RNA mechanisms reveals the potential for complex interaction networks in which the silencing RNAs determine the specificity of the interactions between initiators, nodes and endpoints.

S-34

Does Rhizobium root nodule symbiosis involve a legume specific toolbox? Ton Bisseling¹. ¹Research School Experimental Plant Sciences, lab of Molecular Biology Wageningen University, Wageningen The Netherlands

Rhizobium root nodule symbiosis is a unique property of leguminous plants. The formation of these nitrogen fixing root nodules is set in motion by lipooligosaccharides that are secreted by the rhizobia. The perception and transduction of Nod factors requires a rather small set of genes that are specifically involved in this symbiosis (and in part also in the arbuscular mycorrhizal symbiosis). Nod factor signaling occurs at the onset of the interaction, but most likely also at 2 additional steps during the infection process as well as in the growing root nodule. A similar set of Nod factor signaling genes has been identified by forward genetics in the legumes *Medicago*, *Lotus* and *pea*. This set of legume Nod factor signaling genes provides the means to compare legumes and non-legumes by which a better understanding of the evolutionary origin of legume nodulation can be obtained.

S-35

Recognition of Pathogen Effectors and Activation of Disease Resistance Signaling Pathways in *Arabidopsis thaliana*. Brian Staskawicz¹. ¹University of California, Berkeley, USA

A common feature of most phytopathogens is the ability to directly deliver effector proteins to the plant host to either modulate or suppress host defense signalling. In our laboratory, we are studying effector proteins from both bacterial and oomycete pathogens with the aim of understanding both pathogen virulence during a compatible interaction and effector recognition during an incompatible interaction. Specifically, we have focused on studying the activation of the RPS2 disease resistance signalling pathway after the recognition of the cognate Type Three Secretion Effector protein, AvrRPT2. We will present our most recent model describing the molecular events controlling AvrRPT2 effector recognition and the subsequent activation of the RPS2 disease resistance signalling pathway. In addition, we will describe our progress in the identification and characterization of effector proteins from *Hyaloperonospora parasitica* and our ability to deliver oomycete effector proteins to *Arabidopsis thaliana* via the *Pseudomonas syringae* TTSS. Finally, we will present data to demonstrate that plants have evolved a common mechanism to inhibit growth of pathogens with varied lifestyles.

S-37

Functional analysis of dominant GAF-domain tyrosine mutants of *Arabidopsis* phytochromes in transgenic plants. Yi-shin Su¹, J. Clark Lagarias¹. ¹University of California, Davis, (CA), USA

Light sensing by phytochromes, a family of biliprotein photoreceptors that are widely distributed nature, exploits the reversible photoisomerization of their covalently bound linear tetrapyrrole (bilin) prosthetic groups. Initially undertaken to examine the biological activity of a recently identified class of highly fluorescent, poorly photoactive phytochrome mutants in transgenic plants, the present investigation led to the unexpected discovery of constitutively active phytochrome alleles that possess mutations in a conserved GAF domain tyrosine (YGAF) residue. Most pronounced gain-of-function activities were observed for the Y276H allele of *Arabidopsis* phyB (YHB) whose expression conferred dominant constitutively photomorphogenic (COP) phenotypes as well as constitutive, light-insensitive phyB signaling activities to both dark- and light-grown transgenic plants. YHB-mediated COP development paralleled constitutive nuclear localization of the YHB protein and expression of light-regulated genes in darkness - both of which are consistent with its light-independent activation. Moreover, the COP phenotype was suppressed in bilin-deficient genetic backgrounds, indicating that the YHB allele encodes a bilin-dependent regulator of photomorphogenesis. Phenotypic analysis of transgenic plants expressing YQB, YIB and YRB alleles further revealed that the signaling activity of phyB critically depends on the amino acid at the YGAF position. By comparison with YHB, the COP phenotype conferred by the Y242H allele of phyA (YHA) was less pronounced, and a dominant-negative response to YHA expression was observed in wild-type genetic backgrounds. Taken together, these results implicate participation of this conserved YGAF residue in the transduction of light-driven bilin chromophore isomerization to phytochrome-mediated regulation of plant growth and development. Dominant, constitutively active phytochrome alleles are of potential agronomic significance since their introduction into any transformable crop plant species represents a practical approach to suppress deleterious responses to light quality in field environments.

S-38

Photoreceptor-interacting proteins as immediate facilities for environmental light information processing. Jong Sang Ryu¹, Sung Hyun Hong¹, Hyunmo Choi¹, Tomonao Matsushita², Akira Nagatani², Hong Gil Nam³. ¹Division of Molecular Life Sciences and National Core Research Center for Systems Bio-Dynamics, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea, ²Laboratory of Plant Physiology, Graduate School of Science, Kyoto University, Sakyo-Ku, Kyoto 606-8502, Japan, ³Division of Molecular Life Sciences and National Core Research Center for Systems Bio-Dynamics and The I-BIO Graduate Program, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Plants, being photosynthetic and sessile, exhibit particularly plastic development and growth, depending on the environmental light information. The quality, intensity, duration, and direction of the environmental light provide plants with information not only on the ambient light condition but also on other elements in their environments such as neighboring plants and seasonal changes. Plants perceive light information using specialized photoreceptors, the red (R) and far-red (FR) light-absorbing phytochromes and the blue/UV-A-absorbing cryptochromes and phototropins that ensure optimal responses to their ever-changing environmental conditions. To transmit the responsive light information properly, plants have evolved a set of signaling components that assess and relay the quantitative and qualitative natures of the incident signals to the changes of gene expression that control responses in growth and development. Since protein-protein interactions are fundamental to all cellular signaling processes, we are attempting identification and functional assignment of the proteins that interact with photoreceptors with the aim to understand the very early steps in light signaling. We have identified several putative phyB-interacting proteins, including PAPP5. We will describe how the spatial distribution of PAPP5 is affected by interaction with phyB and in turn affects light signaling. We will also describe the role of a potential cry-interacting transcription factor.

S-39

ZBF3/CAM7 plays an important role as transcriptional regulator in *Arabidopsis* seedling development. Ritu Kushwaha¹, Sudip Chattopadhyay¹. ¹National Centre for Plant Genome Research

Calmodulin (CaM) plays multiple regulatory roles in eukaryotes as Ca⁺ sensor, however its direct function as transcriptional regulator is still unknown. Additionally, the physiological functions of CaM remains largely unknown in plants. In this study, we demonstrate that CAM7 is a unique transcriptional regulator of light signalling that directly binds to the promoters of light inducible genes and promotes photomorphogenesis. The mutational, transgenic and physiological studies illustrate the concerted function of CAM7 and HY5 in *Arabidopsis* seedling development.

S-41

Transposase-Derived Proteins FHY3 and FAR1 From *Arabidopsis* Modulate phyA Signaling Homeostasis Through Direct Activation of FHY1 and FHL Expression. Rongcheng Lin¹, Lei Ding¹, Claudio Casola², Cedric Feschotte², Haiyang Wang¹. ¹Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY, USA, ²Department of Biology, University of Texas, Arlington, TX, USA

Phytochrome A (phyA) is the primary photoreceptor for mediating various far-red light induced responses in higher plants. Following photoactivation, phyA translocates into and accumulates in the nucleus in a process dependent on two homologous proteins FHY1 and FHL. Once in the nucleus, phyA interacts with a set of transcription factors to regulate phyA-responsive gene expression. In this study, we demonstrate that *Arabidopsis* *FHY3* and *FAR1*, which encode two homologous proteins related to Mutator-like transposases, act redundantly to modulate phyA nuclear accumulation through directly binding to and activating the expression of *FHY1* and *FHL*, with *FHY3* playing a more predominant role. We show that *FHY3* and *FAR1* bind directly to the upstream DNA regions of *FHY1* and *FHL* via a zinc finger-like motif most likely acquired from an ancestral transposase. In addition, reporter assays in yeast and *Arabidopsis* protoplasts indicate that *FHY3* possesses an intrinsic capability to mediate transcriptional activation of an adjacent gene. Transcriptional activation is separable from DNA-binding activity, but requires the presence of two residues highly conserved in *Mutator*-like transposases. Constitutive expression of *FHY1* driven by the 35S promoter can largely suppress the mutant phenotypes of *phy3-4*, *far1-2*, and the *phy3 far1* double mutant, supporting that *FHY1* acts downstream of *FHY3* and *FAR1*. Finally, we also show that the expression of *FHY3* and *FAR1* is subject to a negative feedback regulation by phyA signaling. Together these results suggest that *FHY3* and *FAR1* define a novel class of transcription factors that were co-opted from an ancient *Mutator*-like transposase to modulate phyA signaling homeostasis in higher plants.

S-40

Mutation of an enzyme involved in the de novo synthesis of NAD causes early leaf senescence in *Arabidopsis thaliana*.

Jos Schippers¹, Adriano Nunes-Nesi², Roxana Apetrei¹, Alisdair Fernie², Jacques Hille¹, Paul Dijkwel¹. ¹University of Groningen, Groningen, Netherlands, ²Max Planck Institute for Molecular Plant Physiology, Golm, Germany

Leaf senescence is a process of enigmatic beauty that plays its role during the season of change. The rational behind the process is a genetic program aimed at the efficient reuse of valuable nutrients and minerals. In *Arabidopsis* the senescence program is executed in an age-dependent way. By studying mutants with an altered onset of leaf senescence, we aim to reveal the mechanisms that underlie plant ageing. In the presented study we characterized the *old5* (*onset of leaf death5*) mutant. *OLD5* encodes a quinolinate synthase that is involved in the de novo synthesis of Nicotinamide adenine dinucleotide (NAD). Studies on NAD mainly relate to its role in redox biochemistry and energetic metabolism. Recently, the molecule gained a renewed interest with the discovery that nicotinamide cofactors play important roles in cell signaling in animals, yeast and plants. For instance, NAD is used as a substrate for post-translational modification of proteins. Interestingly, the yeast Silent Information Regulator 2 (Sir2) protein has been shown to be an NAD-dependent deacetylase involved in the control of lifespan. Moreover NAD metabolism has been proposed as the mechanism behind calorie restriction.

The *old5* mutant provides an excellent opportunity to answer the question whether NAD metabolism also plays a role in plant ageing. A comprehensive study including results of a physiological and genetical characterization of the mutant are presented as well as results of protein interaction analysis and a broad metabolic profiling of the mutant.

S-42

HSP90, SGT1, and RAR1 form a ternary chaperone complex to regulate resistance-protein dependent plant immunity. Ken Shirasu^{1,2}, Marta Boter¹, Beatrice Amigues³, Jack Peart¹, Christian Breuer¹, Yasuhiro Kadota², Catarina Casais¹, Francoise Ochsenbein³, Raphael Guerois³. ¹The Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK, ²RIKEN Plant Science Center, Suehiro-cho 1-7-22, Tsurumi-ku, Yokohama, 230-0045 Japan, ³SBFM-DBJC, CEA Saclay, 91191 Gif sur Yvette Cedex, FRANCE

SGT1 and RAR1 are highly conserved eukaryotic proteins that interact with HSP90, a molecular chaperone. In plants, SGT1, RAR1 and HSP90 are essential for disease resistance triggered by a number of resistance (R) proteins that contain NB (nucleotide binding site) and LRR (Leucine Rich Repeat). SGT1 and HSP90 also play a crucial role in Nod-like receptors (NLRs)-mediated innate immunity responses in mammals. Here we present structural and functional characterization of plant SGT1. Random mutagenesis of SGT1 revealed that its CS and SGS domains are essential for disease resistance. NMR-based interaction surface mapping of the CS domain combined with mutational analyses showed that the CHORD-II domain of RAR1 and the N-terminal domain of HSP90 interact with opposite sides of the CS domain. Functional analysis of the CS mutations indicated that the interaction between SGT1 and HSP90 is required for disease resistance triggered by Rx, a potato R protein. Biochemical reconstitution experiments revealed that RAR1 functions to enhance SGT1-HSP90 interaction by promoting ternary complex formation.

S-43

Molecular mechanisms underlying the activation of NBS-LRR proteins. Roger Innes¹, Jules Ade¹, Brody DeYoung¹, Thomas Burke¹, Tom Ashfield¹, Lauren Galloway¹, Michelle Metcalf¹. ¹Indiana University, Bloomington, Indiana, USA

This talk will focus on the molecular mechanisms by which plant disease resistance (R) proteins mediate pathogen recognition. In Arabidopsis, the *RPM1* gene mediates resistance to *Pseudomonas syringae* strains that express either AvrB or AvrRpm1, while in soybean recognition of these two proteins is mediated by distinct, but closely linked R genes, *Rpg1-b* and *Rpg1-r*. With the help of several collaborators, we have cloned *RPM1* and *Rpg1-b* and expect to soon clone *Rpg1-r*. Phylogenetic analyses revealed that *RPM1* and *Rpg1-b* are not orthologous and evolved the ability to detect AvrB independently. We were thus interested in determining whether the recognition mechanisms employed by *RPM1* and *Rpg1-b* were the same or different. *RPM1* has been shown to detect both AvrB and AvrRpm1 indirectly, via monitoring the status of a second Arabidopsis protein, RIN4. In addition, it has been shown that a third *P. syringae* protein AvrRpt2 can block *RPM1*-mediated resistance by proteolytically degrading RIN4. To test whether the soybean *Rpg1-b* protein employs a recognition mechanism similar to that of *RPM1*, we identified soybean orthologues of *RIN4*, tested their interaction with AvrB, determined whether they were substrates of AvrRpt2, and assessed whether *Rpg1-b* function was suppressed by AvrRpt2. Our data indicate that *Rpg1-b* likely detects AvrB by a mechanism very similar to *RPM1*, even though it evolved this ability independently. We are also investigating how the Arabidopsis RPS5 protein is activated by the *P. syringae* effector protein AvrPphB. We have previously shown that AvrPphB functions as a protease to cleave the Arabidopsis protein kinase PBS1 and that this cleavage is required to activate RPS5. We are now investigating how this cleavage event triggers RPS5 signaling.

S-45

MOS7 is essential for plant innate immunity. Xin Li¹, Yu Ti Cheng¹, Dongling Bi², Yuelin Zhang² ¹University of British Columbia, ²National Institute of Biological Sciences

Immunity against microbial pathogen infections in plants is a dynamic process. One of the most effective disease resistance mechanisms is mediated by Resistance proteins (R proteins), which play a central role in recognizing pathogens and initiating downstream defense cascades. In Arabidopsis, a dominant mutant, *snc1*, was previously identified that constitutively expresses pathogenesis-related (PR genes and resistance against both bacterial and oomycete pathogens. A point mutation in one of the RPP4 homologs rendered this TIR-NB-LRR-type R protein constitutively active without interaction with pathogens. In screens for suppressors of the constitutive defence responses in *snc1*, a number of loss-of-function mutants were identified. The mutants restore wild-type morphology either completely or partially. We have recently cloned nine of the mutations (*mos1* to *mos9*) using map-based approaches. These suppressors suggest a complicated signalling network downstream of R-protein activation that involves RNA processing, nucleocytoplasmic trafficking, and protein modification, etc. Here we report the analysis of *mos7*. A recessive mutation in *mos7-1* completely suppressed all the autoimmune phenotypes of *snc1*. In *mos7-1* single mutant, basal defense against virulent pathogens along with several R protein-mediated defense are attenuated. *mos7-1* also exhibit defects in systemic acquired resistance (SAR), a key systemic innate immunity pathway in plants. MOS7 localizes to the nuclear rim, consistent with its homology with Nup88. In Drosophila, the Nup88-Nup214 complex was shown to modulate NFκappaB activation, which is essential for activating animal innate immunity pathways. We are currently investigating whether MOS7 is required for NPR1 nuclear translocation, which is a key event in SAR.

S-46

Transcriptional regulators in rhizobacteria-induced systemic resistance (ISR). Sjoerd Van der Ent^{1,2}, Maria J. Pozo¹, Bas W. M. Verhagen¹, L. C. Van Loon¹, Jurriaan Ton¹, Corn M. J. Pieterse¹, ¹Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, P. O. Box 800, 3508 TB Utrecht, the Netherlands, ²Center for Biosystems Genomics, the Netherlands

Plants possess inducible resistance mechanisms through which they can regulate their defense response to pathogen attack. Colonization of Arabidopsis thaliana roots by non-pathogenic *Pseudomonas fluorescens* WCS417r bacteria triggers a jasmonate- and ethylene-dependent induced systemic resistance (ISR) that is effective against a broad range of foliar pathogens. In the roots, the transcriptional activity of a large number of genes, is altered upon colonization by WCS417r. Bioassays revealed that one of the up-regulated genes, the transcription factor (TF) AtMYB72, is essential for activation of ISR. Two independent *myb72* knockout mutants were incapable of mounting WCS417r-mediated ISR against different pathogens. Constitutive expression of AtMYB72 did not result in elevated resistance of the transformants, which demonstrates that AtMYB72 is required, yet not sufficient for ISR. Yeast two-hybrid analysis revealed that AtMYB72 interacts in vitro with EIN3-LIKE 3 (EIL3), a TF that belongs to a family of TFs that play a role in ethylene signaling. Whether EIL3 is a parallel signaling component required for the onset of ISR is currently under investigation.

Micro-array studies revealed that ISR-expressing leaves are primed for augmented expression of predominantly jasmonate- and ethylene-responsive genes. Promoter analysis of the primed genes showed that the ISR-primed genes are enriched for a AtMYC2 binding motif, suggesting a regulatory role for this TF in ISR. Further indications for the involvement of AtMYC2 in ISR arose from a qPCR-based TF profiling, which demonstrated that the systemic expression of several TFs, amongst which AtMYC2, is directly activated upon colonization of the roots by ISR-inducing WCS417r bacteria. Bioassays using *AtMYC2* knockout mutants *jin1-1* and *jin1-2* demonstrated that these *myc2* mutants were not able to mount WCS417r-mediated systemic resistance, indicating that AtMYC2 is indeed required for ISR.

S-44

Durable broad-spectrum powdery mildew resistance in crops and cereals: What can we learn from Arabidopsis? Chiara Consonni¹, Matt Humphry¹, Sandra Noir¹, Ralph Panstruga¹. ¹ Max-Planck Institute for Plant Breeding Research, Cologne, Germany

Recessively inherited loss-of-function alleles (*mlo*) of the barley *Mlo* gene confer resistance that is effective against all known isolates of the barley powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*. In susceptible *Mlo* wild type plants, the fungus potentially manipulates the protein encoded by this gene for plant cell invasion. Although *Mlo* homologs are found in all flowering plants examined to date it was unclear until recently whether *mlo* resistance represents a species-specific phenomenon restricted to barley. Our results demonstrate that *mlo*-based powdery mildew resistance can be induced in the model plant *Arabidopsis thaliana* by inactivation of a particular of the 15 *Mlo* homologs. These data demonstrate that *mlo* resistance is effective in both major clades of flowering plants, suggesting that the role of *Mlo* proteins for colonization by powdery mildews is ancient and evolutionarily conserved. Besides conferring powdery mildew resistance, lack of *Mlo* results in an early senescence-like pleiotropic phenotype in both barley and *Arabidopsis*. The genetic and molecular tool-box available for the dicotyledonous reference species may enable to unravel the molecular basis of *mlo* resistance. In addition, it will help in designing strategies to uncouple the undesired pleiotropic phenotypes from the desired resistance trait.

S-47

The Genetic Basis of Singlet Oxygen-Mediated Signaling of Stress Responses. Klaus Apel¹. ¹ETH-Zurich Switzerland

The evolution of aerobic metabolic processes such as respiration and photosynthesis unavoidably leads to the production of reactive oxygen species (ROS) in mitochondria, chloroplasts and peroxisomes. A common feature among the different ROS types is their capacity to cause oxidative damage by inactivating e. g. proteins, nucleic acids and lipids. These cytotoxic properties explain the evolution of complex arrays of ROS scavengers. In plants chloroplasts and peroxisomes are the major sites of ROS production. Various abiotic stress conditions may limit the ability of a plant to use light energy for photosynthesis. Under such stress conditions hyper-reduction of the photosynthetic electron transport chain and photoinhibition of photosynthesis may occur even at moderate light intensities, often causing damages that have been interpreted as unavoidable consequences of injuries inflicted upon plants by toxic levels of ROS. However, this paradigm needs to be modified. Stress responses triggered by ROS are not only due to physicochemical damages but may also be caused by the activation of genetically determined stress response programs. We'll present results of our work showing that ROS may act as signals during stress whose specificities seem to depend on the chemical identity of a given ROS and its intracellular site of generation.

S-48

Molecular mechanism of chloroplast photorelocation movement. Masamitsu Wada¹, Noriyuki Suetsugu¹. ¹National Institute for Basic Biology, Okazaki, Japan

Chloroplast photorelocation movement is essential for sessile plants. Chloroplasts gather at an area irradiated with weak light to maximize photosynthesis (accumulation response). They move away from an area irradiated with strong light to minimize damage of the photosynthetic apparatus (avoidance response). The process of these chloroplast movements can be divided into three parts; photoperception, signal transduction, and the movement of chloroplasts. The mechanisms are not yet fully understood, but we present in this symposium new findings on the parts of photoperception and chloroplasts movement.

Phototropins (phot1 and phot2) are membrane-associated blue light photoreceptors that function not only in chloroplast relocation, but also in phototropism, stomatal opening, and leaf flattening in *Arabidopsis*. The molecular structure of phototropin is well conserved for the distinct biochemical roles; two LOV domains in its N-terminal half as a photosensory domain and a Ser/Thr kinase domain in its C-terminal half as a signal transducer. Here, we studied the structural roles of the N-terminus of phot2 (P2N) *in vivo* and *in vitro*. Immunoprecipitation and size exclusion chromatography suggested that both phot1 and phot2 exist as multimeric forms. Domain analysis further suggested that the predicted α -helix (from Thr240 to Arg280) was necessary to phot2 tetramer formation.

For the movement of chloroplasts we found the involvement of short actin filaments on chloroplasts (cp-actins). Phototropins regulate the localization and the amount of cp-actins to determine the direction and the motility of moving chloroplasts. CHUP1 and newly identified KAC are necessary for cp-actin generation or maintenance. Because, the lack of cp-actin in chup1 and kac mutants chloroplasts moved rapidly via cytoplasmic streaming and cannot change their position in response to light irradiation. Furthermore, jac1 mutant deficient in the accumulation response was also impaired in cp-actin regulation under the strong blue light, resulting in aberrant avoidance movement. Thus, phototropins and JAC1 mediate chloroplast photorelocation movement via the regulation of cp-actin dependent on CHUP1 and KAC.

S-49

Identification of CO₂ and ABA Signaling Components and Novel Membrane Transduction Mechanisms in Guard Cells. Julian I. Schroeder¹, Yong-Fei Wang¹, Tae-Houn Kim¹, Yingzhen Yang¹, Robert S. Siegel¹, Maria Israellsson¹, Erwin Grill², Jaakko Kangasjärvi³, Hannes Kollist³, Yoshiyuki Murata⁴, Izumi Mori¹. ¹Div. of Biol. Sciences, University of California, San Diego, La Jolla, CA, USA, ²T.U. Munich, Germany, ³U. Helsinki, Finland, ⁴Okayama U., Okayama, Japan

Guard cells have been developed as a model system for dissecting ion channel functions and early signal transduction mechanisms. Genetic loss-of-function mutants in Ca²⁺ sensors that impair Ca²⁺- and abscisic acid-regulated stomatal movements have been lacking. In addition, a Ca²⁺-independent pathway functions in the abscisic acid (ABA) response. We have recently identified two calcium-dependent protein kinases (CDPKs) that function in abscisic acid (ABA) and Ca²⁺ regulation of guard cell anion and Ca²⁺-permeable channels and stomatal closing (I. Mori et al., 2006 PLoS Biol.). Furthermore, several independent CO₂ and abscisic acid signal transduction analyses suggest a new model for how plant cells can achieve specificity in calcium signaling through "priming" and "de-priming" of Ca²⁺ sensitive mechanisms (J. Young et al., 2006 PNAS). Further evidence that correlates with this "Ca²⁺ sensitivity priming" hypothesis will be presented. In addition, evidence for a parallel pathway that functions in the ABA signaling network will also be presented. A central target of ABA signaling is the activation of S-type anion channels in guard cells. Identification of a new membrane protein-encoding gene that is essential for mediating this response will be presented.

Genetic, genomic and signal transduction analyses in a number of laboratories indicate that genetic redundancies and robustness exist within the abscisic acid signal transduction network. To address this complexity we have pursued genomic guard cell expression approaches (e.g. Leonhardt et al., 2004 P1 Cell; Mori et al., 2006 PLoS Biol.). More recently we have developed a chemical genetics approach that allows high-throughput screening for molecules and mutants that affect ABA signal transduction. Progress at isolating a small molecule that blocks ABA responses and identification of mutants in ABA signaling that are insensitive to this compound will be presented.

S-50

ZEITLUPE is a circadian photoreceptor stabilized by a blue-light-enhanced protein-protein interaction. Woe-Yeon Kim¹, Sumire Fujiwara¹, Sung-Suk Suh¹, Jeongsik Kim², Yumi Kim², Linqu Han¹, Hong Gil Nam², David E. Somers¹. ¹Department of Plant Cellular and Molecular Biology/Plant Biotechnology Center, Ohio State University, Columbus, OH 43210, USA, ²Department of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, South Korea

Appropriate regulation of rhythmic cycling of key elements of the clock is essential to maintain robust oscillations. Cyclic oscillation of ZEITLUPE (ZTL) is required to sustain normal circadian cycling via the proteasome-dependent degradation of TOC1, a key element of the central oscillator in plants. ZTL is an F-box protein and its cyclic oscillation is post-transcriptionally regulated by the proteasome. We have identified a factor which regulates ZTL protein abundance. In its absence, the normal four-fold diurnal cycling in ZTL is post-transcriptionally eliminated, resulting in constitutively low ZTL accumulation. Their interaction occurs through the N-terminal ZTL LOV domain, which is necessary and sufficient for the interaction. Further, this interaction is strongly and specifically enhanced by blue light, via the N-terminal flavin-binding LIGHT, OXYGEN OR VOLTAGE (LOV) domain of ZTL. Mutations within the LOV domain that greatly diminish the interactions also lead to strongly reduced ZTL levels. A C82A transition in the LOV domain, implicated in the flavin-dependent photochemistry, eliminates blue-light enhanced binding. These data establish ZTL as a blue-light photoreceptor, by which light absorption facilitates its own stability via a blue-light enhanced protein-protein interaction. Circadian control of message levels of the ZTL-interacting protein, and subsequent cycling of the protein, confers a post-translational rhythm on ZTL protein abundance, defining a novel mechanism to establish and sustain circadian oscillations. Additional progress on this mechanism will be presented.

S-51

Cryptochrome signaling to the plant clock associates with inhibition of COP1-mediated ELF3 ubiquitination. Jae-Woong Yu¹, Vicente Rubio^{2,3}, Sulan Bai², Na-Yeon Lee¹, James A. Sullivan², Sang-Sook Kim¹, Sun-Young Lee¹, Ilha Lee⁴, Nam-Chon Paek¹, Xing Wang Deng^{2,1}. ¹ Department of Plant Science and Research Institute for Agriculture & Life Sciences, Seoul National University, Seoul, Korea, ²Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT, USA, ³Department of Plant Molecular Genetics, Centro Nacional de Biotecnología-CSIC, Madrid, Spain, ⁴ Department of Biological Sciences, Seoul National University, Seoul, Korea

Seasonal changes in daylength are perceived by plant photoreceptors and transmitted to the circadian clock to modulate developmental responses, such as flowering time. Cryptochromes and clock-associated ELF3 regulate this process although a link between them is unclear. Here, we show that COP1 mediates daylength signaling from CRY2 to ELF3 within the photoperiod flowering pathway. We find that ELF3 is ubiquitinated by COP1 in vitro and that this activity decreases in the presence of the constitutively active C-termini of CRY1 (CCT1) and CRY2 (CCT2). Accordingly, rhythmic accumulation of ELF3 protein is altered in both cop1-4 mutants and plants overexpressing CCT2. Remarkably, mutation of COP1 overcomes both the late flowering phenotype and the increased period length of LHY rhythmic accumulation caused by ELF3 overexpression. Therefore, we propose that ELF3 ubiquitination by COP1 is essential for ELF3 function to modulate light signal input from cryptochromes to the circadian clock.

S-52

Positioning *Arabidopsis* EDS1 In pathogen and oxidative stress responses. Jane Parker¹, Lennart Wirthmueller¹, Steffen Rietz¹, Michael Bartsch¹, Enrico Gobbato¹, Ana Garcia¹, Christine Foyer². ¹Max-Planck Institute for Plant Breeding Research, Cologne, Germany, ²School of Agriculture, University of Newcastle, Newcastle upon Tyne, UK

Our interest is in the control of *Arabidopsis* defences once pathogens have overcome resistance at the cell surface (normally triggered by PAMPs) and started to colonize host cells. Low level post-invasive (basal) resistance to biotrophic and hemi-biotrophic pathogens depends on the EDS1 family of nucleo-cytoplasmic proteins (- EDS1, PAD4 and SAG101). These regulators restrict pathogen growth in the absence of cell death and generate signals leading to the priming of systemic defences in uninfected parts of the plant. They also serve as essential components of localised cell death and resistance triggered by intracellular TIR-NB-LRR type immune receptors. In all of these functions, EDS1 controls the production of salicylic acid (SA) and other signal intermediates as well as the fine balance between SA and jasmonic acid/ethylene pathway activation. Several pieces of evidence point to an EDS1/PAD4 activity in transducing ROS-derived signals in pathogen and photo-oxidative stress responses. Characterisation of additional components of the EDS1 pathway (eg. FMO1, a flavin-dependent monooxygenase) and of EDS1-PAD4 dual over expression lines reinforce a connection between EDS1 and the regulation of ROS homeostasis and signalling that impacts significantly on plant development. In order to position EDS1 in TIR-NB-LRR triggered defence and cell death, we have examined RPS4, a prototypical TIR type NB-LRR receptor that recognizes the secreted bacterial effector, AvrRps4. RPS4 protein accumulates to low levels as endo-membrane-associated and nuclear pools in unchallenged cells. Its amounts in either compartment do not change substantially after pathogen challenge. Our data suggest that RPS4 interception of AvrRps4 occurs indirectly and outside the nucleus but that a nuclear activated RPS4 pool is needed to trigger resistance. We present further evidence that EDS1 is an indispensable intermediate between RPS4 activation and RPS4-mediated transcriptional reprogramming in the nucleus.

S-53

Little and large pathogen effectors in plant resistance and susceptibility. Jonathan Jones¹, Kee Sohn¹, Alexandre Robert-Seilhanant¹, Lionel Navarro¹, Rajendra Bari¹, Rita Lei¹, Alejandra Rougon¹, Adnane Nemri¹, Georgina Fabro¹. ¹The Sainsbury Laboratory, JIC, Colney Lane, Norwich NR4 7UH, UK

Plant pathogens use small molecules and also proteins to render their hosts susceptible. Several pathogens either make plant hormones, or perturb host hormone signalling networks by other means. To counteract pathogen activation of auxin signalling, plants induce microRNA miR393 that targets the TIR1 auxin receptor, to attenuate auxin sensitivity. Furthermore, gibberellin made by fungal necrotrophic pathogens leads to attenuation of the defence response to necrotrophs by interference with jasmonic acid signalling. In addition, many bacteria and other pathogens use a specialized secretion system to deliver proteins into host cells that interfere with host defence. We have taken advantage of the bacterial T3SS secretion system to investigate effectors from filamentous pathogens such as oomycetes. Recent data on oomycete effector functions will be reported.

S-54

Penetration Resistance: The First Line of Defense Against Invasive Pathogens. Shauna Somerville¹, Monica Stein¹, Matt Humphry¹, Laurent Zimmerli¹. ¹Carnegie Institution, Stanford, CA, U.S.A.

A majority of host plants are resistant to the majority of pathogens in their environment. The underlying mechanisms of this form of disease resistance are poorly studied. Working collaboratively, the Paul Schulze-Lefert lab (Cologne, Germany), Hans Thordal-Christensen (Copenhagen, Denmark) lab and our lab identified mutants of the model plant *Arabidopsis thaliana* that were compromised in their resistance to the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*, which is pathogenic only on barley. Several *Arabidopsis* genes, not previously implicated in plant resistance mechanisms, have been identified by this approach, including *PEN1* (= *SYP121*), a syntaxin and functional homologue of the barley *ROR2* gene; *PEN2*, a glycosyl hydrolase; and *PEN3* (= *PDR8*), an ABC transporter. In addition, *MLO2* was identified as the functional homologue of the barley *MLO* gene, which confers penetration resistance when inactivated. Several of these mutants permit enhanced penetration success by additional pathogens including *Erysiphe pisi*, a pathogen of pea, and *Phytophthora infestans*, the potato late blight pathogen. The plasma membrane localized *MLO*, *PEN1* and *PEN3* proteins accumulate at penetration sites in inoculated plant tissues. These observations suggest that plants mount effective, broad spectrum and multi-genic defenses designed to block pathogen invasion across the host cell wall and entry into cells. Over evolutionary time, virulent species, such as *Golovinomyces cichoracearum*, a pathogen of *Arabidopsis*, must have acquired the ability to avoid these defenses by stealth or to be insensitive to them.

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S-55

Dissection of the signaling pathway of RPW8-mediated broad-spectrum disease resistance. Wenming Wang¹, Xiaohua Yang¹, Undral Orgil¹, Shunyuan Xiao¹. ¹University of Maryland Biotechnology Institute

The structurally unique resistance (R) gene RPW8 confers broad-spectrum resistance in *Arabidopsis* to powdery mildew pathogens. To understand the molecular basis of RPW8-mediated resistance, we first established that RPW8 activates hypersensitive cell death and defense via a highly conserved signaling pathway also recruited by the TIR-NBS-LRR (Toll-interleukin receptor nucleotide-binding-site and leucine-rich-repeat) R genes. More recently, we identified one isoform of 14-3-3 (GF54a) and a protein phosphatase type 2C (designated DAPP1 for defense-associated protein phosphatase type 2C 1), each of which belongs to a highly conserved gene family, as putative RPW8-interacting proteins in the yeast-two-hybrid system. Our data showed that transcriptional up-regulation of GF54a or down-regulation of DAPP1 in *Arabidopsis* resulted in constitutive expression of PR genes, spontaneous HR-like cell death and enhanced resistance against powdery mildew (*Erysiphe cichoracearum*) and downy mildew (*Hyaloperonospora parasitica*). Genetic analysis indicated that GF54a and DAPP1 may play a positive and a negative regulatory role, respectively, in a conserved salicylic acid-dependent basal defense pathway that is also required for function of RPW8 and TIR-NBS-LRR R genes. Interestingly, we found that expression of GF54a was highly induced in DAPP1-silenced plants, suggesting that DAPP1 may also function as a transcriptional repressor of GF54a. Transient expression of hemagglutinin (HA)-tagged DAPP1 in *Nicotiana benthamiana* and subsequent enzymatic assay demonstrated that DAPP1 is a biologically active phosphatase. By using the bimolecular fluorescence complementation (BiFC) method, we found that both GF54a and DAPP1 appeared to interact with RPW8 in vivo. We thus hypothesize that GF54a may bind to phosphorylated RPW8 to enhance its resistance function, whereas DAPP1 may dephosphorylate RPW8 to down-regulate its function. Both GF54a and DAPP1 may be promising targets for engineering broad-spectrum disease resistance in crop plants.

S-56

Pseudomonas syringae effector AvrPto blocks innate immunity by targeting receptor kinases. Tingting Xiang¹, Yan Zou^{2,1}, Na Zong¹, Yong Wu¹, Jie Zhang¹, Weiman Xing¹, Yan Li¹, Xiaoyan Tang³, Lihuang Zhu², Jijie Chai¹, Jian-min Zhou¹. ¹National Institute of Biological Sciences, ²Institute of Genetics, Chinese Academy of Sciences, ³Department of Plant Pathology, Kansas State University

Receptor kinases perceive Pathogen Associated Molecular Patterns (PAMPs) to initiate plant innate immunity. PAMP-induced immunity is often suppressed by bacterial effectors, resulting in pathogen propagation. To counteract, plants have evolved disease resistance genes to detect the effectors and trigger defenses. The *Pseudomonas syringae* effector AvrPto assists bacterial infection in susceptible plants through an unknown mechanism but triggers disease resistance in tomato plants carrying the cytoplasmic protein kinase Pto. Here we show that AvrPto binds multiple *Arabidopsis* kinases both in vitro and in vivo and block innate immune responses as a consequence. Structural study of the Pto-AvrPto complex and mutational analysis of receptor kinase-AvrPto interaction indicated that Pto and the kinase domain of the receptor kinases are structurally related and bind AvrPto in a competitive manner. The results support a model in which Pto is evolved to "deceive" AvrPto by mimicking the receptor kinases. While the binding of AvrPto to the receptor kinases blocks plant immune responses, the binding to Pto "betrays" the bacterium and triggers disease resistance.

S-57

Genetic and biochemical studies of the auxin receptor reveal a novel mechanism of hormone perception. Mark Estelle¹. ¹Indiana University, Bloomington, IN, USA

The auxin class of plant hormones, including the endogenous auxin IAA, are a relatively heterogeneous collection of molecules. These compounds regulate diverse aspects of plant growth and development by promoting the degradation of transcriptional regulators called Aux/IAA proteins through the action of the ubiquitin protein ligase SCFTIR1. In recent work the F-box protein subunit of SCFTIR1, a protein called TIR1, was shown to function as an auxin receptor. Auxin binds directly to TIR1 to promote binding of the Aux/IAA proteins. Structural studies of the ASK1-TIR1 complex indicate that auxin binding does not produce a conformational change in TIR1. Rather, auxin appears to function as a molecular glue" to stabilize a weak interaction between TIR1 and the Aux/IAA substrates. These studies also suggest how structurally diverse compounds function as auxins. In addition, genetic analysis of TIR1 and other members of the TIR1/AFB auxin receptor family, provide new insight into the complexity of auxin signaling.

S-58

Specific and redundant ARF-GEF functions in membrane trafficking. Gerd Juergens¹. ¹University of Tuebingen, Tuebingen, Germany

Membrane trafficking maintains the endomembrane organisation and mediates the passage of molecules involved in signaling, cell-cell communication and response to abiotic or biotic stress. ARF GTPases are molecular switches that regulate vesicle formation at donor membranes. They are activated by ARF guanine-nucleotide exchange factors (ARF-GEFs), which associate with specific membrane compartments and thus determine when and where ARFs become active. The *Arabidopsis* genome encodes several large ARF-GEFs, which represent the only subfamily of ARF-GEFs conserved between plants, animals and fungi. The best characterised member is GNOM, which is required for the recycling of the auxin-efflux regulator PIN1 from endosomes to the basal plasma membrane. Recent studies on closely related GNOM-LIKE 1 (GNL1) will be presented and the relative roles of GNOM and GNL1 will be discussed.

S-59

AKT1 Regulation and Potassium Uptake in *Arabidopsis*. Jiang Xu¹, Hao-Dong Li¹, Li-Qing Chen¹, Yi Wang¹, Li-Li Liu¹, Liu He¹, Wei-Hua Wu¹. ¹China Agricultural University, Beijing 100094, China

Potassium is an essential mineral element for plant growth and development. Although it is known that plants absorb and transport K⁺ through the membrane transporters, it remains unclear how these transporters are regulated. Here we show that a protein kinase CIPK23, encoded by the LKS1 gene cloned from the low-K⁺ sensitive *Arabidopsis* mutant lks1, significantly regulates K⁺-uptake particularly under low-K⁺ conditions. Lesion of LKS1 significantly reduced K⁺-uptake and caused leaf chlorosis and growth inhibition, whereas overexpression of LKS1 significantly enhanced K⁺-uptake and low-K⁺ tolerance. It was further demonstrated that CIPK23 positively regulated K⁺ transporter AKT1 and two calcineurin B-like proteins CBL1 and CBL9 were upstream positive regulators of CIPK23. The activation of AKT1 by CIPK23 and CBL1 or CBL9 was further confirmed by electrophysiological recordings using *Xenopus* oocyte expression system and also in root cell protoplasts. The AKT1-mediated and CIPK23- and CBL1/CBL9-regulated K⁺-uptake pathway in *Arabidopsis* under low-K⁺ stress will be discussed.

S-60

Molecular control of S-RNase-based self-incompatibility. Lan Zhao¹, Jian Huang¹, Zhonghua Zhao¹, Qun Li¹, Yongbiao Xue¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

The self-incompatibility (SI) response occurs widely in flowering plants as a means of preventing self-fertilization. In these self/non-self discrimination systems, plant pistils reject self or genetically related pollen. In the most widespread SI system, pistil-secreted S-RNases enter the pollen cytoplasm and function as cytotoxins to specifically inhibit growth of the cognate pollen tube. However, the precise role of the S-locus F-box protein, the pollen determinant, is largely unknown. We provide evidence showing that SLF-interacting SKP1-like1 (SSK1) protein is a specific adaptor in an SCFLSLF complex and that SSK1 is essential for pollen to overcome S-RNase cytotoxicity. This pollen-specific SSK1-SLF interaction occurs in Petunia and Antirrhinum, two species from the Solanaceae and Scrophulariaceae, respectively, indicating that this novel SCFLSLF complex is conserved in the two different families with the S-RNase-based SI system. Significant reduction of SSK1 expression level does not impair the pollen tube growth in SI-defective styles, but results in pollen inhibition in cross-pollinations of functional SI-styles. This general incompatibility suggests that the pollen determinant contributes to inhibiting, rather than maintaining the S-RNase activity, at least in solanaceous plants.

S-61

Looking beyond Arabidopsis and angiosperms for biomass crop improvement genes. Clint Chapple¹. ¹Purdue University, West Lafayette, Indiana, USA

One of the most significant barriers to the efficient conversion of biomass to biofuels is the lignin polymer. The biosynthesis of lignin is now relatively well understood in flowering plants, and can be readily manipulated. We have now begun investigations into lignin monomer (monolignols) biosynthesis in lycophytes to determine whether these modern relatives of the earliest tracheophytes might provide new tools for the modification of lignin in biomass crops. Fossil records show that the lycophyte clade arose 400 million years ago, 150-200 million years earlier than the angiosperms. Our interest in the genus *Selaginella* in particular was piqued by the observation that it deposits syringyl lignin, a type of lignin often regarded as being restricted to angiosperms. To gain insight into the evolution of syringyl lignin biosynthesis in *Selaginella*, we cloned candidates for the *S. moellendorffii* homologs of the three phenylpropanoid cytochrome P450-dependent monooxygenases that play essential roles in determining lignin composition in angiosperms. Although genetic approaches and transformation are not currently available in *S. moellendorffii*, complementation experiments in Arabidopsis and enzyme kinetic assays indicate that this *S. moellendorffii* P450 is a ferulate 5-hydroxylase (F5H) that can functionally replace its angiosperm counterpart in syringyl monolignol biosynthesis. Phylogenetic analysis suggests that the identified *Selaginella* F5H is very divergent from all known plant P450s in sequence and appears to have evolved independently from angiosperm F5Hs. This project is thus providing insight into the evolution of phenylpropanoid metabolism in vascular plants, while simultaneously augmenting our lignin modification toolbox.

S-62

Metabolic engineering of terpene biosynthesis in *Arabidopsis* and consequences for plant-environment communication. Harro Bouwmeester^{1,2}, Iris Kappers^{3,2}, Asaph Aharoni^{4,2}, Carolien Ruyter-Spira¹, Zhongkui Sun², Tatsiana Charnikhova¹, Catarina Cardoso¹, Marcel Dicke³. ¹Laboratory for Plant Physiology, Arboretumlaan 4, 6703 BD Wageningen, the Netherlands, ²Plant Research International, P. O. Box 16, 6700 AA Wageningen, the Netherlands, ³Laboratory of Entomology, P. O. Box 8031, 6700 EH Wageningen, the Netherlands, ⁴ Weizmann Institute of Science, P. O. Box 26, Rehovot 76100, Israel

Terpenoids are a class of secondary metabolites that play an important role in the communication of plants with their environment. In the past few years rapid progress was achieved in terpenoid metabolic engineering in plants, including *Arabidopsis*. These engineering experiments have demonstrated that the presence of terpenoid precursors in sub-cellular compartments is not as strictly separated as previously thought and that multi-step pathway engineering, even across cell compartments is feasible [1-4]. Hence it is now becoming feasible to create transgenic plants producing terpenoids on demand. With such engineered plants fascinating results have been obtained that show that insect behaviour is strongly influenced by terpenoids. The nature of these effects varies with terpenoid compound and insect species, and for example concerns repellency of aphids and thrips [1,2], but also attraction of predatory mites, the natural enemies of spider mites [3]. However, also rhizosphere communication with other types of organisms such as parasitic plants and arbuscular mycorrhizal (AM) fungi is mediated by terpenoids. Also here *Arabidopsis*, although not a host of AM fungi, turns out to be a suitable model. For the future, we foresee great progress in the engineering of terpenoid production in *Arabidopsis*. We expect that such transgenic plants will increase our understanding of the biological relevance of these secondary metabolites in the communication of plants with a multitude of other organisms but may also lead to commercial applications.

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S-63

Recent Advances in Plant Cell Wall Biology. geoffrey fincher¹.¹ Australian Centre for Plant Functional Genomics, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia.

Cell walls are central determinants of plant form, growth and development, and change in response to environmental and pathogen-induced stresses. Walls play important roles in the quality of plant-based foods for both human and animal consumption, and in the production of fibres during pulp and paper manufacture. In the future, wall remnants in crop residues could be used as a source of renewable fuel. Cellulose, a range of non-cellulosic polysaccharides, proteins and phenolic compounds are the major components of plant cell walls. They form covalently and non-covalently linked molecular networks that have the strength, flexibility and porosity necessary for cellular function. The fine structures and sizes of wall polysaccharides can be altered following deposition into the wall, to accommodate changing physicochemical requirements as cells grow and differentiate. The chemical structures of most wall polysaccharides have been defined in detail, but the enzymes involved in their synthesis and remodelling remain largely undefined. While there have been real advances in our understanding of cellulose biosynthesis in plants, with few exceptions the identities and modes of action of polysaccharide synthases and other glycosyltransferases that participate in the biosynthesis of the non-cellulosic wall polysaccharides are not known. Emerging functional genomics, molecular genetics and X-ray crystallographic technologies are allowing us to re-examine the central questions related to wall biosynthesis. For example, the availability of the rice, Populus and *Arabidopsis* genome sequences, various mutant populations, high density genetic maps for cereals, high throughput genome and transcript analysis systems, extensive publicly available genomics resources, and an increasing array of analytical systems for the definition of candidate gene function, permit a broad, non-biased approach to the description of wall biosynthesis in plants.

S-65

Secretory peptides that regulate stomatal patterning. Kenta Hara¹, Ryoko Kajita², Keiko Torii³, Dominique Bergmann⁴, Tatsuo Kakimoto¹.¹ Osaka University,² Osaka University,³ University of Washington,⁴Stanford University

Animals use many secretory peptide mediators for control of development. Plants also use peptide mediators, such as phytosulphokine, CLV3 and CLV3-like proteins. To identify novel mediators that regulate plant development, we carried out a genome-scale screen, in which we assayed the effect on *Arabidopsis* development of individually overexpressing 153 genes predicted to encode small (<150aa) secreted peptides. Through this screen, we identified a gene EPI-DERMAL PATTERNING FACTOR 1 (EPF5) and EPF2 that decreased stomatal density when overexpressed. We show that EPF5 is expressed in stomatal cells and precursors and that controls stomatal patterning through regulation of the asymmetric cell division forming a stomatal precursor. EPF5 activity is dependent on the TOO MANY MOUTHS receptor-like protein and ERECTA-family receptor-kinases suggesting that EPF5 may provide a positional cue interpreted by these receptors. On the other hands, EPF2 regulates the density of stomatal lineage.

S-64

GIBBERELLIN INSENSITIVE DWARF5 (GID1), a soluble gibberellin receptor in rice. Makoto Matsuoka¹.¹Nagoya University Bio-Science BioTechnology Center, Nagoya, Japan

GIBBERELLIN INSENSITIVE DWARF5 (GID1) encodes a soluble GA receptor that shares sequence similarity with a hormone sensitive lipase (HSL). Previously, a yeast two hybrid (Y2H) assay revealed that the GID1-GA complex directly interacts with SLR1, a DELLA repressor protein in GA signaling. Recently, we also demonstrated, by a pull-down and Bimolecular Fluorescence Complementation (BiFC) experiments, that the GA-dependent GID1-SLR1 interaction occurs in planta. GA4 was found to have the highest affinity to GID1 in Y2H assays and is the most effective form of gibberellin in planta. Domain analysis of SLR1 by Y2H assay and gel filtration analysis revealed that the DELLA and TVHYNP domains of SLR1 are required for the GID1-SLR1 interaction. To identify the important regions of GID1 for GA- and SLR1-interactions, we used many different mutant versions of GID1, such as the spontaneous mutant GID1s, N- and C-terminal truncated GID1s, and mutagenized GID1 proteins with conserved amino acids replaced with alanine. The amino acid residues important for SLR1-interaction overlapped the residues required for GA-binding, that were scattered throughout the GID1 molecule. When we plotted these residues on the GID1 structure predicted by analogy with HSL tertiary structure, many residues were located at regions corresponding to the substrate binding pocket and lid. Further, the GA-GID1 interaction was stabilized by SLR1. Based on these observations, we proposed a molecular model for interaction between GA, GID1, and SLR1.

S-66

Heterotrimeric G proteins and ABA signaling in *Arabidopsis*. Xigang Liu¹, Wei Li¹, Fangming Wu¹, Ligeng Ma¹.¹National Institute of Biological Sciences, Beijing 102206, CHINA

Abscisic acid (ABA) is an important hormone that mediates many aspects of plant growth and development, particularly in response to the environmental stresses. Several components involved in ABA response were identified, however, the ABA signaling pathways is not well defined. Recent reports revealed that the nuclear RNA-binding protein FCA and the chloroplast protein Mg-chelatase H subunit are ABA receptors, suggesting the existence of multiple ABA receptors in *Arabidopsis*. In addition, previous report verified that the only canonical heterotrimeric G protein α subunit, GPA1, is involved in ABA-mediated seed germination, stomata opening, and inward K⁺ channel in guard cell, suggesting that GPA1 is involved in ABA signaling pathway. Heterotrimeric G proteins coupled with a plasma membrane localized receptor to transduce the extracellular signaling. In the present work, we characterized a putative G protein-coupled receptor. We found that this receptor interacts with both GPA1 and AGB1 (*Arabidopsis* G β subunit), and the interaction between this receptor and GPA1 is dependent on the intrinsic GTPase activity of GPA1. We also observed that mutation in this receptor leads to the defects in ABA responses from seed germination, stomata closure and opening to gene expression. This receptor specific binds to ABA, and the binding between the receptor and ABA follows receptor kinetics. Thus, our results revealed that this receptor is the plasma membrane receptor for ABA, and it mediates all major ABA response via heterotrimeric G proteins in *Arabidopsis*.

S-67

Plastidial oleic acid levels modulate defense signaling by regulating expression of resistance genes. A. C. Chandra-Shekara¹, Ye Xia¹, Srivaths Venugopal¹, Subhankar Barman¹, Aardra Kachroo¹, Pradeep Kachroo¹. ¹Department of Plant Pathology, University of Kentucky, Lexington, KY 40546

Oleic acid (18:1) is one of the major monounsaturated fatty acids (FA) of membrane glycerolipids and its biosynthesis is catalyzed by the soluble stearoyl-acyl-carrier-protein-desaturase (S-ACP-DES). We have previously shown that changes in the levels of 18:1 results in the alteration of salicylic acid (SA)- and jasmonic acid-mediated defense responses (1, 2, 3, 4, 5, 6). This is evident in the *Arabidopsis ssi2* mutant, which encodes a defective S-ACP-DES and consequently accumulates high levels of stearic acid (18:0) and low levels of 18:1. Consequently replenishing 18:1 levels results in restoration of wild-type-like signaling in the *ssi2* mutant (2, 4, 5, 6). Plants carrying low levels of 18:1 exhibit enhanced resistance to virulent pathogens, as well as R-gene specific resistance to viral (Turnip crinkle virus-TCV) and bacterial pathogens (7, 8). We have recently shown that the 18:1-mediated pathway regulates defense signaling by upregulating expression of multiple R genes (9). Normalizing 18:1 levels by second-site mutations restores R gene expression. Intriguingly, TCV inoculation does not activate the 18:1-regulated pathway in resistant plants, instead it results in the induction of several genes that encode 18:1-synthesizing isozymes. Consequently 18:1 levels in the plant remain constant during a resistance response to TCV. These data suggest that the 18:1-regulated pathway may be specifically targeted during pathogen infection and that alterations of 18:1 levels may serve as a novel strategy for promoting disease resistance.

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S-68

The C/S1 network of bZIP transcription factors: Combinatorial control of developmentally and stress regulated transcription by bZIP heterodimers. Friedjof Weltmeier¹, Andrea Ehlert¹, Katrin Dietrich¹, Caroline Mayer¹, Luis Onate-Sánchez², Rosario Alonso², Jesus Vicente-Carbajosa², Wolfgang Dröge-Laser¹. ¹ Albrecht-von-Haller-Institute, University of Göttingen, ²Centro de Biología y Genética de plantas. Dept. Biología, ETSI Agrónomos, Universidad Politécnica de Madrid

In order to sustain optimal plant growth, developmentally regulated gene expression has to be adjusted according to endogenous signals, such as the supply of nutrition, and to exogenous signals such as environmental stresses. By forming specific heterodimers, *Arabidopsis thaliana* group C and S1 basic leucine zipper (bZIP) transcription factors (TFs) might serve this function as a signal integration network. Transcriptome analysis reveals that the group S1 TF AtbZIP53 is involved in regulating seed storage protein genes (2S1) as well as stress related genes, such as the proline dehydrogenase (ProDH) gene involved in degradation of the compatible osmolyte proline. Functional studies in transgenic overexpressing plants (Pro35S:AtbZIP53), T-DNA insertion lines (atbzip53), as well as Chromatin Immunoprecipitation (ChIP) and transient promoter assays in protoplasts reveal that 2S1 and ProDH are direct targets of AtbZIP53. However, heterodimerisation studies performed by yeast two-hybrid (Y2H), protoplast two-hybrid (P2H) 1 and Bimolecular Fluorescence Complementation (BiFC) assays reveal that AtbZIP53 function depends on the formation of heterodimers with group C bZIP TFs. Heterodimerisation leads to a strongly enhanced target gene activation, designated as heterodimer induced transactivation (HIT) 2. Since group C and S1 members are regulated transcriptionally and post-transcriptionally by various stimuli, heterodimerisation might provide a mechanism to integrate signals in order to fine tune gene expression essential for agronomically important traits.

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S-69

A High throughput over-expression pipeline in *Arabidopsis* to discover genes important in phytohormone biosynthesis and regulation. Kenneth Feldmann¹. ¹Ceres, Inc., Thousand Oaks, (CA), USA

Ceres has over-expressed 20,000 plant genes in *Arabidopsis*. The primary transformants have been screened for alterations in phenotype under standard growth conditions. The progeny have been screened in a variety of stress conditions. An analysis of the morphological phenotypes uncovered a number of lines corresponding to genes known to affect hormone biosynthesis and regulation pathways as well as many other genes giving equivalent phenotypes and which may also affect phytohormones. This pipeline will be discussed in general along with details of several interesting over-expression lines.

S-70

Metals, Mutants and Mayhem. Mary Lou Guerinot¹, Suna Kim¹, Joe Morrissey¹, Tracy Punshon¹, Ivan Baxter², Brett Lahner², Antonio Lanzilotti³. ¹Dartmouth College, Hanover, NH USA, ²Purdue University, West Lafayette, IN USA, ³University of Chicago, Chicago, IL USA

We are using ICP-MS [inductively coupled plasma-mass spectroscopy] as a tool to determine the biological significance of connections between a plant's genome and its elemental profile or "ionome." We are screening loss of function mutants in each of the predicted ORFs in the *Arabidopsis* genome and have already identified a number of proteins that dramatically alter a plant's elemental composition, including changes in P, Ca, K, Mg (macronutrients); Cu, Fe, Zn, Mn, Co, Ni, Se, Mo, I (micronutrients of significance to plant and human health); Na, As, and Cd (minerals causing agricultural or environmental problems). We have also been applying our high-throughput ICP-MS screening, in combination with powerful genetic mapping tools such as DNA microarray-based genotyping, to identify loci that drive the natural variation we observe in the plant's elemental profile using accessions of both *Arabidopsis* and rice. Our ionomics project is managed and disseminated using the Purdue Ionomics Information Management System (PiiMS; www.purdue.edu/dp/ionomics), which currently contains publicly accessible ionomic information on over 62,000 *Arabidopsis* shoot samples.

While the ability to quantify the amount of particular metals present in various plant tissues via ICP-MS has proved very informative, we would also like to be able to see where the metals are distributed within various plant organs. We are using X-ray fluorescence micromotography to determine, *in vivo*, the spatial distribution of metals in *Arabidopsis* and rice seed. Examination of various mutants is shedding light on which transporters influence the distribution of important nutrients such as iron in the seed. Such information should aid the development of nutrient-rich seed, impacting not only human health, but leading to agronomic benefits such as increased seedling vigor, crop yields and resistance to disease.

S-72

Metabolites in Motion- Tracking The Dynamic Metabolome with 1H NMR. Jane Ward¹, Aimee Galster¹, Sonia Miller¹, Delia-Irina Coroi¹, John Baker¹, Michael Beale¹. ¹The National Centre For Plant and Microbial Metabolomics, Rothamsted Research, Harpenden, Herts, UK

The model plant *Arabidopsis thaliana* is small, has a rapid growth cycle and the availability of collections of ecotypes, knockout mutants, and transgenic lines make this plant an ideal system for large-scale metabolomic analysis in the context of gene function and whole system analysis.

Experimental protocols for the uniform growth, harvesting and NMR analysis of *Arabidopsis* have been extensively researched and developed, under GARNET. This work has now been extended to a high-throughput screening operation on a hyphenated 600MHz instrument utilising NMR-MS and SPE-NMR to further refine and deconvolute the initial spectral fingerprints. In addition we have (using NMR data from the screening of *Arabidopsis* mutants) developed a fully interactive novel software tool to automate the interpretation of metabolomics results from fingerprint data in terms of true metabolite identities. This approach has clearly shown that 1H NMR is an informative and robust analytical technique in the field of metabolomics.

In order to examine *Arabidopsis* in a further dimension, we have carried out metabolomics studies designed to supercede the single snapshot approach. These range from basic studies on the diurnal variation in metabolites, over light-dark growth cycles, to experiments tracking metabolites through the entire *Arabidopsis* life cycle. Data from these experiments show that [1H]-NMR provides a comprehensive fingerprint that reports rapidly on the status of the more abundant metabolites in the plant. PCA and alternative methods of data analysis demonstrate the utility of the technique in these large scale dynamic experiments and show how the cycling of individual metabolites can be tracked through time-course dataset.

S-73

Brassinosteroids Regulate Organ Boundary Formation and Organ Separation in *Arabidopsis*. Joshua Gendron^{1,2}, Nathan Gendron², Yu Sun², Srinivas Gampala², Dongmei Cao³, Kang Chong³, Zhi-Yong Wang². ¹Stanford University, ²Carnegie Institution, Dept. of Plant Biology, ³Institute of Botany, Chinese Academy of Science

Much is known about how brassinosteroids (BRs) are made, perceived, and transduced as a signal, and it is well established that BRs play a major role in cell expansion. However, little is known about whether or how BRs affect development outside of this role in cell expansion. In this work we show that BRs are involved in establishment of organ boundaries. Organ fusion phenotypes were first noticed in the *bzr1-1D* mutant, which contains a gain of function mutation in a transcription factor that acts as a positive regulator of BR signaling. *bzr1-1D* shows kinking of the main stem towards the lateral branches, and scanning electron microscopy revealed an abnormal fusion of the cauline leaf to the stem. Furthermore, fusions are observed in the *bzr1-1D* mutant between floral organs within and between whorls. Specifically, *bzr1-1D* contains stamen-stamen and stamen-carpel fusions, the latter resulting in bent siliques. Also the *bzr1-1D* mutant shows fused cotyledons similar to those seen in *cuc* and *pin* mutants. Brassinolide (BL) treatment of wildtype plants leads to stamen-stamen fusion, and decreased BR biosynthesis or signaling leads to the appearance of extra valves and replums. A *DWF4p::GUS* transgenic line shows that the BR biosynthesis gene, *DWF4*, is expressed in newly forming organ primordia. A GFP tagged wildtype BZR1 protein accumulates in the nuclei of cells of newly forming organ primordia, and the mutant BZR1 protein accumulates highly in all areas of the meristem and organ primordia, suggesting that restricted localization of BZR1 is required for proper organ separation. Using genetics, semi-quantitative PCR, and chromatin immunoprecipitation we are investigating the interactions between BRs and the auxin and cup-shaped-cotyledon pathways, which both regulate organ fusion. These results demonstrate that BRs not only determine plant size through regulation of cell expansion but are also involved in developmental patterning and organ separation.

S-71

Early Events in Gibberellin Signaling. Tai-ping Sun¹. ¹Duke University, Durham, NC, USA

Plant hormone gibberellin (GA)-induced growth and development is modulated by DELLA proteins, which are major repressors of GA signaling. Recent studies demonstrated that GA, upon binding to its receptor, de-represses its signaling pathway by targeting DELLA for rapid degradation, via the ubiquitin-proteasome pathway. The nuclear-localized DELLA proteins may function as transcriptional regulators, which control target gene expression via interaction with other transcription factors. Our recent microarray analysis has help to identify putative DELLA targets in *Arabidopsis*.

S-74

Plasmodesmata: Pathways for Intercellular Trafficking of Macromolecules. William Lucas¹. ¹University of California, Davis, CA, USA

Plasmodesmata (PD) establish a pathway for cell-to-cell communication within plant tissues and organs. Recent studies have demonstrated that PD evolved the capacity to mediate the cell-to-cell transport of macromolecules, including proteins and RNA. This gave rise to a system in which positional information could be delivered by non-cell-autonomously acting macromolecules, without passage into the extracellular milieu. Initial insight into this unique function was gained through studies on viruses. Plant viruses encode non-structural proteins, termed movement proteins that function in the transport of viral infectious material through PD, thereby allowing the spread of infectious nucleic acids. These findings led to the discovery that many plant proteins, including transcription factors, are able to use this PD cytoplasmic highway to traffic between cells. A number of these endogenous proteins have the capacity to potentiate the cell-to-cell transport of mRNA. Interestingly, proteins that can gain entry into the plant vascular system, and the phloem in particular, are able to move over long distances within the body of the plant. This special property of PD, to mediate the exchange of information molecules, led to the concept that higher plants function as supracellular organisms. Now that it has been unambiguously established that endogenous macromolecules have the capacity to function at a supracellular level, the challenge ahead is to identify the cellular components involved in both mediating and regulating this non-cell-autonomous protein translocation pathway. The focus of this talk will be on emerging principles of PD biology in areas of cell biology, developmental biology and inter-organ signaling. (Funding provided by NSF grant IBN 0444725 and DOE grant DE-FG03-94ER20134).

S-75

A dynamic scaffold: cytoskeletal organization of cellulose synthase. Alex Paredez¹, Ryan Gutierrez¹, Seth DeBold¹, Viktor Kyryk¹, Dorianne Moss¹, Sydney Shaw², John Sedbrook³, Chris Somerville¹, David Ehrhardt⁴. ¹Carnegie Institution, Stanford, CA, USA, ²Indiana University, Bloomington, IN, USA, ³Illinois State University, Normal, IL, USA, ⁴C

The cortical array has long been hypothesized to regulate plant cell morphogenesis by guiding the oriented deposition of cellulose in the cell wall, thus creating the material anisotropy that is responsible for directed expansion of the cell wall during turgor-driven growth. While electron microscopy and pharmacological evidence often supported this hypothesis, several studies had suggested that the relationship between the cytoskeleton and cellulose synthase was more complex than simple models predicted. To shed new light on this question, we created a functional fluorescent protein fusion to CESA6, an isozyme required to support rapid hypocotyl elongation. This tool has permitted visualization of cellulose synthase complexes, revealing their patterns of organization and dynamic behavior as they move through the plasma membrane while creating microfibrils. Co-visualization with labeled microtubules reveals that CESA6 complexes track in a bidirectional fashion along individual elements of the cortical array, and that these trajectories reorient as the cortical array reorients. However, in the absence of cortical microtubules, CESA6 complexes are still able to achieve an organized pattern of trajectories. In addition to guiding CESA complexes in the plasma membrane, the cortical microtubule cytoskeleton also appears to interact with a cytosolic compartment containing CESA protein. This compartment shows a novel form of tip-tracking motility driven by microtubule dynamics. These data suggest there may be a second role for the cortical cytoskeleton in regulating and organizing cellulose synthase. These live cell studies help to reconcile the previous literature, and enable new investigations into the function of cortical cytoskeleton in organizing proteins and macromolecules in the cell membrane and cell wall.

S-76

Control of Arabidopsis Hormone Signaling by the Ubiquitin/26S Proteasome System. Richard Vierstra¹. ¹University of Wisconsin, Madison, (Wisconsin), USA

Plants use a repertoire of methods to control the level and activity of their constituent proteins. One route that plays a prominent role is selective protein breakdown by the ubiquitin (Ub)/26S proteasome system (UPS). In this UPS system, proteins targeted for breakdown are tagged with multiple Ub's; these poly-ubiquitinated species are then recognized and degraded by the 26S proteasome, an ATP-dependent multicatalytic protease complex. Remarkably, analyses of the near complete *Arabidopsis thaliana* genome identified over 1,500 genes, or approximately 5% of the proteome, that are part of the UPS, making it one of the most elaborate regulatory mechanisms in plants. Phylogenetic comparisons to rice also suggest that components of the UPS are rapidly evolving, possibly as a way to fine-tune recognition of specific target classes. Molecular genetic analyses have connected individual components of the UPS to almost all aspects of plant biology, with a particularly important role in hormone signaling. For example, both the synthesis and the response to ethylene are strongly regulated by ubiquitination with two Ub ligases EBF5 and 2 playing key but distinct roles in targeting the ethylene-response EIN3/EIL1 transcription factors for turnover. With respect to abscisic acid (ABA) signaling, regulated degradation of the ABI5 transcription factor is controlled by several factors, including the KEG RING Ub ligase, a family of RAD23 proteins that shuttle ubiquitinated proteins to the 26S proteasome, and the poly-Ub receptor RPN10, which is a core subunit of the 26S proteasome. Through such controls, the UPS is able to dampen signaling at low hormone concentrations, rapidly allow signaling when hormone levels rise, and then enhance recovery when hormone levels dissipate.

S-77

B2-type cyclin-dependent kinase is controlled by protein degradation. Sumiko Adachi¹, Masaaki Umeda¹. ¹Graduate School of Biological Sciences, Nara Institute of Science and Technology, Iksoma, Nara 630-0101, JAPAN

The eukaryotic cell cycle is controlled by cyclin-dependent kinases (CDKs). *Arabidopsis* CDKs have been classified into six types, among which the A- and B-type CDKs are assumed to be crucial for cell cycle progression. The B-type CDKs (CDKBs) are plant-specific CDKs that are expressed specifically from the late S- to the M-phase. While the oscillation of CDKB transcripts during the cell cycle has been well characterized, we have only limited information about the protein stability that may control its kinase activity in response to internal or external stimuli.

CDKBs are further classified into two subtypes, CDKB1 and CDKB2. Here we report that the expression of *Arabidopsis* CDKB2 is under the control of protein degradation machinery. We discovered that *Arabidopsis* CDKB2, but not the A- and B1-type CDKs, contains possible PEST sequences, which are known to target themselves for proteolytic degradation and therefore reduce their half-lives. The β -glucuronidase (GUS) fused to the putative PEST motif of CDKB2 was unstable in tobacco Bright Yellow-2 cells and *Arabidopsis* plants, and the proteasome inhibitor MG132 arrested its degradation. We propose that the kinase activity of CDKB2 is regulated not only at the transcriptional level, but also through proteasome-mediated protein degradation. Such regulatory mechanisms have not been reported so far in yeast and animals, thus they may respond to plant-specific signals in the checkpoint control.

S-78

Plant evolves a unique mechanism of endocytosis. Takashi Ueda¹, Kazuo Ebine¹, Tatsuaki Goh¹, Mariko Sunada¹, Wakana Uchida², Tomoaki Nishiyama³, Mitsuyasu Hasebe⁴, Akihiko Nakano^{1,2}.¹ University of Tokyo, Tokyo, Japan, ²RIKEN, Wako, Saitama, Japan, ³Kanazawa University, Kanazawa, Japan, ⁴National Institute for Basic Biology, Okazaki, Japan

Crucial roles of endocytosis in various plant functions are emerging recently, but its molecular mechanism and physiological significance still remain largely unknown. Using *Arabidopsis thaliana*, we have been studying the molecular mechanism of endocytosis with a special focus on Rab5 GTPases. Three Rab5 members, Ara7, Rha1 and Ara6, are encoded in the *Arabidopsis* genome, which are all involved in endocytosis. Ara7 and Rha1 are orthologs of mammalian Rab5, and Ara6 is a plant-unique type of Rab5 member. Through genetic analysis, we have found that these two subgroups function antagonistically in various developmental stages, although they are all activated by the practically sole GEF, AtVps9a. Bryophytes and lycophytes also have the Ara6-type Rab5, thus this subgroup is well conserved among land plants. These data indicate that land plants have evolved a quite unique mechanism for the regulation of endocytosis, which is essential for the plant life.

S-79

Cell Patterning in the *Arabidopsis* Root Epidermis. John Schiefelbein¹, Su-Hwan Kwak¹, Christa Barron¹, Yana Panciera¹, Christine Bernhardt¹, Yan Lin¹.¹University of Michigan, Ann Arbor, MI, USA

A fundamental aspect of plant development is the specification of distinct cell types. In *Arabidopsis*, root hair and non-hair cells of the root epidermis arise in a position-dependent pattern, which implies that cell-cell communication events play an important role in cell specification. Cellular, molecular, and genetic approaches have been used to define and analyze genes and their corresponding proteins involved in this process. Some of these genes (e.g. GL2, TTG, WER, GL3, and EGL3) encode transcription factors important for non-hair cell specification, whereas others (e.g. CPC, TRY, and ETC1) help to specify the hair cell type. By studying the expression and regulatory interactions between these genes, we have found that transcriptional feedback loops acting within and between adjacent cells are important in establishing the cell type pattern. Specifically, the WER MYB-type protein, the GL3/EGL3 bHLH-type proteins, and the TTG WD-protein appear to interact in a transcriptional complex to positively regulate the GL2, CPC, TRY, and ETC1 genes. The GL2 homeodomain transcription factor is involved in regulating genes that generate the non-hair cell type. The CPC, TRY, and ETC1 proteins are structurally-related small MYB transcription factors that appear to move between cells and inhibit the formation of the WER/GL3/EGL3-TTG complex; this negative regulation represents a type of lateral inhibition mechanism. In another regulatory loop, the GL3/EGL3 proteins negatively affect their own gene expression and move between adjacent cells. The position-dependent pattern relies on a leucine-rich-repeat receptor-like kinase (SCRAMBLED (SCM)), that appears to influence WER gene expression causing an unequal distribution of the transcriptional regulators in the N and H cell positions. Current research is focused on understanding the localization and action of the SCM receptor protein and identifying new cell-fate regulators using genomic and bioinformatics methods. These studies are likely to provide new insights into the basic mechanisms of regulatory gene networks and cell-type patterning during development.

S-80

Stem cell and meristem action in *Arabidopsis*: a numbers game? Ben Scheres¹, Carla Galinha¹, Hugo Hofhuis¹, Marijn Luijten¹, Renze Heidstra¹, Veronica Grieneisen¹, Paulien Hogeweg¹, Jian Xu¹, Stan Maree¹, Ikram Blilou¹, Viola Willemsen¹.¹Dpt of Biology, Utrecht University

Recent data suggest that transport-dependent auxin maxima are important for development not only in the root but also during embryogenesis and in shoot-derived organs. It now becomes an important question how auxin as a patterning cue induces specific downstream pathways to mediate diverse effects. The *Arabidopsis* PLETHORA1 and PLETHORA2 genes encode transcription factors required for stem cell specification and can ectopically induce root identity. PLT expression is auxin-inducible, depends on auxin response factors and follows auxin accumulation patterns during embryogenesis and in post-embryonic root development. PLT genes translate auxin accumulation into region- and cell type specification patterns, and interact with the SHORTROOT-SCARECROW pathway that plays a role in patterning the root stem cells and in providing mitotic potential to the stem cell daughters that form the proximal meristem. This interaction involves the conserved RETINOBLASTOMA-RELATED pocket protein, and we are investigating the interaction between the RBR pathway and patterning genes.

Mutations in new PLT genes reveal that the PLT gene clade extensively regulates expression of the PIN facilitators of polar auxin transport in the root and this contributes to a specific auxin transport route that maintains stem cells at the appropriate position. We are currently investigating the role of graded PLT expression in this control, using genetic and genomic approaches. We are also addressing the properties of the PLT-PIN feedback loop by computational modelling.

S-81

RING-type ubiquitin E3 ligase family in *Arabidopsis thaliana*. Judy Callis¹, Yi-Tze Chen¹, Mandy Hsia¹, Edward Kraft¹, Sophia Stone², Mark Wogulis¹.¹University of CA-Davis, Davis, CA, USA, ²Dalhousie University, Halifax, Nova Scotia, Canada

The ubiquitin pathway catalyzes covalent attachment of the 76-amino acid ubiquitin, typically to epsilon amino groups of substrate proteins, and includes proteins that recognize and catabolize ubiquitylated proteins. Ubiquitylation can affect the activity, localization and/or longevity of the substrate protein. The ubiquitin E3 ligases play an important role in determining specificity of ubiquitylation by interacting with the E2 carrying activated ubiquitin and the substrate. One type of E3 contains a conserved domain called a RING (for Really Interesting New Gene) domain that serves, in part, to interact with the E2. Bioinformatics searches of the predicted *Arabidopsis thaliana* proteome identified over 470 proteins with RING or RING-like domains. Our major goal is to identify the *in vivo* functions for selected RING and RING-like type E3 ligases. cDNAs were isolated and *in vitro* activity assays of recombinant proteins were used to determine whether RING proteins could function as E3 ligases. Publicly available T-DNA insertion lines in over 100 RING domain genes were propagated to isolate homozygous individuals that were subsequently subjected to phenotypic analyses. We have focused detailed studies on three different RING genes that are seedling lethal when the insertion is homozygous. Interacting partners for one group of RING proteins were identified by Y2H analyses and results verified by *in vitro* interaction assays. Altogether, these studies will identify new E3 ligases, their function and aid in our understanding of how ubiquitylation by E2 and RING E3 ligases is regulated. Supported by NSF 2010 program.

S-82

Molecular Mechanism of KNOX Gene Repression by the *Arabidopsis ASYMMETRIC LEAVES1* Complex. Mengjuan Guo¹, Julie Thomas¹, Galen Collins¹, Marja Timmermans¹. ¹Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA

Plant shoots maintain indeterminate growth resulting from the action of a population of stem cells in the shoot apical meristem (SAM). Class I knox homeobox genes are among the key factors that specify indeterminacy in the SAM. Down-regulation of the knox genes is required for leaf initiation and for the establishment of determinant organs. In *Arabidopsis thaliana*, the latter process is mediated by the myb domain protein ASYMMETRIC LEAVES1 (AS1). We found that a fusion of the AS1 non-myb domain to the DNA binding domain of LEAFY, a transcriptional factor required for floral induction, blocked the activation of LEAFY targets, suggesting AS1 is a transcriptional co-repressor that acts directly at the knox targets. Consistent with this hypothesis, we recently showed that AS1 is part of a cellular memory system that also includes the DNA binding protein ASYMMETRIC LEAVES2 (AS2), a predicted RNA binding protein RIK, and a homologue of the chromatin-remodeling protein HIRA (Phelps-Durr et al., 2005). Using biochemical and genetic approaches we have defined the cis- and trans-acting factors that mediate the recruitment of the AS1 complex to the knox targets. Using chromatin immunoprecipitation (ChIP), we have detected three sites in the knox gene BREVIPEDICELLUS (BP) that are bound to AS1 protein complex. Two sites are in the promoter region. Our promoter:reporter constructs resulted in distinct expression patterns in leaves of wild type and as1 mutant background, which verifies the significance of the two ChIP identified sites for AS1-mediated BP silencing. Data from eletrophoretic mobility shift assay indicates that the interaction between AS1 and AS2 is required for their binding to both ChIP identified BP promoter sites, and we have identified the regulatory sequences mediating this binding. Together, our data suggests a molecular framework for maintaining determinacy during organogenesis. Interaction between AS1 and AS2 facilitates their binding to the knox targets and the recruitment of the chromatin-remodeling protein HIRA, which maintains determinacy perhaps by forming a stable repressive chromatin state at the loci.

S-83

Feedback control of leaf polarity by a family of small leucine zipper proteins. Stephan Wenkel¹, John Emery¹, Kathy Barton^{1,2}. ¹Carnegie Institution fo Washington, Stanford, CA 94305, USA

The plant specific class III homeodomain-leucine zipper (HD-ZIPIII) transcription factor proteins are master regulators for polar leaf development. We report the identification of a novel family of plant proteins, the LITTLE ZIPPER (ZPR) proteins, and their role in establishing a negative feedback loop controlling leaf development. HD-ZIPIII proteins cause transcriptional activation of the ZPR genes. The analysis of gain-of-function transgenic plants revealed that ZPR proteins specify abaxial development. ZPR proteins which consist primarily of a short stretch of leucine zipper physically interact with HD-ZIPIII proteins and, when overexpressed, inhibit HD-ZIPIII function.

Bioenergy

P-84

Differences in chemical composition of "Arabidopsis thaliana" seeds and implications for plant-herbivore interactions. Asghar Mosleh Arani¹, Tom J de Jong², H. K. Kim², Nicole M. van dam², Yong H. Choi², Rob Verpoorte², Eddy van der Meijden². ¹Yazd university, Yazd, Iran, ²Leiden university, Leiden, The Netherlands

When plants of "Arabidopsis thaliana" that originated from dune or inland populations were reciprocally transplanted to dune or inland habitats, they were affected differently by the specialist weevils, "Ceutorhynchus atomus" and "Ceutorhynchus contractus" (Curculionidae) which feed on flowers and fruits.

We grew plants in the growth room for one generation and performed a metabolomic analysis on the new seeds produced using NMR spectroscopy and multivariate data analysis. Major differences in chemical composition were found in the water-methanol fractions: more thioglucosinolates and sucrose in dune and more sinapoylmalate in inland populations.

Quantitative analysis of glucosinolates was made with an HPLC analysis, using the same seed batches collected from three sites (inland, dune and growth room). Glucosinolate composition and concentration differed between individual plants, populations and sites.

Fruit damage by adult weevils and their larvae was not significantly correlated with field concentrations of individual glucosinolates, glucosinolate groups and total concentration of glucosinolates in seeds.

We conclude that, given the range of glucosinolate concentrations in dune and inland plants of "Arabidopsis thaliana", other factors must be involved in defense against herbivory by the specialist weevils.

P-85

Structural and functional analysis of SGT1, a unique co-chaperone of HSP90 required for plant disease resistance. Marta Boter¹, Beatrice Amigues², Jack Peart¹, Christian Breuer¹, Yasuhiro Kadota³, Raphael Guerois², Ken Shirasu¹. ¹Sainsbury laboratory, John Innes Centre, Norwich, UK, ²D partement de Biologie Joliot-Curie CEA Saclay, Cedex, France, ³ Plant Immunity Research Group, RIKEN Plant Science Center, Yokohama, Japan

SGT1 and RAR1 are highly conserved eukaryotic proteins that interact with the molecular chaperone HSP90. In plants, SGT1, RAR1 and HSP90 are essential for disease resistance triggered by a number of resistance (R) proteins. To assess the mechanism by which SGT1 functions in disease resistance we screened a series of random point mutations in *N. benthamiana* for defects in Rx mediated resistance against Potato Virus X (PVX). Loss of function and dominant negative causative mutations were distributed broadly over the CS and SGS domain indicating that both domains are involved in SGT1 function in R-gene mediated resistance. Combination of NMR studies with random and site-directed mutagenesis experiments revealed that RAR1 and HSP90 interact with opposite sites of the CS domain. Functional analysis of SGT1 mutants indicated that the interaction between SGT1 and HSP90 is required for SGT1 function in disease resistance triggered by Rx. Finally, we present evidence that SGT1, RAR1 and HSP90 can form a ternary complex.

P-86

Methylation of Histone H3 Lysine 4 Residues in Response to Drought Stress in Arabidopsis. Baogiang Cao¹, Karin van Dijk¹, Yuannan Xia¹, Jean-Jack M. Riethoven¹, Eric P. Moss¹, Jingyi Yang¹, Michael E. Fromm¹, Istvan Ladunga². ¹Center for Biotechnology, University of Nebraska-Lincoln, Lincoln, NE, USA, ²Center for Biotechnology and Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE, USA

Histone modifications are important epigenetic markers for the transcriptional status of genes. In this study, we mapped histone modifications at whole-genome level in control and drought conditions and compared histone remodeling events with changes in gene expression. Histone H3 lysine 4 (H3K4) methylation changes were monitored by immunoprecipitating chromatin in vivo with modification-insensitive H3 antibodies as well as with antibodies specific to mono-, di-, and tri-methylated histone H3K4 residues. Then we used Affymetrix whole-genome tiling arrays to map nucleosome occupancy and histone methylation using over 3 million genomic probes. We found that H3K4 di- and tri-methylation showed significant positive correlation with gene expression ($p < 0.05$, Spearman test). We also investigated the changes in response to drought condition in various genetic regions of each gene, such as 5' untranslated region (UTR), 3' UTR, coding region, up- and downstream 500 base pair regions. We found that 5' UTR of upregulated genes exhibit a significantly higher level of H3K4 trimethylation than in the 3'UTRs of unchanged or downregulated genes. Similar results were obtained for dimethylation in coding regions. This study presents a whole genome view of H3K4 methylation and its changes in response to drought stress in *Arabidopsis*.

P-87

EIN3/EIL1 NEGATIVELY REGULATE BASALDEFENSES THROUGH INHIBITING SAPATHWAY IN ARABIDOPSIS. Huamin Chen¹, Satya Chintamanani², Huiqiong Lin¹, Jie Zhang¹, Xiaoyan Tang², Jian-Min Zhou¹. ¹National Institute of Biological Sciences, Beijing, ²Department of Plant Pathology, Kansas State University

Innate immune responses induced by Pathogen-Associated Molecular Patterns (PAMPs) form a critical line of defense against potential pathogens. However, the regulatory mechanisms involved are poorly understood. Here we show that *Arabidopsis* transcription factors EIN3 and EIL1, known components of the ethylene signaling pathway, negatively regulate PAMP-induced defenses such as callose deposition induced by flg22. Consistent with this, ein3/eil1 double mutant is more resistant to both virulent and nonpathogenic *Pseudomonas syringae* bacteria. Conversely, the ebf5/ebf2 double mutant that overaccumulates the EIN3 protein shows increased disease susceptibility to these bacteria. Surprisingly, a novel ein3 mutant, ein3-5, showed a loss of ethylene-induced triple response but enhanced inhibition of the PAMP-induced cell wall defenses and increased disease susceptibility, uncoupling the ethylene-regulated development and inhibition of the PAMP-signaling pathway. Microarray and northern analyses showed that PR1 several genes involved in SA synthesis, such as isochorismate synthase 1 (ICS1), chorismate synthase, phenylalanine ammonia-lyase 1 (PAL1), phenylalanine ammonia-lyase 2 (PAL2), are constitutively expressed in the ein3/eil1 double mutant. Consistent with a role of EIN3 and EIL1 in SA regulation, the ein3/eil1/sid2 triple mutant shows susceptibility to the bacterium. Together these results indicate that EIN3 and EIL1 negatively regulate basal defenses by negatively regulating the SA pathway.

P-88

Isolation and characterization of oxalic acid insensitive mutant of *Arabidopsis*. Xiaoting Chen¹, Fangfang Chen², Lin Zheng¹, Guodong Lu², Zonghua Wang *³. ¹College of Life Science, Fujian Agriculture and Forestry University, Fujian, China, ²College of Plant Protection, Fujian Agriculture and Forestry University, Fujian, China, ³Overseas College, Fujian Agriculture and Forestry University, Fujian, China

A wide range of fungi can secrete oxalic acid(OA), which facilitates the growth and reproduction of fungi during the interaction between pathogen and host, so OA is considered as one of the ubiquitous pathogenic factors. We screened OA insensitive mutants on modified MS culture medium from *Arabidopsis* mutant library based on the XVE chemical inducible gene expressing system constructed by Professor Jiruo Zuo(about 6000lines). We obtained five mutants which can survive on MS culture medium containing 1.2 mmol/L OA. Based on the sequences we got from TAIL-PCR, we blasted the sequences in TAIR and knew that the T-DNA of mutant D33 was inserted between At2g39720 (Zinc finger) and At2g39730(Rubisco activase), and the T-DNA junctions of the other four mutants were the same, all inserted in the same place of the first intron of At5g10450 (14-3-3 protein GF54 lambda). We try to over expression At5g10440 ,At5g10460 , At2g39720 and At2g39730 , so far we obtained some trans-genic plants and will identify their OA resistance and Sclerotinia sclerotiorum's resistance. Resistance to Sclerotinia sclerotiorum was also tested on OA insensitive mutant by inoculation on detached leaves and found the mutants possess the enhanced resistance. These candidate OA insensitive mutants are under further molecular characterization.

Key words : *Arabidopsis*, oxalic acid insensitive mutant , Sclerotinia sclerotiorum ,chemical inducible gene expressing system

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P-89

An auxin-like protein of *Arabidopsis* controlled disease tolerance to *Sclerotinia sclerotiorum*. Airong Wang¹, Deshu Lin¹, Chunhua Zhang¹, Wenwei Lin¹, Guodong Lu¹, Jie Zhou¹, Zonghua Wang¹. ¹Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002? China

We obtained an activation-tagged mutant showing enhanced disease tolerance to *Sclerotinia sclerotiorum*. Preliminary genetic analysis showed that this phenotype was caused by overexpression of an auxin-like protein. The bioinformatics analysis showed that this auxin-like protein had a J-domain, resembling clathrin-uncoating factor auxilin at its C terminus (Schmid et al., Annu Rev Biochem, 1997, 66: 511-548). It also contained an AsP-Pro-Phe (DPF) adaptor protein-binding motif and FxDxF motif at the N terminus, which is known to bind to the appendage domain of α or β subunits of an adaptor protein complex AP-2 (Brett et al., Structure, 2002, 10: 797 - 809). The bioinformatics data suggested that it may have ATP-binding activity, serine/threonine kinase or phosphatase activity and it might regulate cell cycle or phosphorylation or dephosphorylation of proteins. Subcellular localization by instant expression of GFP fusion protein on onion epidermis showed that it was localized in nuclei, which validated the bioinformatics deduction. Its loss-of-function mutant showed more susceptible to *S. sclerotiorum* than wild type and grew slower on MS medium than wild type. We presumed that the Clathrin-Coated Vesicles may participate in the defense pathway to *S. sclerotiorum*. Further genetic analysis is to confirm the linkage between the molecular or biological functions of the protein and the predicted phenotype.

P-90

The dissection of the WUSCHEL C-terminal domain reveals specificity for the interaction with TOPLESS. Elena Clerici¹, Eric van der Graaff², Thomas Laux², Brendan Davies¹. ¹University of Leeds, UK, ²Institute of Biology III, Freiburg, Germany

An important characteristic of plants is their ability to produce new organs during their entire life cycle. The homeobox gene WUSCHEL (WUS) encodes an important regulator of meristem maintenance in *Arabidopsis*, yet its mechanism of action in meristem regulation is presently unclear. TOPLESS (TPL), previously named WSIP1, a transcriptional co-repressor like protein, interacts with the WUS protein and also plays a role in meristem maintenance. WUS and TPL belong to two small families, other members of which are also capable of interacting with each other in specific combinations. An interaction matrix, testing all combinations of interactions between TPL and WUS proteins shows that specific protein domains are required for interaction. An ERF-like domain, a domain previously associated with transcriptional repression, at the C-terminus of WUS appears to be important for the interaction, suggesting a means by which WUS might repress genes promoting differentiation. A similar domain is also present in AUX/IAA proteins, which are repressor proteins involved in auxin signaling. This suggests that members of the TPL family could mediate the repressive effects of AUX/IAA proteins. Extending the interaction matrix to include AUX/IAA proteins will reveal any specificity in these interactions.

P-91

DELLA proteins regulate trichome initiation and branching through GIS and GL1 in *Arabidopsis*. Yinbo Gan¹, Hao Yu², Jinrong Peng³, Pierre Broun¹. ¹CNAP, Department of Biology, University of York, York YO10 5YW, United Kingdom, ²Department of Biological Sciences and Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117543, Singapore, ³Functional Genomics Lab, Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, 138673, Singapore

Gibberellins (GA) are known to influence vegetative and reproductive phase change in *Arabidopsis*, as well as the development of trichomes, which are faithful epidermal markers of shoot maturation. Downstream of GA production, hormone signaling integrators REPRESSOR OF /ga1-3/ (RGA) and GIBBERELLIC ACID INSENSITIVE (GAI) play a central role in the control of vegetative and reproductive phase change.

In this study, we have probed the relative roles of RGA, GAI, RGA-LIKE 1 (RGL1) and RGL2 in the control of epidermal differentiation and shoot maturation and investigated molecular mechanisms through which they regulate trichome production. We found that RGL1 and RGL2 act synergistically with RGA and GAI in the repression of vegetative and reproductive phase change in *Arabidopsis*. RGL1 and RGL2 also play a significant role alongside RGA and GAI as negative regulators of trichome initiation. In the absence of GA signaling, de-repression of initiation on the inflorescence through loss of DELLA function requires GLABROUS INFLORESCENCE STEMS. When DELLA function is restored, genes encoding activators of trichome initiation, including GIS and GLABROUS1, are rapidly repressed, but this appears to be through an indirect rather than a direct molecular mechanism. In contrast to its relatively minor role in initiation, GAI is a major repressor of trichome branching, a role in which it is antagonized by RGL1 and RGL2.

P-92

The SPT transcription factor acts as an activator in *Arabidopsis* ecotype *Landsberg erecta* (*Ler*) and a repressor in ecotype *Columbia* (*Col-0*) to control seed germination. Yinbo Gan¹, Eve-Marie Josse², Steven Penfield¹, Alison D. Gilday¹, Karen J. Halliday². ¹CNAP, Department of Biology, University of York, York YO10 5YW, United Kingdom, ²Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh, EH9 3JR, United Kingdom

We previously described the role of the basic helix-loop-helix transcription factor SPATULA (SPT) in the control of seed germination by light and temperature (Penfield et al., 2005, Current Biology, 15, 1998-2006). In the current work we show that the role of SPT in seed germination is dependent on the ecotype. In Col-0, SPT acts as a repressor of seed germination while in Ler it acts as an activator. The spt-11 mutation in Col-0 resulted in much higher germination rates of fresh seeds compared to the wild type. Light treatment alone results in 100% germination in spt-11 whereas Col-0 requires both light and cold treatments to reach 100% germination. Constitutive overexpression of SPT in Col-0 results in significantly lower germination rates following either light or cold treatments compared to the wild type. In contrast, the spt-1 and spt-2 mutants in the Ler background exhibit lower germination rates than the wild type following either cold or light treatments, while the overexpressing line results in higher germination rates under both treatments. In agreement with this overexpression phenotype we have found that the spt-10 Ler allele previously reported to be a knockout mutant actually overexpresses SPT transcript despite the fact that the SPT ORF is disrupted by a transposon insertion (Penfield et al., 2005). Consistent with these opposite roles for SPT in germination, we have also found that hypocotyl elongation also responds differently to SPT. Overexpression of SPT in Col-0 results in hypocotyls that are significantly longer than the wild type whereas overexpression in Ler does not result in a significant increase compared to wild type. These results lead us to conclude that the underlying mode of action of the SPT transcription factor in Ler and Col-0 is fundamentally different.

P-93

Networks and Mechanisms of Brassinosteroid Regulated Gene Expression in *Arabidopsis*. Michelle Guo¹, Lei Li¹, Xiaofei Yu¹, Addie Hall¹, Tadao Asami², Shigeo Yoshida², Joanne Chory³, Yanhai Yin¹. ¹Department of Genetics, Development and Cell Biology, Plant Science Institute, Iowa State University, Ames, IA 50011, ²Plant Functions Lab, RIKEN, Wako-shi, Saitama 351-098, Japan, ³Howard Hughes Medical Institute and Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037

Plant steroid hormone brassinosteroids (BRs) play important roles throughout growth and development, regulating diverse processes such as cell elongation, xylem differentiation, ethylene biosynthesis and stress responses. Recent molecular genetic studies established that BRs signal through membrane receptor BRI1 to regulate the protein levels and/or activities of BES1/BZR1 family transcription factors, which function to mediate many of the hormone responses. Little is known about the genetic networks and molecular mechanisms by which BES1 and its homologs regulate target gene expression. To fill these gaps, multidisciplinary approaches have been used to identify BES1 partners, regulators and targets. First, Partner of BES1 (POB1, a putative histone modification enzyme) has been identified by a yeast genetic screen and has been found to interact with BES1 both in vitro and in vivo. Both loss-of-function and gain-of-function mutants of POB1 gene display BR response phenotypes including changed sensitivities to BR biosynthesis inhibitor brassinazole and altered expression of a subset of BR-regulated genes. Second, several BES1 target genes encoding transcription factors and other signaling molecules have been identified by microarray and Chromatin Immunoprecipitation (ChIP) experiments. T-DNA knock-out mutants have been identified and the characterization of the mutant phenotypes will be presented. Finally, we have identified several mutants that have reduced BES1-regulated gene expression and may identify proteins that function with BES1 to regulate BR target gene expression. Our results suggest that BES1 interacts with other transcriptional partners to modify chromatin structure and therefore regulate the expression of different target genes for various BR responses. The research is supported by NSF (IOS 0546503) and a faculty start-up fund from Iowa State University.

P-94

Defense against "Sclerotinia sclerotiorum" in "Arabidopsis" is dependent on JA, SA, ethylene, and ABA signaling. Xiaomei Guo¹, Henrik Stotz¹. ¹Oregon State University, Corvallis, OR, USA

Genotypic differences in susceptibility of "Arabidopsis thaliana" to "Sclerotinia sclerotiorum" have not been reported due to the extreme susceptibility of this cruciferous plant. To overcome this limitation, we have established inoculation conditions that enable evaluation of differences in susceptibility to "S. sclerotiorum" among "Arabidopsis" mutants and ecotypes. Two "coi1" mutant alleles conferred hypersusceptibility to "S. sclerotiorum". The plant defensin gene PDF5.2 was no longer induced after challenging the "coi1-2" mutant with "S. sclerotiorum". Hypersusceptibility of the "coi1-2" mutant to "S. sclerotiorum" was not correlated with oxalate sensitivity. The "npr1" and "ein2" mutants were also hypersusceptible to "S. sclerotiorum". Induction of PDF5.2 and the pathogenesis-related gene PR1 was reduced in "ein2" and "npr1" mutants, respectively. Actigard, a commercial formulation of the systemic acquired resistance inducer benzothiadiazole, reduced susceptibility to "S. sclerotiorum". Based on histochemical analysis of oxalate-deficient and wild-type strains of "S. sclerotiorum", oxalate caused a decrease in hydrogen peroxide production, but no detectable changes in plant superoxide production or gene expression. The "abi1" and "abi2" mutants also conferred hypersusceptibility to "S. sclerotiorum" and they were more oxalate sensitive than wild type plants. Hypersusceptibility of "abi1" and "abi2" mutants to "S. sclerotiorum" were correlated with oxalate sensitivity and wilting symptoms after inoculation with "S. sclerotiorum".

P-95

Alternative oxidase plays a central role in defining the levels of expression of stress induced genes in *Arabidopsis* and is regulated by stress signalling pathways. Lois Ho¹, Estelle Giraud¹, Ryan Lister¹, Rachel Clifton¹, Katharine A Howell¹, Angela Glen¹, David Thirkettle-Watts¹, James Whelan¹. ¹ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, Perth, Western Australia, Australia

The alternative oxidase (Aox) is encoded by small multigene families in plants. In *Arabidopsis* (*Arabidopsis thaliana*), the five members of this family have been shown to be differentially expressed during development and in response to stress. Functional analysis of the *Arabidopsis* Aox1a (*AtAox1a*) promoter indicated that regulation was responsive to stress treatments with hydrogen peroxide and, to a lesser extent, rotenone. Functional analysis of the cis-sequence elements driving the expression of *AtAox1a* identified ten elements responsive to hydrogen peroxide treatment and/or rotenone treatment. Analysis showed that six of these elements were also active in the alternative NAD(P)H dehydrogenase gene, NDB2, previously shown to be co-expressed with *AtAox1a* (Clifton et al 2005, Clifton et al 2006), indicating that co-expression patterns observed were due to co-regulation. Microarray analysis of two independent lines lacking in a functional *AtAox1a* gene revealed that all the transcripts induced and repressed after one hour of treatment with hydrogen peroxide were already similarly changed in the knock-out lines. This reveals that *AtAox1a* plays a role in defining the basal level of expression of these stress induced genes under unstimulated conditions. The induction of *AtAox1a* and NDB2 was also compromised in the defence signalling mutants, *npr1* and *eds4*, and, to a lesser extent, *pad4*. An analysis of the *Arabidopsis* genome for genes with six or more of the cis-elements identified as functional in *AtAox1a*, revealed a set of genes which were involved in stress perception, signal transduction, stress activation of gene expression and other stress induced transcripts. Together, these results indicate that *AtAox1a* is co-regulated with defence and/or stress induced genes and that its activity feeds back to define the expression of stress induced genes.

P-97

Global Identification of Target Genes Regulated by Gibberellin Signaling Repressor RGA during *Arabidopsis* Flower Development. Wen-Wei Hu^{1,2}, Xingliang Hou², Hao Yu^{1,2}. ¹Temasek Life Sciences Laboratory, National University of Singapore, ²Department of Biological Sciences, National University of Singapore

The phytohormone gibberellins (GAs) play crucial roles in regulating many aspects of plant development. Previous studies have shown that the default status for GA signaling is repression that is controlled by DELLA proteins, a family of nuclear growth repressors. The GA signal derepresses its signaling pathway by promoting DELLA protein degradation via 26S proteasome-dependent ubiquitylation pathway. In GA-deficient *ga1-3* mutants, the development of all floral organs was arrested, while various combinations of null mutants of DELLA proteins could gradually rescue floral defects in *ga1-3*, suggesting that DELLA proteins, especially RGA, play critical roles in mediating the GA-signaling pathway in the control of flower development. As a first step to study the target genes of GA signaling mediated by DELLA proteins in flower development, functional *ga1-3 rga rgl2 35S::RGA-GR* transgenic lines were selected for high-throughput microarray analysis. A number of genes whose transcripts were modulated by induced RGA activity were identified. In particular, the RGA-regulated genes included those responsible for synthesis, transport, and signaling of other phytohormones, indicating the presence of potential crosstalk between GA and other hormones. Semi-quantitative RT-PCR and *in situ* hybridization further confirmed that the expression of these identified genes was indeed regulated by RGA in the GA signaling pathway. Characterization of the over-expression transgenic lines and their corresponding loss-of-function mutants is being carried out to further our mechanistic understanding of these target genes downstream of DELLA proteins during flower development.

P-96

ECL1 promotes flowering by the repression of FLC in *Arabidopsis*. Sung Myun Hong¹, Seung Kwan Yoo¹, Jeong Seob Lee², Ji Hoon Ahn¹. ¹Plant Signaling Network Research Center, School of Life Sciences and Biotechnology, Korea University, Seoul, 136-701, South Korea, ²School of Biological Sciences, Seoul National University, Seoul, 151-742, South Korea

Early flowering and Curly leaves 1-1 D (ecl 1-1 D), which showed early flowering and curly leaf phenotype, was isolated by an activation tagging screen. Various approaches showed that earlying of *ecl 1-1 D* was due to the upregulation of *Flowering locus T (FT)*. *ecl 1-1 D co-2* plants flowered earlier than *co-2* plants and *ecl 1-1 D 35 S::CO* flowered earlier than its single overexpression plant suggesting that ECL1 may not act within CO mediated-photoperiod pathway. RT-PCR analysis showed that activation of ECL1 caused downregulation of *FLOWERING LOCUS C (FLC)* and *ecl1-1D FRI FLC* showed early flowering compare with flowering phenotype of *FRI FLC*. *ecl1-1D* showed similar circadian rhythm of FLC mutant by analysis of *CHLOROPHYLL A/B BINDING PROTEIN 2 (CAB2)* expression and leaf movement. Our data suggest that ECL1, which exerts its effect in FLC, is a floral promoter.

P-98

A Role of the C-terminal of AtRGS1 in its Targeting to the Plasma Membrane. Guangzhen Hu¹, Jirong Huang¹. ¹National Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, Shanghai 200032, China

RGS (regulators of G-protein signaling) proteins deactivate G-protein signaling through accelerating GTPase activity of Gα subunits. There is only one protein containing RGS box in *Arabidopsis thaliana*, designated AtRGS1, which also has seven trans-membrane domain at N-terminal. AtRGS1 have been verified to be a plasma membrane protein. Here we present that GPA is unlikely involved in the plasma membrane targeting of AtRGS1 and its C-terminal play a role in this process.

P-99

Identification of a rice QTL regulating grain width and weight
Wei Huang¹, Xian-Jun Song¹, Jun-Xian Shan¹, Min Shi¹, Mei-Zhen Zhu¹, Hong-Xuan Lin¹. ¹National Key Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, The Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China.

We used map-based cloning strategy to identify a QTL, GW2 as the gene encodes a RING-type protein with E3 ubiquitin ligase activity, responsible for rice grain width and weight. GW2 promoter GFP was expressed constitutively in various tissues and organs. Subcellular localization of GFP-GW2 fusion driven by the CaMV 35S showed that GW2 localized to the cytoplasm. We observed an increase in grain width and panicle number per plant in NIL(GW2) carrying GW2 allele of the larger grain size variety (WY3) compared with FAZ1 (the recurrent parent, smaller grain size variety). Histological analyses of spikelet hulls of FAZ1 and NIL(GW2) demonstrated that the increased width of the NIL(GW2) spikelet hull resulted mainly from an increase in cell number, but not in cell size. On the contrary, the increase in NIL(GW2) endosperm size resulted mainly from cell expansion, not from an increase in cell number. We observed the grain milk filling rate in NIL(GW2) and FAZ1 and found that the rate of accumulation of dry matter was faster in NIL(GW2). These data suggest that larger spikelet hull allows greater endosperm growth and provides a greater area of contact for endosperm with the seed coat leading to an accelerated milk filling rate. We examined grain quality traits in NIL(GW2) and FAZ1. Results showed that the WY3 GW2 allele could increase grain size and yield with little influence on appearance and no reduction in cooking or eating quality. Our findings will facilitate breeding efforts to improve grain yield in staple crops.

P-100

The Role of Two Ubiquitin-Like Proteins in *Arabidopsis* Peroxisome Biogenesis and Function. Navneet Kaur¹, Jianping Hu¹, Sheng Quan¹, Jianping Hu¹. ¹MSU DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824. USA

Plant peroxisomes are dynamic organelles that play a vital role in plant growth and development. Peroxins are an eclectic set of proteins necessary for peroxisome biogenesis and import of matrix proteins from the cytosol. PEX2 is a RING family Peroxin which has been shown to be essential for viability and to be involved in the photomorphogenic response. Yeast two hybrid approach was employed to identify PEX2 interacting factors and resulted in the retrieval of an Ubiquitin-Like protein, named AtPXi1 (PEX2 Interactor protein). *Arabidopsis* was found to have a highly conserved paralog of this gene, AtPXi2. AtPXi genes are present in tandem and oriented in head to tail fashion on the chromosome. The AtPXi proteins constitute an N-terminal Ubiquitin-Like (UBL) domain, a C-terminal Ubiquitin Associated domain (UBA) and four chaperonin binding sites in the medial region. Both AtPXIs interact with the RING domain of PEX2. Deletion constructs are being made to delineate the AtPXi domain specific to this interaction. Using fluorescence microscopy we demonstrated that GFP-fusions of AtPXi1 and AtPXi2 localize to the peroxisome. T-DNA insertion lines of AtPXIs were identified and are being investigated to determine the functional role of the AtPXIs. RNAi lines are concurrently being analyzed to further elucidate the functions of the AtPXIs in *Arabidopsis*. UBL-UBA proteins are believed to function as shuttle factors, relaying ubiquitinated proteins to the proteasome. Drawing analogy from the ERAD (Endoplasmic Reticulum Associated Degradation) model, the presence of RING proteins (putative E3 ligases), E2, AAA-ATPases and UBL-UBA proteins in the peroxisomes prompts us to speculate that peroxisomes also have a ubiquitin-proteasome type of proteolytic system associated with them, which regulates peroxisome biogenesis and function by targeting selective peroxisome proteins for degradation.

P-101

CIPK1 Interacts with an *Arabidopsis* Novel Nuclear Protein Similar to Human Activating Signal Cointegrator-1 Yungyong Kim¹. ¹Department of Molecular Biology, Sejong University, Seoul 143-747, Korea

CIPK1 interacts with a couple of calcineurin B-like (CBL) calcium sensors such as CBL1 and CBL3. Therefore it is believed that CIPK1 is also involved in a variety of stress signal transduction pathways which use Ca²⁺ as a second messenger. However little is known about how CIPK1 transmits the signal to downstream components. In efforts to find the *in vivo* substrates of CIPK1, we have performed the yeast-two hybrid screening and discovered a new CIPK1-interacting protein (named K26) in *Arabidopsis*, which possesses a zinc-finger motif found in human activating signal cointegrator-1. Pull-down assays verified formation of the complex between CIPK1 and K26 *in vitro*. Interestingly, K26 exclusively interacted with CIPK1, but not with other CIPK family members including CIPK2, 3, 5, 6, 7, 11, 12 and 23. Deletion analyses revealed that the nonkinase domain of CIPK1 (282 to 451 amino acids) is sufficient and required for the interaction with the K26 region between 147 and 308 amino acid residues. GFP fusion protein analyses indicated that K26 is mainly localized in the nucleus. Currently, we are analyzing a homozygous knock-out *Arabidopsis* mutant that carries a single copy of T-DNA in the K26 genomic locus.

P-102

A conserved role of SHORT VEGETATIVE PHASE (SVP) in controlling flowering time of *Brassica* plants. Hanna Lee¹, Jeong Hwan Lee¹, Soo Hyun Park¹, Jong Seob Lee², Ji Hoon Ahn¹. ¹Plant Signaling Network Research Center, School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea, ²School of Biological Sciences, Seoul National University, Seoul 152-742, Korea

The control of flowering time in *Brassica* plants is an important approach for improving productivity, as vegetative tissues are not produced after the floral transition in *Brassica* plants. In order to determine the feasibility of modulating flowering time in Chinese cabbage plants, genes homologous to *Arabidopsis* SHORT VEGETATIVE PHASE (AtSVP) were isolated from spring-type and fall-type cultivars of Chinese cabbage plants, and their functions were determined. Their deduced amino acid sequences were 91–93% identical with that of AtSVP. The expression of *BcSVP* was ubiquitously detected, and was unaffected by vernalization. Constitutive *BcSVP* expression induced late flowering with additional floral defects. This delayed flowering was attributed to the repression of FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1). *BcSVP* expression under the control of the AtSVP promoter also resulted in the complementation of the *svp* mutation in *Arabidopsis*. These results indicate that *BcSVP* is a functional equivalent of AtSVP and also suggest that *BcSVP* may prove useful for the genetic manipulation of flowering time in *Brassica* plants.

P-103

Chemical genetic Interrogation of protein-protein interaction. Yong-Jik Lee¹, Augusta Jamin¹, Thomas Girke¹, David Carter¹, Natasha Raikhel¹, Zhenbiao Yang¹.¹ University of California, Riverside, CA, USA

The chemical genetics/genomics approach uses small molecules to modify or disrupt the function of specific genes/proteins. This is in contrast to classical genetics, in which mutations disrupt the function of genes. This approach is expected to overcome some limitations associated with genetic approaches, such as the inability to perturb biological processes instantaneously, transiently, and spatially. Given the importance of protein-protein interaction in protein function and regulation, we are interested in identifying chemicals that alter a specific protein-protein interaction. We are testing whether high throughput yeast two-hybrid assay provides a useful method for this purpose. As a proof of concept, we used this assay to screen chemical libraries for chemicals that disrupt the interaction between the ROP2 GTPase and a GAP protein. Strategies to eliminate false positives and identification of several putative positive hits will be discussed.

P-105

An alternative splicing variants of rice pex5p, OsPex5pL, is required for PTS2 protein import. Hye Song Lim¹, Sun Young Kim², Young Jun Jung², Min Gyung Chun², Kyun Oh Lee², Woe-Yeon Kim², Sang Yeol Lee².¹ Environmental Biotechnology National Core Research center, PMBBRC & Division of applied Life Science (BK21), Gyeongsang National University, Jinju 660-701 Korea,² Environmental Biotechnology National Core Research Center, PMBBRC & Division of Applied Life Science (BK21), Gyeongsang National University, Jinju 660-701 Korea

Using the rice PEX14 cDNA as a bait in a yeast two-hybrid assay, two splice variants of the type I peroxisomal targeting signal (PTS1) receptor, OsPex5pL and OsPex5pS, were cloned from a pathogen-treated rice leaf cDNA library. The proteins were produced from a single gene by alternative splicing, which generated a full-length variant, OsPEX5L, and a variant that lacked exon 7, OsPEX5S. OsPex5pL contained 11 copies of the pentapeptide motif WXXXF/Y in its N-terminus, and seven tetra-tripeptide repeats in its C-terminus. Expression of OsPEX5L and OsPEX5S predominantly occurred in leaf tissues, and was induced by various stresses, such as exposure to the pathogen Magnaporthe grisea, and treatment with fungal elicitor, methyl viologen, NaCl or hydrogen peroxide. The Arabidopsis T-DNA insertional pex5 mutant, AtPex5, which does not germinate in the absence of sucrose and was resistant to indole-3-butyrac acid (IBA), was perfectly rescued by over-expression of OsPex5pL, but not by OsPex5pS. Using transient expression of OsPex5pL and OsPex5pS in the AtPex5 mutant, we show that OsPex5pL translocates both PTS1- and PTS2-containing proteins into the peroxisome by interacting with OsPex7p, whereas OsPex5pS is involved only in PTS1-dependent import in Arabidopsis.

P-104

The Arabidopsis Information Resource (TAIR). Donghui Li¹, Eva Huala¹.¹ Carnegie Institution of Washington, Department of Plant Biology, Stanford, (CA), USA

The Arabidopsis Information Resource (TAIR; www.arabidopsis.org) is a community database for *Arabidopsis thaliana* that provides access to curated data about the structure and function of this model plant. The workshop is designed for users who wish to more effectively utilise the curated data and software resources provided by TAIR. Specifically we will address curation of three major data types: gene structure, gene function and metabolic pathway annotation.

Arabidopsis serves as an important resource and potential benchmark for the annotation of other plant genomes. As such, a "gold standard" of gene structure annotation is demanded. We will describe our progress in meeting this aim and give details of the TAIR7 genome release. In addition we will outline our plans for the subsequent TAIR8 release and some of the annotation challenges which remain.

In addition to annotating gene structures, TAIR curators annotate gene function using terms developed by the Gene Ontology (GO) Consortium that describe the molecular function, biological process, and subcellular location of a gene product. We will use TAIR's GO annotations to demonstrate how controlled vocabularies allow for standardization of annotation and assist in comparative genomics. We will describe the process of GO annotation at TAIR and how the annotations can be used to classify large data sets or to facilitate annotation of other sequenced plant genomes. We will demonstrate how to access TAIR's GO annotations in bulk and to derive a global view of the status of functional annotation of the *Arabidopsis* genome.

Plant metabolic databases have been developed to visualize the universe of primary and specialized (secondary) metabolism, to present the flexibility and interconnectivity of biochemical networks and to serve as matrix to predict Pathway/Genome Database's (PGDB's) based on sequenced genomes. We will introduce the contents and curation of the multi-organism database MetaCyc and the *Arabidopsis thaliana* specific database AraCyc. The major functions and applications of MetaCyc and AraCyc will be presented including the visualization of large-scale data on the Omics viewer.

P-106

Differentiation of an unusual post-transcriptional processing mediated by short, direct repeats in response to stress conditions between monocots and dicots. Yongsheng Liu¹, Xiangli Niu¹, Wenjing Zheng¹, Bao-Rong Lu², Di Luo¹, Lijuan Chang¹, Yuguo Wang².¹ College of Life Science, Sichuan University,² Institute of Biodiversity Science, Fudan University

Various abilities to synthesize and accumulate glycine betaine (GB) are crucial for angiosperms to develop salt and drought tolerances. In higher plants, GB is synthesized by a two-step oxidation of choline via an intermediate form of betaine aldehyde, and catalyzed by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH). In this study, numerous truncated and/or recombinant transcripts of two BADH homologues resulted from an unusual post-transcriptional processing were detected in rice (*Oryza sativa*) and other cereal crops, including maize (*Zea mays*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). The observed events took place at the 5' exonic region, and led to the insertion of exogenous gene sequences and a variety of deletions that resulted in the removal of translation initiation codon, loss of functional domain and frame-shifts with premature termination by introducing stop codon. By contrast, the BADH transcripts from dicotyledonous species such as spinach (*Spinacia oleracea*), *Arabidopsis* (*Arabidopsis thaliana*), and tomato (*Solanum lycopersicum*), had correctly processed mRNA. This suggests the differentiation of post-transcriptional processing in BADH genes potentially contributes to the variation of GB synthesizing capacities among various plant species. In addition, comprehensive sequence analyses demonstrated that extensive sequence similarities (named as short-direct repeats, SDRs) are of paired presence surrounding the junctions of both the deletion and/or insertion sites in the unusual BADH transcripts. The sites selection for the deletion/insertion was altered in response to the stress conditions. This indicates that the sequence elements of SDRs are probably required for the recognition of the deletion/insertion sites.

P-107

The Arabidopsis Calcium Sensor CBL3 Inhibits the 5'-MethylthioAdenosine Nucleosidase Activity of C38 in a Calcium-Dependent Manner. Seung-Ick Oh¹, Kyung-Nam Kim¹. ¹Department of Molecular Biology, Sejong University, Seoul 143-747, Korea Abiotic stresses such as cold, drought, and high salt induce changes in cytosolic calcium signatures of plant cells. Calcineurine B-like (CBL) proteins are believed to sense and transmit the calcium signals to a group of serine/threonine protein kinases called CIPKs, thereby activating the kinase activity. In this study, we have identified a new target of the CBL3 protein, designated C38, using a yeast-two hybrid library screening. C38 interacted only with CBL3 but not with other CBL family members such as CBL1 and CBL4. Deletion analysis revealed that the CBL3 region spanning from 109 to 199-amino acids is required and sufficient for the interaction with C38. In the case of C38, it appeared that almost entire sequence is necessary for the interaction with CBL3. Pull-down assays and enzyme activity assays demonstrated that CBL3 associates with C38 only in the presence of calcium, and thereby significantly inhibits the 5'-methylthioadenosine nucleosidase (MTAN, EC 3.2.2.16) activity of C38. Although no striking phenotypic differences were observed between a wild type and a 'c38' mutant in general, the 'c38' mutant was a little more sensitive to high osmotic or salt stresses in terms of germination rate.

P-109

The Overexpression of a Ser/Thr Kinase Protein Altered Ethylene Responses In Arabidopsis. Liping Qiu¹, Chi-Kuang Wen¹. ¹Institute of plant physiology and ecology, Shanghai, China

To uncover new components involved in the ethylene signal transduction pathway, gene expression profiles were analyzed by Affymetrix arrays. Four genes were identified and their overexpressions alter ethylene responses. Here we report that the overexpression of ATR3 (Altered Ethylene Responses 3) conferred constitutive ethylene responses. ATR3 encodes a kinase protein and the kinase domain locates near the C terminus. In vitro assay suggest that ATR3 exhibits ser/thr kinase activity. The overexpression of ATR3 kinase domain conferred ethylene insensitivity, implying that the ATR3 N terminus is a regulatory domain which negatively controls the C-terminal function in the repression of ethylene responses. Possible roles of ATR3 in the ethylene signal transduction pathway were studied by expressing the full-length ATR3 and ATR3 kinase domain in ethylene response mutants. Ethylene responses conferred by ATR3ox was completely masked by the etr1-1 mutation and Ag(I) treatment. However, the ein3-1 mutation and the overexpression of EBF5 only partially masked the overexpression of ATR3. The overexpression of ATR3 kinase domain further rescued the mutant phenotype of etr1 ers1 mutant. Possible roles of ATR3 in the regulation of ethylene responses are discussed.

P-110

Transcription factors as potential tools to alter metabolic pathways in seeds. Armin Schlereth¹, Martin Bringmann¹, Alexander Erban¹, Stefanie Wienkoop¹, Clementina Kakar¹, Mark Stitt¹, Michael Udvardi². ¹Max-Planck-Institute of Molecular Plant Physiology, D-14476 Potsdam-Golm, Germany, ²The Noble Foundation, Ardmore 73401 (Oklahoma), USA

Transcription factors (TFs) exhibit key regulatory functions and are crucial for the regulation of many developmental and physiological processes by influencing gene expression. Furthermore, during seed development and maturation TFs display an essential role in controlling the quality and quantity of the storage compound composition. Since seeds are an excellent and in many human populations the major source for nutrients as well as for animals food it could be beneficial to identify the determinants which control or affect the biosynthesis of the storage compounds. In *Arabidopsis thaliana* almost 1900 TFs are annotated. We designed primer pairs for all these TFs genes (Czechowski et al., 2004) and analysed their expression in comparison to the expression of marker genes either encoding for storage proteins or being relevant concerning the synthesis of storage lipids during seed development using the quantitative Real-time RT-PCR. This allowed the identification of seed specific TFs displaying a developmental expression comparable to that of the marker genes. Therefore, these TFs could have the potential acting as key regulators controlling the storage compound synthesis. For the functional characterization 17 different T-DNA insertion- as well as RNAi- lines of putative key regulators have been selected and will be analysed. To supplement the forward and reverse genetic approaches metabolite, lipid and protein profiling of *Arabidopsis* wild type seeds from various developmental stages from pollination till mature seeds was performed. These uncovered quantitative and qualitative changes in storage compound composition with a high temporal resolution and allowed a seasonal correlation of the expression of TFs- and marker genes with the synthesis of the storage compounds. Moreover, these results can be used as a basis that allows the comparison from WT and transgenic seeds with respect to their differences in storage compound composition. Further studies will transfer the obtained results to grain legume seeds. Czechowski(2004), Plant Journal; 38, 366-79

P-108

A Novel Nuclear Protein Associated with CIPK11 Is Involved in Salt Response of Arabidopsis. Soojin Park¹, Kyung-Nam Kim¹. ¹Department of Molecular Biology, Sejong University, Seoul 143-747, Korea

We have isolated an *Arabidopsis* cDNA, named K50, which encodes a polypeptide interacting with CIPK11 using a yeast two-hybrid system. Deduced amino acid sequence analysis revealed that K50 possesses a bipartite nuclear localization signal and a proline-rich domain, suggesting that it may be a nuclear protein with transcriptional activation activity. In fact, GFP-fusion protein analyses and yeast two-hybrid assays supported that K50 may serve as a transcription activator. Pull-down assays and split-YFP analyses verified formation of the complex between CIPK11 and K50 in vitro and in vivo, respectively. Deletion analyses revealed that K50 interacts with the 363-435 CIPK11 region lacking the CBL3-interacting NAF domain. Interestingly, the CIPK-K50 interaction affinity was enhanced in the presence of Ca²⁺-bound CBL3. Real-time RT-PCR analyses indicated that the K50 gene expression is down-regulated by the salt stress. Interestingly, however, a T-DNA knock-out K50 mutant was more sensitive to the salt stress than the wild-type plants. We have also observed that the stress genes such as RD29A/B and Kin1/Kin2 were highly expressed in the k50 mutant. Anyway, these findings suggest that K50 may play an important role in relaying the cytosolic Ca²⁺ signals induced by salt stress to the nucleus, thereby regulating gene expression.

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The Arabidopsis LIM Protein WLIM1 Binds and Bundles Actin Filaments. Andre Steinmetz¹, Clement Thomas¹, Flora Moreau¹, Stephane Tholl¹, Jessica Papuga.¹, Monika Dieterle¹, Celine Hoffmann¹. ¹Public Research Center-Health, Luxembourg, Luxembourg

Flowering plants express several LIM proteins some of which are specific to mature pollen grains, while others are expressed only in vegetative structures, sometimes in a tissue-dependent pattern. The aims of our studies are to understand the functional differences between the six LIM proteins identified in Arabidopsis by their coding sequences. One of these proteins we have been studying is WLIM1 (At1g10200). To examine its subcellular distribution we expressed it as a GFP fusion under the control of an inducible promoter and analysed its localization in root tissues of Arabidopsis seedlings by laser scanning confocal microscopy. The fusion protein was found to associate with filamentous structures in several cell types. Following incubation of the seedlings with Latrunculin B (LatB) the cortical cytoskeletal structures disappeared while the more internal structures appeared to be more resistant. This sensitivity to LatB of the WLIM1-GFP-labeled cables identified the structures as actin filaments. High- and low-speed centrifugation experiments showed that the bacterially-expressed and affinity-purified protein binds actin filaments *in vitro* without intermediary protein and that this association triggers F-actin bundling. Interestingly also, while in root hairs the cytoskeletal organisation was identical to that observed for fimbrin-GFP-expressing cells, another root cell type showed marked differences in cytoskeletal structure; most notably, the WLIM1-GFP-expressing cells showed thick, more randomly organized actin cables, whereas in fimbrin-GFP-expressing cells the cables were much thinner and longitudinally oriented. These observations identify the Arabidopsis protein WLIM1 not only as an F-actin bundling protein but also as an actin cytoskeleton organizer.

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Developmental control of Arabidopsis seed oil biosynthesis
Hongyun Wang¹, Jinhua Guo¹, Yun Lin¹. ¹University of Illinois, Urbana, IL, USA

Arabidopsis transcriptional factors LEAFY COTYLEDON1 (LEC1), LEAFY COTYLEDON2 (LEC2), FUSCA3 (FUS3), ABSCISIC ACID3 (ABI3), and ABSCISIC ACID5 (ABI5) are known to regulate multiple aspects of seed development. In an attempt to understand the developmental control of storage product accumulation, we observed the expression time course of the five transcripts. The sequential expression of these factors during seed fill suggests differentiation of their normal responsibilities. By extending the expression periods of the two early genes LEC1 and LEC2 in transgenic seeds, we demonstrated that the subsequent timing of FUS3, ABI3, and ABI5 transcripts depends on LEC1 and LEC2. Because a delayed onset or reduced level of FUS3 mRNA coincided with reduction of seed oil content in the transgenic seeds, the role of FUS3 in oil deposition was further examined. Analysis of published seed transcriptome data indicated that FUS3 transcript increased together with nearly all the plastidial fatty acid biosynthetic transcripts during development. The ability of FUS3 to rapidly induce fatty acid biosynthetic gene expression was confirmed using transgenic Arabidopsis seedlings expressing a dexamethasone (DEX)-inducible FUS3 and Arabidopsis mesophyll protoplasts transiently expressing the FUS3 gene. By accommodating the current evidence, we propose a hierarchical architecture of the transcriptional network in Arabidopsis seeds in which the oil biosynthetic pathway is integrated through the master transcriptional factor FUS3.

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The ETR1 N-Terminal Signaling is Primarily Dependent on Subfamily I Receptor and can be Covalent Independent. Fang Xie¹, Qian Liu¹, Chi-Kuang Wen¹. ¹National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese

Arabidopsis ETR1 is an ethylene receptor and plays unique roles in the regulation of ethylene responses. Although ETR1 has the conserved structures and domains of prokaryotic His-kinases of the two-component signaling modules, little is known about the mechanism by which the receptor signal is mediated to downstream components. To further study how the ETR1 signal is mediated to the repression of ethylene responses, we focused on the receptor signal output mediated through the N terminus. It has been shown that the wild-type ETR1 N terminus, the etr1(1-349) protein, cannot repress ethylene responses and that the covalent disulfide linkages are important to the N-terminal signaling. In our study, using genetic and transformation approaches, we found that etr1(1-349) was still capable of the repression of ethylene responses, implying receptor signal output through the ETR1 N terminus. However, the etr1-7 ers1-3, but not etr1-7 ers1-2, mutations completely blocks the etr1(1-349) receptor signal output. Moreover, the dominant N-terminal signaling mediated by Ag (I) and etr1-1(1-349) was also masked in etr1-7 ers1-3, suggesting essential roles of subfamily I receptors in the ETR1 N-terminal signaling. The loss of subfamily II receptors, however, did not perturb the etr1-1(1-349)-mediated ethylene insensitivity. Roles of the disulfide linkages in the ETR1 N-terminal signaling were examined. Mutations that disrupt the formation of covalent dimerization via Cys4 and Cys6 did not perturb the repression of ethylene responses mediated by ETR1, implying that the N-terminal signaling can be non-covalent. Non-covalent protein-protein interaction was examined in yeast two-hybrid assay and the GAF domain may be involved in the ETR1 N-terminal signal output.

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A Study of Arabidopsis Ethylene Signal Transduction Identifies a Transcription Factor and an Ethylene Response Mutant. Qin Wang¹, Xin Zhou¹. ¹Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences

Genetic screen for mutants defective in the seedling triple response has identified genes involved in the ethylene signal transduction in Arabidopsis. Other approaches have been used to isolate more mutants that cannot be identified in the seedling triple-response assay. In an attempt to find more components participating in the ethylene signal transduction pathway, both forward and reverse genetics approaches were used in our study. Here we report the isolation of a transcription factor, ATR2, whose overexpression confers ethylene insensitivity and completely restores the growth of the ctr1-1 mutant. In a genetic screen, we identified a mutant, ain, which exhibits a mild constitutive ethylene response phenotype. Ethylene treatment caused a severer triple-response phenotype in ain than in wild type and the silver treatment did not block the ethylene treatment in ain, suggesting AIN a component downstream of the receptors. In adult stage, the ain mutant was small and dwarf. More genetic analyses for the atr2 and ain mutants are in progress.

P-115

Reduced leaflet pathway controls leaf complexity and leaflet polarity in *Lotus japonicus*. Jun Yan¹, Xuefei Cai¹, Shusei Sato², Satoshi Tabata², Jun Yang¹, Xianglin Cao¹, Wei Ma³, Da Luo^{1,3}. ¹National Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, Shanghai 200032, China, ²Kazusa DNA Research Institute, 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0818, Japan, ³School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200030, China

Leaf forms of seed plants can be simply classified as simple leaf and compound leaf. Compound leaves are normally composed of one petiole and several leaflets, whereas simple leaves have a single lamina. To address the question about the key components in the control of leaf complexity during compound leaf development, we have identified a series of mutants in *Lotus japonicus*: *reduced leaflet 1* (*rel 1*), *rel 3*, *rel 4* and *rel 5*, which display the similar phenotype of reduced leaflet numbers in a compound leaf with defect of abaxial-adaxial (ab-ad) polarity in individual leaflet. Genetic analysis indicates that these four mutants are not allelic to each other, and these genes may act in the same pathway. It is found that the expression patterns of duplicated *LjPhantastica* genes in *Lotus japonicus* are altered in *rel 1* and *rel 3*, accounting for the abnormalities of ab-ad development in the mutant compound leaves, and suggesting that *REL* may act upstream of *LjPhan* genes. Forward and reverse genetic approaches are conducted to analyze the function of *REL* genes and the regulatory network in regulating compound leaf development in *Lotus japonicus*.

P-116

Plasticity of root epidermal cell fate in response to nutrient starvation. Thomas Yang¹, Natasha Savage², Wolfgang Schmidt¹. ¹IPMB, Academia Sinica, Taipei, Taiwan, ²Computational Systems Biology group, Sheffield University, Sheffield, UK

Differentiation of root epidermal cells is controlled by a network of interacting factors, the core of which is a complex of MYB and bHLH transcription factors associated with a WD40 repeat protein. The fate of rhizodermic cells in the ageing root is not irreversibly fixed, but is reached by continuous integration of different signals. Both the stage at which the environmental signals are perceived and the mechanisms underlying the re-programming of epidermal cells have yet to be understood. In the present study, we investigated the effect of P and Fe deficiency on the expression levels of a number of cell specification genes as well as genes known to be up-regulated during RH formation. P-deficient plants produce longer and additional root hairs that are located both in the normal position and in positions normally occupied by non-hair cells. This was not observed in Fe-deficient plants, in which the surface area was increased by the formation of branched hairs. Our results indicate that the respective deficiencies also produced a unique pattern at the gene expression level. Contrary to expectation, the expression of genes determining the non-hair cell fate such as *WER* and *GL 2* was not affected by both deficiencies. However, some cell specification genes, namely *SCM* and *CPC*, and *ETC 1*, were differentially up regulated by either Fe or -P. In addition, some root hair associated genes were found to be induced by both deficiencies in correlation with increase in root hair number. To further investigate the plasticity in root hair development, we have developed a reaction-diffusion model that accounts for previous observations on root epidermal patterning in mature roots in response to P deprivation. The model assumes an additional patterning mechanism that by-passes the theorized cell fate regulatory circuit in the root epidermis. The presumed second mechanism is suggested to allow for plasticity in epidermal cell patterning by changing the cell fate of epidermal cells regardless of their position in a de novo patterning mode.

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The Functional Characterization of OmLEJ and Its Homologs, OsLEJ and AtLEJ2: Those Genes Are Required for Stress Responses and Reproductive Development. Kyoung Shin Yoo¹, Kwang Wook Jung¹, Mei Hua Cui¹, Yun Young Kim¹, Jeong Sheop Shin¹. ¹School of Life Sciences & Biotechnology, Korea University, Seoul, 136-701, Korea

In previous study, we selected twenty candidate clones for functional analysis based on the results of microarray by fungal infection, insect infestation and wound treatment. One of them, of our interest, OmCDCP and its cultivated rice and *Arabidopsis* orthologs were used for functional analyses. Expression of OmCDCP and OsCDCP genes were much induced by wounding, brown plant-hopper and rice blast fungus as well as exogenous JA treatment, in spite of their different intensity and expression pattern. One ortholog in *Arabidopsis*, AtCDCP2, is also characterized. The AtLEJ2 Protein is Localized in Chloroplast. ProAtCDCP2: GUS analysis revealed that the expression of AtCDCP2 is restricted in the pollen grains and endothecium of anther. AtCDCP2 ectopic expression caused developmental delay of floral organ, arrested anther cells on secondary cell wall biosynthesis, and reduced the accumulation of JA in the flower bud clusters, resulting in male-sterility by anther indehiscence, and also those overexpression lines are much more tolerant to various osmotic stresses than wild type. Based on these data, we are trying to characterize the biological roles of novel genes in defense system or developmental process, including regulatory mechanism as well as the effects of gene itself.

P-118

OmDSP1 having novel nuclease activity contributes to resistance against *B. cinerea* in *Arabidopsis*. Min Kyung You¹, Hyung Young Shin¹, Seung-Ick Oh¹, Jeong-Kook Kim¹, Jeong Sheop Shin¹. ¹School of Life Sciences and Biotechnology, Korea University

A blast (*Magnaporthe grisea*)-, brown planthopper (BPH; *Nilaparvata lugens*)- and wounding-responsive OmDSP1 (*Oryza minuta* Diverse Stress responsive Protein 1) was isolated and identified as a novel defense related gene from *O. minuta*. OmDSP1 exists as single copy in its genome and encodes a predicted protein with molecular mass of 39 kDa and a pI of 6.2. OmDSP1 gene was expressed at a basal level under normal condition, and induced by diverse stresses; methyl jasmonic acid (MeJA), ethephon, salicylic acid (SA), abscisic acid (ABA), methyl viologen (MV), NaCl, wounding, blast and BPH. In vivo targeting of OmDSP1-smGFP with onion cells revealed that OmDSP1 was targeted to nucleus.

OmDSP1 was identified as a novel nuclease with DUF551 domain which is found just in plant and bacteria. OmDSP1 shared highly conserved N-terminal region, DUF551 and UVR domains with other plant orthologs. The mRNA expression patterns of OmDSP1 plant orthologs, OsDSPs and AtDSPs genes, were induced also by MeJA, ethephon, SA and ABA, suggesting that orthologs also participate in similar cellular responses under diverse stress conditions.

To investigate the function of OmDSP1, OmDSP1 gene was introduced into *Arabidopsis*. The 35S:OmDSP1 plants showed increased-resistance against attacks of necrotrophic fungal pathogen, *Botrytis cinerea* and germination retardation on media containing ABA. The results of RT-PCR performed with defense-related marker genes (PDF5.2 and Thi2.1) showed to be more sensitively increased by JA or ET in 35S:OmDSP1 plants. AtSAC1b and ABA1 are closely related to callose deposition against pathogen attacks. AtSAC1b and ABA1 are constitutively expressed in 35S:OmDSP1. These responses in 35S:OmDSP1 and atdsp1 plants were suggested to be caused by fine-tuning regulation of crosstalk between JA/ET and ABA signaling pathways. In yeast two hybrid screening, three putative counterparts of OmDSP1 were screened: putative E3 ligase, drought-induced protein Di19 and putative PR10.

P-119

Functional Analysis of A-type ARR_s in Shoot Apical Meristem Regulation. Zhong Zhao¹, Stig Andersen¹, Monika Demar¹, Jan Lohmann¹. ¹Max-Planck-Institute for Developmental Biology

It has been known for decades that some phytohormones, such as cytokinin and auxin, play an essential role in meristem regulation, however, the underlying molecular mechanisms remained largely elusive. In *Arabidopsis thaliana*, stem cell fate in the shoot apical meristem is controlled genetically by a negative feed back loop of CLAVATA3 (CLV3) and the homeodomain transcription factor WUSCHEL (WUS). We have shown previously that WUSCHEL, which positively regulates stem cell fate, represses the transcription of several A-type ARABIDOPSIS RESPONSE REGULATOR genes (ARR5, ARR6, ARR7 and ARR15), in the meristem. Since A-type ARR genes act in the negative feed back loop of cytokinin signaling, this finding provided the first mechanistic link between hormone signaling and plant stem cell regulation.

However, we did not find any visible phenotype in ARR5, ARR6 and ARR7 single mutants, while strong alleles of ARR15 caused female gametophytic lethality. To circumvent the lethality problem, we constructed conditional alleles by inducible artificial microRNAs against ARR15 to further explore its functions in the shoot apical meristem. Our results show that down-regulation of ARR15 or expression of constitutively active forms of ARR15 cause meristematic defects, including disturbed phyllotaxis. Consistent with the observed phenotypes, RNA *in situ* hybridization showed that ARR15 is expressed in the shoot apical meristem. Our data indicate that ARR15 function might be one of the central elements in the regulation of meristem activity.

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Analysis of changes in protein expression during Arabidopsis pollen germination. Jun-Jie Zou¹, Lian-Fen Song¹, Wen-Zheng Zhang¹, Yi Wang¹, Song-Lin Ruan¹, Ji-Dong Feng¹, Wei-Hua Wu¹. ¹State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, National Plant Gene Research Centre (Beijing), China Agricultural University, Beijing 100094, China

To identify candidate proteins involving regulation of *Arabidopsis* pollen germination, proteomic approach was applied to analyze the changes in protein expression during the transition from the desiccated mature pollen to the germinated pollen. By application of 2-DE and silver staining, there were 499 and 494 protein spots resolved from the protein samples extracted from pollen grains and pollen tubes, respectively. Among these proteins, 303 protein spots were analyzed by MALDI-TOF and 213 protein spots representing 189 proteins were identified. A comparative analysis revealed that 40 protein spots showed statistically significant and reproducible changes between mature pollen and pollen tubes. Among these 40 protein spots, 40% of the protein spots were specific to developmental stages. Mass spectrometry analysis allowed the identification of 17 downregulated and 6 upregulated differentially expressed proteins representing 21 diverse proteins. Functional category analysis showed that these proteins were involved in signaling, cellular structure, transport, defense/stress responses, transcription, metabolism and energy. The changed pattern of these proteins indicated extensive changes in the energy metabolism of the pollen tube growth, accompanied by the activation of stress response pathway and modifications of the cell wall. In addition, the detailed methods for collection of *Arabidopsis* pollen, extraction of proteins from pollen and pollen tubes were described and discussed.

P-120

The ETR1 N Terminus is Essential to the RTE1 Function in the Repression of Ethylene Responses Cross Endomembranes

Xin Zhou¹, Qian Liu¹, Fang Xie¹, Chi-Kuang Wen¹. ¹Institute of plant physiology and ecology, Shanghai, China

Arabidopsis RTE1 encodes a membrane protein and functions as a negative regulator of ethylene responses. Through genetic and transformation studies, here we show that the function of RTE1 is primarily dependent on ETR1 dosages and can be independent of the other receptors. To narrow down possible regions that are essential to the function of ETR1 and RTE1, truncated etr1 and rte1 were each expressed ectopically. The ETR1 N terminus is essential to the RTE1 function and ectopic expression of ETR1 N terminus restored ethylene insensitivity in 35S::gRTE1 etr1-7. N terminal deletions in RTE1 also restored ethylene insensitivity in etr1-2 rte1-2. These data suggest that ETR1 and RTE1 may each function through specific domains. Possible interaction between ETR1 and RTE1 was examined by co-immunoprecipitation and our result shows that RTE1 can associate with ETR1 but not ERS1. The RTE1 transcript accumulates upon ethylene treatment but its promoter activity was not ethylene-inducible, implying post-transcriptional regulation of the RTE1 transcript. Sub-cellular localization of RTE1 was studied using GFP-RTE1 fusion and RTE1 could function in Golgi. Possible mechanisms by which ethylene responses are repressed by RTE1 and ETR1 is discussed.

P-122

Molecular genetics of temperature modulation of defense responses. Ying Zhu¹, Yi Wang¹, Jian Hua¹. ¹Cornell University, Ithaca, NY 14853, USA

Temperature regulates growth and development as well as biotic and abiotic responses in plants. However, the molecular mechanisms underlying how temperature modulates these processes are largely unknown. We are particularly interested in how a relatively higher temperature suppresses defense responses and cell death in plant-pathogen interactions. Both *bon* 1-1 and *snc* 1-1 mutants have constitutive defense responses and subsequently dwarf phenotypes at 22 °C and these phenotypes are absent at 28 °C due to an inhibition of defense response by higher temperatures. Using the morphological phenotype as a readout of defense responses, we carried out genetic screens in *bon* 1 and *snc* 1 respectively for mutants defective in this temperature modulation. A number of isolated mutants exhibit dwarf phenotype at 28 °C in *bon* 1 or *snc* 1 but not in the wild type, and these mutants are named ‘int’ for ‘insensitive to temperature’. Some of the int mutants appear to be enhancers of *bon* 1 or *snc* 1 while others appear to confer temperature insensitivity to *bon* 1 or *snc* 1 mutants. We are now in the process of isolating these INT genes through tagging or map-based cloning. One of the int mutants is likely a new *snc* 1 allele that contains a missense mutation in the *R* gene SNC 1, suggesting that temperature modulation of defense responses could occur at the *R* gene level. The revelation of the molecular identities of these INT genes will generate insights on the interplay between defense and temperature responses.

Cell Biology

P-123

Quantitative Proteomic Characterization of Detergent Resistant Domains in the Plasma membrane of Arabidopsis. Sylwia Bem¹, Waltraud Schulze¹. ¹Max Planck-Institute for Molecular Plant Physiology, Golm, Germany

The plasma membrane is a dynamic compartment with key functions in solute transport, cell shape and rigidity as well as communication between cells and the environment. In mammalian and yeast cells, the plasma membrane has clearly been shown to be compartmented into so-called lipid rafts, which are defined by their resistance to treatment with non ionic detergent. The "detergent resistant" regions have unique protein and lipid composition rich in sterols and sphingolipids. In mammalian and yeast cells, it has been shown that these domains are nanometers to one micron in size and they form highly organized microdomains. There are indications that also protein composition and size of detergent resistant domains can change in response to internal and external signals.

Using quantitative proteomic techniques, we characterized the proteome of the detergent resistant domains in Arabidopsis cell cultures. Cell cultures were metabolically labelled with heavy nitrogen and in labelled cell cultures the detergent resistant domains were disrupted by chemical treatment. Preparations of treated cells were then directly compared to preparations of unlabeled cells.

We show that also in plants, similar detergent resistant plasma membrane microdomains are significantly enriched in signal transduction proteins like receptor like kinases, membrane ATPases, transporters, and GPI-anchored proteins. Also, by specific disruption of "detergent resistant domains" by methyl- β -cyclodextrin, we are able to define common contaminating proteins in the "detergent resistant" preparation. Lipid composition of the detergent resistant domain fraction also clearly differs from whole plasma membrane and changes upon methyl- β -cyclodextrin treatment.

P-124

THE ARABIDOPSIS HEAT STRESS INDUCED CO CHAPERONES ROF5 AND ROF2 (FKBPs) ARE DEVELOPMENTALLY REGULATED AND INTERACT WITH HSP90. Adina Breiman¹, Dedi Mei-ri¹, Odelia Farchi -Pisanti¹, Keren Aviezer-Hagai¹, Julia Skovorodnikova¹, Nir Ohad¹. ¹Dept of Plant Sciences, Tel Aviv University, Tel Aviv Israel

The plant co-chaperones FKBPs that belong to the peptidyl prolyl cis-trans isomerases (PPIases) are investigated due to their function in protein folding, signal transduction and chaperone activity. Arabidopsis possesses 22 FKBPs, present in all subcellular organelles. We are studying the large FKBPs ROF5 (FKBP62) and ROF2 (FKBP65). They possess three FKBPs domains followed by TPR and calmodulin binding domains. Arabidopsis and wheat FKBPs interact with HSP90, calmodulin and dynein, participating in the heterocomplex trafficking machinery to the nucleus. The expression of ROF5 and ROF2 is organ specific and developmentally regulated. High expression was observed in the vascular elements in roots, in hydathodes, trichomes and stomata, in stigma and sepals in young flowers, in connective tissues of siliques and in mature seeds. Promoter analysis has revealed heat stress and ABA responsive elements. Both genes are induced at 37°C but only ROF5 is expressed also in plants grown at 22°C. ROF5 and ROF2 were assumed to interact with HSP90 similar to wheat FKBP73 and 77. We have shown that ROF5 coimmunoprecipitates with HSP90. However, when the 5 amino acids defined as essential for interaction between the TPR and HSP90 were mutated, the interaction was abrogated. We have shown that ROF5 binds specifically to HSP90.1 whereas ROF2 did not.

Microarray analysis in knockout mutants reveals a high bias towards genes that are related to response to biotic and abiotic stress, both up and down regulated. We have isolated a T-DNA knockout mutant of rof5-. An increase in expression of genes related to cell wall loosening enzymes and trehalose biosynthesis was revealed in the rof5- mutant by transcriptome analysis. Since no phenotype was observed under normal growth or heat stress conditions, we have produced a double rof5-/rof2- mutant.

We will test the hypothesis that ROF5 plays a role in the ABA regulated pathway associated with inhibition of germination and its capacity to interfere with the expression of cell wall loosening enzymes and trehalose biosynthesis.

P-125

A screen for interaction specificity between RAC/ROP GTPases and their partners in Arabidopsis. Tore Brembu¹, Atle Bones¹, Zhenbiao Yang². ¹Norwegian University of Science and Technology, Trondheim, Norway, ²University of California, Riverside, USA

The RAC/ROP family of small GTPases has emerged as master regulators of numerous cellular and developmental processes in plants, including polarised cell growth, hormone responses and plant defense. Previous screens for RAC/ROP interacting partners have identified four protein families regulating RAC/ROP activity, as well as a number of effector protein families. Several of these protein families appear to be plant specific. The Arabidopsis RAC/ROP family consists of 11 members, and a majority of the RAC/ROP interacting proteins are also encoded by multi-gene families. To date, no studies have been made to investigate whether individual plant RAC/ROP GTPases show differences in binding preferences to their interacting partners. Identifying specific protein-protein interactions will be of great help in elucidating the mechanisms of RAC/ROP signaling pathways. In order to address this issue, we have used a high-throughput protein interaction assay, based on liquid yeast two-hybrid assays performed in 96-well plates. Differences in binding specificity were observed, especially between type I and type II RAC/ROPs. Selected interaction pairs were confirmed using FRET/BIFC and coimmunoprecipitation.

P-126

Towards understanding translational control: Systematic analysis of the *Arabidopsis* cytosolic ribosome proteome provides detailed insights into its protein complement and their post-translational modification. Adam Carroll^{1,2}, Joshua Heazlewood², Jun Ito^{1,2}, Harvey Millar^{1,2}. ¹ ARC Centre of Excellence in Plant Energy Biology, Perth, Western Australia,²University of Western Australia, Perth, Western Australia

The synthesis of polypeptides by cytosolic ribosomes represents a major energy-consuming process of the plant cell and an important point for the control of protein expression. Research aimed at understanding the genetic and molecular mechanisms underlying translation and its control will greatly benefit from accurate knowledge about ribosome structure such as the gene-specific identities of proteins incorporated into ribosomes and the types and sites of covalent modifications on ribosomal proteins. We have used a combination of *in silico* approaches coupled to mass spectrometry analysis to advance the proteomic insight into *Arabidopsis* cytosolic ribosomal composition and covalent modifications. *In silico* digestion of all 409 ribosomal protein sequences in *Arabidopsis* defined gene-specific peptides for each gene family. We then undertook an extensive MS/MS survey of the cytosolic ribosome and used only high confidence matches to gene-specific peptides for calling gene-specific identifications. This has provided an in-depth analysis of the protein composition based on 1413 high quality MS/MS spectra matching to 776 peptide sequences from ribosomal proteins. These have identified peptides from 5 gene families of r-proteins not identified previously, providing experimental data on 79 of the 80 different types of ribosomal subunits. We provide strong evidence for gene specific identification of 93 different ribosomal proteins from these 79 families. We also provided new information on 33 specific sites of co- and post-translational modification of r-proteins in *Arabidopsis* by initiator methionine removal, N-terminal acetylation, N-terminal methylation, lysine N-methylation and phosphorylation. This site specific modification data provides a wealth of resources for further assessment of the role of ribosome modification in influencing the control of translation in *Arabidopsis*.

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Proteomic analysis of the rice (*Oryza sativa*) cell wall. Xiong-Yan Chen¹, Hye Sook Lee², Yeonggil Rim¹, Seonok Kwon¹, Zee Yong Park², Keun-Wu Lee³, Jae-Yean Kim³. ¹ Division of Applied Life Science, PMBBRC/EB-NCRC, Gyeongsang National University, Jinju, 660-701, Korea, ²Deaprtment of Life Science, Gwangju Institute of Science and Technology, Gwangju, 500-712, Korea, ³Division of Applied Life Science, PMBBRC/EB-NCRC, Gyeongsang National University, Jinju 660-701, Korea

Plant cell wall proteins play crucial roles in cell wall structure and architecture, cell wall metabolism, cell enlargement, signaling, responses to biotic and abiotic stress, and many other physiological processes. Despite the importance of cell wall to the biology of plants, whose proteome is less well characterized than those of other subcellular compartments. Here we present a proteomic analysis aimed at generating a proteomic reference map for plant cell wall. Secreted soluble proteins, ionically (weakly-bound) or tightly-bound cell wall proteins from rice (*Oryza sativa*) callus were isolated from culture medium or extracted using CaCl₂ containing buffer or SDS-based buffer, respectively. Cell wall proteins from each fraction were separated by SDS-PAGE. 2 fractions (high and low molecular weight fractions) were separated from polyacrylamide gel, *in-gel* digested with trypsin and analyzed using multidimensional protein identification technology (MudPIT). We have obtained about 500 soluble proteins, about 800 CaCl₂ extracted proteins and about 1900 SDS-solubilized proteins using 3 independent extraction methods mentioned above. Identified proteins include classical cell wall proteins, membrane proteins as well as proteins traditionally considered as cytosolic proteins. Much lower level of cytosolic contamination was observed in secreted or CaCl₂ extracted proteins using intact callus rather than SDS extracted ones from purified cell wall fraction. To our knowledge, this is the first large-scale (>2000 proteins) rice cell wall proteomic analysis, allowing generating a systemic proteomic map for cell walls of rice.

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Study of ROPs and their regulators in root hair development
Xin Chen¹, Matt Smallman¹, Claire Grierson¹. ¹School of Biological Sciences, University of Bristol, Bristol, UK

ROPs are members of a plant specific subfamily of Rho small GTPases. The GTP binding GTPases are active, while the GDP binding forms are inactive. The switch between these two forms requires several different regulators, and they are very important molecular switches in eukaryote signal transduction. Among all these regulators, GDI1 (Guanine nucleotide dissociation inhibitor) was the first one found having a strong phenotype in root hair development in *Arabidopsis thaliana*. In *scn1-1* mutant, where GDI1 can't be fully translated, the root hair cell forms bulges and branches. The aim of my research is to study the affects of structural changes on the function of GDI1 by phenotyping GDI1 point mutated lines, and the interactions between ROPs and GDI1 *in vitro* and *in vivo*. Results of these experiments would reveal how ROPs, GDI1 and the interactions between them contribute to root hair development in *Arabidopsis thaliana*.

P-129

Coordination of transcriptional regulation and chromatin modification of *Arabidopsis* circadian clock genes. Hae-Ryong Song^{1,2}, Ju-Hee Jeong^{1,2}, Bosi Noh^{3,2}, Yoo-Sun Noh^{1,2,†}. ¹Department of Biological Sciences, Seoul National University, Seoul 151-742, Korea, ²Global Research Laboratory for Flowering at SNU and UW, Seoul National University, Seoul 151-742, Korea, ³Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea

Circadian clock genes are regulated through a transcriptional-translational feedback loop. In *Arabidopsis*, LHY and CCA1 transcripts are highly expressed in the early morning. Translated LHY and CCA1 proteins repress the expression of TOC1 transcript which peaks in the evening. TOC1 protein elevates the expression of LHY and CCA1 mRNAs, forming a negative feedback loop that is believed to constitute the oscillatory mechanism of the clock. Recently the rhythmic oscillation of mouse clock genes, mPER1 and mPER2, was shown to be correlated with the regular alteration of chromatin structure through histone acetylation/deacetylation. However, little is known about the chromatin modification-mediated transcriptional regulation of *Arabidopsis* circadian clock genes. Here we propose a possibility that *Arabidopsis* clock-associated genes, LHY, CCA1, and TOC1 might be regulated by rhythmic histone modifications. Our results show that certain type of histone modifications either has positive or negative correlations with the expression of LHY, CCA1, and TOC1 transcripts. Therefore, the rhythmic transcription of these clock genes might depend on regular histone modifications within their chromatin and the fine-tuning of the feedback loop comprising an oscillator in plants might be accomplished by an ordered modification of histones.

P-130

Regulation of Cell Divisions in *Arabidopsis* Posttranslational Control of CDKs. Nico Dissmeyer^{1,2}, Moritz K. Nowack^{1,2}, Stefan Pusch^{1,2}, Hilde Stals³, Dirk Inz³, Paul E. Grini⁴, Arp Schnitger^{1,2}. ¹Max Planck Institute for Plant Breeding Research, Cologne, Germany, ²University of Cologne, Department of Botany III, Cologne, Germany, ³Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, Gent, Belgium, ⁴University of Oslo, Department of Molecular Biosciences, Oslo, Norway

In general, the cell cycle control machinery appears to be highly conserved between the kingdoms: progression through the cell cycle is governed by cyclin-dependent kinases (CDKs). In *Arabidopsis*, one Cdc2+ /Cdc28 homolog (CDKA;1) with the archetypical PSTAIRE hallmark is encoded and due to the specific life style of plants, peculiar mutant phenotypes were expected. In a mutant for cdka;1 that we have isolated, the haploid gametophyte was found to be strongly affected and pollen with only one instead of two sperm cells is produced. This dramatically disrupts flowering plants' key event of double fertilization. Based on this mutant, we explored the regulatory context of CDKA;1 in planta. In yeast two hybrid screens we have identified potential novel interactors and by mutating conserved phosphorylation-sites we found that phosphorylation of a canonical Thr residue is required for high enzyme activity and is essential for its function. Interestingly, this crucial phosphorylation can be partially mimicked by a T>D substitution and restore the primary defect of cdka;1 mutants. Although this variant displayed a dramatically reduced kinase activity with a compromised ability to bind substrates, homozygous mutant plants were recovered. The partially rescued plants, however, displayed various developmental abnormalities: even though flowers were formed, they were completely sterile as a result of the failure of the meiotic program, indicating that different requirements for CDKA;1 function are needed during the course of plant development. In BIFC assays, different mutant variants of CDKA;1 presented a differential substrate binding capability.

Our data shows that the molecular mechanistics of CDK regulation is conserved between yeast, animals, and plants. However, the regulatory circuits controlling CDK activity appear to be strikingly different.

P-131

Functional analysis of AtVamp727 in *Arabidopsis thaliana*. Kazuo Ebine¹, Yusuke Okatani¹, Tatsuaki Goh¹, Emi Ito¹, Tomohiro Uemura¹, Akihiko Nakano^{1,2}, Takashi Ueda¹. ¹University of Tokyo, Tokyo, Japan, ²RIKEN, Wako, Saitama, Japan

While it is getting a consensus for plant scientists that the endocytic pathway plays very important roles in various plant functions of higher order, the molecular mechanism of endocytosis in plant cells still remains almost unknown. We are studying the molecular mechanism of endocytosis and how endocytosis participates in morphogenesis, with a particular focus on SNARE and Rab proteins. We previously examined all SNARE proteins for endosomal localization, and found that only one R-SNARE, AtVamp727, is localized almost exclusively on the Rab5-positive endosomes (Ueda et al., 2004; Uemura et al., 2004). This strongly suggests that AtVamp727 functions in the fusion events of endosomal membranes. To reveal the precise function of this molecule, we carried out genetic and cell biological analysis, to understand the relationship between AtVamp727 and AtVam3/Syp22.

AtVam3/Syp22 is a Qa-SNARE residing on the vacuolar membrane and the prevacuolar compartment/late endosome, and is reported to form a stable *trans*-SNARE complex with AtVti11 and Syp51, Qb- and Qc-SNARE respectively. This *trans*-SNARE complex should also contain another component, R-SNARE, which is not identified to date. The *atvam3/syp22* mutant shows a wide spectrum of phenotypes including wavy leaves, semi-dwarfism, excessive differentiation of idioblasts and late flowering. These phenotypes should be caused by the defect in the endocytic pathway, because *atvam3/syp22* mutation shows strong genetic interactions with *rab5* mutations. We found that the AtVAM3/SYP22 also shows interesting genetic interactions with AtVAMP727, suggesting that these two gene products function cooperatively. Subcellular localizations of these SNARE proteins in the *Arabidopsis* plant also support this idea. In this meeting, we will discuss the function of the possible SNARE complex consisting of AtVamp727 and AtVam3/Syp22 in the vacuolar biogenesis and plant morphogenesis.

P-132

Expression profiles and intracellular dynamics of SYP1s; plasma-membrane localized Qa-SNARE family in "Arabidopsis". Kazuhiko Enami¹, Tomohiro Uemura², Masahiko Sato¹. ¹Kyoto Prefectural University, Kyoto, Japan, ²The University of Tokyo, Tokyo, Japan

Membrane traffic from/to PM is highly organized process within the cells, since plant cells need to respond rapidly to variety of environmental changes, including various stresses, phytohormones and fungal attacks. The numbers of SNARE (Soluble NSF Attachment protein REceptor) genes in "Arabidopsis" genome are much greater than that in any other model organisms. We have previously showed that many SNARE molecules are localized predominantly on the plasma membrane (PM) than the other intracellular compartments in the "Arabidopsis" cell.

To elucidate physiological function of each PM-localized SNARE, we analyzed spatio-temporal expression profiling of 9 PM-resident Qa-SNAREs in "Arabidopsis" to generate transgenic plants expressing GFP-fused Qa-SNAREs under control of their authentic poromotors. The microscopic observations using transgenic plant lines depicted the differences in the expression patterns of 9 PMQa-SNAREs. Developing root section, in particular, showed elaborated regulation of GFP-fused transgenes. One SNARE molecule out of nine expressed constitutively throughout development, whereas expression of other SNAREs limited in the specific cell layer. Furthermore, SYP123 was exclusively expressed in the trichoblasts with elongating root hairs. Deletion of SYP123 expression caused an elongation defect in the root hairs.

These results suggest that SYP123 functions in the elongation step of root hair, not in the bulging phase, and that, furthermore, this Qa-SNARE may control the orientation of polarized vesicular transport by its precise localization on PM of root hair region.

P-133

AtMinE1 is indispensable for chloroplast division in *Arabidopsis* and negatively places the FtsZ ring at the midchloroplast via a balance between the activities of AtMinE1 and ARC11/AtMinD1. Makoto Fujiwara^{1,2}, Haruki Hashimoto², Tomoko Abe¹, Shigeo Yoshida¹, Naoki Sato², Ryuichi Itoh³. ¹RIKEN, Wako, Japan, ²University of Tokyo, Tokyo, Japan, ³University of the Ryukyus, Nishihara, Okinawa, Japan

Chloroplast division comprises a coordinated and irreversible sequence of events that facilitate symmetric binary fission and that involve prokaryotic-like stromal division factors such as tubulin-like GTPase FtsZ and the division-site regulator MinD. In *Arabidopsis*, a nuclear encoded prokaryotic *MinE* homologue, *AtMinE1*, has been isolated and characterized in a limited series of leaf tissues. However, the relationship between *AtMinE1* expression and chloroplast phenotype remains unclear. In our current study using comprehensive microscopic characterizations, we demonstrate that an *AtMinE1* gene-knockout results in a severe inhibition of chloroplast division, whereas its overexpression causes varying degrees of heterogeneity as well as the division-inhibition of chloroplasts per cell. In *AtMinE1* sense plants, dividing chloroplasts possess either single or multiple FtsZ rings located at random intervals and showing constriction depth, mainly along the chloroplast-polarity axis. Quantitative examinations also revealed a preference for symmetric division among the binary-dividing chloroplasts, suggesting that a sole potential division site exists in chloroplasts. The *AtMinE1* sense plants also displayed an equivalent chloroplast phenotypes to *arc11*, a loss-of-function mutant of *AtMinD1* which forms replicating mini-chloroplasts. Furthermore, chloroplast expansion during leaf cell development was unexpectedly found to have an ability to inhibit chloroplast division in both *AtMinE1* sense and *arc11* plants. Our data thus demonstrate that the chloroplast division site placement involves a balance between the opposing activities of *AtMinE1* and *AtMinD1*, which acts to prevent FtsZ ring formation anywhere outside of the midchloroplast. In addition, the imbalance caused by an *AtMinE1*-dominance causes multiple, non-synchronous division events at the single chloroplast level, as well as division-inhibition mediated by chloroplast expansion.

P-134

An *Arabidopsis* chloroplast-targeting MurE homolog has an essential role in chloroplast development. Marlon Garcia¹, Fumiyo Miyouga², Katsuaki Takechi¹, Hiroshi Sato³, Kazuma Nabeshima³, Noriko Nagata⁴, Susumu Takio⁵, Kazuo Shinozaki², Hiroyoshi Takano¹. ¹Graduate School of Science and Technology Kumamoto University Kumamoto 860-8555, JAPAN, ²RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan, ³Faculty of Science, Kumamoto University, Kumamoto 860-8555, Japan, ⁴Faculty of Science, Women's University, Mejirodai, Bunkyo-ku, Tokyo 112-8681, Japan, ⁵Center for Marine Environment Studies, Kumamoto University, Kumamoto 860-8555, Japan

Enzyme encoded by bacterial *MurE* gene catalyzes the ATP-dependent formation of UDP-N-acetylMuramic acid-tripeptide in bacterial peptidoglycan biosynthesis. Genome of *Arabidopsis thaliana* contains one homologous gene of bacterial *MurE* (*AtMurE*). We identified one T-DNA-tagged and three Ds-tagged mutant alleles of the *AtMurE* gene in *A. thaliana*. All four alleles gave the same pale-green phenotype. Generated antisense mutants for the *AtMurE* gene in *A. thaliana* also showed the pale-green phenotype as predicted. These results suggest that *AtMurE* is related with chloroplast biogenesis. In the mutant cells, development of thylakoid membranes and dispersion of plastid nucleoids, those which are normal developmental events of chloroplasts were inhibited. Under the normal conditions, in mature plants, amount of *AtMurE* transcripts was abundant in leaves and flowers, while no transcripts were detectable in roots and stems. Corroborating computer predictions, analysis of the GFP fusion proteins with the N terminus and full-length of *AtMurE* suggests its plastid localization. To analyze functional relationship among *MurE* genes of cyanobacteria, moss and higher plant, complementation experiments for moss *Physcomitrella patens* (Pp) *MurE* knockout line with a small number of macrochloroplasts phenotype were carried out. While *Anabaena* *MurE* fused with N terminal region of Pp*MurE* could complement the macrochloroplasts phenotype in *P. patens*, transformation of *AtMurE* gene could not show recovery of phenotype. This failure may be because of *AtMurE* functional divergence to bacterial and moss *MurE*.

P-135

Molecular characterization of a vesicular transport system inside the chloroplast. Christel Garcia¹, Henrik Aronsson¹. ¹Department of Plant and Environmental Sciences, Goteborg University, Goteborg, Sweden

Thylakoid biogenesis and maintenance require the transfer of many components including galactolipids and carotenoids from the inner envelope where they originate to the thylakoid membrane. But the mechanism by which the thylakoid membrane is formed is still not well understood. A chloroplast localized vesicular transport system similar to the membrane transport between the endoplasmic reticulum and the Golgi apparatus has been proposed to mediate this transfer. Ultrastructural observations have shown the accumulation of vesicles in the aqueous stroma of the chloroplast at low temperatures (Morré et al., 1991, *Plant Physiol* 97: 1558-1564) and biochemical studies have demonstrated that the galactolipid transport to the thylakoid is strongly inhibited at the same temperatures (Andersson et al., 2001, *Plant Physiol* 127: 184-193). The vesicles are also affected by inhibitors of the vesicular trafficking in the secretory pathway (Westphal et al., 2001, *FEBS Lett* 506:257-261).

A recent study, using the *Arabidopsis* genome and web-based localization prediction tools, identified putative chloroplast localized proteins with a high sequence similarity to components of the cytosolic vesicular trafficking (Andersson and Sandelius, 2004, *BMC Genomics* 5:40). Experimental data must now be obtained to define if these proteins are involved in a chloroplast vesicular transport system.

In this purpose, we have first determined the subcellular localization of these proteins using GFP fusion constructs and some of them were detected in the chloroplast. Available *Arabidopsis* T-DNA insertion lines are currently being analyzed and abnormal phenotypes in accordance with a possible role of the affected proteins in chloroplast biogenesis have already been observed. Progress on different aspects of the project will be presented.

P-136

Variation and change in the cell wall glycome of *Arabidopsis thaliana* during growth, development, and differentiation. Michael G. Hahn¹, Glenn Freshour¹, Anathea Albert¹, David Baldwin¹, Ruth Davis¹, Malcolm A. O'Neill¹, Sivakumar Pattathil¹, Sami Tuomiavaara¹, William S. York¹. ¹University of Georgia, Athens, GA, USA

The cell walls of plants play a prominent role in determining the structure and shape of individual cells, and ultimately the morphology of the plant. In addition, cell wall components play active roles in plant development and responses to the environment. Chemical studies have given an overall picture of the structure and organization of the major wall polymers. However, these analyses do not give complete information about wall structure and dynamics at the cellular and subcellular levels. Antibodies are specific and sensitive tools to define the composition of cell walls at the cellular level and to monitor changes in those walls as a function of plant growth and development. An expanded set of over 100 monoclonal antibodies is now available that bind to diverse carbohydrate epitopes residing on all major cell wall polysaccharides in plants, including hemicelluloses, pectins, and arabinogalactans. We have used antibodies generated in our laboratory (CCRC series), and those generated by others (MAC, JIM and LM series), for immunofluorescent and immunogold electron microscopic studies in wild-type *Arabidopsis thaliana*. Tissue sections examined were taken from leaves and flowers, and from different points along roots and stems. The labeling patterns observed show a high degree of diversity in cell- and tissue-specific localizations of carbohydrate structures in cell walls and organelles. In addition, complex patterns of appearance and disappearance of glyco structures in the walls of specific cell types were observed along developmental gradients in the cells and tissues examined. These results provide new insights into the diversity and plasticity of the glycome of plant cell walls. Furthermore, the observed localization patterns serve as a baseline for biochemical and mutagenic approaches toward understanding the functional roles of plant cell wall carbohydrate components in the biology of plant cells. [Supported by grants from DOE (DE-FG02-96ER20220 and DE-FG02-93ER20097) and NSF (DBI-0421683 and RCN-0090281).]

P-137

Functional characterization of the MAP65 family of microtubules associated proteins. Marie-Theres Hauser¹, Roland Baumgartner¹, Vera Vordermaier¹. ¹ BOKU-- University of Natural Resources and Applied Life Sciences Vienna

The phylogenetically conserved family of MAP65s are non-motor microtubule-associated proteins that stabilize microtubules (MT) and regulate their dynamics. Lack of MAP65s causes cell division defects at the anaphase spindle, the central spindle midzone or the phragmoplast in yeast, *C. elegans*, *Drosophila*, human cell lines and plants, respectively. Their MT-binding and -bundling activity has been demonstrated for yeast (*Ase1*), plant (MAP65) and human (PRC1, protein regulating cytokinesis1) homologs. At the N-terminus, MAP65s have a dimerization domain and at the C-terminus a conserved MT-binding domain (Smertenko et al., 2004). Phosphorylation of PRC1/MAP65 at CDK (cyclin dependent kinase) sites of the human homolog and CDK/MAPK (mitogen-activated protein kinase) sites of the tobacco homolog appears to be important for suppressing MT-bundling activity and progression through cytokinesis.

The *Arabidopsis* genome codes for nine MAP65 proteins. We have previously shown that *ple/map65-3* mutants form improper phragmoplasts and exhibit strong cytokinesis defects in roots (Mller et al. 2004). Here we present the results of a reverse genetic approach to determine the function of the other MAP65 members. Since most of the MAP65s are co-expressed during development we hypothesized that they can form homo- and heteromers. Thus, the BiFC (biomolecular fluorescence complementation) system was exploited in transient protoplasts to determine the subcellular localization of homo- and heteromerization and its effect on MT stabilization. The genetic analyses of single mutants show that most of the MAP65 are dispensable for normal development suggesting that the loss of one MAP65 can be substituted by its paralogs. To determine which MAP65s are functionally equivalent and able to replace each other, double mutant analyses have been initiated. The results of the genetic and cell biological analyses will be presented and discussed.

Smertenko et al. (2004) *Plant Cell*, 16, 2035-2047

Mller et al. (2004) *Curr. Biol.* 14, 412-417

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P-138

Interaction and functional characterization of ESCRT-III complex components of *Arabidopsis*. Marie-Theres Hauser¹, Verena Winter¹, Nicole Schlager¹. ¹ BOKU-University of Natural Resources and Applied Life Sciences Vienna

The ESCRT (endosomal sorting complex required for transport) machinery consists of three hetero-oligomeric protein complexes involved in sorting of monoubiquitylated membrane proteins into late endosomal compartments. The ESCRT machinery has been extensively studied in yeast and animal cells and bioinformatic analyses indicate that the components are also present in plants (Winter and Hauser, 2006). Here we present *in vivo* protein-protein interaction analyses using the yeast two hybrid (Y2H) and the bimolecular fluorescence complementation (BiFC) methods. ESCRT-III components interact at vesicular structures in the cytoplasm of protoplasts and partially co-localize with the endosomal marker FM4-64. Moreover, we further show that the previously isolated mutant, *hyade*, which develops root specific cytokinesis defects is a plant specific homolog of an ESCRT-III component. This phenotype is reminiscent to the recently identified *elch* mutant which turned out to be an *Arabidopsis* homolog of the ESCRT-I component *VPS23* (Spitzer et al., 2006). *hyade* mutants stably transformed with a gHYA::GFP construct are complemented and exhibit a wildtype phenotype. gHYA::GFP localizes to similar cytoplasmic structures as the BiFC interactions primarily in the meristematic and cell expansion zone and weakly in the vasculature of mature roots. The link between the ESCRT-machinery and cytokinesis will be discussed.

Winter and Hauser M-T (2006) *Trends Plant Sci* 11, 115-123

Mller et al. , (2002) *Plant Physiol* 13, 312-324

Spitzer et al. , (2006) *Development*. 133, 4679-4689

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P-139

S-acylation: Dynamic control of plant development and signalling by lipid modification of proteins. Piers Hemsley¹, Laura Taylor¹, Claire Grierson¹. ¹ University of Bristol, Bristol, United Kingdom

S-acylation, or palmitoylation, is the only dynamic and reversible lipid modification of proteins. It controls membrane / lipid raft partitioning and sorting of proteins but also affects signalling complex formation, regulation of signal transduction in a similar manner to phosphorylation, affects protein stability and lifespan via ubiquitin mediated proteolysis, enables viral capsule release and acts as a gating mechanism for transmembrane channel proteins. S-acylated proteins in plants include type I and II Rop small GTPases, heterotrimeric G protein gamma subunits, ARA6 Rab GTPase, RIN4 involved in hypersensitive pathogen responses, calcium responsive CDPK/CPKs, CBLS and the alpha-subunits of tubulin. This indicates the varied roles that S-acylation plays in protein function and regulation as well as the range of proteins modified, yet comparatively little is known about it. Prediction of S-acylated proteins is hindered by the lack of a consensus motif for S-acylation and until recently the enzymes responsible were unknown. Recent advances in the field have led to the identification of a family of S-acyl transferases.

S-acyl transferases are represented by 23 different genes in plants, each with differing spatial and temporal expression patterns. Here we present phenotypic data for mutants in S-acyl transferases, with a detailed analysis of those involved in different aspects of root hair development. Plants defective in an S-acyl transferase generally show distinct or partially overlapping phenotypes indicating that different subsets of targets exist for each S-acyl transferase. The first S-acyl transferase to be identified in plants, TIP1 of *Arabidopsis*, will be discussed in detail. Data on whole plant mutant phenotypes, biochemical function, expression profiles, sub-cellular localisation and hormone response have been combined to identify and test potential substrates for TIP1. We also present data obtained from proteomic analysis of the "Tip1-⁻" S-acyl transferase mutants identifying substrates for TIP1.

P-140

Dissecting Plasma Membrane Localization, Vesicular Trafficking and Polar Growth Via Chemical Genomics. Glenn R Hicks¹, N Chary Samboju¹, Stephanie Robert¹, Zhenbiao Yang¹, Natasha V Raikhel^{1,2}. ¹Center for Plant Cell Biology & Dept of Botany and Plant Sciences, University of California, Riverside, CA, USA

The trafficking of proteins to and from the plasma membrane (PM) is an essential process underlying polarized cell growth. The mechanisms and components involved in these processes are poorly understood especially in plants. We are using chemical genomics (the use of chemicals to modify protein function) to identify chemicals affecting protein targeting to the PM. To identify these reagents, a novel pollen-based high throughput chemical screen was developed. The pollen tube is a polarized cell whose growth is strongly dependent upon targeting to the apical PM; thus, it is an excellent model for polar growth. However, genetic approaches would be difficult due to lethality. A 384-well microtiter plate format is used to screen for chemicals that block tobacco pollen germination in vitro. Among the inhibitors are effectors of polar PM targeting. These are identified via a secondary screen using tobacco pollen expressing GFP fused to ROP1-Interacting Partner 1 (GFP-RIP1), a marker displaying focused tip localization. The screen is highly automated using the Atto Pathway confocal microscope permitting a rate of thousands of compounds per week. An initial screen of more than 20,000 compounds demonstrated clearly that the desired classes of compounds can be identified. Among the compounds characterized are those that cause depolarized (isodiamteric) growth and mis-localization of GFP-RIP1 which normally predicts the site of polar tip growth. Recently, several chemicals were examined with success against a panel of endomembrane compartment markers expressed in roots of Arabidopsis seedlings. Interestingly, we have found a chemical that appears to affect late endocytosis of several polar markers (PIN2-GFP, AUX1-YFP) specifically. This indicates that the compound may affect specific pathways and that the target(s) is probably expressed in gametes and sporophytes. Overall, we will use these bioactive chemicals directly to study endomembrane trafficking and as reagents to identify gene networks involved endomembrane trafficking and the control of polar growth.

P-141

Understanding Arabidopsis peroxisomal proteomes. Jianping Hu¹, Andreas Weber¹, Laura Olsen², Pingfang Yang¹, Kalpana Shrestha¹, Sigrun Reumann¹, Sheng Quan¹. ¹Michigan State University, East Lansing, USA, ²University of Michigan, Ann Arbor, USA

Plants contain a large number of structurally similar but functionally diverse peroxisomes that are crucial to various physiological and metabolic pathways in development and during stress responses. However, the protein composition and metabolic and regulatory networks associated with peroxisomes is still poorly understood. Using a combinatorial approach including proteomics, bioinformatics, cell biology, and reverse genetics, we are cataloging the membrane and matrix proteomes of three major peroxisomal variants: leaf peroxisomes from green leaves, glyoxysomes from germinating seedlings, and gerontosomes from senescent tissue. To date we have used mass spectrometry-based shotgun proteomics to identify novel peroxisomal membrane and matrix proteins from mature, wounded, and senescent leaves. Several dozen novel candidate peroxisomal proteins have been identified by this approach. A subset of the novel proteins identified by both proteomic and bioinformatic analyses were subjected to subcellular localization study using fluorescence microscopy of GFP-fusion proteins; a number of them have been confirmed to be peroxisomal. Based on results from a series of physiological and biochemical assays conducted with sequence-indexed T-DNA insertion mutants of some of these genes, hypotheses of novel peroxisomal functions and pathways have been generated. In addition to providing the scientific community with a much-needed comprehensive inventory of plant peroxisomal proteins, we will also disseminate a set of tagged reporter constructs for follow-up studies. This work is funded by the NSF Arabidopsis 2010 program.

P-142

Isolation and characterization of a KN1 binding protein. Lijun Hwang¹, Juyeon Moon¹, Xiaoming Xu¹, Xiong-Yan Chen¹, Jae-Yean Kim^{1,2}. ¹Division of Applied Life Science, PMBBRC/EB-NCRC, Gyeongsang National University, Jinju 660-701, Korea

he intercellular trafficking of the macromolecules via plasmodesmata (PD) plays important roles for developmental and physiological process in plant. PD are intercellular organelles consisted of specialized membrane channels between neighboring cells. However, little is known about PD-associated and regulatory components involved in cell-to-cell trafficking of macromolecules. KNOTTED1 (KN1), a homeobox protein to function in SAM development in maize, is a well studied non-cell-autonomous protein (NCAP) that can traffic cell-to-cell through PD. To screen some trafficking pathway-proteins involved in protein trafficking, we used KN1 protein as a bait for yeast two hybrid screening. We found a protein which can equally interact with KN1 and KNAT1. KBP1 shows very weak homology with a chick caldesmon (24% similarity in whole protein) and includes myosin tail repeats. Blast search for its homologs disclosed only an *Arabidopsis* protein which has 55.2% identity and 64.4% similarity with the KBP1. Deletion assay revealed interacting domains in both KNAT1 and KBP1; N-terminal region of KNAT1 and C-terminal region corresponding exon 26 of KBP1. In silico analysis suggested that KBP1 and its homolog (KBP2) may be plant specific cytoskeletal binding proteins that may play either a role in intercellular protein trafficking of KNOX or a regulatory function of KNOX-related transcription. Here, we present preliminary results obtained and on going research strategy to reveal the function of these proteins.

P-143

Roles of Membrane Traffic in Xylem Differentiation. Jun Ito¹, Tomohiro Uemura², Minoru Kubo³, Makiko Udagawa⁴, Taku Demura⁴, Hiroo Fukuda², Takashi Ueda², Akihiko Nakano^{1,2}. ¹RIKEN Discovery Research Institute, Wako, Japan, ²Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo, Japan, ³Japan Science and Technology Agency, ERATO, Okazaki, Japan, ⁴Plant Science Center, RIKEN, Yokohama, Japan

Establishment and maintenance of the polarity of vascular cells are essential for continuity of the vascular system, where membrane traffic should play crucial roles. Membrane traffic is also expected to participate in critical steps of vasculature development such as tonoplast breakdown during xylem cell differentiation. To understand the precise roles of membrane traffic in vasculature formation, we are studying the functions of Rab GTPases, key regulators in membrane traffic, especially with a focus on xylem cell differentiation. Our expression analysis of all 57 RAB genes in *Arabidopsis* during xylem cell differentiation using a genechip and promoter:GUS transgenic plants revealed that 1) several Rab GTPases, most of which belong to the endocytic Rab GTPases (Rab5, Rab7, Rab11 and Rab18 subgroups), are expressed during xylem cell differentiation specifically, 2) some members of the Rab11 group, which is known to regulate cell polarity in the mammalian system, are expressed at different stages of xylem differentiation, and 3) some vacuole-localizing Rab GTPases (AtRab75, AtRab77, AtRab18-2) are strongly expressed just prior to the drastic change of tonoplast integrity in differentiating tracheary elements. These results strongly suggest that several endocytic Rab GTPases are involved in the xylem formation. We then carried out genetic analysis of *Arabidopsis* mutants, which may have a defect in the endocytic pathway. Some rab7 multiple mutants showed excessive vein formation in their cotyledons and fragmentation of protein storage vacuoles in their dry seeds. The results of the in-depth analysis of these mutants will be presented.

P-144

Genetic analysis of the protein import machinery of Arabidopsis plastids. Paul Jarvis¹. ¹University of Leicester, UK

While plastids retain a fully-functional genetic system, the plastid genome encodes less than 10% of the proteins required to build a fully-functional organelle. The majority of plastidic proteins are encoded in the nucleus and translated on free cytosolic ribosomes. They are synthesized in precursor form, each one bearing an amino-terminal targeting signal, or transit peptide. The transit peptide directs the protein through a post-translational targeting pathway, and is cleaved upon arrival inside the plastid. This targeting or import process is mediated by the coordinate action of two proteinaceous import machines, one in each of the envelope membranes. The import machinery of the outer envelope membrane is called the TOC complex, and that in the inner membrane is called the TIC complex. Over the last decade, several components of the TOC and TIC complexes have been identified using biochemical approaches and isolated pea chloroplasts. Interestingly, many of these components (particularly receptor components of the TOC complex) have been found to have multiple homologues in *Arabidopsis*. We have used genetic approaches to dissect the functional significance of these different TOC protein isoforms. Our results suggest that the different isoforms operate in different import pathways with distinct precursor recognition specificities; i.e., different import pathways exist for different precursor protein classes. The existence of such substrate-specific import pathways might play a role in the differentiation of different plastid types, and act to prevent deleterious competition effects between abundant and non-abundant precursors.

P-146

An Allele-Specific Suppressor of a Defective Brassinosteroid Receptor. Hua Jin¹, Zhenyan Yan¹, Kyoung-Hee Nam¹, Jianming Li¹. ¹University of Michigan, Ann Arbor, MI, USA

BRI1 is a leucine-rich-repeat receptor-like kinase that functions as a cell surface receptor for brassinosteroids (BRs) and its mutations often lead to many developmental defects including dwarfism, delayed flowering, and reduced male fertility. The binding of active BRs to BRI1 triggers a phosphorylation-mediated signaling cascade that regulates the amount, subcellular localization, and/or DNA-binding activities of several plant-specific transcriptional factors. To identify additional components of the BR signaling pathway, we conducted a genetic screen for extragenic suppressors of the *bri1-9* mutation that changes Ser662 to Phe in the newly defined BR-binding domain and identified many wild-type looking plants. One of them, named as *ebs1* for EMS-mutagenized *bri1* suppressor 1, exhibits near wild-type morphology throughout its life cycle, including a large rosette, a long hypocotyl in the dark, and long inflorescence stems at maturity. However, the *ebs1* mutation fails to suppress the phenotypes of *bin2* and *det2* mutants defective in the intracellular signaling and biosynthesis of BRs, respectively, indicating that *ebs1* does not cause a constitutive activation of BR signaling. We suspected that the *ebs1* mutation might directly affect BR perception at the cell surface. Indeed, molecular and physiological assays showed that the *ebs1* mutation restored BR sensitivity to the *bri1-9* mutants, while the double mutant analysis of *ebs1* and several other known *bri1* alleles revealed an allele-specific interaction between *ebs1* and *bri1-9*, implying that *ebs1* does not activates an alternative BR receptor but directly acts on *bri1-9* to restore its receptor function. The molecular identity of the EBS1 gene and the underlying biochemical mechanism by which the *ebs1* mutation restores BR sensitivity to the defective BR receptor will be discussed at the meeting.

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A role of a small G protein Rop2 in ABA-induced stomatal closing movement. Byeong Wook Jeon¹, Jae-Ung Hwang², Miyoung Lee¹, Jiyoung Park¹, Youngsook Lee¹. ¹Division of Molecular Life Sciences, POSTECH, Pohang, 790-784, Korea, ²Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, CA, 92521-0124, USA,

ABA induces stomatal closing, which results in reduction of water loss from plants. We previously suggested that ROP2 is important in guard cell signaling, based on different localization of active and inactive ROP2-fused to green fluorescence protein (GFP); CA-ROP2-GFP is localized predominantly at the plasma membrane whereas DN-ROP2-GFP is at the cytoplasm. Here, we investigated the roles of Rop2 in ABA-induced stomatal closing movement. ABA induced translocation of Rop2 from the plasma membrane to cytoplasm in guard cells of *Vicia faba* expressing ROP2::GFP; the green fluorescence intensity at the plasma membrane decreased upon treatment with ABA. This result suggests that ABA induces inactivation of Rop2. Stomata bordered by guard cells transformed with CA-rop2 closed less than their neighbors bordered by non-transformed guard cells upon ABA treatment. In contrast, stomata bordered by guard cells transformed with DN-rop2 closed more than their controls in response to the same treatment. Mutant *rop2* *Arabidopsis thaliana* guard cells showed phenotypes similar to these transformed *V. faba* guard cells. In addition, CA-rop2 mutant *Arabidopsis* plants were more sensitive than WT and DN-rop2 mutants to drought stress. Thus, we suggest that ABA-induced inactivation of Rop2 contributes positively to stomatal closing and water conservation during drought stress.

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GAP-limited cycling in *Arabidopsis* G protein-coupled sugar signaling. Christopher Johnston¹, Philip Taylor¹, Yajun Gao², Adam Kimple¹, Jin-Gui Chen², David Siderovski¹, Alan Jones¹, Francis Willard¹. ¹University of North Carolina, ²University of British Columbia

Heterotrimeric G-protein signaling is important for cell-proliferative and glucose-sensing pathways in *Arabidopsis*. AtRGS1 is a seven-transmembrane, RGS-domain containing protein that is a putative membrane receptor for D-glucose. Using FRET, we show that D-glucose alters the interaction between AtGPA1 and AtRGS1 in vivo. AtGPA1 is a unique G alpha subunit that is constitutively GTP bound given high spontaneous nucleotide exchange coupled with slow GTP hydrolysis. Analysis of a point mutation in AtRGS1 that abrogates GAP activity demonstrates that regulation of AtGPA1 GTP hydrolysis mediates sugar signal transduction during *Arabidopsis* development, in contrast to animals where nucleotide exchange is the limiting step.

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Dissection of proapoptotic protein Bax-induced plant cell death. Maki Kawai-Yamada¹, Hirofumi Uchimiya¹. ¹The University of Tokyo, Tokyo, Japan

Reactive oxygen species (ROS) are generated under various biotic and abiotic stresses, and trigger cell death. Mammalian pro-apoptotic gene Bax is known to cause cell death when expressed in yeast and plants, even if there is no homologous gene in these organisms. Bax is known to translocate to mitochondria during the initiation of apoptosis in animal cells. To determine the cellular localization of Bax in plant cells, we constructed Bax-GFP plasmid and introduced into tobacco BY-2 suspension cells by particle bombardment. Under confocal laser scanning microscopy, the fluorescent signal of Bax-GFP was observed in punctuated structures that co-localized with the fluorescent signals of Mito-RFP. Furthermore, ROS generation was observed in transgenic Arabidopsis plants expressing mouse Bax, suggesting that Bax may cause oxidative stress-induced plant cell death. Furthermore, we examined transgenic plants expressing both Bax and organelle-targeted green fluorescent protein (GFP) to analyze the cellular events that occur during Bax-induced plant cell death. The results indicated that Bax induced temporal and spatial cell death events at the organelle level. The mitochondria changed morphologically from being bacilli-shaped to being round. The chloroplasts lost membrane function and their contents leaked out, followed by the disruption of the vacuole. Furthermore, ROS production was involved in triggering cell death. To compare Bax-induced cell death and other ROS-mediated plant cell death, Arabidopsis leaves expressing mitochondrial-targeted GFP were treated with ROS-inducing chemicals, such as hydrogen peroxide, paraquat and menadione. After treatment, mitochondria showed morphological changes from a bacillus-like to a round shape. The size of mitochondria decreased by half compared with controls. Such cellular events may cause energy depletion, and resulted in cell death. These results suggest that the Bax-induced plants cell death is a potentially useful heterologous system for studying the regulation of oxidative stress-induced cell death.

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The H subunit of eukaryotic translation initiation factor 3 contributes to the efficient translation of mRNAs with upstream open reading frames. Byung-Hoon Kim¹, Bijoyita Roy¹, Xue Cai¹, Justin N Vaughn¹, Albrecht G von Arnim¹. ¹University of Tennessee, Knoxville, TN, USA

Upstream open reading frames (uORFs) in 5' leader sequences often inhibit translation initiation and can play critical roles in signal-dependent regulation of translation. Although over 30% of mRNAs harbor at least one uAUG in Arabidopsis, factors required to overcome or to regulate them are elusive. Here, we demonstrate that the H subunit of eukaryotic translation initiation factor 3 (eIF3) contributes to the efficient translation of mRNAs with uORFs. eIF3h is considered one of seven non-core subunits with potential regulatory roles in the multifunctional eIF3 complex. The eif3h mutant exhibits a pleiotropic phenotype including a hypersensitive sugar response. Transient and stable transgene expression of luciferase reporter constructs fused to different 5' leader sequences indicated that translation of certain genes, such as the sugar-regulated mRNA AtbZip11, was compromised in the eif3h mutant background. Microarray comparisons of polysome loading in wild-type and eif3h mutant seedlings revealed that eIF3h generally helps to maintain efficient polysome loading of mRNA species harboring multiple uORFs, independent of the mRNA transcript level. Functional classification by MapMan further showed that important regulatory gene classes that are enriched in uAUGs, such as transcriptional regulators and protein modifying enzymes, were translationally compromised in the eif3h mutant while other functional subgroups including ribosomal proteins maintained their polysome loading state. In addition, eIF3h boosted the polysome loading of mRNAs with long coding sequences. Taken together, eIF3h functions in translation initiation by overcoming the repressive effect of uORFs, which is particularly pronounced on mRNAs with a long main ORF.

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Molecular Analysis of a Family of *Arabidopsis* Genes Related to Galacturonosyltransferases. yingzhen kong¹, Michael Hahn^{1,2}. ¹Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602-4712, USA,². Department of Plant Biology, University of Georgia, Athens, GA 30602

Pectin is a major component of the cell wall polysaccharides of all land plants and is made up of a range of galacturonic acid (GalA)-containing polysaccharides. Pectic polysaccharides play important roles in plant cell growth and development, and responses to pathogens. A complete understanding of the role(s) of pectic polysaccharides in plant biology will require a complete understanding of their biosynthesis. We are studying a gene family in *Arabidopsis thaliana* that was identified bioinformatically as being closely related to a group of 15 genes (GAUT1-15), one of which (GAUT1) has been shown to encode a functional galacturonosyltransferase. The proteins encoded by the ten GATL (GAlecturonosylTransferase-Like) genes are smaller than those encoded by the GAUT genes and appear to lack a transmembrane domain, but do contain a signal sequence that suggests the targeting of GATL proteins into the endomembrane/secretory pathways. Expression studies (RT-PCR) demonstrate that all ten GATL genes are transcribed, albeit to varying degrees, in *Arabidopsis* tissues. Promoter : -b-glucuronidase studies provided high resolution information about cell-specific locations of GATL gene expression. Nine of the ten GATL genes are almost expressed in all major plant organs. However, GATL4 expression appears confined to pollen grains. Most of the GATL genes are expressed strongly in xylem cells in both stem and root sections. T-DNA insertion mutants in several GATL genes display reduced GalA content in total cell wall material and two show abnormalities in seed coat mucilage, which is composed primarily of pectin. The sub-cellular localization of several GATL proteins has been investigated by using green fluorescent protein (GFP) tagging, and the results provide evidence supporting Golgi localization for these proteins. These data suggest that GATL genes are involved in the biosynthesis of pectin by acting as galacturonosyltransferase. [This work is supported by grants from the NSF Plant Genome Program (DBI-0421683) and DOE (DE-FG02-96ER20220).]

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Dual localization of the Whirly 1 protein in plastids and in the nucleus. Karin Krupinska¹, Ying Miao¹, Evelyn Grabowski¹, Isabell Kilbienski¹, Maria Mulisch². ¹ Institute of Botany, University of Kiel, Germany, ²Central Microscopy, University of Kiel, Germany

The Whirly1 protein of *Arabidopsis thaliana* (AtWHY1, gene locus At1g14410) belongs to a small family of single stranded DNA binding proteins. It has been shown to bind to a specific DNA element called ERE (elicitor response element) which is present in the promoter of the PR10a gene of potato (Desveaux et al. 2000) as well as to the telomeric heptanucleotide TTTAGGG (Yoo et al. 2007). A mutant with a T-DNA insertion in the Atwhy1 gene was shown to possess longer telomeres (Yoo et al. 2007) suggesting that AtWHY1 inhibits telomerase activity.

When a GFP fusion protein of the complete AtWHY1 protein of *Arabidopsis thaliana* was analyzed for its subcellular location by transient transformation assays, it was detected exclusively in plastids (Krause et al. 2005). When the mature AtWHY1 protein without the plastid target peptide was fused to GFP, the protein was however located in the nucleus. Immunological analyses with an antibody against the Whirly1 protein of barley revealed that in barley primary foliage leaves the protein was predominantly present in chloroplasts as well as in the nucleus. When the WHY1 protein is located in the nucleus it is part of high molecular weight protein complexes. Bimolecular fluorescence complementation assays and yeast 2-hybrid analysis showed that the AtWhy1 protein might form homooligomers and co-localize in the nucleus when the plastid transit sequence was cleaved off. The high molecular weight complexes are extremely stable and do contain DNA. By DNase treatment the monomeric form of AT-WHY1 may be released from the complex. Immunological analyses indicated that during leaf senescence when chloroplasts were dismantled the proportion of WHY1 located in the nucleus increased.

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P-152

GNOM acts during *Arabidopsis* stomatal development. Jie Le¹, Steffen Vanneste², Tom Beeckman², Fred Sack¹. ¹Department of Botany, University of British Columbia, 6270 University Blvd, Vancouver, BC V6T 1Z4, Canada, ² Department of Plant Systems Biology, VIB, Ghent University, B-9052 Ghent, Belgium

Arabidopsis GNOM/EMB30 is a guanine-nucleotide exchange factor (GEF) for the ADP-ribosylation factor (ARF) family of small GTPases. Mutations in GNOM induce well-studied defects in embryonic divisions, root growth, and vascular patterning. Some of these abnormalities are likely related to the function of GNOM in vesicular trafficking, especially in endosomal recycling of targets such as PIN proteins which are membrane-associated auxin efflux carriers.

The role of GNOM in stomatal development has not been studied, except for brief mention of adjacent stomata in *gnom* alleles (Mayer et al. , 1993, Development). We found a range of stomatal defects in *gnom*, with phenotypic severity following an allelic series. These results suggest that stomatal development requires GNOM-mediated vesicular trafficking and might involve PINs and polar auxin transport as well.

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***Arabidopsis* ABP30.6, a novel actin-bundling protein, is phosphorylated by AtMAPK6 * .** Yuan Li¹, Yongduan Xie¹, Lihui Zhang¹, Guoqin Liu¹, Dongtao Ren¹. ¹China Agricultural University, College of Biological Sciences, Beijing 100094, China

The actin cytoskeleton is a highly organized and dynamic structure present in all eukaryotic cells where it plays important role in many processes, including intracellular transport, cell growth, division and morphogenesis, or responses to environmental stimuli. In cells, the formation of higher-order structures, such as bundles and cables, is regulated by specific families of actin-binding proteins (ABPs). However, only a few ABPs with actin-bundling activity were found in plant cells. Here we reported the identification and characterization of ABP30.6, a novel actin-bundling protein in *Arabidopsis*. Low-speed and high-speed cosedimentation assays shown that the recombinant ABP30.6 was binding to F-actin with high affinity ($K_d = 0.41 \mu M$) and inducing actin-bundles formation. Using truncated proteins, the N-terminus of ABP30.6 protein, was shown to be required for actin bundles formation. The effect of ABP30.6 on depolymerization of pyrene-labeled F-actin indicated that, ABP30.6 stabilized actin filaments in a concentration-dependent manner. Interestingly, we found that ABP30.6 was strongly phosphorylated by activated mitogen-activated protein kinase, AtMAPK6 in *in vitro* protein phosphorylation assay system. The phosphorylated-ABP30.6 shown a higher actin-bundling activity. Using transient transformation system, ABP30.6 was found to accumulate when co-expressed ABP30.6 with the active mutant of AtMEK5, an upstream MAPK kinase of AtMAPK6. The results suggested that phosphorylation of ABP30.6 may be required for fully activation of its function and ability of stabilization *in vivo*.

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MAIGO2 is involved in exit of seed storage proteins from the ER in "Arabidopsis thaliana". Lixin Li¹, Tomoo Shimada¹, Hidemuki Takahashi¹, Haruko Ueda¹, Yoichiro Fukao², Maki Kondo³, Mikio Nishimura³, Ikuko Hara-Nishimura¹. ¹Graduate School of Science, Kyoto University, Kyoto, Japan, ²Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan, ³Department of Cell Biology, National Institute for Basic Biology, Okazaki, Japan

In higher plants, seed storage proteins are synthesized on the endoplasmic reticulum (ER) as precursors and then are transported to protein storage vacuoles, where they are processed into mature forms. We isolated and investigated *Arabidopsis* mutants ("maigo") that abnormally accumulated the precursors of storage proteins in dry seeds (1). "maigo2" mutant abnormally accumulated precursors of two major storage proteins, 2S albumin and 12S globulin, in dry seeds (2). "mag2" seed cells contained many novel structures with an electron-dense core composed of the precursors of 2S albumin. 12S globulins were localized in the matrix region of the structures together with ER chaperones, BiP and PDI, which were more abundant in "mag2" seeds. MAG2 protein had a RINT-1/TIP20 domain in the C-terminal region. Some MAG2 molecules were peripherally associated with the ER membrane. MAG2 had an ability to bind to two ER-localized t-SNAREs (At Sec20 and At Ufe1). Our findings suggest that MAG2 functions in the transport of storage protein precursors between the ER and the Golgi complex in plants.

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- (2) Li et al. , Plant Cell (2006) Dec;18 (12) : 3535-3547.

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SWA2 encodes a nucleolar protein which is essential for female gametophyte development in *Arabidopsis*. Na Li¹, Li Yuan¹, Jie Liu¹, Weicai Yang¹.¹Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Datun Road, Beijing 100101, China

Female gametogenesis is a fundamental step in sexual plant reproduction. One of the four meiotic products differentiates to form functional megasporangium which undergoes three consecutive rounds of mitotic division to produce an eight-nucleate embryo sac. Subsequently, cytokinesis occurs simultaneously in the coenocytic embryo sac to form a seven-celled female gametophyte. Often the embryo sac development within a single pistil is coordinated and synchronous. Mechanisms controlling these division cycles and the coordinated development are not yet known. Here we report that SWA2 is essential for the progression of gametophytic division cycles in *Arabidopsis thaliana*. In swa2 mutant, the progression of the mitotic division cycles and the synchrony of female gametophyte development were impaired, causing arrest of mutant female gametophyte at two-, four- or eight-nucleate stages. Delayed pollination test showed that some of these mutant ovules were able to develop into functional embryo sacs and could be fertilized. SWA2 encodes a nucleolar protein which can physically interact with a putative *Arabidopsis* NOC2 homologue in yeast two-hybrid assay. SWA2 is expressed ubiquitously throughout the plant, at high level in meristems and gametophytes. These data suggest that SWA2 is essential for the mitotic progression of the female gametophyte.

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The thylakoid membrane protein ABC2/CYO1, a cotyledon-specific chloroplast biogenesis factor of *Arabidopsis thaliana*, has protein disulfide isomerase activity. Hiroshi Shimada¹, Mariko Mochizuki², Kan Ogura¹, Yumiko Shirano³, Daisuke Shibata⁴, Ken-ichiro Takamiya¹.¹Tokyo Institute of Technology, Yokohama, Japan,²Tokyo Institute of Technology, Yokohama, Japan,³Boyce Thompson Institute for Plant Research, N. Y., USA,⁴Kazusa DNA Research Institute, Chiba, Japan

The development of chloroplasts in cotyledons differs in several aspects from that in true leaves, but the cotyledon-specific program of chloroplast biogenesis has not been clarified yet. The abc2/cyo1 mutant in *Arabidopsis thaliana* exhibits albino cotyledons but normal green true leaves. The abc2/cyo1 mutant plants developed abnormal chloroplasts under light but normal etioplasts under dark. Because of its T-DNA tag, we isolated the ABC2/CYO1 gene and verified it was responsible for the albino cotyledon phenotype by complementation. The ABC2/CYO1 protein has a C4-type zinc finger domain similar to that in Dnaj of *E. coli*, and ABC2/CYO1 appears to be the higher plant specific protein and is highly conserved among not only dicotyledonous but also monocotyledonous plants. The ABC2/CYO1 gene was expressed in only young plants under light conditions, and the ABC2/CYO1 protein localizes to the thylakoid membrane in chloroplast. The ABC2/CYO1 mutation did not affect transcription of photosynthetic genes but decreased the amount of the photosynthetic proteins. Recombinant ABC2/CYO1 protein accelerates the reduction of a disulfide pair in insulin and renatures the RNase by oxidizing its dithiol pairs, indicating that the ABC2/CYO1 protein has the protein disulfide isomerase activity. These results suggest that ABC2/CYO1 has a chaperone-like activity that is crucial for rapid assembly of the thylakoid membrane proteins during chloroplast biogenesis in cotyledons.

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AtAzg1 is a high-affinity purine and cytokinin cell importer in *Arabidopsis thaliana*. Veronica G. Maurino¹, Esther Grube¹, Benjamin S. Schumacher², Ondrej Novak³, Miroslav Strnad³, Ulf-Ingo Flügge¹, Marcelo Desimone².¹ Botanisches Institut, Universität zu Köln, Gyrhofstr. 15, D-50931, Köln, Germany,²Zentrum für Molekularbiologie der Pflanzen, Universität Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen, Germany,³Laboratory of Growth Regulators, Institute of Experimental Botany ASCR and Palacky University, Slechtitelu 11, 783 71 Olomouc, Czech Republic

The mechanisms governing the transport of purines and the phytohormone cytokinins in plants are poorly understood. A high-affinity purine transporter of *Aspergillus nidulans* has been recently identified as a member of a novel protein family with members in prokaryotes, fungi and plants. Two orthologous proteins are encoded in the *A. thaliana* genome. In this work, AtAzg1 (*Arabidopsis thaliana* Aza-guanine resistant) was functionally expressed in a yeast mutant deficient in adenine uptake to study transport features. AtAzg1 mediated a H⁺-gradient-dependent high-affinity transport of adenine (KM = 1.62 M) with a broad substrate specificity including adenine, hypoxanthine, guanine, cytokinins and toxic purine analogs. In contrast, other structurally related compounds, such as pyrimidines, nucleosides, caffeine and degradation products of purines (xanthine, uric acid, allantoin) were not transported. Transient expression of AtAzg1-GFP fusion proteins in cultured *A. thaliana* cells and in onion epidermal cells revealed that AtAzg1 is localized to the plasma membrane indicating a function as cell importer. Azg1 knock-out mutants presented a conditional phenotype resistant to purine analogs and toxic concentrations of transzeatin (tZ) and, in contrast to the wild-type, azg1 knock-out seedlings did not accumulate cytokinin conjugates after treatment with tZ. On the contrary, *A. thaliana* overexpressing lines showed hypersensitivity to purine analogs and tZ and presented uptake rates for adenine and tZ several times higher than the wild-type. These results indicate that AtAzg1 functions as a purine and cytokinin importer *in vivo*. Promoter activity analysis showed that AtAzg1 gene expression is modulated by cytokinins suggesting a role for AtAzg1 in metabolism or signaling of cytokinins.

P-156**Microtubule arrays at the cytokinesis-interphase transition**

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Plant microtubule arrays undergo major changes during the cell cycle, but transitions between the arrays are not well defined. During cytokinesis, almost all microtubules are associated with the phragmoplast array. But after cytokinesis microtubules are found primarily in the cell cortex. At the transition from cytokinesis to interphase, existing data suggest that the first interphase microtubules form at the nuclear surface in suspension cells (Yoneda and Hasezawa 2003, Eur J Cell Biol and references therein). But relatively little is known about how the cortical array initiates in cells in tissues.

To observe how microtubules repopulate the cortex during late cytokinesis, we visualized microtubules in living *Arabidopsis* leaf epidermal cells harboring a constitutively expressed alpha-tubulin-GFP construct. The fine-staging of cells late in cytokinesis was facilitated by the predictable polarity of division in this tissue. Our results indicate that microtubule nucleation in the cell cortex plays a major role in the re-establishment of the cortical array at the cytokinesis-G1 transition.

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Sterol function in polar PIN2 auxin efflux carrier positioning during *Arabidopsis* root gravitropism. Shuzhen Men¹, Yohann Boutté¹, Thomas Moritz¹, Markus Grebe¹. ¹Umeå Plant Science Centre, Dept. of Forest Genetics & Plant Physiology, SLU, Umeå, Sweden

Plant membrane sterols are crucial for diverse cellular processes during plant growth and development including cell polarity [1, 2]. The directional growth of plant roots along the gravity vector requires correct membrane sterol composition [2] and is mediated by polar auxin transport which involves the *Arabidopsis* PIN2 auxin efflux carrier [3-6]. Previous studies suggested that polar membrane localisation of the auxin efflux carrier protein PIN1 in root tip cells requires correct sterol biosynthesis [2], and that PIN2 co-traffics with sterols along a common transport route [7]. However, a mechanistic link between sterol and PIN protein in a tropic growth process has not been provided. We have extended our previous studies and functionally analysed the dependence of polar PIN2 protein positioning on sterol composition, employing a combination of pharmacological and new genetic tools for modulation of sterol composition. We provide subcellular localization data for one component of the sterol biosynthesis machinery and employ cell biological approaches to demonstrate that sterol composition in meristematic cells just after cytokinesis is required for establishment of PIN2 polarity. Our results suggest that sterol biosynthesis in dividing cells and post-cytokinetic cells is critical for polar PIN2 partitioning and root gravitropism. We further report more detailed results that post-cytokinetic polarity acquisition of PIN2 requires sterol-dependent endocytosis, providing a mechanism for sterol action on auxin efflux carrier positioning during root gravitropism.

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MIPS, the missing link between cell cycle and cell death
Ping-Hong Meng¹, Sophie Blanchet¹, Cécile Raynaud¹, Catherine Bergounioux¹. ¹CNRS, Université Paris-sud, IBP, France

The Proliferating Cell Nuclear Antigen (PCNA) functions as a sliding clamp for the DNA polymerase, and is thus a key actor of DNA replication. It is also involved in DNA repair, maintenance of heterochromatic regions throughout replication, cell cycle regulation and Programmed Cell Death. We have previously identified two *Arabidopsis* SET-domain proteins that interact with PCNA: ATXR5 and ATXR6. ATXR5 possesses a double localisation in plastids and in nucleus and its subcellular localization is regulated via the presence/absence of its chloroplast-targeting sequence. ATXR6 is solely nuclear, up-regulated during S-phase, a direct target of the AtE2Fb transcription factor and is required for S-phase progression. The over-expression of either ATXR5 or ATXR6 causes male sterility due to the degeneration of defined cell types. A second round of two hybrid screen using ATXR5 as a bait has allowed the identification of ATMIPS as one of the ATXR5 partners. MIPS myo-inositol phosphate synthase and IMP myo-inositol monophosphatase catalyse reactions in the de novo inositol biosynthesis pathway. Scavenge inositol phosphates from the phosphotidylinositol signaling pathway and IP6 breakdown also provide inositol for intracellular pools. In this study, we have investigated the function of the AtMIPS1 gene in *Arabidopsis*. We confirmed the existing of interaction between AtMIPS1 and ATXR5/6 in Yeast and in living plant cells. We found that two mutants Atmips alleles display a same cell death phenotype. This cell death phenotype was molecularly biochemicaly and cytologicaly analysed. Our data provide new insight to link cell cycle to cell death in plant.

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Identification and characterization of components of G beta/gamma signaling in *Arabidopsis thaliana*: NDR gene family. yashwanti mudgil¹, Alan Jones¹. ¹University of North Carolina, Chapel Hill, USA

In animals, the basic units of the heterotrimeric G-protein signaling network include a cell-surface receptor (GPCR), a coupling complex comprised of alpha, beta, and gamma subunits (G protein), and a downstream target (effector). Sequential activation of these G protein components result in changes in cellular activities such as ion flux, protein trafficking, and gene expression. Plants also utilize signaling through G proteins but there are few candidates for the upstream GPCRs and the downstream effectors. In the current study, we are seeking downstream effectors of G protein signaling. Toward this, we performed a yeast three-hybrid screen using *Arabidopsis* G beta1 and G gamma1/or gamma2 subunits as baits to screen for physical interactors in an *Arabidopsis* cDNA expression library. We found and confirmed interaction between the G beta-gamma2 dimer and a protein of unknown function designated NDR (N-Myc Down Regulated) in animals. The sunflower NDR homolog, designated SF21, is expressed the highest in pollen and in the transmitting tissues of pistils, and it shows multiple alternative and organ-specific splicing transcripts. *Arabidopsis* has three NDR proteins (AtNDR1, 2 and 3) which are highly similar, between them NDR2 and 3 are predominately expressed in pollen. All three members interact with G beta-gamma2 in the yeast 3-hybrid configuration. Over expression studies indicate its role in vegetative branching and flower development. Biochemical characterization and down regulation studies by micro RNA in wild type and other G protein component mutant background are in progress to further dissect out its function in G beta-gamma signaling.

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Molecular analysis of cell death suppressor (AtBI-1) interacting factors. Minoru Nagano¹, Yuri Ihara-Ohori¹, Hirofumi Uchimiya^{1,2}. ¹Institute of Molecular and Cellular Biosciences, The University of Tokyo, ²Iwate Biotechnology center

In organisms including animals and plants, programmed cell death (PCD) is crucial for the maintenance of their lives and is highly regulated by various factors. *Arabidopsis* Bax Inhibitor-1 (AtBI-1) is proposed as one of such factors. BI-1 was first identified from human cDNA library as a suppressor of yeast cell death-induced by mammalian proapoptotic protein, Bax. Although no homologs of Bcl-2 family proteins including Bax have been identified in plants, BI-1 is widely conserved in not only animals but plants. BI-1 is about 26 kDa protein with 7 transmembrane domains and is localized in the membrane of endoplasmic reticulum (ER). AtBI-1 is rapidly up-regulated in plants during wounding or pathogen challenge and overexpression of AtBI-1 suppresses H2O2-, salicylic acid-, elicitor-, heat- and cold-induced cell death in plant cells, suggesting that AtBI-1 plays an important role in responses to various environmental stresses. Moreover, there is a coiled-coil region in the C-terminus of AtBI-1, in which AtBI-1 interacts with calmodulin, and AtBI-1 affects calcium homeostasis in cell death regulation. However, the mechanism of AtBI-1-mediated cell death suppression has not been elucidated. To investigate the function of AtBI-1 in detail, we attempted to identify interacting factors of AtBI-1 by the screening of *Arabidopsis* cDNA library. By using split-ubiquitin yeast two-hybrid system, *Arabidopsis* cytochrome b5 (AtCb5) was isolated as a candidate of such factor. Cb5 is known as a ubiquitous electron transfer protein found in animals, plants and yeasts and is localized in ER or mitochondrial outer membrane. Here, we show the interaction between AtBI-1 and AtCb5, and the role of AtCb5 on the cell death suppression by AtBI-1.

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The "SGR5" and "SGR9" genes are synergistically involved in amyloplasts movement in endodermal cell of inflorescence stem. Moritaka Nakamura¹, Masao Tasaka¹, Miyo Terao Morita¹. ¹Nara Institute of Science and Technology

Our previous research using *Arabidopsis* "shoot" "gravitropism" ("sgr") mutants have demonstrated that shoot endodermis is essential for shoot gravitropism, and that amyloplasts sedimentation in the endodermal cells is important in gravity sensing. However, molecular entities that control amyloplasts sedimentation remain unclear. Each "sgr" and "sgr9" single mutant exhibits weak gravitropic response in the inflorescence stem. Interestingly, the "sgr5sgr9" double mutant completely loses the response. The "SGR9" and "SGR" genes encode a RING finger protein and a putative transcription factor, respectively. Both genes play their roles in gravitropism within the endodermis.

Here, we analyzed the distribution and movement of amyloplasts in living endodermal cells of each single or double mutant by using vertical microscope system. In contrast to biased location of amyloplasts to the direction of gravity in wild-type, amyloplasts were dispersed throughout cells in both single and double mutant. The movement of amyloplasts was severely restricted in double mutant, while that in each single mutant was similar to that in wild-type. These results suggest that complete loss of gravitropic ability in "sgr5sgr9" double mutant due to restriction of amyloplasts movement in addition to dispersed amyloplasts.

We have previously reported that "sgr2" loses gravitropic ability as well as "sgr5sgr9". Although amyloplasts movement is severely restricted in both "sgr2" and "sgr5sgr9", distribution of amyloplasts in "sgr2" is obviously different from that in "sgr5sgr9". Since SGR2 is involved in vacuolar function, impairment of vacuolar membrane dynamics is likely to affect amyloplasts distribution and movement in "sgr2" endodermis. Then, we observed vacuolar membrane dynamics in "sgr" and "sgr9" single and the double mutant. Our results suggest that defect of amyloplasts movement in "sgr5sgr9" is unlikely to be due to impairment of vacuolar membrane dynamics.

Taken together, "SGR" and "SGR9" seem to be involved in distribution and movement of amyloplast via novel action that is independent of vacuolar function.

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Transgene-induced Phytochrome A silencing and epiallele formation in 'Arabidopsis' 'thaliana'. Scott Nicholson¹, Rekha Chawla², Vibha Srivastava¹. ¹University of Arkansas, ²Indiana University-Purdue University Indianapolis

Strong silencing of Phytochrome A ('PHYA') was found among a population of '*Arabidopsis thaliana*' transformed with a t-DNA construct not intended to produce silencing. Two non-transgenic epimutant lines were isolated from a transgenic line. Several analyses were conducted upon the transgenic and epimutant lines to determine the basis of silencing. Both transgenic and epimutant lines were screened for the transgene; the epiallele was found to have segregated away from the transgene while the silenced transgenic lines each contained several transgene loci. 'PHYA' transcript level was much reduced in transgenic and epimutant lines compared to wild type (wt); the greatest reduction (~80%) was in the epimutants. 'PHYA' siRNA were detected in several of the transgenic lines, indicative of post-transcriptional silencing (PTGS). Methylation analysis revealed promoter and coding sequence methylation in transgenic lines, also indicative of PTGS, while the epimutants displayed methylation only within the coding region, not in the promoter as typical in transcriptional silencing (TGS). Reduction of coding region methylation by crossing the epimutant with a CG methyltransferase-deficient 'met1' line resulted in recovery of wild-type progeny. Further, heterochromatic markers were found to be associated with 'phyA' exon 1 in the epimutant, but not with other regions of the gene. Nuclear run-on analysis showed a lower level of 'PHYA' transcript in the epimutants than in wt, confirming that TGS was the cause of silencing in the epimutant. As these findings deviate from previous descriptions of TGS, a novel method of TGS maintenance may have been discovered.

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The ECHIDNA protein is required for cell elongation and represents a novel component of the endocytic pathway in "Arabidopsis". Jaesung Oh¹, Robert Nilsson¹, Delphine Gendre¹, Julien Schmidt¹, Errin Johnson¹, Alan Marchant¹, Malcom J Bennett², Markus Grebe¹, Rishikesh Bhalerao¹. ¹UPSC, ²University of Nottingham

We studied an "*Arabidopsis thaliana*" T-DNA insertion mutant which exhibits a severe dwarf phenotype. Hypocotyl and root length in this mutant are reduced by 68% and 44%, respectively, compared to wild type (WT). The mutant is also unable to form root hairs, together suggesting a defect in differentiation and cell elongation. In support of this, the localization of ROP proteins, which are integral in root hair initiation, are unchanged in the mutant, implying that the mutation acts downstream of the hair initiation pathway. Moreover, root hair formation in the mutant can be restored by the addition of the ethylene precursor ACC to the growth medium, indicating that the ethylene response pathway is intact. The gene, "ECHIDNA", disrupted by this mutation encodes a protein with similarities to a yeast protein involved in vesicular trafficking. Interestingly, immunolocalization studies reveal that ECHIDNA partially co-localizes with VHA-a1 and ARF5, which reside in trans-Golgi/endocytic compartments. In addition, ECHIDNA associates with FM4-64 labeled endosomes, while FM4-64 internalization is altered in "echidna" roots. Taken together, these results suggest that ECHIDNA is possibly involved at multiple stages of the endocytic pathway. Furthermore, an immuno-dotblot assay using a suite of antibodies directed against specific cell wall epitopes showed that some RG-I type pectins are more abundant in the mutant relative to WT plants, suggesting that ECHIDNA may function in the transport and/or recycling of cell wall compounds, the absence of which impairs cell elongation and root hair formation.

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Class XI myosin motors are required for optimal tip growth of root hairs. Eunsook Park¹, Nilou Soltanian¹, Andreas Nebenf hr^{1,1}.¹ University of Tennessee, Department of Biochemistry, Cellular and Molecular Biology Knoxville, TN 37996, USA Email: epark3@utk.edu http://www.bio.utk.edu/cellbiol

Tip growth is the key growth mechanism on root hairs and pollen tubes. Many kinds of vesicles are trafficking toward (or backward) the apical dome of root hairs to supply membrane and cell wall material as well as energy for growing tips along the shaft of the hair with velocities around 2 to 3 m/s for *Arabidopsis thaliana*. It suggested that fast movements along actin network are responsible for those trafficking. The relationship of tip growth and acto-myosin cytoskeleton has been extensively studied with pollen tubes. To investigate the role of these organelle movements in the delivery of secretory vesicles to the root hair tip, we have isolated mutants in myosin genes. Arabidopsis genome encoded 13 class XI myosin genes. Mutations of the most conserved two genes resulted in reduced root hair elongation. Disruption of the XI-K gene by T-DNA insertion resulted in shorter root hairs with only 70% the length of WT. Another mutant, mya1, did not show the shorter root hair phenotype by itself, however, in combination with xi-k caused even shorter root hairs (45% of WT). It suggested that organelle movements in the root hairs of the mutants might be impaired. Interestingly, cytoplasmic streaming velocities in single and double mutant root hairs are within in the same range as in wild type when observed with DIC optics. This preliminary observation suggested that only the movements of specific organelles might be impaired in mutant cells. To identify which organelle movements and distributions are defect in mutants, we have transformed a number of fluorescent organelle markers, for example Golgi-YFP, into the mutants and wild type. Preliminary evidence suggests that the distribution of YFP-RabA4b-labeled vesicles is abnormal in the mutants. A detailed observation and analysis of the distribution of several other organelles in WT and mutant root hairs might be able to give the clue for the role of organelle trafficking on tip growth of root hair cells. This work is supported by NSF grant MCB-0416931.

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Evolutionary and functional analysis of two recently duplicated *Arabidopsis* kinesin genes, ATK1 and ATK5 : roles in the reproductive development. Li Quan¹, Rong Xiao^{1,2}, Wuxing Li^{1,2}, Sung-Aeong Oh³, David Twell³, Hong Ma^{1,2}.¹ Department of Biology and the Huck Institutes of the Life Sciences, the Pennsylvania State University, University Park, PA 16802, USA,² Plant Physiology Program, the Pennsylvania State University, University Park, PA 16802, USA,³ Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, United Kingdom

Gene duplication plays an important role in evolution, allowing functional and expression divergence. However, relatively few studies have been reported on functional divergence of recently duplicated genes. Here, we report that two kinesin genes, ATK1 and ATK5, are paralogs of high overall sequence similarity derived from the most recent *Arabidopsis* genome-wide duplication, which occurred after the separation of *Arabidopsis* and poplar. Nevertheless, parts of the N-terminal sequences of these proteins and their corresponding DNA sequences are significantly divergent. Additionally, ATK1 has higher expression level than other close ATK homologs in anthers near the time of meiosis. Genetic study indicates that the atk1 and atk5 mutations cannot be transmitted through the gametophytes simultaneously, probably due to abnormal gametophyte mitosis. Moreover, although ATK1 was previously found to be only required for male meiosis, our cytological experiments revealed that ATK1 and ATK5 together are important for both the male and female reproductive development. Therefore, our results suggest that the duplicate genes, ATK1 and ATK5, have retained redundant functions in female meiosis and gametophyte development, but have experienced functional divergence in male meiosis. In male meiosis, the fact that ATK1 seems to play a major role and has a greater extent of coding sequence divergence suggests that it may have undergone neofunctionalization. In gametophyte development, the fact that both ATK1 and ATK5 are needed suggest that they may both experienced subfunctionalization. The analysis of ATK1 and ATK5 provides a demonstration of functional evolution of duplicate genes and a scenario for maintenance of duplicated genes.

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Overexpression in At1g23540 gene suppresses inflorescence growth. Youngmin Park¹, Indeok Hwang¹, Hyeyonsoon Cheong^{1,1}.¹ Department of Biotechnology, Chosun University, Gwangju, Korea

Genetic approach in mutants screening is an important tool for studying gene function in plant. We got phenotypically distinct plants in the activation tagged lines and isolated a mutant, igi1 (Inflorescence Growth Inhibitor 1). Segregation ratio of the F2 generation, TAIL-PCR walking and genotyping PCR results indicated a single T-DNA insertion at the 200bp upstream of the At1g23540 coding region in the BAC F5O8. We referred to At1g23540 gene as IG1 and confirmed overexpression of the gene by Real time PCR. The expression level for IG1 gene was increased approximately by 8,000 to 10,000 fold in igi / igi homozygous line. igi / igi homozygous line was sterile and severely defective in growth and differentiation. igi / IG1 heterozygous mutant showed smaller siliques, bumpy stem and shortened inflorescence. We generated IG1 overexpression lines (IG1-OX) for recapitulation phenotypes of the igi1 mutant. IG1-OX lines displayed similar phenotypes with igi / IG1 heterozygous mutant. These results indicated IG1 gene overexpression is related to bumpy stems and shortening of inflorescences.

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A Functional and structural characterization of plant lipid rafts as membrane signalling platforms. Sylvain Raffaele¹, Fabienne Furt¹, Béatrice Satiat-Jeunemaitre², Jean-Jacques Bessoule¹, Jean-Pierre Carde³, Sébastien Mongrand¹. ¹Lab de Biogénie Membranaire, Bordeaux, France, ²ISV, Gif-sur-Yvette, France, ³Lab de Physiologie & Biotechnologie Végétales, Villenave d'Ornon, France

Evidences are accumulating suggesting that the plasma membrane (PM) of eukaryotic cells is not homogenous. Dynamic clustering of sphingolipids and sterols give rise to domains insoluble in non-ionic detergents named lipid rafts. These years, a growing interest was devoted to their study in PM of plant cells. We showed that lipid rafts exist in PM isolated from tobacco leaves, BY2 cells, and *Medicago truncatula* roots. Similar domains were found in *Arabidopsis thaliana*. Nevertheless, little is known about the architecture of these structures, and their functional relevance. With aim to gain insights on these questions, we developed a strategy taking benefit from the different model plants *Medicago*, *Nicotiana* and *Arabidopsis*.

In a first step, we carried out lipidomic and proteomic studies on plant lipid rafts. Interestingly, microdomains are able to recruit specific sets of PM proteins. Therefore, we investigated the association with microdomains of stress-related enzymes enriched in rafts.

Another striking result from proteomic studies is the enrichment in lipid rafts of a plant-specific protein, hydrophilic although associated with PM, called Remorin (REM). Remorins are a protein family present in various plant species: Potato REM1 is known to bind OGA and pectin, and its *Arabidopsis* homolog, AtDBP, forms filaments *in vitro*. To understand the basis of the association of REM with lipid rafts, we first performed *in silico* 3D modelling to propose a putative structure for AtDBP. Putative functional domains were tested using GFP fusions for their ability to bind the membrane. Then we adopted multiple microscopy strategies to study the localisation of REM1 *in situ*, and on isolated PM and rafts. In parallel, we exploited *Arabidopsis* large set of data to identify biological processes that may rely on REM. Wild type and over-expressers were assayed for alteration in these processes. Results will be discussed in term of cell biology and putative function of Remorins.

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The mitotic checkpoint in *Arabidopsis thaliana*. Joseph Ramahil¹, Jodi Stewart¹, Simon Chan¹. ¹UC Davis, Davis, California, USA

The mitotic checkpoint (also known as the spindle assembly checkpoint) monitors the progress of mitosis, delaying the onset of anaphase until each chromosome is correctly attached to the spindle. Unattached kinetochores use the Mad1 and Bub3 proteins to bind the mitotic checkpoint components Mad2 and BubR1. A Mad2/Bub3/BubR1 complex is then released in a form that can inhibit the anaphase promoting complex cofactor Cdc20.

Although the mitotic checkpoint has been studied extensively in budding yeast, yeast kinetochores require sequence-specific interactions with a 125bp centromere DNA and are thus very different from plant and animal kinetochores that assemble on 100s of kB of DNA and are epigenetically specified. Mouse mutants in Mad1, Mad2 and BubR1 are embryonic lethal, making it difficult to genetically analyze the mitotic checkpoint in an organism with a large, complex centromere.

In *Arabidopsis thaliana*, MAD1 and MAD2 are encoded by single genes. Interestingly, homozygous mad1 and mad2 T-DNA mutants are viable and fertile, demonstrating that the mitotic checkpoint can be dispensable for normal development in a multicellular eukaryote (bub3 and bubR1 mutants are also viable and fertile, but *Arabidopsis* has multiple homologs of these proteins). Viable mitotic checkpoint mutants present the opportunity to study how epigenetic changes in a complex centromere/kinetochore affect the mechanism of chromosome segregation. The effects of mad1 and mad2 mutants on mitotic chromosome dynamics, sensitivity to microtubule destabilizing drugs, and genetic interactions with epigenetic mutants will be presented.

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Kinesin-related proteins and a NIMA-related kinase regulate epidermal cell morphogenesis in *Arabidopsis*. Tatsuya Sakai¹, Miki Nishioka¹, Yukiko Uehara¹, Miho Takahashi¹, Noriko Fujisawa¹, Kensuke Saji², Motoaki Seki¹, Kazuo Shinozaki¹, Kiyotaka Okada². ¹RIKEN Plant Science Center, ²Kyoto University

The involvement of kinesin motor proteins in both cell tip growth and cell shape determination has been well characterized in various organisms. However, the functions of the kinesins during cell morphogenesis in higher plants remain largely unknown. Recent transcriptome analyses have suggested that *MRH2*, which encodes an armadillo repeat-containing kinesin-related protein, is involved in root-hair morphogenesis in *Arabidopsis thaliana*, although the details of the underlying mechanisms have not been elucidated. In our current study, we have isolated a cell morphogenesis mutant of *Arabidopsis* root hairs, *antlers1-1*, and demonstrate that *ANTLERS1* is allelic to *MRH2*. For clarity, we have renamed the gene *Arabidopsis thaliana ARMADILLO REPEAT KINESIN1* (*AtARK1*). The *AtARK1* gene has two homologs in the *Arabidopsis* genome, *AtARK2* and *AtARK3*, and our present results show that *AtARK2* is involved in cell morphogenesis in the root epidermis. We further reveal that a NIMA-related protein kinase, *AtNEK6*, binds to the *AtARK* family proteins and has pleiotropic effects upon epidermal cell morphogenesis, suggesting that *AtNEK6* is involved in cell morphogenesis in *Arabidopsis* via microtubule functions associated with these armadillo repeat kinesins.

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Chloroplast protein targeting with emphasis on the role of Hsp93. Lars Sjogren¹, Henrik Aronsson¹, Adrian Clarke¹. ¹Department of Plant and Environmental Sciences, Gteborg University, Sweden

Most chloroplast proteins are encoded in the nucleus and imported post-translationally into chloroplasts. A protein complex in the chloroplast envelope, called the translocon, mediates import. Components of the translocon are called Toc or Tic, for translocon at the outer/inner envelope membrane of chloroplasts. Many Toc and Tic components have been identified and characterised, but for most their function remains undefined. Proteins imported by the translocon have a transit peptide that targets them to the main receptors Toc33 and Toc159. Thereafter the incoming protein is transferred over the envelope membranes through the Toc75 and Tic110 channels. The transit peptide is removed upon transfer across the envelope membranes.

Hsp93 (also known as ClpC) works in close proximity with Tic110 and the co-chaperone Tic40 at the latter stage of import. Tic110 recruits Tic40 when a precursor is present in the channel. Tic40 then releases the precursor from Tic110 and stimulates ATP hydrolysis of Hsp93 which is suggested to be the motor for the translocation process. In the mitochondrial protein transport systems Hsp70 is the known motor. Although no connection has been established between the envelope associated Hsp70 and the TIC complex, a motor function for chloroplast Hsp70 cannot yet be excluded. In Arabidopsis two paralogs of Hsp93 exist (Hsp93-III and Hsp93-V) with Hsp93-V being more highly expressed. Loss of the more abundant Hsp93-V causes a chlorotic phenotype and less developed chloroplasts, but only at most a minor decrease in import rate. Thus, it appears the function of Hsp93-V is not primarily that of a motor. Indeed, stromal Hsp93 plays an important role in protein degradation as part of the Clp protease, and so alternative roles for Hsp93 at the envelope need to be considered. We have postulated that Hsp93-V performs a quality control function of the import and this will be further discussed.

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Topography of phytochrome A action. Jutta Rösl¹, Ilse Klein¹, Mathias Zeidler¹. ¹University Giessen, Plant Physiology, Giessen, Germany

Plants use the photoreceptor family of phytochromes to sense their light environment in the red/far-red region of the spectrum. Phytochrome A (phyA) is the primary photoreceptor that regulates early seedling development. phyA mediated seedling de-etiolation is the critical developmental transition from heterotrophic to photoauxotrophic growth. Irradiation with high fluence rate far red provides a way to assess specifically the role of phyA in this process and was used to isolate phyA signaling intermediates. fhy1-3 is an insertion mutant of a gene encoding a phyA signal transduction component. FHY1 is a small 24 kDa protein with little similarity to known proteins, besides a small conserved sepiin-related domain at the C-terminus, a nuclear localization signal (NLS) and a nuclear exclusion signal (NES). The NLS and NES of FHY1 are indeed involved in its nuclear localization and exclusion (Zeidler et al. 2004). Nuclear localization of FHY1 is needed for the execution of responses downstream of phyA. FHY1 has one homologous gene in Arabidopsis, FHL (FHY1 Like), for which overlapping functionality with FHY1 has been demonstrated (Zhou et al. 2005). We generated double fhl/fhy1 mutant lines and analysed the physiological response in HIR (high irradiance response) and VLFR conditions. FHY1 and FHL both are specifically impaired in phyA signaling. The double knockout lines show a very strong phenotype, comparable to the phyA null mutant. This phenotype is not due to changes in phyA levels. In CFP:FHY1 and phyA:GFP cotransformed protoplasts light dependent intracellular distribution was analysed and co-translocation as well as co-localization of phyA and FHY1 could be observed. Phenotypical analysis of fhl/fhy1 revealed novel cytoplasmic phyA functions.

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Arabidopsis pri-miRNA processing proteins HYL1 and DCL1 define a nuclear body distinct from the Cajal body. Liang Song¹, Nina Fedoroff². ¹Plant Biology Program, The Pennsylvania State University, University Park, PA, USA, ²Biology Department and Huck Institute of the Life Sciences, The Pennsylvania State University, University Park, PA, USA

Small regulatory miRNAs are encoded in long precursors and released from them during processing by cleavage within partially duplexed stem-loop structures. In the present work, we investigated the role of the Arabidopsis nuclear RNA-binding protein HYL1 and the nuclear RNase III enzyme DCL1 in processing of pri-miR171a. The miR171a gene is complex, with multiple transcription start sites, as well as alternative splicing of exons and alternative polyadenylation sites. Both HYL1 and DCL1 proteins are required for processing of the major pri-miR171a, spliced and polyadenylated forms of which accumulate in plants homozygous for mutations in either gene, but not in wildtype plants. In transiently transfected Arabidopsis protoplasts, HYL1-mCherry and YFP-DCL1 fusion proteins co-localize to small nuclear bodies similar to Cajal bodies, but lacking the Cajal body marker Atcoolin or U2B⁺. The HYL1-DCL1 bodies do not co-localize well with SE bodies, nor do SE bodies co-localize with AtCoolin or U2B⁺ labeled Cajal bodies. The distinct HYL1- and DCL1-containing nuclear bodies may be miRNA precursor processing sites. Alternatively, they may be assembly and storage sites for the miRNA precursor processing machinery.

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Canonical SRP components can be bypassed for post-translational protein targeting in chloroplasts. Tzvetelina Tzvetkova-Chevolleau¹, Claire Hutin¹, Laurent D. Noel¹, Robyn Goforth², Jean-Pierre Cardé³, Stephano Caffarri¹, Neil E. Hoffman⁴, Ralph Henry², Michel Havaux¹, Laurent Nussaume¹. ¹OEA, ²University of Arkansas, ³INRA Centre de Bordeaux, ⁴APHIS/USDA

The chloroplast Signal Recognition Particle (cpSRP) and its receptor (cpFtsY) targets proteins both co-translationally and post-translationally to the thylakoids. This dual function enables cpSRP to utilize its posttranslational activities for targeting a family of nuclear encoded light-harvesting chlorophyll-binding proteins (LHCPs), the most abundant membrane proteins in plants. Previous *in vitro* experiments indicated an absolute requirement for all cpSRP pathway soluble components. In agreement, a cpFtsY mutant in Arabidopsis exhibits a severe chlorotic phenotype resulting from a massive loss of LHCPs. Surprisingly, a double cpftsY/cpsrp54 mutant, recovers to a great extent from the chlorotic cpftsY phenotype. This establishes that in plants a new alternative pathway exists that can bypass cpSRP post-translational targeting activities. Using a mutant form of cpSRP43 that is unable to assemble with cpSRP54, we complemented the cpSRP43-deficient mutant and found that this subunit is required for the alternative pathway. Along with the ability of cpSRP43 alone to bind the ALB3 translocase required for LHCp integration, our results indicate that cpSRP43 has developed features to function independently of cpSRP54/cpFtsY in targeting LHCPs to the thylakoid membranes.

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Identification and characterization of systemic RNA interfering mutants in *Arabidopsis thaliana* by double strand luciferase. Nazim M Uddin¹, Yeonggil Rim¹, Chun-Lin Shi¹, Juyeon Moon¹, Jae-Yean Kim¹. ¹Division of Applied Life Science, PMBBRC/EB-NCRC, Gyeongsang National University, Jinju 660-701, Korea.

Post-transcriptional gene silencing (PTGS) is a plant RNA-induced gene silencing surveillance mechanism providing a defense against invasive nucleic acids such as transgenes, virus and transposons. Small RNAs generated from long dsRNA can be associated into the RNA-induced silencing complex (RISC), and then guide the cleavage of target mRNA at a single site in the region of complementarity. Previous studies showed that RNA-induced silencing of endogenous and exogenous gene expression spreads locally and systemically, respectively. This mobile signal in systemic RNAi might be a complex consisted with small RNAs and proteins, and could traffic through plasmodesmata (PD) and phloem system in plants. However, little is known about this mobile signal, and the PD associated proteins functioning in this pathway. To isolate protein components functioning in this process, we developed a genetic mutant screening system in which the firefly luciferase (Luc) reporter was used. The transgenic plants carrying 35S-Luc construct showed high luciferase activity, whilst the transformants containing both 35S-Luc and pSuc2-dsLuc showed no or little luciferase activity. These results suggested that the luciferase gene was silenced by pSuc2-dsLuc and the silencing signal induced in the vascular cells was able to move into the neighboring cells through PD. This silencing line was treated with EMS, generating around 4000 M1 plants. Several candidates were found, showing significant luciferase recovery. Genetic Studies including mapping are being carried out to identify the candidate gene. So far, two mutants were roughly mapped on the long arm of chromosome 1 and 3, by using a series of SSLP and CAPS markers. Here, we present the current progress on the screening of siRNA movement mutants and their characterization.

P-177**Analysis of trans-Golgi network (TGN) dynamics in plants**

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In all eucaryotic cells, the post-Golgi organelles, such as the trans-Golgi network (TGN), endosomes, vacuoles and the plasma membrane, are connected by vesicular traffic. This complex network plays a critical role in several higher-order functions. The TGN is one of the most important organelles for protein transport at the post-Golgi network, and functions as a sorting spot that directs cargo proteins to a variety of post-Golgi compartments. However, the TGN of plant cells has not been well understood yet. In order to elucidate the structure, function and dynamics of plant TGN, we focused on Syp41, the ortholog of Tlg2/syntaxin16 which is localized to the TGN in yeast and mammalian cells, as a TGN marker. We established the transgenic plants expressing GFP-Syp41 under the control of the native promoter. The observation by CLSM (confocal laser scanning microscopy) showed that the TGN is a dot-like BFA (brefeldin A)-sensitive and Wm (wortmannin)-insensitive compartment, which is partially stained with FM4-64. Next, we addressed the relations between the TGN and the Golgi apparatus by analyzing the transgenic plant expressing GFP- or mRFP-tagged Syp31 (cis-Golgi marker) or ST (sialyl transferase, trans-Golgi marker) with Syp41 tagged with GFP or mRFP. The fluorescence patterns indicated that TGN was mainly located at the trans-side of Golgi apparatus, but some population was independent from the Golgi apparatus. Taken together, we suggest that TGN does not always behave together with the Golgi apparatus, and functions in endocytic pathways in addition to the biosynthetic pathways. We will also discuss dynamic movement between TGN and the Golgi apparatus.

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Temperature-sensitive allele of RSW4 links the putative plant separase to cell division and cell expansion in the root of *Arabidopsis*. Shuang Wu¹, Wolf-Ruediger Scheible², Tobias I. Baskin¹. ¹Biology Department, University of Massachusetts, Amherst, Massachusetts, USA, ²Max-Planck-Institute for Molecular Plant Physiology, Golm, Germany

The temperature-sensitive mutant, radially swollen4 (*rsw4*), was isolated previously as root morphogenesis mutant, in which expansion becomes less anisotropic. Map-based cloning revealed that a point mutation occurs in At4g22970. This gene encodes a homologue of separase, an essential protein in eukaryotes involved in separating replicated chromatids at the onset of anaphase. RSW4 is the single copy of separase in *arabidopsis* and T-DNA insertion alleles are lethal. The connection between reduced separase function and root swelling remains unclear.

Here I report the confirmation of mitotic defects from reduced separase function by means of immuno-fluorescence staining, TEM, and observations of histone-GFP in living cells. The transition from metaphase to anaphase in the mutant at the restrictive temperature (30° C) is severely affected. Chromosome bridges and un-separated sister chromatids are common in the mutant. Although affected cells proceed into cytokinesis, new cell walls are often incomplete, characterized by cell wall stubs and gapped cell walls. With longer time at 30° C, the meristem area of the mutant decreases, corresponding to a decreased rate of cell production.

To explain root swelling in *rsw4*, we first quantified the angular distribution of cortical microtubules in root epidermal cells, imaged with immuno-fluorescence and in living cells expressing GFP-tubulin. Cortical microtubules become disorganized and the extent of the disorganization correlates with the time course of root swelling. This suggests that separase could act at interphase on microtubule behavior. However, we also found that with time at 30° C, cyclinB-GUS staining is enhanced in the mutant suggesting the cell cycle progression is impaired. To explore the regulatory state of cells in *rsw4* further, we have initiated a large analysis of mRNA using micro-array and real time PCR methods. Our hypothesis is that orderly progression through M-phase plays an essential role in the determination of cell and organ polarity.

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The Kingdom- and Tissue-specific Anchorage of Plant RanGAP to the Nuclear Envelope Involves a Novel Family of Plant Nuclear Pore Associated Transmembrane Proteins. Xianfeng Morgan Xu¹, Tea Meulia², Iris Meier¹. ¹ Plant Biotechnology Center and Department of Plant Cellular and Molecular Biology, The Ohio State University, Columbus, Ohio 43210, USA,² Molecular and Cellular Imaging Center, The Ohio State University/Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, USA

Ran is a multifunctional small GTPase of the Ras superfamily involved in nucleocytoplasmic transport, mitotic spindle assembly, cell cycle control, and nuclear envelope (NE) formation. Its roles are accomplished by the asymmetric distribution of its GTP- and GDP-bound forms, enabled by the specific localization of Ran accessory proteins, the Ran GTPase-activating protein RanGAP and the nucleotide exchange factor RCC1. Mammalian RanGAP1 is targeted to the NE during interphase and to the spindle and kinetochores during mitosis via a sumoylated C-terminal domain and interaction with the nucleoporin Nup358/RanBP2. In contrast, Arabidopsis RanGAP1 is associated with the NE during interphase and the cell plate during cytokinesis, mediated by an N-terminal, plant-specific domain (WPP domain). Here, we have identified in a yeast two-hybrid screen a novel plant-specific protein that binds to the WPP domain. WPP-domain Interacting Protein 1 (WIP1) and RanGAP1 interact in vivo and co-localize at the NE and cell plate. WIP1 has two homologs in Arabidopsis (WIP2 and WIP3) with a similar domain organization of extended coiled-coil domain and C-terminal transmembrane domain. WIP1 co-localizes with a nuclear pore marker and its nuclear pore association was confirmed by immunogold-labeling. The targeting of WIP1 requires the transmembrane domain which is also sufficient for the NE (presumably nuclear pore) association. In a wip1/wip2/wip3 triple mutant, but not in single or double mutants, RanGAP1 is dislocated from the NE in undifferentiated root tip cells, while NE targeting in differentiated root cells and targeting to the cell plate remain intact. We propose that WIP family members are novel plant nuclear pore proteins involved in RanGAP1 NE anchoring in specific cell types. Our data support a separate evolution of RanGAP targeting mechanisms in different kingdoms.

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Functional Identification of Sugar-1-P Kinases in Arabidopsis
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The salvage pathway is an essential metabolic process required for plant development specifically during seed germination, pollen growth and embryo development where storage carbohydrates are converted to simple sugars. These sugars need to be activated into nucleotide-sugars in order to facilitate cell division and cell growth. The function of the salvage pathway is also essential during normal growth to recycle sugars generated from breakdown of diverse glycoconjugates (glycoproteins, glycolipids, and small metabolite-glycosides). A typical plant cell requires generating over 25 different types of nucleotide-sugars. Sugar-1-P kinases are a group of enzymes that phosphorylate free sugars to sugar-1-P. Pyrophosphorylases (PPases) are a group of enzymes required to convert the sugar-1-P with nucleotides (NTP) such as ATP, GTP, TTP, CTP, or UTP to form the corresponding NDP-sugar.

Galactokinase (GalK) phosphorylates D-galactose with ATP to D-galactose-1-phosphate. Little is known about this and other sugar-1-P kinases, which are required to phosphorylate GlcA, GalA, Rha, Xyl, Ara, etc. Here we report the functional identification of many of these kinases at the biochemical and gene levels. We developed a new HPLC-based assay in conjunction with NMR analysis. Current work involves the i) determination of domains involved in sugar recognition, ii) wall analysis of mutants in these kinase genes, iii) determination of the expression and regulation of these kinase genes. The complete identification of all the sugar-1-P kinases is significant if we wish to understand the flux of carbon contributing to carbohydrate storage, wall synthesis, gene regulation in sugar synthesis and other regulatory mechanisms in salvage pathway.

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Existence of the cells without detectable plastids in the crumpled leaf mutant of *Arabidopsis thaliana*. Yasushi Yoshioka¹, Yuling Chen², Tomoya Asano³, Makoto Fujiwara⁴, Shigeo Yoshida⁵, Yasunori Machida¹. ¹Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ., Nagoya, Japan, ²Col. Life Sci., Hebei Norm. Univ., Shijiazhuang, China, ³Adv. Sci. Res. Center, Kanazawa Univ., Kanazawa, Japan, ⁴Dept. Life Sci., Grad. Sch. Arts Sci., Univ. Tokyo, Tokyo, Japan, ⁵Plant Science Center, RIKEN, Yokohama, Japan

The plastid is an essential organelle to the plant cell. Plant cells that lack plastids have not been found even in the plants of which plastid division is severely suppressed. Absence of chloroplasts in stomatal guard cells was reported in accumulation and replication of chloroplasts 6 (arc6) of *Arabidopsis thaliana* (Robertson et al., J. Cell Sci. 108, 2937-2944, 1995). However the presence of plastids without chlorophyll has not been investigated in these cells. We investigate whether a plastid division mutants of *A. thaliana*, crumpled leaf (*crl*), contain the cells lacking plastids using a plastid-targeted yellow fluorescent protein. Our data indicated that leaf epidermis including stomata and cotyledon primordia of mature embryos contained the cell having no detectable plastids in *crl*. Staining of the stomata and the cotyledon primordia of the mature embryos with 4',6-diamidino-2-phenylindole (DAPI) also indicated the existence of the cells without detectable plastids DNA in *crl*.

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sec28 is an intracellular membrane trafficking mutant defective in the cell wall formation. Hugo Zheng¹, Gill Dean², George Haughn², Ljerka Kunst². ¹McGill University, Montreal, Canada, ²University of British Columbia, Vancouver, Canada

Intracellular membrane trafficking links the cellular organelles such as the endoplasmic reticulum (ER), Golgi, and the plasma membrane (PM) in eukaryotic cells. In plants, it plays crucial roles in the delivery of proteins, lipids and polysaccharides to vacuoles, PM and cell walls, which are among most biological significant and commercial important structures. While proper sub-cellular change in these structures is central to many aspects of plant developmental processes and cellular adaptation to environmental stresses, food, oil, cotton, wood and plant fibers rely on the formation of plant ER, vacuoles and cell walls. It is therefore important to understand how intracellular membrane trafficking is regulated in plant cells.

In *Arabidopsis*, forward genetics is a powerful approach, some of mutants such as gnom, knolle, keule and vac1, which were identified by virtue of their developmental phenotypes, have subsequently been found to have mutations in genes encoding regulators of plant membrane trafficking. Systematic isolation of mutants defective in various membrane trafficking steps has, however, yet to be applied in *Arabidopsis* owing to the lack of common reporter markers and suitable screens. We have recently developed a secretory GFP (secGFP)-based visual screen and biochemical assay system in *Arabidopsis* for isolating potential mutants with perturbed membrane trafficking, aiming at identifying novel genes controlling plant specific membrane trafficking (Zheng et al., 2004). Based on the intracellular accumulation of secGFP in a compartment reminiscent of Golgi apparatus in a mutagenized secGFP transgenic line, we have recently identified sec28 as a potential post-Golgi trafficking mutant. Further biological and cellular characterization indicated that sec28 is associated with many physiological and developmental phenotypes, including defects in cell wall formation, cuticular wax secretion, and apical dominance. The sec28 mutation has been mapped to a 300 kbp region on chromosome 1; the fine mapping of the mutation is currently in progress. Zheng, H., et al. (2004). Plant J. 37, 398-414

Developmental Mechanisms**P-183**

Targets of CINCINNATA, a gene involved in leaf morphogenesis in *Antirrhinum*. Pooja Aggarwal¹, Utpal Nath¹. ¹Indian Institute of Science, Bangalore (Karnataka), India

Although much is known about how plant organs are patterned at the meristem by the action of developmental genes in specific domains, we are only beginning to understand how organs such as leaves, once initiated, grow to their final shape and size. Since cell migration is absent from the plant, cell proliferation is likely to play a more important role in plant organogenesis than it does in animal. The recently identified TCP family of transcription factors has a key role in controlling organ morphogenesis through regulation of cell division. For instance, it has been shown in *Antirrhinum* (Nath et al., Science 299: 1404-1407, 2003) that the TCP gene *CINCINNATA* (*CIN*) controls leaf shape and surface curvature by regulating cell proliferation in the young leaf. It has been proposed that *CIN* regulates cell proliferation by altering the expression of the genes involved in cell division. However, the molecular targets of *CIN* are largely unknown.

As a first step to identify the direct targets of *CIN*, we have determined the consensus DNA binding sites of the *CIN* protein using Random Binding Site Selection Assay (RBSS). The consensus site, GT-GGTCCC, is identical to that determined for TCP4, the *Arabidopsis* orthologue for *CIN* (our unpublished results). We, then, have carried out differential display PCR (DD-PCR) to identify the transcripts differentially expressed between the wild type and the *cin* mutant. We have got the differentially displayed bands cloned and sequenced to know the identity of the genes. To further identify the genes differentially expressed in the *cin* mutant, we have carried out micro-array analysis using 44K *Arabidopsis* chips as well as 13K custom-made *Antirrhinum* chips. Since majority of the orthologous genes between *Antirrhinum* and *Arabidopsis* have considerable sequence homology, and because the consensus binding sequence of *CIN* and TCP4 are identical, it is likely that the *Arabidopsis* chip can be used for *Antirrhinum* probe with appropriately modified hybridization conditions. Using these bio-chemical approaches, we have identified several direct targets of *CIN*.

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Analysis of the "BRC1" gene promoter. José Antonio Aguilar-Martínez¹, Pilar Cubas¹. ¹Dpto. Genética Molecular de Plantas, Centro Nacional de Biotecnología-CSIC, UAM, Madrid, Spain

Plant branching patterns depend mainly on the control of axillary meristem development. Axillary meristems form axillary buds that can grow out to generate new shoots or become arrested, depending on endogenous and environmental factors. We have shown that the *Arabidopsis* TCP transcription factor "BRANCHED1" ("BRC1") controls axillary bud development and acts as an integrator of branching signals within axillary buds (1). We have also shown that "BRC1" transcription is affected by developmental and external signals (1). To identify important genomic regions responsible for the regulation of "BRC1" we have carried out a promoter deletion analysis and a phylogenetic comparison of 5 flanking regions of the "BRC1" gene in several Brassicaceae species. Results obtained in this study have been used as a starting point to perform a one-hybrid screening to look for upstream regulators of "BRC1".

1 Aguilar-Martínez et al. 2007 Plant Cell 19:458-472

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Identification of downstream genes of *Arabidopsis CUP-SHAPED COTYLEDON* genes. Mitsuhiro Aida¹, Yuka Tsubakimoto¹, Ayano Kariya¹, Kenichiro Hibara¹, Masao Tasaka¹. ¹Nara Institute of Science and Technology (NAIST), Nara, Japan

The *Arabidopsis CUP-SHAPED COTYLEDON* genes CUC1, CUC2, and CUC3 encode transcriptional activators of the NAC family and are redundantly required for shoot meristem formation and organ separation. Double mutant combinations in any two of these genes cause severely fused cotyledons and lack of an embryonic shoot meristem. Conversely, ectopic overexpression of CUC1 induces ectopic shoot meristem formation on the surface of cotyledons. To identify downstream genes of CUC1 and CUC2, we carried out microarray experiments. We compared expression profiles in RNA extracted from wild-type and cuc1 cuc2 double mutant embryos at the torpedo stage and selected candidate genes that were downregulated in cuc1 cuc2. In addition, another set of microarray experiments identified genes ectopically activated in cotyledons of plants ectopically expressing CUC1. We are now analyzing expression patterns of these candidate downstream genes in wild type and cuc1 cuc2 embryos by *in situ* hybridization. The results from these experiments will be presented.

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BET bromodomain protein GTE4 affects embryonic and post-embryonic root development in "Arabidopsis". Maria Maddalena Altamura¹, Chiara C. Airoldi², Giuseppina Falasca¹, Federica Della Rovere¹, Sandra Citterio³, Lucia Colombo². ¹Dipartimento di Biologia Vegetale, Sapienza Università di Roma, Rome, Italy, ²Dipartimento di Biologia, Università degli Studi di Milano, Milan, Italy, ³Dipartimento di Scienze dell'Ambiente e del Territorio, Università di Milano Bicocca, Milan, Italy

The bromodomain was first discovered in the "*Drosophila*" Brahma protein (Tamkun et al., 1992) and is present in a broad range of chromatin-modifying proteins (Yang, 2004 and references therein). BET proteins form a separate group of bromodomain proteins which all share a unique additional domain named extra terminal (ET) domain (Pandey et al., 2002). Plant BET proteins are distinguishable from the yeast and animal ones for the presence of only one bromodomain instead of two (Pandey et al., 2002). In the "*Arabidopsis*" genome 12 BET encoding genes have been identified. Until now only two of these were characterized, "IMB1" and "GTE6". IMB1 is a transcriptional activator and promotes seed germination regulating abscisic acid and phytochrome A (Duque and Chua, 2003). GTE6 positively regulates the "myb"-domain gene "AS1", which is involved in leaf axis specification (Chua et al., 2005). We are now investigating "GTE4" (At1g06230) using an integrated analysis of molecular biology and histology on the homozygous knock-out mutant. RT-PCR experiments showed that "GTE4" is expressed in all the organs of the plant. Results about embryonic and post-embryonic root development are presented. Anomalies in the development of the root pole cells are present in the mature embryo of the knock-out mutant. A significant reduction in primary root length in comparison with the wild type was also observed during the first week after seed germination. The histological analysis suggests that the abnormal root growth is due to an anomalous increase in size of the meristematic cells in the root apex. In conclusion, GTE4 seems to be involved in the regulation of root dome formation and development from the embryo to the seedling.

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Stamen and carpel development in *Arabidopsis* and *Antirrhinum*. How proteins evolution changes mechanisms of male and female organ development. Chiara A. Airoldi¹, Barry Causier¹, Sara Bergonzini¹, Brendan Davies¹. ¹Centre for Plant Sciences University of Leeds, Leeds, United Kingdom

The development of male and female floral organs has been one of the most studied processes in plant development; it has a key role for plant reproduction and therefore a high agronomical importance. To study stamen and carpel development we are using two plants that played a fundamental role in the study of floral organ development: *Arabidopsis* and *Antirrhinum*. In these two plants the evolution of genes that regulate stamen and carpel development has followed two different paths. In *Arabidopsis*, development of both male and female floral organs is determined by the presence of the MADS-box transcription factor AGAMOUS (AG). In *Antirrhinum* two genes, which are phylogenetically related to AG, are responsible for the same process: FARINELLI (FAR) and PLENA (PLE). PLE and FAR differ from AG in their ability to determine the development of stamens and carpels. Indeed, during the course of evolution they have undergone a process of subfunctionalisation such that FAR is more responsible for the development of male organs and PLE is more involved in the formation of female organs. These differences can be observed when PLE, FAR and AG are overexpressed in *Arabidopsis*. Whereas AG overexpression converts sepals into carpelloid structures and petals into stamens, FAR overexpression only converts petals into stamens, and PLE mainly converts sepals into carpelloid structures. AG, PLE and FAR are all expressed in stamens and carpels, indicating that their different functions are driven by differences at the protein level. To identify if these protein differences are located in a specific domain of the protein, we overexpressed different domain fusions between FAR, AG and PLE in *Arabidopsis*. From an analysis of the flowers of these plants we demonstrated that the C terminal domain is responsible for the differential ability of AG PLE and FAR to induce female or male development.

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The use of FRUITFULL versions with modified transcriptional activity reveals the possible role of FUL in meristem maintenance. Vicente Balanza¹, Shussei Sato², Martin Yanofsky², Cristina Ferrandiz¹. ¹Instituto de Biología Molecular y Celular de Plantas IBMCP (CSIC-UPV), Valencia, Spain, ²Department of Biology and Center for Molecular Genetics, University of California San Diego, La Jolla, CA 92093-0116, USA

FRUITFULL is an *Arabidopsis* MADS box gene involved in multiple aspects of plant development. FUL controls flowering time and cauline leaf development, acts redundantly with APETALA1 and CAULIFLOWER in the specification of floral meristem identity and plays a major role in carpel and fruit development. To gain further insights on FUL mechanisms of action and to identify FUL molecular targets, we have generated new alleles of FUL with modified transcriptional activity by traditional fusion of the FUL coding sequence to the VP16 activation domain under the control of FUL regulatory sequences. The phenotypes of the transgenic plants have an effect on different aspects of plant development. The FUL:VP16 plants show altered shoot apical meristem phenotypes. FUL:VP16 lines, after initiating between 10 and 30 normal flowers, form a terminal structure at the shoot apex consisting of a spiral arrangement of >15 stamens and carpels. FUL:VP16 also show weak floral meristem indeterminacy and strongly enhances agamous, clavata or crabs claw indeterminacy phenotypes. Recently generated FUL:EAR plants show phenotype in the shoot apical meristem too. FUL:EAR shoot apical meristems often terminate producing 4-5 flowers and a terminal carpel. A possible role of FUL in the regulation of meristem maintenance under the view of current models will be discussed.

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The BELL homeodomain proteins ATH1 and PNY are redundantly required for vegetative shoot apical meristem function in *Arabidopsis*. Dongping Bao¹, Bas Rutjens¹, Evelien van Eck-Stouten¹, Sjef Smeekens¹, Marcel Proveniers¹. ¹Utrecht University, Utrecht, The Netherlands

In animals, TALE homeodomain (HD) proteins play fundamental roles in development, in particular in the specification of body plan and pattern formation. In plants, pattern formation is an ongoing process that starts during embryogenesis and lasts the entire life span. Most of the plant adult body is formed post-embryonically by continuous organogenic potential of the root and shoot apical meristems. Proper shoot apical meristem (SAM) function requires maintenance of a delicate balance between the depletion of stem cell daughters into developing primordia and proliferation of the central stem cell population. Mutations in the KNOX-class TALE HD protein SHOOTMERISTEM-LESS (STM) cause defective initiation and maintenance of the SAM and results in early developmental arrests. STM is able to physically interact with members of a distantly related second class of TALE HD transcription factors, the BELL class. In animals the formation of such heterodimers is often indispensable for TALE HD functionality. One of the STM-associating BELL proteins, PENNYWISE (PNY) enhances meristem defects of weak to intermediate *stm* loss-of-function mutants. PNY itself, however, is not essential for meristem function, possibly due to redundancy. Here we show that the BELL-class protein ARABIDOPSIS THALIANA HOMEobox1 (ATH1) fulfills a functional redundant role with PNY and STM during initiation and maintenance of the vegetative SAM. Like PNY and STM, ATH1 is highly expressed in the vegetative SAM, but loss of ATH1 function seems not to be detrimental to meristem function. However, *ath1* mutations enhance weak and intermediate *stm* alleles. Moreover, combined loss of ATH1 and PNY results in a *stm* mutant phenocopy during the embryonic and vegetative phases, whereas generative development appears unaffected. Finally, we present data implying that both KNOX and BELL protein function are regulated through their subcellular distribution by a mechanism highly conserved in animals and plants.

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SEUSS and AINTEGUMENTA Regulate Carpel Margin and Ovule Development. Fang Bao¹, Sridevi Azhakanandam¹, Staci Nole-Wilson¹, Huda Bhatti¹, Robert Franks¹. ¹North Carolina State University, Raleigh, (NC), USA

In *Arabidopsis*, ovules arise from meristematic placental regions that reside on the adaxial margins of the carpel. Our work indicates that SEUSS and AINTEGUMENTA are required for the specification or early development of the placentae and for initiation of ovule primordia. We show that the patterning genes PHABULOSA and CRABS CLAW are mis-expressed in *seu* and *aintegumenta* double mutant carpels and that *seu* and *aintegumenta* mutants completely fail to initiate ovule primordia. As SEUSS and AINTEGUMENTA encode transcriptional regulators, we are working to determine if CRABS CLAW or PHABULOSA are direct targets of SEUSS regulation. Additionally, we are using genetic means to determine if mis-regulation of these patterning genes in the *seu* and *aintegumenta* double mutant is responsible for the failure to initiate ovule primordia.

We have also initiated genetic screens to identify novel regulators of ovule and carpel margin development. We have identified several suppressor of *aintegumenta* (*saint*) mutants that can partially rescue the integument growth defect of a hypomorphic loss-of-function *ant-3* allele. The *ant-3* *saint* double mutants display rescued integument and female gametophyte phenotypes and have a 4 to 8 fold increase in fertile seeds per pod relative to *ant-3*. Progress on map-based cloning of one of the *saint* mutants (*saint49*) will be presented. In a second screen, we identified *seu* modifier (*sum*) mutants that enhance the carpel and ovule defects of *seu-1* single mutants. One such mutant, *sum63-1*, displays wild type ovule development, but acts as a synergistic enhancer of the *seu-1* ovule phenotype. The *sum63* *seu-1* ovule phenotype is characterized by a strong loss of the outer integument, while inner integument and female gametophyte development are not disrupted. The *sum63* mutation maps to a portion of the genome with no previously identified ovule mutants and thus may represent a novel regulator of ovule development.

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NEW TRANSCRIPTION FACTORS AFFECTING DORMANCY IN ARABIDOPSIS SEEDS. JM Barrero¹, AA Millar¹, T Czechowski², WR Scheible², M Udvardi², D Burgess³, RL Jones³, J Jacobsen¹, F Gubler¹. ¹CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia, ²Max-Planck Institute of Molecular Plant Physiology, Am M hlenberg 1, 14476 Golm, Germany, ³Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA

Seed dormancy is a very important trait which maximizes the survival rate of the seed in nature, and whose control is essential in many crop species. We have used transcription factor analysis to help identify new genes which are involved in the dormancy maintenance or release in *Arabidopsis thaliana*. RNA was isolated from imbibed dormant and non-dormant C24 seeds and screened by qRT-PCR for differentially expressed transcription factors. We have identified 31 which are differentially expressed during the first few hours of imbibition. After analyzing T-DNA insertions lines for these genes, 4 of them displayed an increased or reduced dormancy level compared with wild type Columbia. RNAi lines have been generated to study the role of the selected genes in the high dormancy ecotype C24. Work is in progress to determine the spatial expression pattern of these genes in the seed tissues. One of the genes that we have identified encodes a RING finger protein which is involved in ABA biosynthesis during seed development, promoting the expression of *NCED* genes in the seed and generating a complete loss of dormancy when mutated.

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A systems approach to understand cell cycle regulation during leaf development. Gerrit Beemster^{1,2}, Frederik Coppens^{1,2}, Nubia Barbosa Eloy^{1,2,3}, Steffen Vanneste^{1,2}, Joanna Boruc^{1,2}, Roland Merks^{1,2}, Tom Beeckman^{1,2}, Lieven De Veylder^{1,2}, Geert De Jaeger^{1,2}, Dirk Inze^{1,2}, Pierre Hilson^{1,2}. ¹VIB, Ghent, Belgium, ²Ghent University, Ghent, Belgium, ³Universidade Federal do Rio de Janeiro; Rio de Janeiro, Brazil

The molecular regulation of the plant cell cycle plays a crucial role in the regulation of the growth of multi-cellular organs. The sequencing of the *Arabidopsis* genome has enabled us to identify 61 "core" cell cycle genes (including CDKs, Cyclins and directly interacting proteins (Vandepoele et al., 2002; Plant Cell 14, 903 - 916). Transcriptional analysis of genes that are specifically expressed in proliferating tissues during leaf development and in the root tip implicated several hundreds of additional genes, many still fully unknown, that also appear to regulate aspects of the cell cycle process (Beemster et al., 2005; Plant Physiol. 138, 734-743). We have functionally analyzed the function of a selection of these genes. The results indicate that the link between cell cycle regulation and whole organ rate is complex, due to the large numbers of genes involved, their non-linear regulatory relationships and the lack of knowledge about the relationship between the cell cycle regulated events cell division and endoreduplication on the one hand and cell growth on the other.

To unravel the nature of the regulatory of the cell cycle in the context of organ growth regulation we are taking a systems biology approach. To this end we have cloned the promoters of 51 of the core cell cycle genes and analyzed the expression pattern of during leaf and root development. These data allowed us to develop a "phenocustering" approach to find genes with similar expression patterns as well as regulatory promoter elements that are shared between the members of such clusters.

By means of matrix yeast-2-hybrid analysis, tandem affinity purification and split GFP analysis we are also constructing the cell cycle interactome and localisome. To integrate this wealth of data, we have developed a combination of top-down and bottom-up modeling approaches that can be used to identify regulatory relationships and to test their dynamic behavior.

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Unique, Shared, and Redundant Roles for the *Arabidopsis* SWI/SNF Chromatin Remodeling ATPases BRAHMA and SPLAYED. Staver Bezhani¹, Cara Winter¹, Steve Hershman¹, John D. Wagner¹, John F. Kennedy¹, Chang Seob Kwon¹, Jennifer Pfluger¹, Yanhui Su¹, Doris Wagner¹. ¹University of Pennsylvania

SWI/SNF chromatin remodeling ATPases are conserved in the animal and plant kingdom and regulate transcriptional programs in response to endogenous and exogenous cues. In contrast with their metazoan orthologs, null mutants in two *Arabidopsis thaliana* SWI/SNF ATPases, BRAHMA (BRM) and SPLAYED (SYD), are viable, facilitating investigation of their role in the organism. Previous analyses revealed that syd and brm null mutants exhibit both similar and distinct developmental defects, yet the functional relationship between the two closely related ATPases is not understood. Another central question is whether these proteins act as general or specific transcriptional regulators. Using global expression studies we find overlapping functions for the two SWI/SNF ATPases. This partial diversification may have allowed expansion of the SWI/SNF ATPase regulatory repertoire, while preserving essential ancestral functions. Moreover, only a small fraction of all genes depends on SYD or BRM for expression, indicating that these SWI/SNF ATPases exhibit remarkable regulatory specificity. Our studies provide a conceptual framework for understanding the role of SWI/SNF chromatin remodeling in regulation of *Arabidopsis* development.

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The peroxin ABSTINENCE BY MUTUAL CONSENT is required for gamete-gamete recognition during pollen tube reception Aurélien Boisson-Dernier¹, Sabine Frietsch², Tae-Houn Kim¹, Marie Beverley Dizon¹, Julian Schroeder¹. ¹UCSD, La Jolla, California, USA, ²UNR, Reno, Nevada, USA

In animals and plants, fertilization relies on complex and specialized mechanisms that achieve the precise delivery of the male gamete to the female gamete and their subsequent union. In plants, the male gametophyte or pollen tube carries two sperm cells over a long distance through the maternal tissues to the female gametophyte or embryo sac. During this long assisted journey, a multitude of signal exchanges between the pollen tube, the maternal diploid tissues and the haploid embryo sac take place that culminate in pollen tube reception, the process through which the pollen tube release the sperm cells into the female gametophyte. Here, we report the isolation and characterization of the *Arabidopsis* mutant *abstinence by mutual consent* where pollen tube reception is impaired only when an *amc* pollen tube reaches an *amc* embryo sac leading to pollen tube overgrowth and the absence of sperm release. Moreover, *AMC* is strongly expressed in both male and female gametophytes during fertilization but strongly down-regulated during subsequent embryo development. We further show that YFP-AMC fusion localized to peroxisomes and that *AMC* functions as a peroxin that mediates protein import into peroxisomes. The identification of *AMC* as the first gene required for pollen tube reception with essential roles in both male and female gametophytes, points towards a key role for peroxisomes in gamete recognition and successful sperm release.

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The role of YABBYs in leaf patterning. Oliver Bonaccorso¹, Melissa Stahle¹, John Golz¹. ¹Department of Genetics, University of Melbourne, Parkville, VIC 3010. Australia

Patterning along the adaxial (topside)-abaxial (bottom side) axis of a leaf requires the activity of several families of transcription factors that include the abaxially expressed YABBYs (YABs). We present data showing that YABs also have a role in promoting adaxial cell identity. Using the Yeast two hybrid assay, we show that two closely related transcriptional co-repressors LEUNIG (LUG) and LEUNIG-HOMOLOGY (LUH) interact with three leaf expressed YABBYs FILAMENTOUS FLOWER (FIL), YAB3 and YAB5, suggesting these proteins might form a complex in planta. Consistent with this possibility, we find that lug enhances the leaf polarity defects of fil yab3 mutants. To further investigate the role of LUG and LUH in leaf development, we attempted to isolate lug luh double mutants. While our analysis indicates that the double mutants are embryonically lethal, we found that lug luh/+ plants produce narrow leaves and occasionally abaxialized lotus or needle-like leaves. These polarity defects become more frequent when either fil or fil and yab3 mutations are crossed into the lug luh/+ background. Finally we also show that like fil yab3, lug luh/+ enhances the polarity defects of kan1 kan2 double mutants.

The partial loss of adaxial cell identity in some lug luh/+ leaves is incongruous with the reported abaxial promoting function of the YABs. To address this issue further we isolated yab2 and yab5 mutants and generated triple and quadruple combinations with fil and yab3. Both fil yab3 yab5 and fil yab2 yab3 yab5 plants produce radially symmetric leaves lacking adaxial cell identity. These observations suggest that YABBYs promote adaxial identity and that this function is highly redundant. Using microarrays we found that auxin response genes are up regulated following ectopic FIL expression as are KANADI1 and PHABULOSA. Using cycloheximide we show that increased KAN1 expression is a direct result of FIL activity whereas changes in PHB expression are not. Based on these findings we propose a model in which YABs promote adaxial cell identity non cell-autonomously and that this pathway is part of a system that allows leaf development to proceed independently of an adaxial promoting signal from the meristem.

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Downregulation of RETINOBLASTOMA-RELATED PROTEIN (*RBR*) expression in *A. thaliana* leads to enhanced cell division and defects in cell specification. Lorenzo Borghi¹, Ruben Gutzat¹, Johannes Füller¹, Wilhelm Gruissem¹. ¹ Institute of Plant Sciences, ETH Zürich, Switzerland

In animal cells, the RETINOBLASTOMA protein (pRB) plays a key role in controlling the transition from G1 to S phase by modifying the activity of E2F/DP transcription factor complexes. In *A. thaliana*, RBR seems to perform a similar function (1,2,3). Here we report that a constitutive down regulation of *RBR* expression via RNA interference (RNAi) or co-suppression leads to enhanced cell division during embryogenesis and altered post embryonic development. Cloning of an RNAi inducible system against RBR, based on the pOp/LhG4 binary system (4), gave us a tool to perturb *RBR* expression levels at later stages of plant development. Interestingly, newly formed organs after RNAi induction are characterized by disturbed control of cell division and organ growth and also show defects in certain differentiation pathways. Our preliminary results are presented here.

1. J Virol. 2007 Apr;81(8):4177-85
2. Plant Physiol. 2006 Jan;140(1):67-80
3. Cell. 2005 Dec 29;123(7):1337-49
4. Plant J. 2005 Mar;41(6):899-918

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The Jagged Lateral Organ (*JLO*) gene regulates meristem maintenance and organ development by controlling auxin transport. Marina Bureau¹, Lorenzo Borghi², Madlen Rast¹, Rüdiger Simon¹. ¹ Institut für Genetik, Heinrich-Heine Universität, Universitätstr. 1, Düsseldorf, Germany, ² Swiss Federal Institute of Technology (ETH) Zurich, Institute of Plant Sciences, Universitätstr. 2, CH-8092 Zürich, Switzerland

We have started to address the role of meristem-to-organ boundaries in the control of stem cell activities in meristems. To do this, we began with the analysis of the *Arabidopsis JLO* gene, a member of the LBD gene family of transcription factors. In *Arabidopsis*, LBD genes were shown to play a role in boundary establishment and communication between meristem and initiating lateral organs. RNA in situ hybridisation experiments showed that *JLO* is expressed in boundaries between meristem and organ primordia. Overexpression *JLO* causes formation of lobed leaves and meristem arrest, suggesting that *JLO* is involved in organ patterning and maintenance of stem cells. One role of *JLO* is to increase expression of meristem specific KNOX genes. Furthermore, we found that *JLO* reduces auxin transport by inhibiting the expression of the auxin export carrier PIN1. *JLO* loss-of-function mutants revealed embryo-lethality when homozygous, demonstrating a prominent role of *JLO* in the early steps of development. We propose that *JLO* is required during the globular stage to control auxin distribution and embryo patterning.

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Control of AHP6, a central player of vascular development in the *Arabidopsis* root. Ana Campilho¹, Anthony Bishop¹, Hanna Help¹, Ykä Helariutta¹. ¹ Institute of Biotechnology, University of Helsinki, Finland

During vascular development in the *Arabidopsis* root, cytokinins promote pluripotent cell as well as phloem identity and inhibit protoxylem cell identity. Protoxylem cell fate is dependent on the localised inhibition of cytokinin signalling by AHP6, a pseudo-phosphotransfer protein that acts to inhibit the phosphorelay associated with cytokinin signalling. AHP6 is expressed specifically in both protoxylem cell files. Conversely, cytokinin signalling negatively regulates the spatial domain of AHP6 expression. Consequently, a negative regulatory feedback loop operates where cytokinin signalling counteracts expression of its inhibitor facilitating protoxylem formation. The identity of either the negative regulatory (cytokinin mediated) or promotive factors which converge on AHP6 is unknown. To identify and characterize upstream factors controlling AHP6, a forward genetic screen was performed to identify altered expression patterns of pAHP6::GFP within an EMS mutagenized line. A set of novel mutants was identified and the phenotypic description of these genetically interacting loci will be presented. Further functional and molecular characterization of those loci can reveal the basic genetic mechanisms underlying vascular development.

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HERMIT regulates Petunia inflorescence architecture and plays part in floral transition. R Castel¹, E Kusters¹, A Rebocho¹, I Roobeek¹, A Procissi¹, R Koes¹. ¹Vrije Universiteit, Amsterdam, the Netherlands

All flowering plants are built of very similar organ types, but the positions and numbers of these organs differ between species. This is most obvious in the inflorescences, which are the structures that bear the flowers. In some species the inflorescence consists of a single solitary flower, whereas in other species it consists of an infinite number of flowers arranged in different branching patterns. Two major types of multi-flower inflorescences are cymose (*Petunia*) and raceme (*Arabidopsis*). The molecular mechanisms that underlie these divergent branching patterns are still largely unknown.

We have identified three inflorescence branching loci in *Petunia*: EVERGREEN (EVG, WUSCHEL-related homeobox), EXTRAPETALS (EXP, MADS-box) and, the latest, HERMIT (HER). evg mutants have fasciated inflorescences, resulting in a bushy phenotype with few flowers. Both exp and her mutants lack an inflorescence meristem and the multi-flower cyme has transformed into a single, solitary flower. Transposon display and revertant analysis demonstrated that the her phenotype is caused by a dTph1 transposon insertion in the third exon of a class 1 KNOTTED1-like homeobox gene. Judged by RT-PCR, her is considered a null mutant. Phylogenetic analysis showed that HER is closely related to SHOOTMERISTEMLESS (STM) of *Arabidopsis*. Strong stm mutants completely lack a shoot apical meristem. The *Petunia* genome contains at least four other KNOX class 1 genes, one of which has no clear counterpart in *Arabidopsis*.

Like STM, HER is expressed throughout all meristems, and over-expression of STM and HER in *Petunia* or *Arabidopsis* gives identical phenotypes. Moreover, at least in *Petunia*, HER and STM are exchangeable, as the genomic fragments of both HER and STM can complement the hermit phenotype. Thus, in contrast to *Arabidopsis*, *Petunia* shoot apical meristem development is not dependent on HER/STM. Instead, *Petunia* needs HER/STM for inflorescence meristem development.

Although her mutants flower at the same time as wild type *Petunias*, double mutants with a late flowering *Petunia* mutant reveal an additional role for HER in floral transition.

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The TEMPRANILLO genes of the *Arabidopsis* RAV family act in the photoperiod pathway to directly repress *FT* expression. Cristina Castillejo¹, Soraya Pelaz^{1,2}. ¹Laboratori de Genetica Molecular Vegetal, CSIC-IRTA. Barcelona, Spain,² ICREA. Barcelona, Spain

Floral induction is probably the most important process in plant development since it takes to the formation of the reproductive structures and, therefore, the species perpetuation. In the *Arabidopsis* photoperiod depending pathway, the first step of the floral induction is the activation of *FT* by CO. Activation of *FT* occurs specifically in the vascular bundles of leaves so it must travel to the shoot apex where flowering occurs. Once there, *FT* interacts with FD to activate the expression of the floral meristem identity genes, such as AP1.

Our results suggest that two partially redundant transcription factors of the RAV family, TEMPRANILLO1 (TEM1) and TEMPRANILLO2 (TEM2) are involved in the regulation of *FT*. Silencing of these genes results in an early flowering phenotype and their constitutive expression extremely delays flowering as a consequence of *FT* repression. Moreover, the constitutive expression of *FT* completely rescues the 35S::TEM1 late flowering phenotype. In addition, the consensus RAV binding sequence is present in the *FT* 5'UTR and we found that TEM1 protein binds this sequence in vitro. These observations strongly suggest that TEMPRANILLO is a direct repressor of *FT* transcription. Furthermore, *FT* repression by TEM1 occurs both in the vascular bundles and in the shoot apex as revealed by the late flowering phenotype obtained when expressing TEM1 under the control of specific phloem or shoot meristem promoters. Accordingly to the role of TEM in repressing *FT*, the expression of TEM and *FT* genes along development is antagonistic. Besides, TEM gene expression is regulated, like CO, by light and the circadian clock. We propose that TEM may be acting in the photoperiod pathway in parallel with CO to regulate *FT* and that the balance between CO and TEM activities would allow a sharp control of flowering time. In agreement with this, the progeny of the cross between early flowering 35S::CO plants and the very late flowering 35S::TEM1 plants shows a wild type flowering time.

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The role of auxin in stamen development. Valentina Cecchetti¹, Andrea Raganelli², Giuseppina Falasca³, Maria Maddalena Altamura³, Paolo Costantino¹, Maura Cardarelli². ¹Dipartimento di Genetica e Biologia Molecolare, Sapienza Universita' di Roma, Rome, Italy,² IBPM-CNR, Sapienza Universita' di Roma, Rome, Italy,³Dipartimento di Biologia Vegetale, Sapienza Universita' di Roma, Rome, Italy

In angiosperms, the late phase of stamen development consists of three main processes -filament elongation, pollen maturation and anther dehiscence - and is coordinated with the development of the pistil. We previously demonstrated a role for auxin on stamen and pistil development, by means of the localised expression of *rolB*, an *Agrobacterium* oncogene that enhances the sensitivity to this hormone. Tobacco plants expressing *rolB* driven by the promoter of the gene DMC1 display shorter filaments as compared to controls, a severe delay in anther dehiscence and alterations in male and female meiosis; these developmental alterations could be phenocopied by exogenous auxin (1). To verify the role of auxin in stamen development in *Arabidopsis* flowers we analysed auxin accumulation and synthesis in the meiotic and postmeiotic stages of stamen development. The phenotype of *tir/afb* mutants defective in auxin perception was analysed with respect to anther dehiscence, filament elongation and pollen maturation. Results demonstrate the role of auxin in controlling the timing of different processes in stamen development. In addition, we isolated a tobacco gene, NtROX1, acting downstream of *rolB*, overexpressed in pDMC1::rolB anthers. We demonstrated a role for ROX1 in filament elongation, by controlling the balance between procambial cell proliferation and xylem differentiation during stamen development in tobacco flowers (2). The sequence of ROX1 shares conserved elements with three genes of *Arabidopsis*. We are currently analysing the expression pattern of these genes during stamen development in *Arabidopsis* and characterising monogenic mutants.

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"DORNROESCHEN" ("DRN") and "DRN-LIKE" control the change from radial to bilateral symmetry and act upstream of auxin in the "Arabidopsis" embryo. John Chandler¹, Melanie Cole¹, Wolfgang Werr¹. ¹University of Cologne, Cologne, Germany

Embryonic patterning is a fundamental process of plant development, leading to the formation of the SAM and RAM necessary for organogenesis and subsequent growth. A key aspect of this process is the transition from radial to bilateral symmetry. The AP2 domain-encoding transcription factor "DRN" and its parologue "DRNL" are expressed from very early globular stages and act redundantly to control cotyledon ontogeny and stereotypic hypophyseal cell divisions via interaction with proteins from the class III HD-ZIP family such as PHAVOLUTA, and from the bHLH transcription factor family. The transition from radial to bilateral symmetry involves auxin signalling and response and is pre-patterned by "CUC" gene expression. Both "DRN" and "DRNL" are implicated in this transition; genetic analyses show that "DRN" and "DRNL" function in different "CUC" pathways, a finding which addresses the redundancy within both gene families. A disruption in radial symmetry is reflected in single "drn" and "drnl" mutants which show mono- or polycotyledony and initiate leaves with an altered phyllotaxis and "drn drnl" double mutants completely lack cotyledons, have radialised pin-like embryos which initiate leaves directly and are disturbed in all subsequent developmental stages. "drn drnl" double mutants also show an asymmetry of "STM" and "CUC" gene expression, demonstrating a loss of positional information and placing "DRN" and "DRNL" gene functions in pathways leading to the establishment of bilateral symmetry. Polar localisation of PIN1 is important in establishing the RAM and cotyledon initiation and creating bilateral symmetry. Consistent with hypophyseal and cotyledon defects in the dm mutant, DR5::GFP and PIN1 expression are also altered, placing "DRN" function upstream of auxin transport and/or response. "DRN" expression is also positively regulated by auxin: mutational analysis of several auxin response elements in upstream and downstream "DRN" regulatory regions reveals a differential contribution of elements to the tissue-specific expression of "DRN" during embryo development.

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Developmental Steps in Acquiring Competence for Shoot Development in Arabidopsis Tissue Culture. Ping Che¹, Sonia Lall¹, Stephen Howell¹. ¹Iowa State University, Ames IA, USA

Arabidopsis shoots regenerate from root explants in tissue culture through a two-step process requiring preincubation on an auxin-rich callus induction medium (CIM) followed by incubation on a cytokinin-rich shoot induction medium (SIM). During CIM preincubation, root explants acquire competence to respond to shoot induction signals. During CIM preincubation, pericycle cells in root explants undergo cell divisions and dedifferentiate, losing the expression of a pericycle cell-specific marker. These cells acquire competence to form green callus after only one day CIM preincubation and to form shoots after 2-3 days CIM preincubation. Reversible DNA synthesis inhibitors and auxin response mutants (*axr1* and *axr4*) interfered with the acquisition of competence to form shoots. Genes requiring CIM preincubation for upregulation on SIM were identified by microarray analysis and included RESPONSE REGULATOR 15 (ARR15), POLYGALACTURO-NASE INHIBITING PROTEIN 2 (PGIP2) and WUSCHEL (WUS). These genes served as developmental markers for the acquisition of competence because the CIM preincubation requirements for ARR15 and PGIP2 upregulation correlated well with the acquisition of competence to form green callus, and the CIM preincubation requirements for WUS upregulation matched those for shoot formation. Unlike ARR15, another cytokinin inducible, A-type ARR gene, ARR5, was upregulated on SIM, but the induction did not require CIM preincubation. These findings indicate that competencies for various events associated with shoot regeneration are acquired progressively during CIM preincubation, and that a set of genes, normally upregulated on SIM, are repressed by a process that can be relieved by CIM preincubation.

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Genetic control of male gametogenesis in Arabidopsis. Li-Qun Chen¹, Xue-Qin Zhang¹, Ke-Zhen Yang¹, Xiao-Lei Liu¹, Chuan Xia¹, Yi Deng¹, Wen-Qing Li¹, Xiao-Yun Tan¹, Wei Wang¹, Dong-Jie Jia¹, Xi Cao¹, Xuan Ma¹, Lu-Yuan He¹, Wen Dui¹, De Ye¹. ¹State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing, China

We are focusing on understanding the genetic mechanisms that control the cell differentiation and interaction in the plant male gametogenesis. As the first step, we largely isolate and characterize the mutants and corresponding genes that are involved in the male gametogenesis and pollen tube growth in Arabidopsis by phenotypic screens of the enhancer-and gene-trap dissociation(Ds) insertion lines in *Arabidopsis thaliana* ecotype Landsberg erecta (Sundaresan et al., 1995, G & D 13: 2108-2117). So far, 107 male gametophytic mutants have been isolated and are being characterized. 89 out of 107 mutations have been successfully mapped on 5 different chromosomes using TAIL-PCR. All mutations mainly or only affect the male gametophytic function. 15 genes that play key roles in controlling the pollen formation and pollen tube growth have been cloned and are being characterized. Molecular and functional characterization of the genes show that they may control the cell differentiation and interaction in male gametogenesis and pollen tube growth via taking part in signal transduction, transcription regulation and metabolism. (The correspondence is addressed to De Ye, E-mail: yede@cau.edu.cn)

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Defective of Tapetum Development and Function1 (TDF1) is essential for tapetal development and plays a vital role in tapetum gene regulation network in *Arabidopsis*. Hui Chen¹, Jun Zhu¹, Hui Li¹, Yao Song¹, Ju-Fang Gao¹, Zhong-Nan Yang¹

¹Shanghai Normal University

Tapetum plays important roles in anther development and is essential for pollen development by providing enzymes for callose dissolution, and materials for pollen wall formation in *Arabidopsis*. In this study, we reported the molecular analysis of a defective of tapetum development and function1 (tdF5) mutant which is essential for tapetum development and function. It has two layers of tapetal cells suggesting that TDF5 negatively regulates tapetal periclinal division. TDF5 was isolated using a map-based cloning strategy and was confirmed by genetic complementation. It encodes a R2R3 MYB transcription factor predominantly located to the nucleus, and highly expresses in meiocytes, tapetum, and microspores in anther development. Callose staining and RT-PCR analysis suggest TDF5 regulate microspore release from tetrad and pollen wall formation. A preliminary regulation network containing nine regulation genes essential for tapetum formation, development and function has been constructed with TDF5 playing a key role in the network. The function investigation of TDF5 and the gene regulation network of tapetal development in this work should provide a roadmap for the investigation of molecular mechanism of tapetal development in plant.

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"Arabidopsis" homologs of components of SWR1 complex regulate flowering and plant development. Kyuha Choi¹, Chulmin Park¹, Jungeun Lee¹, Mijin Oh¹, Bosi Noh^{2,3}. ¹National Research Laboratory of Plant Developmental Genetics, Department of Biological Sciences, Seoul National University, Seoul, 151-742, Korea., ²Global Research Laboratory for Flowering at SNU and UW, Seoul, 151-742, Korea., ³ Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea.

The 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 thaliana*". Mutations in "*Arabidopsis* SWC6 (AtSWC6)", "SUPPRESSOR OF FRIGIDA3 (SUF3)" and "PHOTOPERIOD INDEPENDENT EARLY FLOWERING1 (PIE1)", homologs of "SWC6", "ARP6" and "SWR1", respectively, caused similar developmental defects, including leaf serration, weak apical dominance, flowers with extra petals, and early flowering by reduction in expression of "FLOWERING LOCUS C (FLC)", a strong floral repressor. Chromatin immunoprecipitation assays showed that AtSWC6 and SUF3 bind to the proximal region of the "FLC" promoter, and protoplast transfection assays showed that AtSWC6 colocalizes with SUF3. Protein interaction analyses suggested the formation of a complex between PIE1, SUF3, AtSWC6, and AtSWC2. In addition, H2AZ, a substrate of SWR1C, interacts with both PIE1 and AtSWC2. Finally, knockdown of the H2AZ genes by RNA interference or artificial micro RNA caused a phenotype similar to that of atswc6 or suf3. Our results strongly support the presence of SWR1C-like complex in *Arabidopsis* that ensures proper development, including floral repression through full activation of "FLC".

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Cooperation between localized auxin biosynthesis and polar transport is required for organogenesis in *Arabidopsis*. Youfa Cheng¹, Xinhua Dai¹, Yunde Zhao¹. ¹Section of Cell and Developmental Biology, University of California San Diego

Auxin is essential for many aspects of plant development. In the past few years, much progress has been made in auxin biosynthesis, polar auxin transport, and auxin signaling. However, the underlying mechanisms by which the distinct auxin pathways are integrated to control plant development are largely unknown. Here we analyze interactions between polar auxin transport and YUC-mediated localized auxin biosynthesis during plant development using *Arabidopsis* as a model system. We demonstrate that both localized auxin biosynthesis and polar auxin transport are necessary for proper auxin distribution that is required for *Arabidopsis* organogenesis. We suggest a mechanism that integrates localized auxin biosynthesis and polar auxin transport to control auxin dynamics and developmental processes.

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CHICORY1(CHI1), A C2H2 ZINC FINGER PROTEIN OF ARABIDOPSIS, IS INVOLVED IN DEVELOPMENT. Kyung Sook Chung¹, Joonki Kim¹, Jong Seob Lee², Ji Hoon Ahn¹. ¹Plant Signaling Network Research Center, School of Life Sciences and Biotechnology, Korea University, Seoul, 136-701, Korea., ²Crop Functional Genomics Center, School of Biological Sciences, Seoul National University, Seoul 152-742, Korea

From activation tagging screening, we isolated a dominant mutant, which has a serrated leaves and named *chicory1-1D* (*chi1-1D*). In addition, *chi1-1D* showed pleiotropic phenotypes such as, reduced height, delayed growth, thick stems, open floral buds, decreased silique size, and increased indehiscent fruits. The 35S enhancers of cauliflower mosaic virus (CaMV) were inserted into the promoter region of the CHI1 gene and resulted in gain-of-function of CHI1 gene. CHI1 encodes a novel type of C2H2 zinc-finger protein and has a putative nuclear localization signal. CHI1::GFP fusion protein was localized in nuclear in the transgenic root epidermal cells. Loss-of-function mutants of CHI1, *chi1-2* and *chi1-3* were decreased a silique size and the number of seeds. these were due to decreased seed set in proximal regions of the silique. After fertilization, the fruit size increases dramatically and *FUL* gene was known to be important to cell expansion after fertilization in fruit development. The expression of *FUL* was up-regulated in *chi1-2* , but was down-regulated in *chi1-1D* . It shows the possibility that CHI1 may function as a negative regulator of *FUL* .

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An Evolutionarily Conserved Mechanism Delimiting SHR movement Defines a Single Layer of Endodermis In Plants. Hongchang Cui¹, Mitchell P. Levesque¹, Teva Vernoux¹, Jee W. Jung¹, Alice J. Paquette¹, Kimberly L. Gallagher¹, Jean Y. Wang¹, Ikram Blilou², Ben Scheres², Philip N. Benfey¹. ¹Duke University, Durham, NC, USA, ² Utrecht University, Padualaan, Utrecht, the Netherlands

SHR and SCR are key regulators of radial patterning in the *Arabidopsis* root. Produced in the stele, the SHR protein subsequently moves into an adjacent cell layer where it controls SCR transcription and endodermis specification. SCR is required for the asymmetric cell division that gives rise to the cortex and endodermis. Despite of its ability to move between cells, SHR movement is limited to only one cell layer. There was indication that SCR plays a role in restricting SHR movement, but the underlying molecular mechanism has been unknown.

Using a ChIP-PCR assay, we find that SCR directly regulates its own transcription through a positive feedback loop. SCR also directly controls the transcription of other known SHR targets, and loss of SCR abolishes the ability of SHR to regulate these genes. Moreover, microarray experiments indicate that SHR and SCR share a large set of downstream genes. Protein immunoprecipitation and yeast two-hybrid assays show that SCR forms a heterodimer with SHR through the GRAS domain. Based on these results we proposed a model whereby SCR blocks SHR movement by sequestering it into the nucleus through protein-protein interaction and by a SHR/SCR dependent feedback loop for SCR transcription that prevents SHR escape. Supporting this model, SHR becomes largely cytoplasmic in scr mutant, and reduction in SCR transcript level by RNAi causes ectopic movement of SHR and a multi-layer endodermis phenotype.

Finally, we find that functional homologs of SHR and SCR in rice also interact with each other and have expression patterns analogous to those of their *Arabidopsis* counterparts. Since nearly all plant species examined so far have a single layer of endodermis, we suggest that the mechanism we have uncovered in this study by which SCR restricts SHR movement is likely to be evolutionarily conserved.

* Note: this work has been accepted by Science.

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The roles of dsRNA binding proteins in *Arabidopsis thaliana*. Shaun Curtin^{1,2}, Neil Smith¹, John Watson¹, Paul Roffey², Chris Blanchard², Peter Waterhouse¹. ¹CSIRO Plant Industry, Canberra, (ACT), Australia, ² Charles Sturt University, Wagga Wagga, (NSW), Australia

Double-stranded binding proteins (dsRBP) are proteins that specifically bind to dsRNA. In humans and *Drosophila* they have been shown to be important components of the RNA silencing pathways of these organisms. We have obtained T-DNA insertion lines for each of the five dsRBP homologs in *Arabidopsis* (DRB1-5) and have investigated their roles in the miRNA, trans-acting siRNA, DNA methylation, mobile silencing signal and viral defence pathways. A comprehensive analysis using double and triple DRB mutants was undertaken and the results from this study will be presented.

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***Arabidopsis thaliana* trichomes as defense organs.** Manli Davis¹, Kengo Morohashi¹, Erich Grotewold¹, Rebecca Lamb¹. ¹The Ohio State University, Columbus, OH, USA

Control of gene transcription is of central importance in the development of any organism. Hierarchical arrangements (networks) of transcription factors (TFs) provide the information necessary to deploy genes with particular spatial and temporal patterns. It has been predicted that there are over 1,700 TFs encoded in the model plant species *Arabidopsis thaliana*. Many of these TFs are involved in various developmental processes but only a handful of them have known function. As part of our research on TFs, we are studying TFs involved in trichome initiation: GLABROUS3 (GL3)/ENHANCER OF GLABROUS3 (EGL3), GLABROUS1 (GL1) and TRANSPARENT TESTA GLABRA1 (TTG1). These genes encode bHLH, MYB and WD repeat proteins respectively; gl3 egl3 double mutants and gl1 or ttg1 single mutants are glabrous. These proteins have been shown to form a transcriptional complex necessary for trichome initiation. The identification of the targets of these genes will provide information about the developmental processes of trichome development and the regulatory network involved in this process. As a complement to this avenue of investigation, we are also comparing gene expression between gl3 egl3, gl1 and ttg1 mutants and wild type in order to identify other trichome-enriched genes: 88 genes have significantly reduced expression in all three mutant backgrounds. Several of these genes had been previously identified as genes with high expression in trichomes. Identification of such genes will provide information on what function(s) the mature trichomes play in *Arabidopsis*. The proteins encoded by the trichome-enriched genes are involved in biotic and/or abiotic responses in plants, based on both bioinformatics and functional data. We conclude that *Arabidopsis* trichomes function as defense organs by serving as both a physical and a molecular barrier.

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Involvement of the Histone Acetyltransferase AtHAC1 in the Regulation of Flowering Time via Repression of FLC in *Arabidopsis thaliana*. Weiwei Deng¹, Chunyan Liu¹, Yanxi Pei², Xian Deng¹, Lifang Niu¹, Xiaofeng Cao¹. ¹State Key Laboratory of Plant Genomics and Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China, ²College of Life Science and Technology, Shanxi University, Taiyuan, China

Histone acetylation is an important post-translational modification correlated with gene activation. In *Arabidopsis*, the histone acetyltransferase AtHAC1 is homologous to animal p300/CBP (CREB-binding protein) proteins, which are the main histone acetyltransferases participating in many physiological processes, including proliferation, differentiation and apoptosis. The functions of p300/CBP in animals are well characterized, whereas little is known about the roles of AtHAC1 in developmental control in *Arabidopsis*. Lesions in AtHAC1 caused pleiotropic developmental defects, including delayed flowering, a shortened primary root and partially reduced fertility. Analysis of the molecular basis of late-flowering in hac1 mutants showed that the hac1 plants respond normally to day-length, gibberellin acid (GA) treatment and vernalization. Furthermore, the expression level of the flowering repressor *FLOWERING LOCUS C* (*FLC*) is increased in hac1 mutants, indicating that the late-flowering phenotype of hac1 mutants is mediated by *FLC*. Since histone acetylation is usually associated with the activation of gene expression, histone modifications of *FLC* chromatin are not affected by mutations in HAC1 and expression levels of all known autonomous pathway genes are unchanged in hac1 plants, we propose that HAC1 affects flowering time by epigenetic modification of factors upstream of *FLC*.

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A common factor involved in root and shoot meristem development. Anja van Dijken¹, Ben Scheres¹, Renze Heidstra¹. ¹ Utrecht University, Dept. Molecular Genetics, Faculty of Sciences, Utrecht, The Netherlands

Plant post-embryonic development takes place in the meristems, where sets of stem cells self-renew and produce daughter cells that differentiate giving rise to different organ structures. Stem cell maintenance is dependent on organizing cells within the stem cell niches in the shoot and root meristems. To identify genes involved in the root organizer (or quiescent center, QC) and stem cell specification and maintenance we screened an EMS mutant population generated in a transgenic *Arabidopsis* line expressing two independent fluorescent QC markers for altered expression patterns.

Many isolated mutants displayed reduced expression of both QC markers in the early stages of seedling development. In several of these mutants columella stem cell identity was lost; suggesting disturbed signaling between QC and stem cells. Interestingly, the recessive mutant QC19-3, displays both a root and shoot meristem phenotype.

As early as globular embryo stages altered divisions in the root meristem occasionally occur. Root growth is also retarded; mutant root length is ~50% reduced and displays shorter meristem size. QC marker expression is reduced and frequently re-patterning of the root stem cell niche within the vasculature is observed indicating disturbed QC/stem cell maintenance. In later stages occasionally the root splits into two root tips that continue to grow slowly.

Mature embryos exhibit a reduced shoot apical meristem compared to wild type. After germination the QC19-3 mutant shoot meristem appears arrested; seedlings up to 11 days old do not show any leaf primordia before retarded shoots are initiated, resembling the *wus* mutant phenotype. Mature mutant plants do form small, serrated, pale leaves and develop small fertile flowers. Introduction of markers and genetic interaction studies are in progress to determine the genes involved.

In addition, a map based cloning approach is running to identify the gene responsible. Cloning the gene will shed light on its function in organizer/stem cell specification and maintenance and whether it is a common factor involved in root and shoot meristem development.

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Comparison of *Arabidopsis* growth analysis using traditional methods versus imaging techniques. Gonzalo Estavillo¹, Daniel Harris-Pascal¹, Robert Furbank², Barry Pogson¹. ¹The ARC Centre of Excellence in Plant Energy Biology, The Australian National University, Canberra, Australia.² CSIRO Plant Industry, Canberra, Australia

Growth analysis using simple primary data can be used to study plant morphology and function. The classical, destructive method involves tissue harvesting and estimation of different plant physical parameters such as leaf area and weight at regular intervals. An alternative method is the use of non invasive techniques based in image recording and software analyses that allows for periodical sampling in a non destructive way. In theory, this approach requires less starting material than the former, and the growth of the same individual can be documented through the life cycle or experimental conditions. It allows also for analysis of many other structural and morphological traits of a large number of plants.

Although the intrinsic advantages of the imaging system, the results should be compared with those of traditional techniques to assess its utility in specific cases. Here we compare the results of plant growth analyses of wild type and mutant *Arabidopsis* plants performed with the traditional method versus morphological phenotyping with Scanalyzer (Lemnatec).

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Analysis of compensation reveals three different modes that modulate cell expansion in determinate organs. Ali Ferjani¹, Naoko Ishikawa², Tetsuya Hisanaga³, Ushio Fujikura⁴, Minoru Kubo⁵, Taku Demura⁶, Hiroo Fukuda¹, Gorou Horiguchi¹, Hirokazu Tsukaya^{1,2}. ¹Graduate School of Science, The University of Tokyo,² National Institute for Basic Biology,³Faculty of Sciences, The University of Tokyo,⁴The Graduate University for Advanced Studies,⁵JST, ERATO,⁶RIKEN Plant Science Center

Leaf size is determined by the number and the size of leaf cells. Therefore, cell division and expansion processes are fundamental for leaf-size regulation. Organ-wide cross-talks between these two processes have been suggested, because inhibited cell division in leaves is compensated by increased cell size. This intriguing phenomenon that we named compensation has been frequently reported, however, it is not yet understood how, when and where it occurs. In this study, to answer these questions, we isolated five new *Arabidopsis thaliana* mutants (*fugu1-fugu5*) that exhibit compensation. *fugu* mutants were characterized together with *an3*, *erecta*, and a *KRP2 o/e*, previously reported to exhibit compensation. Time-course analyses of leaf development revealed that compensation in *fugu2-1*, *fugu5-1*, *an3-4*, and *er-102* mutants is induced post-mitotically, indicating that cell enlargement is not caused by the uncoupling of cell division from cell growth. In each of the mutants, either the rate (*fugu2-1*, *er-102*, *an3-4*) or duration (*fugu5-1*) of cell expansion was selectively enhanced. Interestingly, DNA microarray analysis in *fugu2* and *fugu5* revealed that the expression level of substantially different gene sets was affected, confirming that compensation in these mutants is regulated differently. In contrast, we found that compensation in *KRP2 o/e* occurs during cell proliferation. Compensation did not occur in roots despite decreased cell numbers. Flow cytometric analyses revealed that increases in ploidy level are not always required to trigger compensation, suggesting that compensation is only partially mediated by ploidy-dependent processes. Therefore, compensation indicates the presence of mechanisms that are specific to determinate organs and regulate their size by coordinating cell proliferation and expansion at least by three different modes.

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MAB4, a NPH3-like protein, is involved in polar auxin transport during *Arabidopsis* organogenesis. Masahiko Furutani¹, Takahiro Kajiwara¹, Takehide Kato¹, Masao Tasaka¹.¹ Graduate School of Biological Sciences, NAISt, Ikoma, Nara, Japan

In higher plants, aerial organs such as leaves and flowers are formed from the shoot apical meristem (SAM). Organogenesis is mediated by the localized concentration of phytohormone auxin. The local auxin accumulation is established by PINOID (PID), a Ser/Thr kinase, through the control of cellular localization of PIN-FORMED1 (PIN1) functioning as an auxin efflux carrier, in the SAM. To investigate molecular mechanisms by which auxin controls organogenesis, we analyzed the *macchi-bou 4* (*mab4*) mutant identified as a *pinoid* (*pid*) enhancer mutant. While *mab4* and *pid* single mutants displayed relatively mild cotyledon phenotypes, *pid mab4* double mutants completely lacked cotyledons. MAB4 encoded a novel protein, which belongs to the NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3) family supposed to function as a signal transducer. MAB4 mRNA was detected in the protodermal cell layer of the embryo and the meristem L1 layer at the site of organ initiation. In *mab4* embryos, the abundance of PIN1:green fluorescent protein (GFP) was severely reduced on the plasma membrane of the protodermal cell layer. In *pid mab4* embryos, PIN1-GFP polarity was completely reversed in the protodermal cell layer and auxin maxima were not established in the apex. In addition, subcellular localization analyses indicated that MAB4, localized in a subpopulation of endosomes and unidentified intracellular compartments, in part overlapped with PID in cultured *Arabidopsis* cells. These results suggested that MAB4 is involved in polar auxin transport in organogenesis in concert with *PID*.

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Isolation and characterization of novel transcriptional repressors of seed storage protein genes in "Arabidopsis". Ming-Jun Gao¹, Derek J Lydiate¹, Dwayne D Hegedus¹, Kevin Rozwadowski¹.¹ Molecular Genetics Section, Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada S7N 0X2

Progression through embryo development to seed maturity and the transition to germination and vegetative growth is subject to tight regulation by the cooperation of many factors. These include transcriptional activators such as ABI3, FUS3, LEC1 and LEC2, transcriptional repressors such as PKL, HSI2 and HSL1, the competing effects of phytohormones including ABA and GA, and metabolite signals such as sugars. Given that the transcriptional activators regulating seed development also play central roles in regulating physiological response and development in the growing plant, effective means of repressing expression of seed-specific genes exist to inhibit their ectopic expression and prevent negative pleiotropic effects. To date only a limited number of such repressors have been identified. "PKL" encodes a putative CHD3 chromatin remodelling factor and acts in conjunction with GA to repress embryonic identity during early stage of germination. HSI2 and HSL1 are B3- and EAR-domain containing transcriptional repressors of sugar-inducible expression of seed maturation genes during seedling growth. Here, we report the isolation and characterization of two novel transcriptional repressors of seed storage protein (SSP) genes isolated using yeast one-hybrid screening with putative negative cis-acting elements present in the promoters of SSP gene. Although disruption of either of the two repressor genes did not significantly affect seedling growth, their inactivation dramatically resulted in the expression of SSP genes in seedlings and increased accumulation of SSPs in developing seeds. Their mode of action in regulating the transition from vegetative to reproductive growth and seed development will be discussed.

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SCL15 regulates floral transition through an autonomous-independent pathway. Ming-Jun Gao¹, Abdelali Hannoufa¹.¹ Molecular Genetics Section, Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK S7N 0X2, Canada

The shoot apical meristem (SAM) gives rise to the aboveground portion of the plant, and the maintenance of SAM requires key signaling factors including WUSCHEL (WUS) and CLAVATA (CLVs). Floral meristem (FM) is formed on the flanks of the SAM and produces flowers after the plant has been induced to transit from vegetative to reproductive development. The WUS/CLV signaling pathway maintains the stem cells in the FM, and transition to flower development requires the activity of FM identity genes such as "LEAFY" ("LFY") and "APETALA1" ("AP1") and their antagonistic interaction with shoot identity gene "TERMINAL FLOWER 1" ("TER1"). Compared to the mode of action of homeotic ABC genes, little is known about upstream events leading to the regulation of the SAM and FAM identity genes. We have isolated a "Brassica napus" auxin responsive SCARECROW-like transcriptional activator, BnSCL1, based on its interaction with the histone deacetylase HDA19 (Gao "et al"., 2004, Plant Mol Biol, 55: 417). Here, we report that SCL15, an ortholog of BnSCL1, regulates floral transition through an autonomous-independent pathway. Disruption of SCL15 results in extremely delayed flowering and down-regulation of expression of the FM identity genes ("LFY", "WUS" and "UFO"), their upstream floral pathway integrator "FT", and their downstream homeotic genes ("AP1", "AP3", "PI", "AG" and "SEP3"). However, the expression of the homeotic floral organ identity gene "AP2" was not affected in the "SCL1" mutant. We demonstrate that SCL15 acts as a recruiting factor for HDA19 to regulate expression of reporter genes through recruitment of HDA19, and this repression is accompanied by an increase in histone deacetylation in the target promoter region, as determined by chromatin immunoprecipitation. We propose that SCL15 may function in recruiting HDA19 to repress a transcriptional repressor that is required to regulate the integrator "FT" and to control the transition from vegetative to flower development.

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The *Arabidopsis* Dof transcription factor DAG1 Is Involved in PhyB- and GA- mediated seed germination. Stefano Gabriele¹, Julie Martone¹, Gianluca Ragone², Luciana Corso¹, Paolo Costantino¹, Paola Vittorioso¹.¹ Dept. Genetics and Molecular Biology, Univ. La Sapienza, Rome, (Italy),² Istituto Dermatologico Italiano, Via dei Monti di Creta, Rome, (Italy)

In *Arabidopsis* there is a clear relationship between light and gibberellin signaling with respect to seed germination. It has been demonstrated that phytochrome B acts on seed germination through transcriptional regulation of both GA biosynthetic genes (*AtGA3ox1-2*) and GA deactivation genes (*AtGA2ox2*). We have previously shown that inactivation of the DAG1 gene, encoding a zinc finger transcription factor belonging to the Dof family, considerably alters the germination potential of seeds. In fact, our data clearly indicated that the *dag1* seeds have a higher sensitivity to red light and an altered level/sensitivity to endogenous GAs.

In order to unravel the role of DAG1 in PhyB- and GA- mediated seed germination, we produced double mutant of *dag1* with respectively the *phyB* null mutant and with *rgl2*, the master negative regulator GA-mediated seed germination.

Moreover we produced plants overexpressing PhyB in a *dag1* background compared to a Wt (Ws) background. Analyses of germination properties of both double mutants *dag1, phyB* and *dag1, rgl2* as well as of *dag1* overexpressing PhyB transformants have been performed.

Data arising from these analyses and a putative model of DAG1 function will be presented.

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THE AGAMOUS TARGET GENE ATH1 IS REQUIRED FOR DEVELOPMENT OF THE BASAL REGION OF THE FLOWER. Concepcion Gomez-Mena¹, Robert Sablowski¹. ¹ John Innes Centre, Norwich, NR4 7UH UK

Floral organs are initiated in the flanks of the floral meristems in concentric regions known as whorls. The identities of these primordia are specified by the combinatorial activity of 3 types of floral homeotic genes. These master genes must be central nodes in complex networks of genes that control the development of different floral organs. Despite many efforts, little is known about these gene networks functions.

In *Arabidopsis*, the floral homeotic gene *AG* specifies stamen and carpel identity. We have used an *AG* inducible system to identify genes activated by *AG* in early stages of flower development (Gómez-Mena et al 2005). Here, we report the functional characterization of one of these early targets of *AG*: *ATH1* (At4g322980), which belongs to the TALE superfamily of homeodomain proteins that also includes *BEL1*. We have identified an insertional mutant in the *ATH1* gene that initially appeared to be defective in floral organ abscission. In wt plants, intact floral organs are shed soon after anthesis, whereas in the *ath1* mutant they remained attached to the developing fruit indefinitely. However, a closer look revealed multiple defects in the basal region of the *ath1* mutant flower, including not only a failure to develop the abscission zone, but also partial organ fusions and small or absent nectaries. Ectopic expression of *ATH1* resulted in a compressed inflorescence that resembled the phenotype of mutants with defects in the synthesis or sensitivity to gibberellins. The *ath1* mutation modified the pattern of expression of a gibberellin 3-oxidase GUS reporter in young floral buds, supporting the idea that *ATH1* may control gibberellin biosynthesis. Together these results indicate that *ATH1* is required for the proper formation of multiple structures in the basal region of the flower, possibly through localized regulation of gibberellin biosynthesis.

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HAWAIIAN SKIRT -an F-box gene that regulates organ fusion and growth in *Arabidopsis*. Zinnia H Gonzalez-Carranza¹, Unchalee Rompa¹, Janny L Peters², Anuj Bhatt³, Carol Wagstaff⁴, Anthony Stead⁵, Jeremy A Roberts¹. ¹School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom., ²Faculty of Science, Radboud University Nijmegen, Nijmegen, The Netherlands., ³Department of Plant Sciences, University of Oxford, Oxford, United Kingdom., ⁴School of Biological Sciences, University of Southampton, Southampton, United Kingdom., ⁵School of Biological Sciences, Royal Holloway, University of London, Surrey, United Kingdom.

The *hawaiian skirt* (*hs*) mutant of *Arabidopsis* was identified from a fast neutron-mutagenised population of Col-0 WT. The site of the mutation has been mapped to chromosome 3 and found to be the consequence of a 28bp deletion that introduces a premature amber termination codon into the ORF of an F-box gene. The principle phenotypic feature associated with the mutant is the failure of floral organs to abscise and this is due to the fusion of the sepals along the lower part of their margins. By introgressing the abscission marker ProPGAZAT, GUS into the mutant it was possible to demonstrate that the differentiation of the abscission cells takes place but that cell separation is delayed in *hs*. Spatial and temporal characterisation of *HS* expression, using ProHS:GUS or ProHS:GFP fusions, has identified expression to take place at discrete sites throughout the plant. By comparing the *hs* mutant, Col-0 WT, and Pro35S:HS ectopically expressing lines we have demonstrated that the silencing of *HS* results in plants with larger leaves, roots and seeds, whilst over-expressing the gene produces the opposite effect. These results are discussed in the context of the role of *HS* during plant development.

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A role for the BAH-PHD containing protein SHORT LIFE (SHL) in the regulation of the floral transition in *Arabidopsis*. Leticia López-González¹, Laura Narro-Diego¹, José Miguel Martínez-Zapater², José A. Jarillo¹, Manuel Piñeiro¹. ¹ Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) Dpto. Biotecnología, Edificio Z. Carretera de La Coruña km 7. 28040 Madrid, Spain., ²Dpto. Genética Molecular de Plantas. Centro Nacional de Biotecnología-CSIC. C/ Darwin 3, Cantoblanco. 28049 Madrid, Spain

We are interested in understanding chromatin remodeling mechanisms involved in the repression of the floral transition, one of the most dramatic phase changes in plant development. Previously, we have identified *EARLY BOLTING IN SHORT DAYS* (*EBS*), a locus involved in the repression of flowering and the regulation of other developmental processes in *Arabidopsis* (Gómez-Mena et al., 2001). *EBS* is required to repress the expression of the floral integrator *FT* under non-inductive photoperiodic conditions short days and encodes a nuclear protein with a BAH and a PHD domain (Piñeiro et al., 2003). Both types of domains are thought to mediate protein-protein interactions and are present in chromatin remodeling factors. Proteins with the same modular architecture as *EBS* have been found in *Arabidopsis* and other plant species, but not in other organisms, suggesting that these proteins are part of a plant-specific family of transcriptional regulators. By analysing loss-of-function alleles we show that another member of this family in *Arabidopsis*, *SHL*, also has a role in the repression of flowering. Moreover, double mutant analyses suggest that *SHL* has partially overlapping functions with *EBS* in the control of the floral transition in *Arabidopsis*. Progress in the genetic and molecular analysis currently underway in our laboratory to understand the role of *SHL* in the control of flowering time will be presented. In addition, we will discuss natural variation data with implications in the evolution of this family of plant specific proteins likely involved in chromatin remodeling processes.

Gómez-Mena, C., et al. (2001) *Plant Cell* 13, 1011-1024.

Piñeiro, M., et al. (2003) *Plant Cell* 15, 1552-1562.

P-223

Delayed-fluorescence, a potential universal tool for the measurement of circadian rhythms in plants. Peter Gould¹, James Hartwell¹, Anthony Hall¹. ¹University of Liverpool

The endogenous 24 hour oscillator, the circadian clock, plays an important role in plants, enhancing performance and increasing vegetative yield. Much of our current understanding of the mechanism and function of the circadian clock in plants has come from the development of *Arabidopsis thaliana* as a model circadian organism. Key to this rapid progress has been the development of robust circadian markers, specifically clock driven promoter luciferase fusions. Studies of the clock in crop species and non-model organisms are currently hindered by the absence of a simple high-throughput universal assay for, clock function, accuracy and robustness. Delayed-fluorescence (DF) is a fundamental process occurring in all photosynthetic organisms. It is luminescence produced post-illumination as photosynthetic reaction centres decay to a ground state. Here, we report that the amount of DF oscillates with an approximately 24h period and in *Arabidopsis* is under the control of the circadian clock. Thus, delayed-fluorescence provides a simple clock output that potentially allows the clock to be assayed in-vivo in any photosynthetic organism. This simple high-throughput, non-transgenic assay could be integrated into crop breeding programmes, allowing the selection of plants that have robust and accurate clocks. It can also be used to rapidly characterise the role and function of *Arabidopsis* mutants in the circadian system.

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Investigation of HOX-Domain Containing Transcription Factors of Arabidopsis and Cotton Involved in Trichome Development. Xue-Ying Guan¹, Na Yu¹, Xiao-Xia Shanggu¹, Xiao-Ya Chen¹. ¹Institute of Plant Physiology and Ecology, Shanghai, China

Arabidopsis trichomes are single-celled epidermal hairs. Trichomes provide a valuable system for investigation of cell differentiation and cell-to-cell communication in plants. Several types of transcription factors that are involved in regulating trichome initiation and development have been identified, including a MYB protein GL1 (AT3G27920), a bHLH protein GL3 (AT5G41315), a WD40 domain-containing protein TTG1 (AT5G24520), and a HD-ZIP III protein GL2 (AT1G79840). Another three closely-related MYB like proteins, TRY (AT5G53200), CPC (AT2G46410) and ETC1 (AT1G01380), are known as negative factors to suppress trichome formation in the neighbor cells. Cotton fibers are trichomes of *Gossypium* ovules (seeds). Previously, we isolated a MYB gene, GaMYB2 (AY626160), from *G. arboreum*. GaMYB2 is highly expressed in fiber cells at early developmental stages; when expressed in gl1 mutant plants of Arabidopsis, it rescued trichome development to a wild-type level. We further analyzed three GL2-like genes of cotton (*G. arboreum*), namely GaHOX1, GaHOX2 and GaHOX3. These three genes show different expression patterns in cotton tissues, and they could rescue the Arabidopsis gl2 mutant phenotype either completely or in certain conditions. We found that the HD-ZIP III proteins interact with each other to exert their functional activity.

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Monitoring root elongation and lateral root generation in *Arabidopsis* via regulating *GNOM* expression by an inducible anti-sense system. Jingzhe Guo¹, Jun Wei¹, Mengxiang Sun¹. ¹Key Laboratory of MOE for Plant Developmental Biology, College of Life Sciences, Wuhan University, Wuhan 430072, China

The *Arabidopsis GNOM* gene encodes an ARF GDP/GTP exchange factor. Studies of strong *gnom* mutants show that *GNOM* is involved in embryonic axis formation and polar localization of the auxin efflux carrier PIN1. Subsequent analysis of weak *gnom* mutants also reveals its important role in post-embryogenesis. We established a *GNOM* inducible anti-sense system to further study the role of *GNOM* during plant growth. It enables to suppress *GNOM* expression in specific tissues and at specific stage of plant development. Thus it offers a unique opportunity to seek how auxin transport and polar distribution could regulate root generation and development in terms of their normal pattern and level are temporally and spatially interrupted.

Our data show that root elongation of induced seedlings was obviously less than that of control, and the main reason for it is that the root cell elongation in root differentiation zone was greatly inhibited, but not the meristem activity was reduced. Such inducible phenomenon is found dose-dependent. When grew on plates containing increasing concentration of NAA, inhibition of root elongation in induced seedlings was getting severer, but this was not the case for 2, 4-D. This confirms that *GNOM* does regulate auxin efflux transport. However, when inducer was removed from medium, normal root elongation was observed again. The cell elongation can restore similar to wide type after withdrawing the inducer. Thus, root elongation was reversibly controlled by regulating *GNOM* expression and interrupting the auxin distribution temporally and spatially. Lateral roots formation was also greatly inhibited when *GNOM* was down regulated. However, there was almost no emergence of lateral roots after withdrawing the inducer. The lateral roots could only generate again in the presence of exogenous NAA.

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Isolation and Characterization of A Male Sterile Mutant Which Has Defect in Exine Patterning of *Arabidopsis thaliana*

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A novel mutant of *Arabidopsis thaliana* was isolated from an activation tagging mutant pool. The homozygous mutant exhibited dramatically reduced fertility with otherwise normal vegetative development. Megagametogenesis and male meiosis of mutant is not affected. Whereas, inhibited stamen extension and aborted pollen development was observed after microspore release. SEM (Scanning Electron Microscopy) analysis revealed that sporopollenin deposited randomly on surface of most of the pollen grains, resulting a spotted exine pattern instead of reticulate pattern.

To identify the corresponding gene, we performed thermal asymmetric interlaced PCR (TAIL-PCR) to obtain the genomic flanking sequences adjacent to T-DNA. Sequencing of flanking sequence indicated that the T-DNA inserted in the last intron of a gene encoding a putative transmembrane protein. GFP fusion and tissue specific RT-PCR indicated that the gene was expressed high specifically in reproductive organ of *Arabidopsis*, especially in anther. Our results provide further insight into the molecular basis of plant male reproductive development.

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Role of the MNP subunits in COP9 signalosome assembly and activity and their functional interaction with CUL3-based E3 ligases. Giuliana Gusmaroli¹, Pablo Figueiroa², Giovanna Serino³, Xing Wang Deng^{1,1}Yale University, New Haven, (CT), USA,²University of Delaware, Newark, (DE), USA,³Università La Sapienza, Roma , ITALY

The COP9 signalosome is an evolutionarily conserved multisubunit protein complex involved in the regulation of several developmental processes. Among its eight subunits, CSN5 and CSN6 contain a characteristic MPN domain and, in Arabidopsis, are encoded by families of two genes. Here we report the characterization of both MPN subunits using a series of single and double mutants within each gene family. Our results indicate that, while CSN6A and CSN6B retain mostly redundant functions, CSN5A and CSN5B have functionally diversified. Loss of CSN5A triggers severe pleiotropic developmental defects, whereas the complete depletion of either of the two MPN members results in CSN disassembly and instability of several subunits, along with the complete loss of CUL1, CUL3 and CUL4 de-rubylation. Furthermore, we demonstrate that CSN interact with CUL3, in addition to CUL1 and CUL4, and that the lack of CSN activity differentially affects the stability of those three cullins. In fact, while CUL1 levels remain unchanged, CUL4 is up regulated and CUL3 is strongly down regulated. Interestingly, we also show that optimal CUL3 activity is required to maintain the cellular pool of CSN5, through a post-transcriptional mechanism. In fact partial loss of CUL3 activity results in CSN5 accumulation and suppression of the pleiotropic phenotype of a weak *csn5* mutant. Our data suggest the existence of a reciprocal regulation between CUL3 and CSN5 accumulation. The present study thus completes the genetic analysis of all CSN subunits, and confirms the structural interdependency between PCI and MPN subunits in functional CSN complex formation. Finally, since all the lethal *csn* null mutants lack the entire CSN complex, we were interested in establishing if cullin de-rubylation is the only CSN essential function. We generated transgenic *csn* mutants carrying a fully assembled COP9 signalosome complex specifically lacking the cullin de-rubylation activity. The effect of this "catalytically dead" complex on *csn* mutant viability will be presented.

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BLADE-ON-PETIOLE1 and 2 Control Arabidopsis Lateral Organ Fate Through Activation of LOB-Domain Genes and Regulation of Organ Polarity Genes. CHAN MAN HA^{1,2}, JI HYUNG JUN¹, HONG GIL NAM², JENNIFER FLETCHER^{1,1} USDA/UC BERKELEY, PLANT GENE EXPRESSION CENTER, 800 BUCHANAN STREET, CA 94710, USA,² DEPARTMENT OF LIFE SCIENCE, POSTECH, PO-HANG, KYUNGBUK 790-784, KOREA

Continuous lateral organ formation is critical for higher plants to produce their characteristic architectures, but the regulatory pathways that specify organ cell fate are still poorly understood. Here, we present a novel function for the putative transcriptional regulators BLADE-ON-PETIOLE1 (BOP1) and BOP2 in the control of LATERAL ORGAN BOUNDARIES (LOB)-domain (LBD) and adaxial-abaxial polarity gene expression during *Arabidopsis* lateral organ development. 35S:BOP1 and 35S:BOP2 plants exhibit a very short and compact stature, hyponastic leaves, and downward-orienting siliques.

We show that three LBD genes, ASYMMETRIC LEAVES2 (AS2), LOB and LBD36/ASL1, are up-regulated in 35S:BOP and down-regulated in bop mutant plants. Ectopic activation of BOP1 or BOP2 also results in repression of class I knox gene expression. In addition, some 35S:BOP phenotypes are eliminated in the *as2* and *as1* mutant backgrounds. We demonstrate a role for BOP1 and BOP2 in the establishment of the adaxial-abaxial polarity axis in the leaf petiole, where they regulate PHB and FIL expression and overlap in function with AS1 and AS2. Interestingly, we find that KANADI1 (KAN1) and KAN2 promote adaxial organ identity as well as abaxial organ identity.

Our data indicate that BOP1 and BOP2 act at the lateral organ boundary to repress genes that promote meristem cell fate and to induce genes that promote lateral organ fate and polarity, thereby restricting the developmental potential of the organ-forming cells and facilitating their differentiation.

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Manipulating cell cycle components during male gametogenesis using pollen cell-specific molecular tools. Said Hafidh and David Twell Department of Biology, Univers¹.¹ UNIVERSITY OF LEICESTER

Double fertilization is one of the defining characteristics of angiosperms and depends upon the production of twin sperm cells. The formation of two sperm cells is controlled by two division events that are essential for correct cell fate specification and successful fertilization. We are interested in identifying core cell cycle genes involved in the control of cell division during male gametogenesis. However, lack of tools for manipulating gene expression during pollen development has been a hindrance for the study of gene function in the male germline.

RNA interference (RNAi) is currently being used as an approach for studying gene function. This pathway is conserved in eukaryotes mediating sequence specific transcriptional silencing and degradation of cytoplasmic RNA through short interfering (si) RNAs. We showed that some of the core components involved, Argonaute-like (AGO)s and Dicer-like (DCL) genes, are expressed throughout pollen development. We have developed pollen cell-specific molecular tools for the production of hairpin double stranded RNA to down regulate target genes, and pollen cell-specific over-expression vectors.

The progression through G2/M transition is regulated by different classes of B-type cyclins in combination with CDKA. To identify the B-type cyclins potentially involved in sperm cell formation, we have applied the tools we have generated to manipulate expression of Cyclin B1 family during pollen development. Our data support an essential role of AtCycB1 family for the completion of pollen mitosis I and pollen mitosis II. Moreover, using AtCycB1; 1 translational-reporter fusion construct, we could show cycling of CYCB1; 1 protein during the two pollen cell division events, highlighting the existence of anaphase promoting complex activity in the male gametophyte.

We further illustrate that AtDUO1, an R2R3-MYB transcription factor regulating cell fate specification, is also required for AtCycB1; 1 expression in the generative cell. Therefore, we demonstrate an important link between cell fate specification and core cell cycle components regulating G2/M transition in the plant male germ line.

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A heat shock transcription factor involved in *Arabidopsis* root stem cell patterning and maintenance. Colette A. ten Hove¹, Wouter J. de Vries¹, Ben Scheres¹, Renze Heidstra¹.¹ Department of Molecular Genetics, Utrecht University, Utrecht, The Netherlands

In the heart of the root meristem of the plant *Arabidopsis thaliana* resides a group of stem cells that generate all the cells that make up the root and sustain growth. These stem cells surround a group of mitotically inactive cells called the quiescent center (QC) that is required for stem cell maintenance. Together the QC and stem cells form a so-called stem cell niche. The combinatorial action of the plant specific auxin dependent AP2 transcription factors the PLETHORA (PLT) genes, in parallel with the GRAS family transcription factors SHORTROOT (SHR) and SCARECROW (SCR) specify the stem cell niche. To find missing links and additional genes involved in stem cell patterning and maintenance we have performed an EMS mutagenesis screen in a double fluorescent QC-marker bearing background. The *schizoriza* mutant was identified because of its disturbed fluorescence QC-marker expression and positional cloning revealed that the affected gene encoded a heat stress transcription factor. SCHIZORIZA appears to be a cell fate determinant as the *schizoriza* mutant shows a loss of QC and columella stem cells and misspecification of lateral root cap/epidermis, cortex/endodermis stem cells leading to major defects in root pattern formation. Until now not much was known about other functions of heat stress transcription factors besides their role in the heat stress response. Here we provide evidence for a role in development.

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PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. Hugo Hofhuis¹, Carla Galinha², Marijn Luijten¹, Viola Willemsen¹, Ikram Blilou¹, Renze Heidstra¹, Ben Scheres¹.¹ Utrecht University, ²University of Oxford

Factors with a graded distribution can program fields of cells in a dose-dependent manner, but no evidence has hitherto surfaced for such mechanisms in plants. In the *Arabidopsis* root, two "PLETHORA (PLT)" genes, encoding AP2-domain transcription factors, have been shown to maintain the activity of stem cells. Here, we show that a clade of four "PLT" homologues is necessary for root formation. Promoter activity and protein fusions of PLT homologues display gradient distributions with maxima in the stem cell area. PLT activities are largely additive, dosage dependent and their functions in separate expression domains can be uncoupled: high levels of PLT activity promote stem cell identity and maintenance; lower levels promote mitotic activity of stem cell daughters; and further reduction in levels is required for cell differentiation. Our findings indicate that PLT protein dosage is translated into distinct cellular responses in plant development.

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Functional Characterization of STICHEL gene in *Arabidopsis thaliana*. Cho-Chun Huang¹, Martin Huelkamp¹.¹ Department III of Botanical Institute, University of Cologne, Germany

The STICHEL (STI) gene plays an important role in the regulation of branch number of the unicellular trichomes in *Arabidopsis* (Ilgenfritz et al., 2003). GFP-STI protein fusions in trichome cell indicate that the localization of STI in the cortex and that it is concentrated at the tip of trichomes. Mutation analysis with the conserved residue of the AT-Pase domain showed that the domain is essential for STICHEL protein (STI) to regulate trichome branching. Interestingly, yeast two hybrid assay and BiFC analysis showed that STI interacts with itself and might act as oligomers. Progress on this work will be presented.

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The Interaction of LATERAL ORGAN BOUNDARIES (LOB) with basic-Helix-Loop-Helix Transcription Factor bHLH048 Inhibits DNA-Binding Activity. Aman Husbands¹, Elizabeth Bell², Harley Smith¹, Patricia Springer¹. ¹UC Riverside, Riverside, CA, USA, ²John Innes Centre, Norwich, UK

LATERAL ORGAN BOUNDARIES (LOB) is the founding member of the LOB DOMAIN (LBD) gene family, a novel plant-specific family of transcription factors. This family is defined by the conserved LOB domain, a stretch of approximately 100 aa, shared by each member of the LBD family. Originally isolated from an enhancer trap screen, the function of LOB is still unknown, although there is some data suggesting roles in boundary specification and brassinosteroid signalling. To determine whether LOB had the ability to bind DNA, a Selection and Amplification Binding (SAAB) assay was performed and identified the nucleotide sequence GCGGCC as a cis-element bound by the LOB domain. Electrophoretic Mobility Shift Assays (EMSA) confirmed that this sequence is bound by the LOB domain of LOB, and by other LBD proteins, supporting the hypothesis that this family of transcription factors binds the same cis-element. A yeast-2-hybrid screen identified bHLH048, a member of the basic-helix-loop-helix (bHLH) family of transcription factors, as an interacting partner of LOB. This bHLH is predicted to be a non-DNA binding protein, but preliminary results suggest that bHLH048 homodimers recognize a G-box motif, the previously characterized binding site for the bHLHs. When complexed with LOB, bHLH048 appears to reduce the affinity of LOB for the LBD binding site. Related bHLH genes have been shown to be involved in brassinosteroid signalling, and preliminary data from the Springer lab shows that LOB also regulates BR responses. Interaction of these two transcription factors at the protein level, and the concomitant inhibition of DNA-binding activity, may represent a control point whereby LOB's downstream effect on brassinosteroid signalling is modulated by bHLH048.

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The *Arabidopsis BEATNIK* gene acts as a repressor of auxin gradient-directed polar root hair positioning. Yoshihisa Ikeda¹, Karin Ljung¹, Markus Grebe¹. ¹Ume Plant Science Centre, Dept. of Forest Genetics & Plant Physiology, SLU, SE-90183 Ume , Sweden

The polarity of single cells is commonly coordinated within the plane of a single tissue layer. In animals, this phenomenon is referred to as planar polarity and has been highly successfully investigated e. g. during *Drosophila* wing hair positioning. We study plant-specific planar polarity of root hair positioning in the *Arabidopsis* root epidermis, where hairs are strictly formed close to basal ends of hair-forming cells [1-3]. Hair position along the trichoblast is directed toward the maximum concentration of an auxin gradient in the root tip [2,4,5]. We recently demonstrated that shaping this gradient requires combined function of the auxin influx carrier AUX1, ETHYLENE INSENSITIVE2 (EIN2), and GNOM genes which converges prior to polar Rho-of-plant (ROP) recruitment to the hair initiation site. Moreover, local auxin gradients can coordinate planar root hair positioning [5]. In screens for mutants with altered hair placement, we have identified the recessive *beatnik* (*btk*) mutation. *btk* causes root-specific phenotypes, including long hair and hyperpolar hair initiation at basal-most ends of trichoblasts. We show that in *btk*, F-actin prematurely accumulates in the hair bulge and ROPs localize to the hair initiation site at basal-most ends of cells with enhanced polarity. The *btk* phenotype can be suppressed by *act2*, weak *gnom*, by *aux1* and *ein2* mutations, indicating that *BTK* acts as a repressor of coordinated polarity upstream of these genes. The auxin concentration gradient and auxin biosynthesis rates are enhanced in *btk* root tips, suggesting that *BTK* acts on planar polarity as a repressor of auxin gradient function. We report progress on identification of the *btk* mutation and analysis of *btk* loss-of-function mosaics to study its local effect on planar polarity.

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P-234**Identification of SUF4 protein complex in *Arabidopsis***

Hyun-Ju Hwang¹, Kyuha Choi¹, Sanghee Kim², Youngmi Lee¹, Ilha Lee¹. ¹Department of Biological Sciences, Seoul National University, Korea, ²Laboratory of Plant Molecular Biology, Rockefeller University, New York, USA.

We previously reported that PPRESSOR OF FRIGIDA4 encoding a C2H2-type zinc finger protein, represses flowering by transcriptional activation of *Arabidopsis* FLOWERING LOCUS C (2006, The Plant Cell, 18, 2985-2998). *FLC* encodes a MADS box transcription factor to act as a floral repressor. *FRI*, encoding a coiled-coil protein, is an *FLC* activator. 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 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 this complex in diverse background by gel filtration. Also, we generated the epitope tagged transgenic plants and double mutants among them for immunoprecipitation.

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Identification and analysis of functional domains of the bi-functional transcription factor, WUSCHEL. Miho Ikeda¹, Masaru Ohme-Takagi^{1,1}. Advanced Industrial Science and Technology, Central 4, Higashi 1-1-1, Tsukuba, Ibaraki, Japan

WUSCHEL is known to be a multiple functional gene, which is involved in the maintenance of shoot apical meristem (SAM), transition of developmental phase and phytohormone signaling. *WUSCHEL* is an activator of *AGAMOUS* but acts as a repressor for *ARR* genes. This suggests that *WUSCHEL* is a bifunctional transcription factor, which acts as activator or repressor depending on the target gene. But the functional mechanism of *WUSCHEL* gene was not fully analyzed. Transient expression assay showed that *WUSCHEL*, as it is, acted as a repressor and a series of mutation analysis revealed that *WUSCHEL* contained two independent repression domains. One is the EAR-like motif that was previously reported as a repression domain conserved in various transcriptional active repressors, and the other was a novel domain that was conserved among *WUSCHEL*-related homeobox genes and all of the known *WUSCHEL* ortholog genes. On the other hands, mutation analyses showed that *WUSCHEL* had an activation domain. Analysis of the transgenic plants that respectively expressed a series of mutated *WUSCHEL* gene revealed that the maintenance of SAM and the induction of embryonic gene expressions in germinated plants were regulated by the repression activity of *WUSCHEL* in addition to the regulation of *ARR* gene expressions. We also identified that the novel repression domain mainly regulated cytokinin signaling, maintenance of SAM and developmental phase transition (embryonic to vegetative), while the EAR-like domain has a supportive function of these physiological phenomena.

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Functional analysis of TEBICHI in meristem maintenance and organ development in *Arabidopsis thaliana*. Soichi Inagaki¹, Kenzo Nakamura¹, Atsushi Morikami². ¹Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, ²Department of Agrobiological Resources, Meijo University, Nagoya, Japan

TEBICHI (TEB) of *Arabidopsis thaliana* is homologous to *Drosophila* MUS308 and mammalian POLQ with DNA helicase and DNA polymerase domains. Loss-of-function mutant, *teb*, shows various morphological defects such as short roots, asymmetric leaves, and fasciated stems, that are accompanied with defects in cell division patterns and cell differentiation in embryo and meristems. In *teb* mutants, response to DNA-damage is constitutively activated, and cells expressing *cyclinB1;1:GUS* are accumulated in meristems, suggesting that DNA damage accumulated in *teb* mutants activates cell cycle checkpoint, leading to a defect in G2/M cell cycle progression. To further analyze the function of TEB, we analyzed the genetic interaction of *TEB* with *ATR*, which is involved in cellular responses to defects in DNA replication. We observed that accumulation of cells expressing *cyclinB1;1:GUS* in *teb* was rather suppressed by *atr* mutation, suggesting that the ATR-dependent G2/M cell cycle checkpoint is activated in *teb*. However, *atr* mutation enhanced morphological phenotypes of *teb* mutants such as defects of growth of roots and aerial parts, as well as abnormal leaf shapes. These results suggest that defects which occur in association with DNA replication, rather than defects in cell cycle progression, are important in causing the morphological defects in *teb* mutants. *teb atr* double mutants frequently developed needle-like leaves, suggesting that *teb atr* is defective in adaxial-abaxial polarity of leaves. Histochemical analysis of expression of abaxial-specific *FILAMENTOUS FLOWER* showed that leaves of *teb* and *teb atr* were partially abaxialized, suggesting that TEB is involved in the establishment of adaxial-abaxial polarity of leaves. Altogether, our results suggest that proper replication of genome in the S-phase is critical for the proper expression of developmentally regulated genes.

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Cytokinins Determine *Arabidopsis* Root-Meristem Size by Controlling Cell Differentiation. Raffaele Dello Ilio¹, Francisco Scaglia Linhares¹, Emanuele Scacchi¹, Eva Casamitjana-Martinez², Renze Heidstra², Paolo Costantino², Sabrina Sabatini¹. ¹University ? La Sapienza?, Rome, Italy, ²Utrecht University, Utrecht, The Netherlands

Plant postembryonic development takes place in themeristems, where stem cells self-renew and produced daughter cells that differentiate and give rise to different organ structures. For the maintenance of meristems, the rate of differentiation of daughter cells must equal the generation of new cells: How this is achieved is a central question in plant development. In the *Arabidopsis* root meristem, stem cells surround a small group of organizing cells, the quiescent center. Together they form a stem cell niche, whose position and activity depends on the combinatorial action of two sets of genes-*PLETHORA1* (*PLT1*) and *PLETHORA2* (*PLT2*) and *SCARECROW* (*SCR*) and *SHORTROOT* (*SHR*)-as well as on polar auxin transport. In contrast, the mechanisms controlling meristematic cell differentiation remain unclear. Here, we report that cytokinins control the rate of meristematic cell differentiation and thus determine root meristem size via a two-component receptor histidine kinase-transcription factor signaling pathway. Analysis of the root meristems of cytokinin mutants, spatial cytokinin depletion, and exogenous cytokinin application indicates that cytokinins act in a restricted region of the root meristem, where they antagonize a noncell-autonomous cell-division signal, and we provide evidence that this signal is auxin.

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A semi-dominant mutation in a ribosomal protein L10 gene suppresses the dwarf phenotype of the *acl5* mutant. Akihiro Imai^{1,2}, Mio Komura¹, Eri Kawano¹, Taku Takahashi¹. ¹Okayama University, Okayama, Japan, ²National Institute for Basic Biology, Okazaki, Japan

Disruption of the *Arabidopsis thaliana* ACAULIS5 (ACL5) gene, which encodes spermine synthase, results in a severely dwarfed phenotype. A previous study showed that *sac51-d*, a dominant extragenic suppressor mutant of *acl5-1*, has premature termination codon in an upstream open reading frame (uORF) of *SAC51*, which encodes a putative transcription factor, and suggested the involvement of its uORF-dependent translational control in the *ACL5*-mediated stem growth. Here we report the identification of a gene responsible for the second suppressor mutant of *acl5-1*, *sac52-d*. *SAC52* encodes a ribosomal protein L10 (RPL10A), which is highly conserved among eukaryotes and thought to be a translational regulator. Transgenic insertion of a genomic fragment containing the semi-dominant *sac52-d* allele into *acl5-1* mutants rescued the dwarf phenotype of *acl5-1*. GUS reporter activity under the control of the *SAC51* promoter with its uORFs was higher in *acl5-1 sac52-d* than that in *acl5-1*, suggesting that the suppression of the *acl5-1* phenotype by *sac52-d* is attributable, at least in part, to the enhanced translation of *SAC51*.

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Role of TTG2 in genetic network of epidermal cell differentiation in *Arabidopsis*. Tetsuya Ishida¹, Sayoko Hattori¹, Kiyotaka Okada^{1,2}, Takuji Wada¹. ¹RIKEN PSC, Yokohama, Kanagawa, Japan,²Graduate School of Science, Kyoto Univ., Kyoto, Japan

Root epidermal cells differentiate into either root hair cell or hair-less cell in *Arabidopsis*. In current model, a transcriptional complex consisting of WER (R2R3-MYB transcription factor), GL3/EGL3 (bHLH transcription factor) and TTG1 (WD40 repeat protein) promotes GL2 (encoding an HD-Zip transcription factor) expression, which results in differentiation into hair-less cell. CPC (R3-MYB protein) inhibits formation of the WER-GL3/EGL3-TTG1 complex to repress GL2 expression, resulting in differentiation into root hair cell.

TTG2, encoding a WRKY transcription factor, regulates trichome development. TTG2 is expressed in atrichoblasts of roots as well as in trichomes. We found that TTG2 expression in roots was directly promoted by the WER-GL3/EGL3-TTG1 complex and was repressed by CPC. Transgenic *Arabidopsis* expressing chimeric TTG2 protein fused with transcription repressor domain (TTG2:SRDX) formed root hairs from all epidermal cells of roots. In TTG2:SRDX lines, GL2 expression were repressed in roots, suggesting a possibility that TTG2 promotes GL2 expression.

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The ASYMMETRIC LEAVES2 gene regulates adaxial cell proliferation involved in the development of symmetric and flat leaf laminae. Hidekazu Iwakawa¹, Mayumi Iwasaki¹, Hiroo Takahashi², Takeshi Kobayashi^{1,3}, Yasunori Machida⁴, Chiyo Machida^{1,3}. ¹Plant Biology Research Center, Chubu Univ., Kasugai, Japan,²Grad. Sch. of Eng., Nagoya Univ., Nagoya, Japan,³College of Biosci. and Biotech., Chubu Univ., Kasugai, Japan,⁴Grad. Sch. of Sci., Nagoya Univ., Nagoya, Japan

Leaves of angiosperms exhibit remarkable diversity in terms of both shape and complexity. Nonetheless, plants appear to exploit common mechanisms that are responsible for the establishment of three axes during leaf development. Leaves are generated as lateral organs from a shoot apical meristem and develop along the axes, proximal-distal, adaxial-abaxial and medial-lateral axes. The AS2, a member of the AS2/LOB family, and AS1 play important roles to establish these axes. Here we report the patterns of expression of these genes and the importance of the sites of such expression in leaf development. Transcripts of both genes accumulated in entire leaf primordia at early stages but patterns of accumulation changed as leaves expanded. AS2 and AS1 transcripts were detected, respectively, in the adaxial domain and in the inner domain between the adaxial and abaxial domains of leaves. Ratios of numbers of adaxial cells to abaxial cells in cotyledons of corresponding mutants were larger than ratios in wild-type cotyledons. Low level expression of AS2 under the control of AS1 promoter in as2 restored an almost normal phenotype in some cases but also resulted in flatter leaves than those of wild-type. Strong expression of the construct in wild-type and as2, but not as1, resulted in the formation of upwardly curled leaves. Our results indicate that AS2 represses cell proliferation in the adaxial domain in the presence of AS1 and that adaxial expression of AS2 at appropriate level is critical for development of flat and symmetrical laminae. Real-time RT-PCR analysis revealed that mutation of either AS2 or AS1 resulted in increased levels of transcripts of ETT/ARF3, KAN2 and YAB5. Thus, AS2 and AS1 might negatively regulate these genes in the adaxial domain, which might be related to the development of flat and expanded leaves.

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Development of tapetum-specific organelles involved in the formation of pollen coats in *Arabidopsis*. Sumie Ishiguro¹, Toshiya Suzuki¹, Yuka Nishimori¹, Michika Sassa¹, Kenzo Nakamura¹. ¹Grad. Sch. Bioagricultural Sci., Nagoya Univ., Nagoya, Japan

The pollen coat, which is a surface component of pollen grains, consists mainly of proteins and lipidic compounds such as sterol esters and hydrocarbons. To study how the pollen coat is produced, we identified and characterized a conditional male-sterile mutant, flaky pollen-1 (*fkp1-1*), whose pollen grains lack the pollen coat. *FKP1* encodes 3-hydroxy-3-methylglutaryl-coenzyme A synthase, an enzyme of mevalonate pathway required for the biosynthesis of terpenoids including sterols. The *fkp1-1* possesses a T-DNA insertion 550 bp upstream of the initiation codon. RT-PCR and promoter analyses revealed that the T-DNA insertion results in knockdown of *FKP1* predominantly in tapetum. The tapetal cells contain two classes of specific organelles, elaioplasts and tapetosomes. The elaioplasts are plastids that accumulate sterol esters, whereas the tapetosomes derived from endoplasmic reticulum show a structural similarity to seed oil bodies, which contain vast amount of triglycerides. These organelles are partially degraded immediately after the rupture of tapetal cells and become components of pollen coats. Electron microscopy showed that the *fkp1-1* mutation affected the development of both organelles, which caused the coatless phenotypes of *fkp1-1* pollen grains. These results suggest that not only the elaioplasts but also the tapetosomes, which contain less-amount of sterols, require the mevalonate pathway for development. In order to visualize the development of these organelles, we constructed a GFP-fusion of *FBP1*, an elaioplast-localized fibrillin-like protein, and a GFP-fusion of *GRP17*, a tapetosome-specific oleosin protein. When we expressed these proteins under their own promoters, many fluorescent particles are observed in developing tapetal cells. Then only the *GRP17*-fused GFP was deposited on the surface of pollen grains, which is consistent with the data from pollen coat proteome. We concluded that the GFP fluorescence *in planta* reflects the development and degradation of two organelles as well as the fates of two proteins.

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The ASYMMETRIC LEAVES2 regulates leaf polarity by repressing ETTIN in Arabidopsis. Mayumi Iwasaki¹, Hidekazu Iwakawa¹, Yoshihisa Ueno², Hiroo Takahashi³, Shoko Kojima^{1,4}, Takeshi Kobayashi^{1,4}, Yasunori Machida², Chiyoko Machida^{1,4}. ¹Plant Biology Research Center, Chubu Univ., Kasugai, Japan, ²2 Grad. Sch. of Sci., Nagoya Univ., Nagoya, Japan, ³Grad. Sch. of Eng., Nagoya Univ., Nagoya, Japan, ⁴College of Biosci. and Biotech., Chubu Univ., Kasugai, Japan

The ASYMMETRIC LEAVES2 (AS2) and ASYMMETRIC LEAVES1 (AS1) genes of *Arabidopsis thaliana* are required for symmetrical and flat lamina expansion. AS2 encodes a plant specific protein that contains AS2/LOB domain, and AS1 encodes a myb (SANT) domain protein. AS2 and AS1 are thought to act as a transcriptional regulator of certain genes including class 1 KNOX genes. To identify the downstream target of AS2, microarray analysis of mRNA from shoot apices was performed. Expression levels of several genes involving abaxial identity were altered in as1, as2, and pAS1::AS2 plants. We examined the expression of ARF2, ETT/ARF3, ARF4, four KANADI genes, five YABBY genes and the class 1 KNOX genes by quantitative RT-PCR. ETT, KAN2, YABBY5, as well as BP, were upregulated in as1 and as2 and downregulated in pAS1::AS2 plants. AS2 and AS1 might regulate adaxial identity by repressing these genes. To assess whether AS2 acts directly regulates ETT, KAN2, YABBY5, and BP, we used glucocorticoid receptor (GR)-mediated inducible system. After the treatment 35S:AS2-GR plants with DEX, expression of ETT and BP were decreased, but those of KAN2 and YABBY5 were not changed within 12 hours. ETT is known to be a target of tasiR-ARF. We report how AS2 regulates the ETT expression in the process of leaf development.

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Identification of STM protein interactors involved in flowering. Ricardo Junqueira¹, Siddhartha Kanrar¹, Harley M. S. Smith¹. ¹University of California, Riverside, CA, USA

In higher plants, shoot development is characterized by the continuous production of organs initiated on the flanks of the shoot apical meristem (SAM). The activity of the SAM is differentially regulated throughout plant development and the identity of initiated lateral organs corresponds to the plant developmental phase. The homeodomain transcription factor SHOOTMERISTEMLESS (STM) is essential for meristem maintenance. In addition, the weak allele *stm-10* displays a late flowering phenotype with defects in floral specification during inflorescence development, suggesting that STM is involved in promotion of flowering. To gain further insights into the role of STM, a yeast two-hybrid screen was performed in order to identify proteins involved in flowering that associate with this homeodomain protein. The results will be discussed. Supported by NSF Grant IOB-0615774 and CAPES

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Molecular Genetic analysis of cell division effects in leaf morphogenesis. Sang Eun Jun¹, Kiu-Hyung Cho¹, Yoon-Ah Byun¹, Masaaki Umeda², Gyoung-Tae Kim^{1,3}. ¹Division of Molecular Biotechnology, Dong-A University, Busan 604-714, South Korea, ²Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara 630-0101, Japan, ³Environmental Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, South Korea

As plants grow, cell division plays an important role in which plants undergo normal developmental process and form appropriate shape. There are 7 inhibitors of cyclin-dependent kinase (ICK/KRP) which inhibit CDK activity by tight interaction with cyclin-CDK complexes in *Arabidopsis*. To investigate how KRPs affect leaf morphology, we construct transgenic plants overexpressing KRPs under the control of the CaMV 35S promoter.

In our study, common phenotypes of each transgenic plant which overexpresses KRPs, such as reduced size of whole plant, reduced size and serration of leaves and floral organs, and production of small siliques with fewer seeds, were characterized.

Transgenic plants overexpressing KRP1 and KRP5 show similar serration phenotypes. However, transgenic plants overexpressing KRP1 (Group I) have round leaves whereas those of KRP5 (Group II) show narrow leaves. Interestingly, the phenotype of bifurcate leaves with serration was observed only in transgenic plants overexpressing Group III KRPs. In addition, rolling up phenotype of leaves, and rough and curvature phenotype on leaf surface were observed in transgenic plants overexpressing KRP6.

Taken together, we will discuss about the roles of KRPs and cell division in leaf morphogenesis from our results of genetic and anatomical analysis of each transgenic plant overexpressing these KRPs.

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Study on "AtMYB23" in the "Arabidopsis" Root Epidermal Cell Fate Specification. Yeon Hee Kang¹, Kook Hui Ryu¹, John Schiebelbein², Myeong Min Lee¹. ¹Department of Biology, Yonsei University, Sichon 134, Seoul, Korea, ²Department of Molecular Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA

AtMYB23 is a member of the MYB transcription factor family. It has been suggested that several MYB-related transcription factors are involved in cell fate specification in "Arabidopsis". The "WER" gene is expressed in non-hair cells in the root and is proposed as a master regulator in root epidermal cell fate patterning. A WER homologue, the "GL1" gene is expressed in developing trichomes in the leaves and regulates in trichome development. AtMYB23 protein shows high similarity in amino acid sequence with WER and GL1 protein. "AtMYB23" is expressed both in non-hair cells of the root epidermis and in trichomes of the leaves. It has been reported that "AtMYB23" is involved in the trichome morphogenesis as well as its initiation. However, its function in root epidermal cell fate specification is not clear yet. To clarify the role of "AtMYB23" in the root, we analyzed two T-DNA insertion mutant lines. The position-dependent cell fate specification was not significantly disrupted in these mutants. However, mutation in "AtMYB23" gene affects the expression of some genes which are involved in this cell fate specification. Based on our results, "AtMYB23" may not be critical in the cell fate specification in the "Arabidopsis" root epidermis, but may regulate the efficiency of this patterning process.

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Low germination of brassinosteroids-deficient mutant can be overcome by the treatment of the reducing reagent, dithiothreitol. Sun Young Kim¹, Beg Hab Kim¹, Kyoung Hee Nam¹. ¹Division of Biological Science, Sookmyung Women's University, Seoul, 140-742, Korea

Several environmental factors including light, temperature, and moisture and plant hormones, especially gibberellin (GA), abscisic acid (ABA), ethylene, and brassinosteroids (BR) affect seed germination. BR has been known to rescue the germination of GA-deficient and GA response mutants seeds, implicating that BR probably acts downstream of GA. BR-deficient mutant, det2, also has low germination phenotype in addition to dark green dwarfed stature. Det2 seeds germinated only 50 % after 3 to 4 days, when the wild type seeds germinated almost completely. We found treatment of the reducing reagent, dithiothreitol (DTT), to the media induced the increased germination in det2, which is not substituted by treatment of GA. To investigate whether the effect of DTT on the stimulation of seed germination would be specific to det2 mutation, we observed the germination rate of other mutants involved in the metabolism and signaling of GA and ABA. Each mutant exhibited its characteristic early or late germination phenotype, and DTT treatment was not induced the difference of the seed germination. We also examined the germination aspects in brassinazole (BRZ)-treated wild type seeds on the DTT-containing media. In contrast to det2, BRZ-treated wild type seeds showed normal germination compared to the untreated seeds and DTT did not affect germination. This suggests that exogenously induced BR-deficiency may not exert the same influence on the reduced germination as shown in det2, in which continuous low levels of BR were kept in seeds throughout the formation of the mature seeds during embryogenesis. We further wanted to know the genes differentially regulated in germinating seeds of wild type and det2 with or without DTT and performed the full screening of differentially expressed genes using ACP-based PCR method. Several genes were fished out as their expression patterns were fluctuating and were analyzed.

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A genetic understanding of aging and senescence of leaf organ in Arabidopsis. Jin Hee Kim¹, Hyo Jung Kim¹, In Chul Lee¹, Seung Hee Choi¹, Hong Gil Nam^{1,2}. ¹Division of Molecular Life Sciences and National Core Research Center for Systems Bio-Dynamics, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea. ²The I-BIO graduate program, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Leaf development ends with senescence, a process consisting of deterioration events that ultimately led to death. Since leaf senescence is well coordinated developmental process, it is well expected there are many genes that influence leaf senescence. However, in spite of the biological and practical importance, the genetic mechanisms of senescence are largely unknown. Through forward and reverse genetic approach, we have identified several delayed leaf senescence mutant. From chemical - mutantized pool of *Arabidopsis*, we found ORE1, a transcription factor and ORE12, a cytokinin receptor. And to identify upstream or downstream component of ORE12, we screened EMS-mutagenized suppressors of the ore12-1 mutant. We have also screened delayed leaf senescence mutants from activation tagging lines to identify the negative regulators of leaf senescence. The recent progress on characterization of mutants and identification of the mutated genes is presented. From reverse genetic approach, RAV1 that is up regulated in an early senescence stage and RPK1, receptor-like kinase were isolated. The overexpressor lines exhibit early senescence symptoms, revealing a possible positive regulator of leaf senescence. Some of our results suggest that unique mechanisms for leaf senescence control leaf longevity by hormone, stress, and ROS. Although we are still seeing only a glimpse of the regulatory genes to occur during leaf senescence, we anticipate that we will be able to reconcile or organize general mechanisms relevant to leaf senescence in near future.

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Regulation of plant development by chemicals, identified through the chemical genetic approach. Junyoung Kim¹, Suna Jeong¹, Jaeki Min², Young Tae Chang², Hong Gil Nam³. ¹Division of Molecular Life Sciences, POSTECH, Pohang, Kyungbuk, 790-784, Republic of Korea. ²Department of Chemistry, New York University, New York, New York 10003, USA. ³Division of Molecular Life Sciences, POSTECH, Pohang, Kyungbuk, 790-784, Republic of Korea. National Core Research Center for Systems Bio-Dynamics and The I-BIO graduate program, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Chemical genetics is to use small molecules that alter the protein functions to which they bind. Among various chemical libraries, some ligands could inactivate or activate certain protein functions. Until now, there are many reports that various ligands modulate functions of proteins with binding their partner molecules. Thus, using chemical genetic strategy, we want to identify new small molecules that act like conditional mutagen regulating the protein functions. Thereafter, these chemicals will be used to study the functional mechanism by identifying the protein targets of the chemicals. In this study, we have attempted to probe chemicals which can regulate a variety of plant phenotype such as seed germination, growth, development and aging through a chemical library - whole plant screening system using 24 well plate. Also we are focusing on finding chemicals that affect flowering time in *Arabidopsis*. A flowering key regulator gene, GIGANTEA(GI) identified from our laboratory is screened using GI promoter and luciferase fused transgenic plant.

Among about 60,000 chemical libraries obtained from department of chemistry at NYU and Korea Chemical Bank, we found 48 chemicals that affected plant growth and development. Plump3 is one of the them which affect plant development. Now, we are trying to describe action mode of plump3 and analyze hit chemical inducing different transcription pattern of GI pro: :luciferase. Finally, we would like to elucidate plump3 function by identifying its binding partner in *Arabidopsis*. Also, we hope to regulate flowering time using identified small molecule through the chemical genetic screening.

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Natural variation of flowering-time gene "CONSTANS". Yasushi Kobayashi¹, Detlef Weigel^{1,2}. ¹Department of Molecular Biology, Max Planck Institute for Developmental Biology, D-72076 Tbingen, Germany, ²Salk Institute, La Jolla, CA 92037, USA

Plants normally flower in response to seasonal cues, such as daylength and temperature. "*Arabidopsis thaliana*" has a broad range of habitat in the Northern hemisphere, and extensive flowering-time variation has been observed among natural populations. "FRIGIDA" and "FLC" genes have been shown to act as major determinants controlling flowering-time variation. The large effect of the epistatic "FRIGIDA-FLC" module suggests that potentially significant other genetic variation could be easily overlooked. To gain a clue of potentially hidden genetic variation, we have combined a sequence-based reverse quantitative genetic approach and a QTL mapping-based forward quantitative genetic approach. We will present the functional analysis of natural variation on flowering-time gene "CONSTANS", which we expect to have significant effects after exposure to vernalization, which reduces the action of the "FRIGIDA-FLC" induced flowering delay.

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Senescence associated members of the ALA gene family in *Arabidopsis thaliana*. Tilbert Kosmehl¹, Alexis Kasaras¹, Christine Rausch¹, Reinhard Kunze¹. ¹Freie Universität Berlin, Institut für Biologie / Angewandte Genetik, Albrecht-Thaer-Weg 6, 14195 Berlin, Germany

Senescence is the genetically programmed last phase of leaf development and responsible for the recycling of nutrients before the cells die. It is a highly regulated process that involves complex morphological and metabolic changes and extensive alterations in gene expression. The mobilization of metabolites and nutrients out of senescent tissues into juvenile, storage or reproductive organs is mostly dependent on the activity of transmembrane transporters. However, to date only very few senescence-associated transporters (SATPs) have been reported. By a genome-wide transcriptional analysis of *Arabidopsis thaliana* developmental leaf senescence many novel, previously unrecognized, SATPs were identified.

Among these are three putative aminophospholipid translocases that show increased transcript levels in rosette leaves during senescence (ALA1, ALA10 and ALA11). They belong to the P4-type ATPases, a new subfamily of the P-type ATPase ion pump superfamily with 12 members. ALA10 and ALA11 are highly similar (86% identity), whereas ALA1 is more distantly related (33% identity).

T-DNA insertion lines for ALA1, 10 and 11 were identified and, to recognize overlapping or redundant functions, double and triple mutant lines were generated. Mutant lines ALA1 and 11 exhibit weakly reduced growth and enter senescence slightly earlier compared to wild type, whereas the ALA10 mutant line shows no obvious phenotype. The ALA10/11 double mutant line starts to senesce earlier than wild type and shows a stunted growth. Remarkably, in the ALA1/10/11 triple mutant line premature senescence is even more pronounced and accompanied by developmental abnormalities. In all mutant lines cold treatment promotes precocious senescence and reduced growth, whereas these symptoms disappear when plants grow at elevated temperatures. These characteristics are consistent with a role of the ALA proteins in membrane reorganization by transport of phospholipids or modification of the lipid bilayer composition during development and stress response.

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A Novel Transcriptional Cascade Regulating Expression of Heat Stress Proteins during Seed Development of *Arabidopsis*. Sachin Kotak¹, Elizabeth Vierling², Helmut Blumlein³, Pascal von Koskull-Dörring¹. ¹Institute of Molecular Biosciences, Biocenter N200/R306, Goethe University, D-60439 Frankfurt, Germany, ²Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, Arizona 85721, ³Institute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, Germany

Within the *Arabidopsis thaliana* family of 21 heat stress transcription factors (Hsfs), HsfA9 is exclusively expressed in late stages of seed development. Here, we present evidence that developmental expression of HsfA9 is regulated by the seed-specific transcription factor ABSCISIC ACID INSENSITIVE3 (ABI3). Intriguingly, ABI3 knockout lines lack detectable levels of HsfA9 transcript and protein, and further ectopic expression of ABI3 conferred the ability to accumulate HsfA9 in response to abscisic acid in transgenic plantlets. Consequently, the most abundant heat stress proteins (Hsps) in seeds (Hsp17.4-CI, Hsp17.7-CII, and Hsp101) were not detectable in the ABI3 knockout lines, but their expression could be detected in plants ectopically expressing HsfA9 in vegetative tissues. Furthermore, this seed-specific transcription factor cascade was reconstructed in transient -glucuronidase reporter assays in mesophyll protoplasts by showing that ABI3 could activate the HsfA9 promoter, whereas HsfA9 in turn was shown to be a potent activator on the promoters of Hsp genes. Thus, our study establishes a genetic framework in which HsfA9 operates as a specialized Hsf for the developmental expression of Hsp genes during seed maturation.

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TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in *Arabidopsis*. Tomotsugu Koyama^{1,2}, Masahiko Furutani³, Masao Tasaka³, Masaru Ohme-Takagi^{1,2,†} National Institute of Advanced Industrial Science and Technology (AIST)², Core Research for Evolutional Science and Technology (CREST)³, Nara Institute of Science and Technology

Plants form shoot meristems in the so-called boundary region and these meristems are necessary for normal morphogenesis of aerial parts of plants. However, the molecular mechanisms that regulate the formation of shoot meristems are not fully understood. We report here that expression of a chimeric repressor from TCP3 (TCP3SRDX), a member of TEOSINTE BRANCHED1, CYCLOIDEA and PCF (TCP) transcription factors in *Arabidopsis*, resulted in various developmental defects that include the formation of ectopic shoots on cotyledons, the wavy surfaces and margins of shoot lateral organs, the irregular pattern of vasculature and lack of the specific differentiation of epidermal cells. Expression of TCP3SRDX induced ectopic expression of boundary-specific genes, namely the CUP-SHAPED COTYLEDON (CUC) genes, and suppressed the expression of miR164, whose product cleaves the transcripts of CUC genes. This abnormal phenotype was substantially reversed on the cuc1 mutant background. By contrast, to gain function of TCP3, we generated the miR319/JAW-resistant version of TCP3 (mTCP3). Expression of mTCP3 suppressed the expression of CUC genes and resulted in the fusion of cotyledons and defects in formation of shoots. Our *in situ* hybridization assay showed that the TCP3 transcript was present in cotyledons, but not at the cotyledon boundary where CUC genes are functioning. In addition, we found that eight TCPs had functions similar to that of TCP3. Our results demonstrate that the TCP transcription factors play a pivotal role in the control of morphogenesis of shoot lateral organs by negatively regulating the expression of boundary-specific genes.

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Petunia LEAFY and UFO: a switch in the switch to flowering
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In flowering plants such as Petunia and *Arabidopsis*, the inflorescence meristem (IM) at the apex of an inflorescence shoot consists of a group of undifferentiated cells that repeatedly generates new floral meristems (FMs), the initials from which flowers develop. FMs differ from IMs in their pattern of organogenesis and the identity of the organs that are formed. Petunia has a cymose inflorescence structure; at the time that the IM converts into an FM, a new IM is formed laterally.

Two Petunia meristem identity (MI) genes that specify the floral fate of the FM have been identified: ABERRANT LEAF AND FLOWER (ALF) and DOUBLE TOP (DOT), which are the apparent orthologs of the *Arabidopsis* genes LEAFY (LFY) and UNUSUAL FLORAL ORGANS (UFO), respectively. alf and dot mutants have a strong flower-to-shoot phenotype; both completely lack flowers and have a bushy phenotype due to the repetitive bifurcation of the IMs. In contrast to lfy mutants, alf and dot mutants are not late-flowering. *In situ* hybridization shows that ALF is already expressed in the vegetative phase and later in the FM and all organs of the flower. DOT expression is first visible in the developing FM and later in a pentagonal domain on the adaxial side of the emerging sepal primordia.

Strikingly, when ALF or LFY is overexpressed in Petunia, no phenotype is observed, whereas overexpression of either of these genes in *Arabidopsis* leads to early flowering and the formation of single, terminal flowers. In contrast, ectopic expression of DOT or UFO in Petunia leads to a shoot-to-flower phenotype, comparable to overexpression of LFY or ALF in *Arabidopsis*, whereas overexpression of either of these F-box genes in *Arabidopsis* does not lead to early flowering or changes in the inflorescence architecture.

Apparently, Petunia and *Arabidopsis* have evolved different ways of floral MI specification by these key MI genes. To examine whether the difference between the two species is caused by cis- or trans-acting factors, ALF, DOT, LFY and UFO promoter::GUS constructs were stably transformed to both Petunia and *Arabidopsis*. Results shed light on the divergent evolution of MI regulation.

P-255

The RETINOBLASTOMA-RELATED PROTEIN (RBR) interacts with sucrose and auxin signalling to control cell division and cell differentiation. Yechan Laizet¹, Ruben Gutzat¹, Johannes Fitterer¹, Wilhelm Gruissem¹. ¹ETH - Institute of Plant Sciences, Zürich, Switzerland

Plant organogenesis in shoot and root meristems relies on a tightly regulated balance between cell division and cell differentiation. One of the key regulators of this process is the Retinoblastoma-related protein (RBR), acting at least in part through the DP/E2F pathway to regulate cell cycle activity (reviewed in 1). *Arabidopsis* plants carrying a constitutive RBR loss-of-function allele are gametophytic lethal. To investigate the role of RBR during post-embryonic development, we constructed a conditional RBR loss-of-function allele, using a CRE-lox system inducible by heat shock (2). Excision of RBR after induction is detectable through the transcriptional activation of a green fluorescent protein (GFP) reporter. In plants induced 3 days after germination, loss of RBR triggers defects in cell division and differentiation in leaves; epidermal cells do not acquire the typical jig-saw shape; trichomes and stomata hardly differentiate. Reiterative heat shocks arrest organ production, and previously initiated leaves show additional cell division. Growth signals like sucrose and hormones are known to play a role in cell division control. In rbr^{-/-} roots, the hormone auxin together with sucrose triggers a strong cell hyper-proliferation. Moreover, these roots do not show the typical differentiation pattern but rather develop into undifferentiated callus tissue. A similar hyperproliferation is observed in RBR co-suppression line grown on sucrose and gene expression analysis reveal that genes involved in cell cycle regulation are deregulated. Our results confirm the role of RBR on cell division control, but also show that organ development requires RBR for a proper cell differentiation.

1. *Plant Molecular Biology* (2006) 60: 947-961.
2. *Cell.* 2005 Dec 29; 123(7): 1337-49.

P-256

Evidence that plant miRNPs ribonucleoproteic complexes are associated with translated mRNAs in polysomes. Elodie Lanté¹, Etienne Delannoy¹, Rodnay Sormani¹, Christophe Robaglia¹, Patrice Crété¹. ¹Laboratoire de Génétique et Biophysique des Plantes, Marseille, France

MicroRNAs (miRNAs) regulate gene expression post-transcriptionally by a conserved mechanism from plants to animals called RNA silencing. The common hypothesis is that most animal miRNAs affect gene expression by blocking mRNAs translation while most plant miRNAs trigger mRNAs cleavage prior to translation. However this view is certainly far from being complete. Therefore we are studying the link between the translational machinery and plant miRNAs.

Results: Cytoplasmic extract fractionation on sucrose gradient reveals that although miRNAs are found in light fractions from the top of the gradient, some miRNAs are linked with heavy fractions of polysomes. The level of co-sedimentation varies for different miRNAs, and miR168 is strongly associated with polysomes. In contrast, miR168*, the non-functional partner of the miRNA in the duplex miR/miR*, is only found within light, untranslated, fractions. Interestingly, miR173, which triggers cleavage of the TAS2 non coding RNA, is also weakly associated with polysomes. We conclude that the association of miRNAs to polysomes is dependent on the function of regulatory RNA and is linked to the presence of a translatable mRNA target. In addition, protein analysis reveals that Argonaute 1 (AGO1) protein is in part associated with polysomal fractions and that miR168 association with polysomes is lost in ago1-4 mutants. So we conclude that miRNAs are present in a ribonucleoprotein complex associated with polysomes. Further we show that association of miR168 with polysomes is sensitive to micrococcal nuclease, suggesting that miRNAs association with polyribosomes is mRNA mediated.

In conclusion this work shows that plant miRNAs, like animal miRNAs, are associated with polysomes both in *Arabidopsis thaliana* cell culture and plants. This association depends on a ribonucleoproteic complex called miRNP, containing at least intact miRNA and AGO1. Further work will address the effect of miRNAs on mRNA translation and provide a better understanding of the spatio-temporal relationship between RNA cleavage and translational regulation.

P-257

Mutations in *Arabidopsis* homologs of proteins of the SWR1 complex accelerate flowering time. Ana Lazaro¹, M. Angeles Gmez-Zambrano¹, Leticia Lpez-Gonzlez¹, Manuel Pfeiro¹, Jose A. Jarillo¹. ¹Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Department of Biotechnology, Madrid, Spain

Our interests is focused on the analysis of the molecular mechanisms involved in the regulation of flowering time and, particularly, on those factors required for the repression of flowering until plants are in optimal environmental conditions or reach the appropriate developmental stage to flower. Initially, we characterized early in short days (*esd1*) mutations, which cause early flowering and several vegetative and reproductive developmental defects. *esd1* abolishes the *FLC*-mediated late flowering phenotype of plants carrying active alleles of *FRI* and of mutants of the autonomous pathway (Martin-Trillo et al., 2006). We found that *ESD1* is required for the expression of *FLC* and other members of the *FLC*-like/MAF gene family to levels that inhibit flowering. The *ESD1* locus encodes *ARP6*, a homolog of the actin-related protein family that is part of the SWR1 chromatin-remodeling complex in yeast, which catalyzes the exchange of histone H2A for the histone H2A.Z variant in nucleosome arrays ensuring full activation of underlying genes. Now, we have isolated *Arabidopsis* mutants in homologs of others SWR1C components. Mutations in *AtSWC6* cause similar developmental defects as *esd1*, including serrated leaves, weak apical dominance, flowers with altered number and size of organs and early flowering by reduction in expression of *FLC* and other MAF genes. *swc6* suppresses late flowering phenotype of *FRI* and of *fve* and *fca* mutant plants. Protein interaction analyses suggest the formation of a complex between *ESD1* and *AtSWC6*. In addition, we will present evidence that *AtSWC6* is needed to achieve both the levels of H3 acetylation and H3-K4 methylation required for high *FLC* expression, on the same manner as *ESD1* does. These observations are consistent with the presence of a SWR1C-like complex in *Arabidopsis* that may regulate plant development controlling gene expression, being required for modulation of chromatin structure.

Martin-Trillo et al. (2006). *Development*, 13, 1241-1252.

P-258

Many Ways Regulating the Floral Repressor FLC in *Arabidopsis*. Ilha Lee¹. ¹Seoul National University

Flowering is regulated by a complex genetic network sensing environmental cues and endogenous signals. FLOWERING LOCUS C (FLC), a strong floral repressor, is one of central players in such a complex genetic network. To understand the regulatory mechanism of FLC, we screened early flowering mutants from late-flowering winter annual plants after fast neutron mutagenesis. The map-based gene cloning approach using the selected mutants revealed molecular mechanism of FLC regulation. The expression of FLC is regulated by diverse mechanisms as FRIGIDA-mediated activation, autonomous pathway, vernalization, PAF5 complex mediated histone modification, and ATP-dependent chromatin remodeling. Here, we present molecular evidence how FLC is transcriptionally activated and repressed by diverse mechanisms and how each mechanism interact one another.

P-259

SHORT ROOT regulates vascular tissue patterning in *Arabidopsis* roots. Ji-Young Lee¹, Annelie Carlsbecker², Yka Helariutta³, Philip Benfey¹. ¹Duke University, ²Uppsala University, ³University of Helsinki

Vascular tissues in *Arabidopsis* roots develop from initial cells above the quiescent center (QC) and form a bilaterally symmetric structure (diarch) constituted with xylem, phloem, and procambium. In wild type roots, the protoxylem, two outer xylem strands characterized by their spirally patterned secondary cell wall, differentiates earlier than the metaxylem, inner xylem strands with the reticulate secondary cell wall. SHORT ROOT (SHR), a GRAS family transcription factor, that was shown to regulate the asymmetric cell division of endodermis/cortex initials and maintain the stem cell niche around the QC, is transcribed in the xylem and the procambium, an undifferentiated tissue in the boundary of xylem and phloem. Its proteins subsequently move out of those tissues into the outer tissues, QC and the endodermis, to play roles as described above. However, genome-wide gene expression profiling of shr mutant showed significant change in the expression of genes enriched in the stele, indicating an additional role of SHR played in the stele. To find out whether SHR is also involved in the specification of cell types in the stele, a systematic analysis was performed using stele cell-type specific GFP marker lines in shr mutant. We found that the absence of SHR not only affects the proliferation of stele cells but also affects the cell type specification, xylem in particular. In shr, the ectopic metaxylem forms instead of the protoxylem. This ectopic metaxylem formation was found to happen via the activation of class III HD-ZIP transcription factors in shr-2. In particular, PHABULOSA (PHB) was found to be critical for the proper protoxylem patterning via SHR regulation. This regulation seems to occur indirectly via the regulation of SHR on microRNA 165/166.

P-261

The flowering pathway integrator SOC1 directly regulates LFY by interacting with AGL24. Jungeun Lee¹, Mijin Oh¹, Ilha Lee¹. ¹Department of Biological Sciences, Seoul National University, Seoul, Korea

The transition to flowering is controlled by complex genetic networks in response to environmental and endogenous signals in *Arabidopsis*. The genetic and molecular analyses of *Arabidopsis* revealed that the photoperiod pathway, autonomous/vernalization pathway and GA pathways can act distinctly to promote flowering, converging on common downstream integrators; *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR of OVEREXPRESSION of CONSTANS* (*SOC1*) and *LEAFY* (*LFY*). These regulators integrate inputs from the multiple flowering pathways and make plants decide when to flower by inducing floral meristem identity genes at the shoot apex. The previous studies have proposed that the MADS-box transcription factor, *SOC1* might regulate *LFY* expression and another MADS-box transcription factor *AGL24* would act together with *SOC1* or mediate the signal from *SOC1* to *LFY*. However, there are still little data to support these hypotheses. In this study, we present empirical evidences to verify the hypotheses. Chromatin Immunoprecipitation assay shows *SOC1* directly binds to the *LFY* promoter, and transient expression assay shows *AGL24* acts as interacting partner of *SOC1*. In addition, through the suppressor mutagenesis of the transgenic lines overexpressing *SOC1* protein, we found that MADS domain is important for *SOC1* function and contributes to make *SOC1-AGL24* complex.

P-262

ROL5: a potential regulator of reactive oxygen species during cell wall formation. Ruth Maria Leiber¹, Christoph Ringli¹. ¹Inst. of Plant Biology, Zurich, Switzerland

Cell walls are complex structures that confer shape to plant cells and ultimately to the whole plant. The basic structure of cell walls is composed of cellulose microfibrils, which are interconnected by hemicellulose and embedded in a pectin matrix. Numerous structural proteins also play a role in interconnecting the polysaccharides of the cell wall. How the process of wall development and expansion and thus cell growth is controlled, still remains largely elusive.

In the model plant *Arabidopsis thaliana* the proteins of the LXR gene family encodes extracellular LRR-extensin proteins that are good candidates for a regulatory function during cell wall development. The LXR1 protein is expressed in root hairs and strongly influences root hair cell wall formation. As a consequence, root hairs of the lxr1 mutant are miss-shaped, swell, form bulges and often burst.

To investigate the developmental process in which LXR1 is involved, we have performed a suppressor screen on the lxr1 mutant. In this screen we isolated a number of rol (repressor of lxr1) mutants that suppress the lxr1 root hair phenotype (Diet A, et. al. *Plant Cell* 18, 1630-41). rol5 represents a new suppressor mutant of lxr1. Although the encoded ROL5 protein is of unknown function, the characterization conducted so far revealed that ROL5 is a mitochondrial protein, which is predominately expressed in the elongation zone of roots. ROL5 is homologous to the yeast protein NCS6 and partially complements the function of NCS6 in yeast, where NCS6 is implicated in cell growth and morphology. Further the characterization indicates that ROL5 is involved in a process related to reactive oxygen species (ROS). Rol5 mutant plants are hypersensitive to ROS, which are produced by the cells and have been shown to be important for the cell growth process. The characterization of ROL5 will shed light on the function of this protein during cell growth and may even provide new insights into the mechanisms of growth regulation in plants.

P-260

Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis*. Jeong Hwan Lee¹, Seong Jeon Yoo¹, Soo Hyun Park¹, Ilwoo Hwang², Jong Seob Lee³. ¹Plant Signaling Network Research Center, School of Life Sciences and Biotechnology, Korea University, Seoul, 136-701, Korea, ²Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang 790-784, Korea, ³School of Biological Sciences, Seoul National University, Seoul, 151-742, Korea

Plants must perceive and rapidly respond to changes in ambient temperature for their successful reproduction. Here we demonstrate that *Arabidopsis* SHORT VEGETATIVE PHASE (SVP) plays an important role in the response of plants to ambient temperature changes. The loss of SVP function elicited insensitivity to ambient temperature changes. SVP mediates the temperature-dependent functions of FCA and FVE within the thermosensory pathway. SVP controls flowering time by negatively regulating the expression of a floral integrator, *FLOWERING LOCUS T* (*FT*), via direct binding to the CArG motifs in the *FT* sequence. We propose that this is one of the molecular mechanisms that modulate flowering time under fluctuating temperature conditions.

P-263

Comparative interactome analysis of tomato MADS-box proteins. Charles H. Leseberg¹, Christie L. Eissler¹, Xiang Wang², Hanzi Guo², Liqing Ma², Bo Wei², Aili Li², Long Mao^{1,2}. ¹Department of Biological Sciences, Northern Illinois University, DeKalb, IL 601115, USA, ²Institute of Crop Sciences, Chinese Academy of Agricultural Sciences and National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Beijing 100081, China

MADS-box genes are key transcription factors for plant development. The MIKCC subfamily of MADS-box genes has arisen due to its diversification in land plants. Large-scale protein interaction data is limited to *Arabidopsis* and petunia. We conducted a comprehensive protein-protein interaction study in tomato (*Solanum lycopersicum*), a model plant for fruit abscission, development and ripening. Approximately half of the tomato MIKCC subfamily representing the subclades APETALA1/FRUITFUL, APETALA3/PISTILLATA, AGAMOUS, SEPALLATTA, STMADS11, and SUPPRESSOR OF CONSTANS1 was included. A comparative analysis between existing MADS-domain protein networks and other species including *Arabidopsis* was conducted. In addition, we tested potential higher order interaction complexes as shown to exist in *Arabidopsis* while attempting to find new complexes amongst the tomato MADS-domain proteins. Details will be presented.

P-265

Comparative and functional characterization of candidate regulators of wood and fiber differentiation in "Arabidopsis thaliana" and "Populus trichocarpa". Eryang Li¹, Juergen Ehrling², Michael Friedmann¹, Weiya Qiang³, Carl J. Douglas¹. ¹Department of Botany, University of British Columbia, Vancouver BC, Canada V6T 1Z4, ²Institute for Plant Molecular Biology, Centre National de la Recherche Scientifique, 67000 Strasbourg, France, ³School of Life Science, Lanzhou University, Gansu, P. R. China

In "*Arabidopsis*", stages of interfascicular fiber differentiation can be identified along the axis of bolting stems. In "*Populus*", different stages of xylem differentiation can be visualized along the axis of internodes development at the shoot tip, and different stages of cellular differentiation during secondary xylem formation can be dissected after re-activation of the vascular cambium following winter dormancy. Using tissue samples representing different stages of xylem and fiber differentiation in "*Populus*" and "*Arabidopsis*", we assayed global changes in gene transcription over the course of stem and xylem development and interfascicular fiber differentiation to gain insights into the developmental and regulatory events that control these patterns of xylem and fiber differentiation in both species, and to identify genes conserved in "*Arabidopsis*" and "*Populus*" that may regulate fiber and xylem differentiation including lignin deposition. These studies employed an "*Arabidopsis*" full genome longmer microarray (>26,000 genes) and a poplar cDNA microarray. A subset of "*Arabidopsis*" transcription factors (TF) were identified that are associated with fiber development and/or secondary wall formation and lignification in the root stele and other tissues, making them targets for functional studies.

We have identified putative orthologues of many of the "*Arabidopsis*" candidates in *Populus* genome and many of these are differentially expressed in concert with xylem development, as assayed by global expression profiling of *Populus* cDNA microarray. Examining the functions of these *Populus* genes will allow greater insight into the potential roles played by conserved genes regulating secondary wall and wood formation, and provide a novel way to increase secondary wall differentiation and lignification.

P-266

A Cre/loxP-Mediated Cell Autonomous Gene Excision System to Produce Marker Gene Free Transgenic Soybean Plants

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Marker-gene-free transgenic soybean plants were produced by isolating a developmentally regulated embryo-specific gene promoter app1 from *Arabidopsis* and developing a cell autonomous gene excision system using the P1 bacteriophage Cre/loxP recombination system. To accomplish this, the Cre recombinase gene was placed under control of the app1 promoter and, together with a selectable marker gene (hygromycin phosphotransferase), were cloned between two loxP recombination sites. This entire sequence was then placed between a constitutive promoter and a coding region for either β-glucuronidase (Gus) or glyphosate acetyltransferase (Gat). Gene excision would remove the entire sequence between the two loxP sites and bring the Gus or Gat coding region to the constitutive promoter for expression. Using this system marker gene excision occurred in over 30% of the stable transgenic events as indicated by the activation of the Gus reporter gene or the Gat gene in separate experiments. Transgenic plants that have 1 or 2 copies of a functional excision-activated gat transgene and are free of any marker gene were obtained in T0 or T1 generation. This demonstrates the feasibility of using developmentally controlled promoters to mediate marker excision in soybean.

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The NAC transcription factor GORGON enhances the pid-8 phenotype in Arabidopsis. Wei Li¹, Hicham Zegzouti¹, Mingtang Xie², Roman Deniskin¹, Sioux K. Christensen¹. ¹University Of California-Los Angeles, Los Angeles, CA, USA, ²University Of California, Riverside, CA, USA

Auxin is an essential phytohormone that regulates numerous mitogenic and morphogenic processes during plant development. PINOID (PID), a previously identified plant specific serine/threonine kinase, has been shown to regulate auxin signaling. In Arabidopsis, among the phenotypes associated with the strong allele of pinoid (pid-9) is a pin-like inflorescence characterized by the absence of buds and flowers, and the weak allele pid-8 produces sterile flowers and other various auxin signaling or transport related abnormalities rather than pins. To identify other genes affecting the establishment or maintenance of polar auxin flow, we generated an activation tagged insertion lines in the pid-8 background to isolate pinoid enhancer mutants. Transformed plants were screened for the pin-like inflorescence characteristic of pid strong alleles phenotype. Gorgon (gon-ID) was identified as a dominant enhancer of the pid-8 phenotype that produces both pin and sterile flowers. Also, this mutant produced a thickened and often bent or twisted stems, reduced fertility, and delayed bolting and senescence. Sequences flanking the T-DNA insertion have been cloned using plasmid rescue, and the ORF upstream of the insertion is identified as a gene that encodes a NAC transcription factor family of proteins. The GON gene was showed by RT-PCR to be overexpressed in the gon-1D mutant. The resulting phenotypes have been recapitulated by overexpressing the GON gene in pid-8. Here, we present a histological examination of the mutant and the expression pattern of GON.

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Specification of Arabidopsis floral meristem identity by repressing flowering time genes. Chang Liu¹, Jing Zhou¹, Keren Bracha-Drori², Shaul Yalovsky², Toshiro Ito¹, Hao Yu¹. ¹Department of Biological Sciences and Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117543, Singapore, ²Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Flowering plants produce floral meristems in response to intrinsic and extrinsic flowering inductive signals. In Arabidopsis, the floral meristem identity genes LEAFY (LFY) and APETALA1 (AP1) are activated to play a pivotal role in specifying floral meristems during floral transition. We show here that the emerging floral meristems require AP1 to specify partly their floral identities by repressing directly a group of flowering time genes, including SHORT VEGETATIVE PHASE (SVP), AGAMOUS-LIKE 24 (AGL24) and SUPPRESSOR OF OVEREXPRESSION OF CO1 (SOC1). In wild-type plants, these flowering time genes are normally down-regulated in emerging floral meristems. In the absence of AP1, these genes are ectopically expressed transforming floral meristems into shoot meristems. By post-translational activation of an AP1-GR fusion protein and chromatin immunoprecipitation assays, we further demonstrate the repression of these flowering time genes by induced AP1 activity and in vivo AP1 binding to the cis-regulatory regions of these genes. These findings indicate that once AP1 is activated during the floral transition, it acts partly as a master repressor in floral meristems by directly suppressing the expression of flowering time genes, thus preventing the continuation of the shoot developmental program.

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Arabidopsis DNA ligase I exerts a maternal gametophytic effect on seed development. Jing Li¹, Sébastien Andreuzza^{1,2}, Anne-Elisabeth Guittot¹, Jean-Emmanuel Faure^{1,3}, Frédéric Berger¹. ¹Temasek LifeScience Laboratory, 1 Research Link, National University of Singapore, 117604 Singapore, ²Ecole Normale Supérieure de Lyon, 46 Allée d'Ulm, F-69364 Lyon cedex 07, France, ³Current address: European Commission, Directorate General for Research

Flowering plant reproduction is characterized by a predominant maternal involvement in embryogenesis and production of seeds. However, only a limited number of maternal effect genes have been identified. Most of these genes are implicated in a single genetic pathway and the associated maternal effects are the consequence of imprinting mechanisms resulting in the sole expression of the maternal allele during seed development. Other mechanisms linked to maternal effects have been suspected but never demonstrated in a few other cases of non-imprinted genes.

In this study, we characterize a new mutant displaying maternal effect on seed development. The maternal effect results from loss-of-function of the gene encoding the Arabidopsis thaliana DNA ligase I (AtLIG1). Genetic analyses show that atlig1 mutants display a complex array of phenotypes, causing some degree of lethality during male and female gametogenesis, embryogenesis and endosperm development. Maternal effects associated with atlig1 inheritance from the ovule primarily cause defects in endosperm development. Interactions between atlig1 and mutations affecting the maintenance DNA methyltransferase MET1 indicate that AtLIG1 may play a role in parental genomic imprinting.

P-270

TORMOZ Like1 is essential for orientating cell divisions during early embryogenesis in Arabidopsis. Nai-You Liu¹, Hong-Ju Li¹, Jie Liu¹, Wei-Cai Yang¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Precise control of orientating cell divisions is critical for early embryogenesis in Arabidopsis. Here we report the characterization of an Arabidopsis Ds-insertion mutant tormoz like1 (tol1), which causes the early arrest of embryo development. Aberrant cell division patterns occur in the embryo proper, but not in the suspensor. The zygote of the mutant embryo in tol1 elongates and undergoes an asymmetric cell division normally, but further divisions of the apical cell are impaired. The apical cell divides transversely or obliquely but not longitudinally as that in wild type. In addition, the majority of the mutant embryos were arrested before the 16-celled dermatogen stage. TOL1 encodes a nucleolus localized U3 SnRNP-associated WD-40 protein which is highly conserved in almost all eukaryotic species. TOL1 is expressed early in the ovule primordial, the mature embryo sacs, young embryos, and tissues undergoing rapid growth including root tip and shoot apex. Genetic complementation experiment indicates that the mutant phenotype observed is indeed the result of a Ds insertion within TOL1. Together, our findings suggest an essential role of TOL1 to direct longitudinal cell divisions in Arabidopsis early embryo development.

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Characterization of Mutants Defective in Female Gametogenesis and Embryogenesis in Arabidopsis. Man Liu¹, Naiyou Liu¹, Li Yuan¹, Dongqiao Shi¹, Jie Liu¹, Weicai Yang¹. ¹The Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Datun Road, Beijing 100101, China

Female gametophyte development and embryogenesis are essential for plant sexual reproduction. However, little is known about the relationship between these two subsequent stages and the functions of essential genes in these developmental stages. We identified two *Arabidopsis thaliana* mutants, sgt13018 and 77B, which are defective in these steps. In sgt13018, both female gametogenesis and embryogenesis were impaired. The female gametophyte development was paused at FG5 stage, but they eventually could develop into mature embryo sacs and attracted pollen tubes to enter them, and the mutant embryos were mainly arrested at zygote or 1-cell stage. Molecular analysis indicates that sgt13018 encodes a putative U5 snRNP-specific protein, and is predominantly expressed in the young organs. In 77B, a gene, which encodes a putative DEAD/DEAH box helicase protein, was disrupted with a Ds insert. Loss of 77B gene function cause arrest of embryo sacs at four-, five- or eight-nucleate stage within the same pistil. The gene is expressed ubiquitously throughout the plant. Together, these data suggest that the essential genes, sgt13010 and 77B, play an important role in the regulation of sexual reproduction.

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Seed dormancy requires histone H2B monoubiquitination. Yongxiu Liu¹, Kazumi Nakabayashi¹, Maarten Koornneef¹, Wim Soppe¹. ¹Max Planck Institute for Plant Breeding Research, Carl-von-Linne-weg 10, 50829 Cologne, Germany

Seed dormancy has an adaptive role in nature by optimizing germination to the most suitable time, and a tight control of dormancy is very important in crop plants. Extensive genetic and physiological studies have identified the involvement of several factors, but the molecular mechanisms underlying this process are still largely unknown. With the molecular identification of the *Arabidopsis* HISTONE MONOUBIQUITINATION 1 (HUB1) gene, we have revealed a role for chromatin remodeling in the seed dormancy mechanism. The hub1 mutant (originally identified as rdo4) has reduced dormancy levels and mild pleiotropic phenotypes. HUB1 encodes a C3HC4 RING finger protein, which is required for monoubiquitination of histone H2B. The *Arabidopsis* genome contains one HUB1 homologue, which we named HUB2. The hub2 mutant also has reduced seed dormancy and is not redundant with hub1. The ubiquitinated form of histone H2B could not be detected in the hub1 and hub2 mutants. Furthermore, we found altered expression levels of dormancy related genes in the hub1 mutant.

In yeast and human, monoubiquitination of histone H2B is a prerequisite for histone H3 methylation at Lys-4 and -79 and is associated with actively transcribed genes. We are studying this relation in *Arabidopsis* and aim to identify the genes that are direct targets of HUB1. Furthermore, we are analyzing the relation between the HUB1 and HUB2 proteins and try to understand more about the role of histone H2B monoubiquitination on seed dormancy by analyzing other components that affect histone H2B monoubiquitination.

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Direct Interaction of AGL24 and SOC1 Integrates Flowering Signals in Arabidopsis. Chang Liu¹, Hongyan Chen¹, Hong Ling Er¹, Hao Yu¹. ¹National University of Singapore

During the transition from vegetative to reproductive growth, the shoot meristem of flowering plants acquires the inflorescence identity to generate flowers rather than vegetative tissues. An important regulator that promotes the inflorescence identity in *Arabidopsis* is AGAMOUS-LIKE 24 (AGL24), a MADS-box transcription factor. By using a functional estradiol-inducible system in combination with microarray analysis, we identified AGL24-induced genes including SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1), a floral pathway integrator. Chromatin immunoprecipitation (ChIP) analysis of a functional AGL24-6HA tagged line revealed *in vivo* binding of AGL24-6HA to the regulatory region of SOC1. Mutagenesis of AGL24 binding site in SOC1 promoter resulted in the reduction of SOC1 expression. Our results show that SOC1 is one of the direct targets of AGL24, and that SOC1 expression is upregulated by AGL24 mainly at the floral transitional stage. ChIP analysis using a functional SOC1-9myc tagged line and promoter deletion analysis also revealed *in vivo* binding of SOC1-9myc to the regulatory regions of AGL24 and upregulation of AGL24 by SOC1. Furthermore, we found that like other flowering genetic pathways, the gibberellin pathway independently controls AGL24 and SOC1. These observations suggest that during floral transition a positive regulatory feedback loop conferred by direct transcriptional regulation between AGL24 and SOC1 integrates flowering signals.

P-274

Towards the understanding of the function of CLV3 and CLE19 peptides. Chun-Ming Liu¹. ¹Institute of Botany, Chinese Academy of Sciences

Over the past ten years studies in *Arabidopsis* provided concrete evidence showing that stem cells are indeed present in higher plants. Stem cells, positioned in the central zone of the shoot apical meristem (SAM), are the source of totipotent cells that serve as the founders of all newly formed aboveground organs during the plants life cycle. The maintenance of a constant pool of stem cells requires tightly regulated machinery to sustain a dynamic equilibrium between cells in an undifferentiated state and cells that are destined to differentiate into various tissues and organs. At the molecular level, the balance is controlled by a feedback regulation loop consisting of a WUS homeobox transcription factor and a CLV1/CLV2/CLV3 receptor complex. WUS, which is expressed in the L3 layer of the central zone in the SAM organizing center, promotes the stem cell identity. CLV3 encodes a putative extracellular protein with 96 amino acids and is mainly expressed in the L1 and L2 layers of the central zone of the SAM, above the CLV1 domain. We have showed previously that the conserved C-terminal CLE motif is the functional cue of CLV3 and CLE19. Most likely the endogenous CLV3 consists of 12 amino acids (RTVPhSGPhDPLHH), with hydroxyl groups attached to two of the three proline residues [Kondo, 2006]. Using molecular genetic tools we have further studied the function of CLV3 and several other CLE genes. The results showed that most of these CLE genes are functionally redundant.

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FT RNA Act as a Systemic Floral Initiator. Kuan-Ju Lu^{1,2}, Yu-Chen Liu¹, Nien-Chen Huang¹, Tien-Shin Yu¹. ¹Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan,²Department of Life Science, National Central University, Taiwan

The idea that FT RNA or protein may act as florigen-like molecules to regulate floral initiation is one of the most exciting discoveries in recent plant biology. However, the original paper described FT RNA is a florigen has been retracted recently. Therefore, whether FT RNA is a mobile floral initiator remains to be further investigated. From classic physiological experiments, it has been established that florigen is transported through phloem translocation stream. To test whether FT RNA is localized in the phloem sap, RNA were extracted from the phloem extrudes of excised *Brassica oleracea* inflorescences. RT-PCR analysis indicated that the transcript of FT is detected in Broccoli phloem sap. To further examine the long-distance movement of FT RNA, the *Arabidopsis* grafting experiments were next conducted. Our results indicated that the FT RNA moves long-distance to the scion apices, in addition, the floral initiation of scion tissues are accelerated. Interestingly, the long-distance movement of FT-GFP chimera RNA is dramatically impaired after the insertion of GFP in the 3' end of FT, suggesting the movement of FT RNA is determined by the intact structure of RNA. Consistent with this finding, the results performed with tomato grafting indicate that tomato SFT full-length RNA, an ortholog of FT, moves long-distance to the scion apices. Taken together, our results support the hypothesis that FT RNA may act as a systemic floral initiator in diverse plant species.

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Genetic evidence for a role of cation/H⁺ transporters in male fertility. Yongxian Lu¹, Xiyan Li¹, Senthilkumar Padmanaban¹, Heven Sze¹. ¹Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA

Pollen development and tube growth are critical for male fertility and seed production, though little is known about the molecular and cellular bases governing these processes. A family of *Arabidopsis thaliana* CHX genes was found to be preferentially expressed in pollen, but their specific role in pollen biology is unknown (Sze et al, 2004). AtCHXs are thought to be K⁺(Na⁺)/H⁺ exchangers with a role in osmoregulation and K⁺ homeostasis in vegetative tissues (Padmanaban et al. 2007).

A reverse genetics approach was used to determine the function of two related genes CHX-A and CHX-B. Single T-DNA insertion mutants (aa or bb) showed similar *in vitro* pollen germination and tube growth to that of wild-type. However, no double mutants (aabb) were recovered in over 300 progeny plants from the AaBb parent. Reciprocal crossing of Aabb with wild-type (AABB) plants suggested a block in male-specific transmission when both A and B genes are defective. When wild-type pistil was pollinated with a limited number of pollen grains from Aabb plants, seed set was reduced about half of that from AAAb plants. These results demonstrate that CHX-A and CHX-B perform similar functions, and that either A or B is essential for male fertility. The cellular roles of both genes in reproduction are under investigation. Reference: Sze H, Padmanaban S, Cellier F, Honys D, Cheng NH, Bock KW, Conejero G, Li XY, Twell D, Ward JM. (2004) Expression pattern of a novel AtCHX gene family highlight potential roles in osmotic adjustment and K⁺ homeostasis in pollen development. *Plant Physiology* 136: 2532-2546 Padmanaban S, Chanroj S, Kwak JM, Li XY, Ward JM, Sze H (2007) Participation of Endomembrane Cation/H⁺ Exchanger AtCHX20 in Osmoregulation of Guard Cells. *Plant Physiology* (In press)

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Regulation of Flowering Time by a Protein Arginine Methyltransferase, AtPRMT10. Falong Lu^{1,2}, Lifang Niu^{1,2}, YanXi Pei^{1,3}, ChunYan Liu¹, XiaoFeng Cao¹. ¹State Key Laboratory of Plant Genomics and Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, 100101, China,²Graduate School, Chinese Academy of Sciences, Beijing, 100039, China,³College of Life Science and Technology, Shanxi University, Taiyuan, China, 030006.

Histone methylation is involved in modulation of chromatin structure, gene expression, heterochromatin formation, imprinting, genome stability and other important cellular processes. In plants, it has been reported that histone H3 methyltransferases play important roles in gene silencing and developmental regulation. However, little histone H4 methyltransferase has been identified and roles of histone H4 methylation in plant development remain to be elucidated. Here, we purified a histone methyltransferases from cauliflower by a conventional biochemical approach. PHRMT10, a dimerized plant specific protein arginine methyltransferase, was identified to methylate histone H4 specifically *in vivo*. *Arabidopsis thaliana* protein arginine methyltransferase 10 (AtPRMT10), the *Arabidopsis* homolog of PHRMT10, was demonstrated to be a type I protein arginine methyltransferase which preferentially methylate histone H4 at R3 *in vitro*. Genetic disruption of AtPRMT10 resulted in late flowering by the up-regulated *FLOWERING LOCUS C* (*FLC*) transcriptional level, indicating that wild-type function of AtPRMT10 is required for promoting flowering in *Arabidopsis* through repressing the expression of *FLC*. This work adds an extra layer of complexity in flowering time regulation and also sheds light on the importance of arginine methylation in plant development.

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Control of Floral Dorsoventral Asymmetry and Lateral Petal Morphogenesis in *Lotus japonicus*. Yonghai Luo¹, Da Luo^{1,2,*}. ¹Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Graduate School of the Chinese Academy of Sciences, Chinese Academy of Science, ²School of Life Science and Biotechnology, Shanghai Jiao Tong University

Floral zygomorphy is a morphological novelty and considered to have evolved several times independently in flowering plants. It has been shown that in *Antirrhinum majus*, two TCP genes, *CYC/DICH*, control both the floral DV asymmetry and petal organ asymmetry. To view a general Evo-devo scenario of the origin of zygomorphic flowers, we investigate the relevant genetic mechanisms involved in *Lotus japonicus*, a model plant in legumes, with independent-origin zygomorphic flowers.

Here we isolated a key component of floral development, *KEW*, by map-based cloning in *Lotus japonicus* and found it was sufficient and necessary for lateral petal development. Genetic data displays hierarchical epistatic relationships among dorsal, lateral and ventral identity and the ventral identity is a ground one.

Furthermore, we show that *KEW* is likely involved in two processes, establishment and maintenance of floral DV asymmetry and morphogenesis of lateral petals. We characterize a putative global transcriptional inhibitory mechanism regulating the asymmetric expression of *KEW*, which might help to establish and maintain the floral DV asymmetry subsequently, and another local transcriptional regulation system mediates the morphogenesis of lateral petals. Our other relevant data confirm these conclusions and indicate a putative conserved, general regulation model of this kind of genes. In addition, we described the development of lateral petals based on the analysis of epidermal cell morphology. By doing that, we think that *KEW* is likely to communicate separable different genetic networks in different spatio-temporal points during lateral petal development. We identified one kind of these responsive factors, namely *MOK* (*modifier of KEW*) and found that loss of its functions resulted in a semidominant phenotypes of *KEW1/kew1*. Taken together, we integrated the genetic, molecular and morphological data to discuss a hypothetical process of lateral petal development.

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Expression activation of a gene encoding an unknown protein with a DUF5723 domain affected siliques development in *Arabidopsis*. Liqing Ma¹, Aili Li¹, Xiaomei Tan¹, Hanzi Guo¹, Xiang Wang¹, Bo Wei¹, Yuanfang Zhu¹, Charles H. Leseberg², Long Mao^{1,2}. ¹Institute of Crop Sciences, Chinese Academy of Agricultural Sciences and National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Beijing 100081, China, ²Department of Biological Sciences, Northern Illinois University, DeKalb, IL 601115, USA

Insertion of a T-DNA construct at the promoter region of a gene encoding an unknown protein caused shortened stature with smaller, partially fertile siliques and curly cauline leaves in *Arabidopsis* plants. The gene contained a DUF5723 domain that is conserved in the rice genome. Genotypic analysis showed that heterozygous plants exhibited the most significant phenotypes whereas only a portion of homozygous transgenic plants displayed mutant phenotypes. The gene's expression, as measured by RT-PCR, was significantly up-regulated in plants displaying a mutant phenotype when compared to the control. This suggests that the over expression of the unknown gene may be responsible for the observed phenotypes. As expected, preliminary analysis of loss-of-function plants from the ABRC did not find obvious phenotypic changes. Over-expression experiments showed that only 5 out of 30 transgenic plants displayed similar phenotypes to the plant originally discovered. We deduce that the expression of the gene is negatively regulated, maybe in a stage-specific manner. Results from promoter analysis using the GUS reporter gene, interacting proteins from yeast two-hybrid screening and further genetic analysis will be presented.

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Role of polar auxin transport in orchestrating plant root growth and patterning. Ari Pekka Mahonen¹, Veronica Grieneisen¹, Jian Xu¹, Paulien Hogeweg¹, Ben Scheres¹. ¹Utrecht University, Utrecht, The Netherlands

The phytohormone auxin has been implicated in patterning of the root meristem. From embryogenesis onwards, an auxin response maximum is formed and maintained in the distal region of the root. The auxin maximum is achieved by PIN auxin efflux facilitators, which collectively control auxin distribution and form auxin reflux loop to regulate cell division and cell expansion in the primary root. The PLETHORA1 (PLT1) and PLT2 genes encode double AP2-domain transcription factors expressed in the embryonic root pole and later in the root stem cell region. Auxin induces transcription of PLT genes, which are redundantly required for stem cell maintenance in the root meristem. Furthermore, PIN proteins have an important role in restricting the expression domain of PLT genes. Conversely, PLT genes are required for PIN gene transcription to stabilize the auxin maximum at the distal root tip. These data reveal an interaction network of auxin transport facilitators and root fate determinants that control patterning and growth of the root primordium. In order to dissect this complex interaction network, we have developed *in silico* system to model auxin transport. The auxin flux pattern in our model yields to stable accumulation of auxin in a distal maximum, without local auxin production or decay. From the maximum auxin concentration decreases in graded manner. Both the auxin maximum and the gradient are robust towards parameter settings, root growth and noise. Next, we will use genetic tools to manipulate PIN localization in cell-type-specific manner. These local interruptions of the auxin transport route should result in altered auxin accumulation in the root meristem and in the *in silico* model, and consequently, in global repatterning in the root meristem.

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Function of DNA-repair genes in meiosis. Hong Ma¹, Wuxing Li¹, Rong Xiao¹, Asela Wijeratne¹, Wei Zhang¹, Changbin Chen¹. ¹The Pennsylvania State University

Plants face a variety of stresses, including DNA-damaging radiation and chemicals. We have studied the function of *Arabidopsis* homologs of DNA repair genes, including the members of the RAD51 gene family. We found that some of these genes are involved in DNA repair during mitotic growth, whereas others genes have no detectable effects on the growth of plants in response to DNA-damaging agents. Furthermore, many of these genes have critical functions during meiosis. We present results from detailed analysis of the meiotic functions of these RAD51 homologs. In addition, we have performed genetic analysis to test for possible interactions among members of this gene family. We have also identified novel meiotic genes using expression profiling. Two of these genes are homologs of known DNA repair genes. Our studies support the idea that homologs of DNA repair genes play essential functions during meiosis to ensure proper chromosome segregation, and that they also seem to play a role in DNA repair during mitotic growth.

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Activation of the TAS3-derived tasiRNA pathway in the root system of *Arabidopsis thaliana*. Alexis Maizel¹, Martin Crespi¹. ¹CNRS Plant Science Institute, Gif-sur-Yvette, France

Plant and animals use small RNAs (microRNAs and siRNAs) as guide for post-transcriptional and epigenetic regulation. In plants, miRNAs and trans-acting siRNA (tasiRNA) result from different biogenesis pathways but both interact with target transcripts to direct their cleavage. Three ta-siRNA gene families (TAS1, TAS2, and TAS3) are known in *Arabidopsis thaliana*. TAS gene transcripts are cleaved by miRNAs; the cleavage products are copied into dsRNA by RDR6, and diced into tasiRNAs by DCL4. Biogenesis of TAS3-derived tasiRNAs involves miR390 and they target mRNAs encoding AUXIN RESPONSE FACTORs ARF3/ETTIN and ARF4. Specific degradation of ARF3 in the leaves by TAS3-derived tasiRNA is critical for leaf development and phase transition. In a screen for large non-protein coding RNAs in *Arabidopsis* using a dedicated micro-array, we identified that TAS3 gene is highly expressed in root tissues. Using reporter constructs for TAS3 and miR390 loci as well as analysis of the accumulation of their derived RNAs, we have characterized the expression pattern of the TAS3 pathway during root development. We present evidence that this pathway can be linked to the architecture of the root system.

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Identification of the cis-acting sequences required for KNOX regulation of LATERAL ORGAN BOUNDARIES. Amanda Mangeon¹, Bin Shuai¹, Patricia S. Springer¹. ¹Department of Botany and Plant Sciences, Center for Plant Cell Biology, University of California, Riverside

LATERAL ORGAN BOUNDARIES (LOB) is the founding member of the plant-specific *LBD* gene family of hypothetical transcription factors. *LOB* was isolated from an enhancer-trap screen based on its expression pattern in the boundaries of lateral organs, including the adaxial boundaries between the shoot apical meristem and leaf primordia. Class I *KNOX* transcription factors are expressed in the meristem and excluded from leaves. The *KNOX* genes *BREVIPEDICELLUS (BP)* and *SHOOT MERISTEMLESS (STM)* are required for meristem maintenance and are responsible for the repression of leaf-expressed genes, such as *ASYMMETRIC LEAVES1 (AS1)* and *AS2*, in the meristem. In turn, *AS1* and *AS2* function to repress *KNOX* gene expression in leaves, indicating an antagonistic relationship between genes expressed in these adjacent domains. As *LOB* is expressed at the boundary between these domains, we are interested in whether *LOB* might function to mediate communication between the meristem and organ primordia. *LOB* expression is positively regulated by both *KNOX* transcription factors and by *AS1/AS2*, consistent with this idea. Using promoter deletion analysis we have mapped the regulatory regions responsible for *LOB* expression pattern and for *KNOX* regulation of *LOB*. These results will be presented.

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HYL1 regulates Leaf Form through Coordination of MIR166 and MIR319 genes. Yanfei Mao¹, Lin Yu¹, Liguo Jia¹, Zhongyuan Liu¹, Guixuan Song¹, Feijie Wu¹, Yuke He¹. ¹ Shanghai Institute of Plant Physiology & Ecology, Chinese Academy of Sciences, Shanghai, China

During leaf differentiation from shoot apical meristem, the three-dimensional form of leaves is specified along proximodistal (base-to-tip), dorsoventral (top-to bottom) and mediolateral (middle-to-margin) planes. Null mutation of *Arabidopsis HYPONASTIC LEAVES1*, which participates in microRNA (miRNA) biogenesis, changed the three-dimensional form of leaves as the *hy1* leaves become abnormally lengthy, narrow, sessile and transversely incurved. Despite the recent progress in understanding of *HYL1*-controlled miRNA processing, it remains uncertain whether or how the shofar-shaped leaves of *hy1* mutants is caused by impaired miRNA processing. To clarify the role of *HYL1* in development of leaf form, we crossed *hy1* with the gain-of-function mutants of *MIR166* and *MIR319a*, respectively, and made a series of double or triple mutants containing the mutant alleles of the miRNA-targeted genes. Genetic analysis, in combination with expression analysis, showed that in *hy1* leaves the loss of polarity in the lateral area was caused by the impaired *MIR166* processing whereas unbalanced division of the distal cells and the marginal cells was related to the reduced accumulation of *MIR319*, both contributed to the specification of the shofar-shaped leaves. In rosette leaves of *jba-1d jaw-1D* double mutants, biogenesis of either *MIR166* or *MIR319* is enhanced when defects in polarity and cell division became severe, thus demonstrating mutual enhancing interactions between *MIR166g* and *MIR319a* genes. These results suggest that *HYL1* regulate leaf form through coordination of *MIR166* and *MIR319* genes.

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The DAG1 transcription factor from *Arabidopsis thaliana*: study of its transcriptional/post-transcriptional regulation Julie Martone¹, Patrizia Cirelli¹, Stefano Gabriele¹, Annalisa Rizza¹, Gianluca Ragone², Paolo Costantino¹, Paola Vittorioso¹. ¹Dept. of Genetics and Molecular Biology, Univ. La Sapienza, Rome, (Italy), ²Istituto Dermatologico Italiano, Via dei Monti di Creta, Rome, (Italy)

DAG1 (*Dof Affecting Germination 1*) is a DOF transcription factor of *Arabidopsis thaliana* involved in the control of seed dormancy and germination. In fact, seeds of the *dag1* mutant show an increased germination potential compared to the *Wt*. Most interestingly, the pattern of segregation of the *dag1* phenotype showed that the effect of the disruption of *DAG1* is maternal and the gene is expressed only in the vasculature of the mother plant but not in the seed or at any stage of embryo development. This pointed to maternal control acting on the developmental fate - dormancy vs. germination - of the mature seed. A study on the *DAG1:GFP* cellular localization allowed us to reveal a potential action of this TF as a non-cell autonomous protein, as it appears to be present in tissues where the *DAG1* promoter is inactive and the mRNA is absent. Moreover, our data indicates that this phenomenon could be developmentally regulated. With respect to the maternal effect of *DAG1* inactivation we decided to investigate whether the *DAG1* protein could move to the seed. Genetic and molecular data will be presented on *DAG1* localization and on its transcriptional and post-transcriptional regulation.

Moreover, to further characterize the role of *DAG1* we performed an analysis of the *dag1* transcriptome. We have identified several putative targets that are up/down-regulated in the *dag1* mutant, and some of them seemed to be clearly related to *DAG1*.

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Movement of FT protein from the vasculature to the apex is sufficient to induce flowering in *Arabidopsis thaliana*. Johannes Mathieu¹, Frank K tner¹, Norman Warthmann¹, Markus Schmid¹. ¹Max Planck Institute for Developmental Biology, Department of Molecular Biology, Spemannstrasse 37-39, D-72076 Tbingen, Germany

Correct timing of flowering is a crucial factor for reproductive success in plants, and the time at which the shoot apical meristem (SAM) switches fate from vegetative to reproductive growth is tightly regulated by intrinsic and extrinsic factors. An important environmental input is day length. More than 70 years ago grafting experiments provided evidence that plants produce in their leaves a signal that translocates to the SAM to induce flowering. The nature of this signal, termed "florigen", has long eluded discovery. Recently, studies in *Arabidopsis* have shown that two genes, *CONSTANS (CO)* and *FLOWERING LOCUS T (FT)*, that are predominately expressed in leaves, are central for the plant to respond to inductive photoperiod. It was further shown that *FT* is the primary transcriptional target of *CO* in the leaf and that *FT* mRNA can be detected at the SAM after heat shock induced expression in the leaves. However, whether movement of the *FT* mRNA is necessary and / or sufficient to induce flowering is unclear.

Here we show that expression of a functional artificial miRNA targeting *FT*-mRNA (*amiR-FT*) under control of the *FD-* (SAM-specific) promoter does not suppress flowering. In contrast, plants expressing *amiR-FT* from either the *35S* - or the *SUC2* -promotor mimic the late-flowering of the *ft* mutant phenotype. Together, these results suggest that *FT* mRNA is required in the vasculature but might not be necessary at the apex to induce flowering. To assess the role of *FT* protein movement during floral transition, we developed a novel assay in which a large *FT* fusion protein is retained in the tissue where it is expressed. Upon exposure to a protease the mature *FT* protein is released and free to act. Using this assay we demonstrate that release of *FT* protein in the vasculature is sufficient to induce flowering.

These results suggest an important role for the movement of *FT* protein in the induction of flowering by photoperiod.

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BLADE-ON-PETIOLE is Required for Abscission Zone Formation. Sarah M. McKim¹, Grethe-Elisabeth Stenvik², Wenche Kristiansen², Melinka A. Butenko², Sung Ki Cho³, Shelley R. Hepworth¹, Reidunn B. Aalen², George W. Haughn¹. ¹ Department of Botany, University of British Columbia, Canada, ²Department of Molecular Biosciences, University of Oslo, Norway, ³Division of Biological Sciences, University of Missouri, USA

The *Arabidopsis* "BLADE-ON-PETIOLE" ("BOP") genes encode redundant transcription factors that are involved in leaf and flower development. Loss-of-function "bop1bop2" mutants display a range of defects characterized in detail, including ectopic leaf blade growth and extra floral organs. Also, "bop1bop2" mutants show a loss of floral organ abscission although to date this has received little attention. Abscission occurs along specialised cell files, called Abscission Zones (AZs), which develop at the junctions between the leaving organs and main plant body. We have thoroughly characterized the "bop1bop2" abscission phenotype to assess the role of "BOP1/BOP2" in the known abscission developmental framework. Microscopic and quantitative analyses of "bop1bop2" mutants show no differentiation of either floral AZs or vestigial caudine leaf AZs suggesting BOP proteins are essential to establish AZ cell identity. In support of this hypothesis, BOP activity was required for precocious floral organ abscission and ectopic abscission at the pedicel and caudine leaves promoted by ectopic expression of the "INFLORESCENCE DEFICIENT IN ABSCISSION" ("IDA"). The "IDA" gene acts late in the abscission program to trigger the final separation step if there is a differentiated AZ present (1, 2). Interestingly, expression of several abscission-related markers, including "IDA", were relatively unperturbed in "bop1bop2" mutants, indicating that these genes respond to positional cues independent of BOP activity. We also discovered that BOP1/BOP2 activity is essential for formation of nectary glands which normally develop at the receptacle proximal to AZs. This suggests that "BOP1/BOP2" genes are involved in determining multiple cell identities at the receptacle.

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Molecular and physiological characterization of TF71, a member of the *Arabidopsis* AP2/EREBP transcription factor family Mohammad Mehrnia¹, Erwan Michard^{1,2}, Maria Ines Zanor³, Bernd Moeller-Roeber^{1,3}. ¹University of Potsdam, Institute of Biochemistry and Biology, 14476 Potsdam-Golm, Germany, ²Present address: Centro de Biologia do Desenvolvimento, Instituto Gulbenkian de Ciencia, 2780-156 Oeiras, Portugal, ³Max Planck Institute of Molecular Plant Physiology, 14476 Potsdam-Golm, Germany

AP2/EREBP transcription factors are known to be involved in many aspects of cellular and developmental processes as well as various responses to environmental stimuli. To date, most of the members of the superfamily have yet to be analysed. This study investigates the function of one member (named TF71) of the *Arabidopsis* AP2/EREBP family. TF71 gene expression was analysed using promoter-reporter (-glucuronidase) fusions. Expression was detected in root tips, at positions of lateral root formation, in various flower tissues, in pedicels and the abscission zone of siliques, and in stipules. In leaves, GUS activity was generally only marginal, but strongly, within 6 hours, induced by wounding. TF71 expression was also found to be induced by auxin treatment, and by the addition of phosphate to phosphate-starved seedlings. TF71 expression is remarkably strong during the S phase of the cell cycle. In addition, strong TF71 activity was observed during callus formation. Ectopic expression of TF71 under the control of the CaMV35 S promoter strongly reduces apical dominance and leads to a reduction of overall plant size. Our data indicate that TF71 is a transcription factor particularly important during cell proliferation. To identify direct target genes of TF71 we have generated transgenic plants expressing it under the control of a chemically inducible promoter. Transcriptome profiles will be obtained from plants shortly after treatment with the chemical inducer. The data obtained will be presented and the function of identified target genes discussed.

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An *Arabidopsis* ARM repeat protein, ARCP1, is necessary for the anther dehiscence. Yu Mei¹, Hong-Wei Xue¹. ¹Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Armadillo (Arm) repeat proteins are a large family of proteins containing tandem copies of an approximately 40 amino acid long sequence motif. Animal Arm repeat proteins function in various processes, including intracellular signalling and cytoskeletal regulation. Over 100 Arm repeat proteins are predicted in *Arabidopsis*, but only few of them have been well characterized. Our study focused on ARCP1, an *Arabidopsis* Arm repeat protein which contains sixteen Arm repeats at the N terminus and a C2 domain at the C terminus. Studies through semi-quantitative RT-PCR and promoter-GUS gene fusion showed that ARCP1 was constitutively expressed in various tissues with the strongest expression in roots, stigma and pollen grains. A T-DNA knockout mutant of ARCP1 was further characterized and analyzed to examine its physiological functions. The homozygous mutant displayed a male sterile phenotype. Further analysis through light microscopy and scanning electron microscopy revealed that the sterility was caused by the defect of the mutant in the right time of anther dehiscence. Exogenous MeJA and expression of only the C2 domain could not rescue the sterile phenotype, suggesting that the multiple Arm repeats containing in ARCP1 were of great importance to its physiological functions and that the defect in dehiscence of anther at the correct time may be a result of MeJA insensitivity.

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NAC transcription factors NST1, NST2 and NST3 are key regulators of secondary wall formation in *Arabidopsis*. Nobutaka Mitsuda¹, Akira Iwase¹, Hiroyuki Yamamoto², Masato Yoshida², Motoaki Seki³, Kazuo Shinozaki³, Masaru Ohme-Takagi^{1,1}. Advanced Industrial Science and Technology (AIST), Tsukuba, JAPAN,²Nagoya University, Nagoya, JAPAN,³RIKEN Plant Science Center, Yokohama, JAPAN

Wood is the largest terrestrial biomass and widely used in our life. However, the key factor that regulates wood formation remained still obscure. Wood is formed by successive addition of secondary xylem whose cells have heavily thickened secondary walls mainly composed of lignin and cellulose. In our comprehensive study of *Arabidopsis* transcription factors, we identified that NAC domain transcription factors, NST1 (NAC Secondary wall Thickening promoting factor1), NST2 and NST3, are master regulators of secondary wall formation in *Arabidopsis*. NST1 and NST2 are expressed in anther and disruption of both genes resulted in indehiscent anther due to loss of secondary walls in anthers, which is required to generate tension for dehiscence. On the other hand, NST1 and NST3 are expressed in xylem of stem and hypocotyl, which has similar structural feature to a trunk of tree. The double mutant of these genes (*nst1-1 nst3-1*) cannot stand erect due to loss of secondary wall formation in fiber and secondary xylem of hypocotyls except vascular vessel. Conversely, over-expression of NST's induced ectopic secondary wall formation in various aboveground tissues. Genes that are involved in the biosynthesis of the components of wood, namely lignin and cellulose, clearly down-regulated in the knockout plants while they are upregulated in the plant over-expressing NST1. We concluded that the NST transcription factors are master regulators of secondary wall formation in various tissues except vascular vessels.

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Promoter Analysis of PRESSED FLOWER (PRS) Necessary for Development of the Marginal Domain of Lateral Organs in *Arabidopsis*. Miyuki Nakata¹, Shunji Funaki¹, Noritaka Matsumoto¹, Ryuji Tsugeki¹, Kiyotaka Okada^{2,1} Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan,²National Institute for Basic Biology, Aichi, Japan

Lateral organs of vascular plants, including leaves and floral organs, develop various tissues in a position-dependent manner. For example, in marginal domain of lateral organs, especially leaves and sepals, margin-specific epidermal cells differentiate and form the boundary between adaxial and abaxial epidermal cells. Some of *WUSCHEL*-related homeobox genes, *Arabidopsis PRESSED FLOWER (PRS)* and maize *NARROW SHEATH1 (NS1)/NS2* are specifically expressed in marginal domain of lateral organ primordia. *PRS* and *NS1/NS2* are necessary for formation of the marginal domain (1-3). These suggest that the margin-specific expression of *PRS* and *NS1/NS2* is an important step for development of marginal domain of lateral organs.

To investigate the mechanism of the margin-specific expression, we carried out promoter analysis of *PRS*. The 838 bp upstream region of *PRS* was sufficient for the margin-specific expression in lateral organs at the early developmental stage. We further dissected the 838-bp promoter and identified the 140-bp region necessary for the early margin-specific expression of lateral organ primordia. We are screening for the transcription factors which specifically bind to the 140-bp region by yeast one-hybrid system.

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The pre-replication factor AtMCM2, is involved in the maintenance of the columella root meristem initials. Di-An Ni¹, Cecile Raynaud¹, Rossangela Sozzani², Rino Celli², Sophie Blanchet¹, Diego Albani³, Catherine Bergounioux^{1,1}. CNRS, Universit Paris-sud, IBP, France,²Depart. of genetics , University of Pavia, Pavia, Italy,³Depart. of Botany , University of Sassari, Italy

Initiation of DNA replication is a crucial decision point during cell proliferation and lies at the point of convergence of complex networks of signaling molecules that have evolved to specify when and where cells divide in an organism. This process is precisely regulated during G1 phase of the cell cycle by the pre-replication complex (pre-RC) (Dresselhaus et al., 2006). Compared to budding yeast, animal and human, *Arabidopsis* possesses all the homologues of MCM proteins including two recently identified non-MCM2-7 proteins (MCM8 and MCM9). The *Arabidopsis* AtMCM2 encodes a homologue of replication licensing factor MCM2. *Arabidopsis* mutant analysis indicated that AtMCM2 is embryo lethal. Transgenic *Arabidopsis* overexpressing AtMCM2 (MCM2OE) remain small in size and smaller cells are observed in MCM2OE plants. In addition, leaf DNA content analysis shows that endoreduplication is also inhibited, thus confirming AtMCM2 involvement in DNA replication. Moreover, in agreement with the behavior of almost all factors of the pre-replication complex, AtMCM2 up and down regulation depends on E2F transcription factors. Expression studies show that AtMCM2 is developmentally regulated. Interestingly, during root development AtMCM2 expression is high in secondary root primordia and becomes later restricted to the central cylinder above the root quiescent center and excluded from the cortical cells of the dividing zone of root meristems. Down-regulation of AtMCM2 by RNAi severely affects root growth. Conversely, over-expression of AtMCM2 induces the formation additional stem cells in the root meristem. Moreover, over-expression of MCM2 rescues the phenotype of pit mutants in which the root meristem stops functioning, by allowing the emergence of a lateral root close to the apical meristem. Therefore, the present work may open new prospects on the involvement of the pre-RC in root stem cell specification.

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Mutations in the Type II protein arginine methyltransferase AtPRMT5 result in pleiotropic developmental defects in *Arabidopsis thaliana*. LiFang Niu^{1,2}, YanXi Pei^{1,3}, FaLong Lu^{1,2}, Chun-Yan Liu¹, JiXian Zhai^{1,2}, XiangFeng Kong^{1,2}, XiaoFeng Cao¹.¹State Key Laboratory of Plant Genomics and Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China 100101,²Graduate School, Chinese Academy of Sciences, Beijing, China, 100039,³College of Life Science and Technology, Shanxi University, Taiyuan, China, 030006

Human protein arginine methyltransferase 5 encodes a type II protein arginine methyltransferase and its orthologs in animals and yeast have been shown to play important roles in the regulation of RNA processing, signal transduction and gene expression. However, PRMT5 homologs in higher plants have not yet been reported and biological significance of these proteins in plant development remains elusive. Here, using conventional biochemical approach, we purified a plant histone arginine methyltransferase from cauliflower that was nearly identical to AtPRMT5, an *Arabidopsis* homolog of human PRMT5. AtPRMT5 symmetrically di-methylated histone H4R3, H2A and myelin basic protein (MBP) *in vitro*. Mutations in AtPRMT5 caused pleiotropic developmental defects, including growth retardation, dark green and curled leaves, and *FLC*-dependent delayed flowering. This is the first time demonstration that the type II protein arginine methyltransferase is involved in repression of vegetative growth and *FLC*-dependent flowering time in *Arabidopsis*.

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Investigating the Role of *Arabidopsis thaliana* Genes At1G14120 and At1G14130 in Adventitious Rooting. Colm Nolan¹, Thomas Gallagher¹. ¹School of Biology and Environmental Science, University College Dublin, Dublin, Ireland.

Adventitious rooting is a complex process, the control mechanisms of which are still poorly understood. It is mediated by several factors, both light and hormones (especially auxin) play a central role and may interact with other endogenous factors or environmental stimuli. *Arabidopsis* genes At1g14120 and At1g14130 show sequence homology to Apple (*Malus domestica*) ARRO-1 (adventitious rooting related oxygenase), which is up-regulated during auxin-induced adventitious rooting, encoding a dioxygenase activity.

Full length gene nucleotide sequences under the control of the cauliflower mosaic virus 35S promoter have been expressed in lines of transgenic *Arabidopsis* plants to cause constitutive and ectopic gene expression. For underexpressors/knockouts, hairpin loop-forming RNAi constructs have similarly been engineered, to promote cleavage by the plant cell of both the transgene-derived and the endogenous messenger RNA, reducing/obliterating endogenous gene expression. A gene knockout line (by T-DNA insertion) for AT1G14130 has been obtained and the gene sequence disruption verified. Real time PCR will be carried out with short PCR products and comparisons made between different tissues of the plant which will complement results from microarray experiments. Absolute mRNA copy number in over-expressors/ knockouts and RNAi plants can also be estimated using this method. In gene promoter studies, At1G14120 and At1G14130 full length promoter fragments have been generated with an in-frame fusion to the GUS reporter gene, to colorimetrically display potential spatial and temporal patterning in gene expression. Cell and plant level gene product transportation and localisation is also being examined by the expression of the gene nucleotide sequences fused in-frame to the EYFP reporter gene.

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Gene expression profiling of stomatal precursors. Nalini Odpalli¹, Jeanette Nadeau¹. ¹Department of Biology, University of Central Florida, Orlando, FL 32816-2368, USA

Stomata are structures that act as valves to control water loss and gas exchange through the plant epidermis. They are non-randomly spaced through cell-cell signaling that controls the position, frequency, and polarity of asymmetric divisions of stem cell-like precursors that form stomata. In leaves, mutation of the TOO MANY MOUTHS (TMM) LRR receptor-like protein causes patterning defects that lead to overabundant and clustered stomata. In contrast, the *tmm* mutation prevents development of stomata in other organs such as the inflorescence stem. In *tmm* stems, stomatal precursors (meristems) are produced but stop dividing and differentiate as epidermal pavement cells. Also in contrast to leaves, stem stomata form in a morphogenetic gradient so that mature stomata and guard mother cells are spatially separated from meristems. We chose to exploit these characteristics to identify genes that may play a role in stomatal stem cell maintenance and biogenesis using microarray gene expression profiling. The apical region of the inflorescence stem tip containing only meristems (but no stomata) was harvested from both mutant and wild-type plants. Resulting RNA was hybridized to the Affymetrix ATH1 genechip. A Bayesian statistical method (RACE) following robust multichip averaging was used to identify 352 genes that showed significant changes in expression level between mutant and wild-type tissues. No other known regulators of stomatal development, with the exception of *TMM* itself, were differentially expressed. Although a large proportion of these genes are probable components of signal transduction cascades, no functional class was statistically over-represented in the dataset. 11 genes were also differentially regulated by the YODA MAPKKK that controls stomatal development (Bergmann et al. (2004) *Science* 304:1494). Analysis of insertional mutants in candidate genes is currently underway and stomatal phenotypes have been observed in several, validating this approach to identification of new regulators of stomatal biogenesis.

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Repression of AUXIN RESPONSE FACTOR10 by microRNA160 Is Critical for Plant Development. Hiroyuki Nonogaki¹, Po-Pu Liu¹, Taiowa Montgomery^{2,3}, Noah Fahlgren^{2,3}, Kristin Kasschau^{2,3}, James Carrington^{2,3}. ¹ Department of Horticulture, Oregon State University, Corvallis, OR 97331, USA, ² Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331, USA, ³ Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA

AUXIN RESPONSE FACTORS (ARFs) are transcription factors involved in auxin signal transduction during many stages of plant growth development. ARF50, ARF56 and ARF57 are targeted by microRNA160 (miR160) in *Arabidopsis thaliana*. Here, we show that negative regulation of ARF50 by miR160 is critical for normal plant development. Transgenic plants expressing a miR160-resistant form of ARF50, which has silent mutations in the miRNA target site (termed mARF50), exhibited developmental defects such as serrated leaves, curled stems, contorted flowers and twisted siliques. These phenotypes were not observed in wild-type plants or plants transformed with the targeted ARF50 gene. During seed germination and seedling establishment, mARF50 mutant seeds and plants were hypersensitive to ABA in a dose-dependent manner. ABA hypersensitivity was mimicked in wild-type plants by exogenous auxin. In contrast, overexpression of MIR160 (35S:MIR160) resulted in reduced sensitivity to ABA during germination. These results indicate that repression of ARF50 by miR160 plays a critical role in seed germination and postembryonic developmental programs.

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Investigation of possible DME-downstream pathway genes using gene chip microarray. Hyonhwa Ohr¹, Jean Choi¹, Robert Fischer², Yeonhee Choi¹. ¹Department of Biological Sciences, Seoul National University, Seoul, 151-742, Republic of Korea, ² Department of Plant and Microbiology, University of California, Berkeley, CA94720, USA

DME (DEMENTER) DNA glycosylase is an active demethylase excising 5-methylcytosine and required for the maternal allele expression of the imprinted genes such as MEDEA, FWA and Fertilization-Independent seed2 (FIS2). Temporal and spatial expression of DME in the central cell of the female gametophyte, not in pollen, establishes gene imprinting by hypomethylating the maternal allele of the imprinted genes. While the biological relationship between DME and the target genes are well investigated, it is still unknown whether there are other genes regulated by DME. To gain insights of the possible DME-downstream pathway genes, we generated transgenic plants showing ectopic DME expression under the CaMV 35S promoter in pollen. In this study, we analyzed the microarray gene profile from pollen and stamen tissues comparing CaMV::DME transgenic plants to wild type. We found only 91 genes were induced by ectopic DME expression in male tissues. Some of the possible DME target genes are involved in cell cycle and transcriptional regulation. Semi-quantitative RT-PCR was performed to confirm and narrow-down the candidate genes of gene chip analysis. The possible candidate genes in this study will be useful for the further research on DME DNA glycosylase regulatory mechanism.

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Differential requirement for the function of SRD2, an snRNA transcription activator, in various stages of plant development. Misato Ohtani¹, Taku Demura¹, Munetaka Sugiyama². ¹Plant Science Center, RIKEN, ² Bot. Garden., Grad. Sch. Sci., The Univ. Tokyo

snRNA (small nuclear RNA) is a class of eukaryotic non-coding RNAs, which have essential roles in pre-mRNA splicing and rRNA processing. As these functions are fundamental to cell activities, the regulation of snRNA transcription should be a vital issue for all eukaryotes. Here we address developmental control of snRNA transcription and its significance through the analysis of arabidopsis SRD2 gene, which encodes an activator of snRNA transcription. In young seedlings, a high level of SRD2 expression was observed in shoot and root apical meristems, leaf primordia, and root stele tissues, where a large amount of snRNA accumulated. In grown-up plants, SRD2 was highly expressed in developing leaves and flowers as well as apical meristems. Mutations in the SRD2 gene interfered with many but not all aspects of development in the regions that showed strong expression of SRD2. Of note, establishment of the fully active state of apical meristems in the seedling stage was very sensitive to the srd2 mutation, while maintenance of the established meristems was substantially insensitive. These results demonstrated differential requirement for the SRD2 function in various stages of plant development.

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Dek1-a key player in a general model for epidermis formation in plants? Lene Olsen¹, Qing Tian^{2,3}, Stein Erik Lid¹, Betty Lemmon⁴, Roy Brown⁴, Fred Gruis⁴, Kjetil Fosnes¹, Marisa Otegui⁵, Odd-Arne Olsen^{2,3}, Hilde-Gunn Opsahl-Sorteberg¹. ¹Norwegian University of Life Sciences, 1432 Aas, Norway, ²Pioneer Hi-Bred International, A DuPont Company, Johnston, IA, USA, ³ present address: Monsanto Company, Chesterfield, MO, USA, ⁴University of Louisiana, Lafayette, LA, USA, ⁵University of Wisconsin-Madison, Madison, WI, USA

Cell fate specification and development of the maize endosperm aleurone layer, the endosperm epidermis, involve Dek1, Cr4 and Sal1. DEK1 (240 kDa), consisting of a membrane spanning (DEK1-MEM) domain and a cytosolic calpain-like cysteine proteinase (DEK1-CALP), is essential for position- (surface) dependant formation of aleurone cells. In "Arabidopsis thaliana", the dek1 lethal phenotype is complemented by the AtDek1 gene. In contrast, DEK1-CALP is unable to complement the mutant. Interestingly, expression of DEK1-MEM leads to a dominant negative phenotype similar to dek-RNAi phenotypes, including lack of protoderm in the embryo and failure to form an apical meristem. These results suggest that Dek1 is activated by a ligand and that the calpain proteinase is regulated by the membrane domain, and that Dek1 is essential for epidermis formation throughout the plant. Embryo cytoskeleton studies show that despite a functional microtubule apparatus, the dek1 embryos have asymmetrical division planes, misplaced phragmoplasts and they appear tiered with protodermal defects. In addition to results from "Arabidopsis thaliana", we will present data from maize endosperm *in vitro* organ cultures that show that Dek1, Cr4 and Sal1 co-localizes to endosomes, possibly suggesting that these molecules may functionally interact and that a proper concentration of Dek1 and Cr4 in plasma membranes is maintained by internalization and degradation "via" traffic through Sal1-positive endosomes. Based on the effect of dek1 and cr4 mutations in a variety of epidermal cell types of both monocots and dicots, we propose that the Dek1 pathway described here is responsible for surface recognition, and hence epidermal cell fate specification in all plants.

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Functional analysis of a rice gene homologous to the Arabidopsis FVE. Hyo Young Park¹, Il-Sun Baek¹, Yun-Hee Jang¹, Soon-Kap Kim¹. ¹School of Life Sciences and biotechnology, Korea university

A full length rice cDNA clone (AK111794, OsFVE) showing significant similarity to the Arabidopsis FVE gene was isolated from a Japanese rice cultivar. The FVE is a chromatin remodeling complex protein which belongs to the autonomous pathway of flowering time control. The amino acid sequence identity of the OsFVE clone with FVE1 (MIS4) and FVE2 (MIS5) are 75% and 69%, respectively. The OsFVE gene is composed of fourteen introns and fifteen exons. RT-PCR analysis indicated that the OsFVE mRNA was detectable in all tissues examined, but showed higher expression in mature leaves than in leaves of young rice seedlings.

We made transgenic Arabidopsis fve-3 mutant plants expressing the OsFVE cDNA driven by 35S promoter in order to examine if the OsFVE could rescue the late flowering phenotype of fve-3 mutant. The T1 transgenic Arabidopsis plants showed a wide spectrum of flowering times from one similar to that of Col-0 to the other showing later flowering time than the fve-3 mutants. About one third of total T1 lines showed partially-rescued phenotype. We examined the expression patterns of flowering time genes such as FLC and SOC1 in the T2 lines showing partially-rescued phenotype. Intermittent cold treatment delays the flowering of Col-0 but not that of fve-3 plants. This phenotype was not rescued in the T2 transgenic lines. This is consistent with observation that the FLC mRNA level of the T2 transgenic lines were not changed by intermittent cold treatment.

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A role for LMI2, a MYB transcription factor, in the Meristem Identity Transition. Jennifer Pastore¹, Natasha Chavdaroff¹, Doris Wagner¹. ¹University of Pennsylvania, Philadelphia, U.S.A.

Plants go through distinct developmental phases throughout their life cycle. The transition from vegetative to reproductive phase, known as the meristem identity transition, is vital to species survival and important for our economical and agricultural resources. In *Arabidopsis thaliana*, this transition is initiated by the plant specific transcription factor LEAFY (LFY). We have shown that LFY directly activates two other meristem identity regulators APETALA1 (AP1) and CAULIFLOWER (CAL). Using a microarray approach combined with chromatin immunoprecipitation (ChIP) and RT-PCR, our lab has identified five additional direct targets of LFY that are candidate meristem identity regulators. One of these targets is a MYB transcription factor known as MYB17 (At3g61250). MYB transcription factors have been identified in all major eukaryotic organisms. In animals, their function is to control cell differentiation and proliferation. In plants, their role is more diverse, with roles in processes including secondary metabolism, cellular morphogenesis, signal transduction in growth, abiotic stress and pathogen defense.

Is MYB17 involved in the meristem identity transition? To address this question we performed phenotypic analyses using two T-DNA insertion lines. Mutations in MYB17 cause the strongest meristem identity phenotype of all the newly identified LFY targets. The severity of the single mutant phenotype is similar to that of AP1 loss-of-function mutants. We named this meristem identity regulator LMI2 (Late Meristem Identity 2). Preliminary expression analysis places LMI2 upstream of AP1. Phenotypic analysis of ap1lmi2 double mutants indicates both genes likely act in the same pathway. These and additional findings that will be presented demonstrate that LMI2 plays an important role in the meristem identity pathway.

P-302

A new view on the reduction of petals and stamens in cruciferous flowers. Aleksey Penin¹, Maria Logacheva¹. ¹Moscow State University, Moscow, Russia

The studies on floral mutants of *Arabidopsis thaliana* and on wild type flowers of related species evidence that reduction of floral organs affects more frequently petals and stamens, not sepals and carpels. However, the genetic mechanism of this remains unclear. We report here the results of the studies on a new floral mutant of *A. thaliana* that is characterized by the reduction of petals and a part of stamens. Flower structure of this mutant is similar to those of some species of Lepidium thus it has been called *lepidium-like* (*lel*).

In order to clarify the role of *LEL* in a genetic network controlling flower development we have constructed double mutants *ag lel*, *ap2-1 lel*, *ap3-1 lel*, *ap1-20 lel*, *clv1-1 lel*, *ptl-1 lel* and analyzed the expression of genes involved in flower development using real time RT-PCR.

Double mutant *lel ag* shows indeterminate flowers with normal petals, suggesting that *LEL* is not a gene that acts specifically to promote petal development. In support of that gene expression analysis has revealed no alteration in the expression of A and B class genes. At the same time, *lel* shows altered expression of genes, controlling stem cell activity. Strong decrease in *WUS* expression suggests that *LEL* is a positive regulator of *WUS*.

With regard to the fact that *lel* develop normal carpels and sepals its action on stem cell activity is confined to second and third floral whorls, i. e. the middle of the flower. The epistasis of *AG* over *LEL* indicates that *AG* acts at the earlier stages of flower development. Thus, the gene responsible for the formation of the meristem zone where petals and stamens develop is acting later than the gene responsible for carpel development. Floral morphology of *ap2-1 lel*, *ap3-1 lel*, *ap1-20 lel*, *clv1-1 lel* also supports the suggestion that during flower development the zones of sepal and carpel formation are activated before the zone which gives rise to petals and stamens and that the activity of stem cells in this zone is mediated by *LEL*. This provides a possible mechanism for the reduction of petals and stamens as the decrease of the stem cell activity.

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Prediction and characterization of genes exclusively expressed in the female gametophyte of *Arabidopsis thaliana*

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The female gametophyte of *Arabidopsis thaliana* is contained within the ovule and composed of seven cells: 3 antipodal, 2 synergids, the egg cell, and a binucleated central cell. We have used a combination of publicly available microarray data and Massively Parallel Signature Sequencing (MPSS) information to identify candidate genes exclusively expressed in the female gametophyte. Initially, the patterns of expression of MPSS-detected genes that were active in wild-type but not nozzle/spore-cysteless ovules were identified in ATH1 microarray experiments conducted by Twell. A group of 13 genes, not detected in other MPSS collections or in the overall microarray data available, were selected for genetic and molecular characterization. Some of these weakly expressed genes encode transcription factors or signaling proteins. Conventional and real-time reverse-transcription polymerase chain reaction (RT-PCR) confirmed that 4 are expressed in wild-type but not in ovules lacking the female gametophyte. A genetic screen identified 2 insertional lines showing semi-sterile phenotypes, suggesting a role at the gametophytic level. Whole-mount *in situ* hybridization is being conducted to determine the pattern of mRNA localization of these genes and confirm unambiguously their gametophytic nature.

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SCHIZORIZA is a regulator of the stem cell niche development. Monica Pernas¹, Eoin Ryan¹, Paul Linstead¹, Liam Dolan¹. ¹Department of Cell and Developmental Biology. John Innes Centre, Norwich, UK.

The root meristem contains a group of stem cells responsible for the generation of the different cell layers of the root during the life of the plant. These stem cells or initials surround a group of slowly dividing cells known as the quiescent centre (QC). Together the stem cells and the QC constitute the stem cell niche of the root. The QC acts as a source of signals that position and maintain the stem cells during development. We have discovered that SCHIZORIZA (SCZ) is a putative transcription factor that is required for the maintenance of the stem cell niche in the arabidopsis root. The phenotype of the "scz" mutant and the pattern of SCZ gene expression support this role. The patterns of cell division in the stem cells niche are altered in "scz" mutants compared to wild type indicating a requirement for SCZ in the maintenance of the stem cell niche organization. Furthermore SCZ positively regulates the differentiation of the columella initials given that these cells types do not develop in "scz" mutants. SCZ regulates also the activity of ground tissue initials since these cells undergo a pericinal division in "scz" mutants instead of an anticinal division that occur in wild type. This results in the formation of extra layers of ground tissue with mixed endodermal and cortical identity in the "scz" mutant. To verify the role of SCZ in the function of the stem cell niche we show that SCZ is expressed in the QC, ground tissue initials, endodermis and cortex layers. SCZ is therefore an important regulator of the root stem cell nice and we are currently characterising its interaction with other genes that control the development of this structure.

P-305

IRONIC, a new iron deficiency-responsive mutant defective in root epidermal patterning. Paula Perry¹, Wolfgang Schmidt¹. ¹IPMB, Academia Sinica, Taipei, Taiwan

Roots are the site of nutrient uptake, the supply of which is unevenly distributed in soils and varies with time. Hence, phenotypic plasticity of roots is essential for effective soil exploration. During growth, changes in root architecture and cell fate acquisition occur according to the appropriate environmental conditions. Thereby, post-embryonic plant development is affected by environmental cues, overriding endogenous developmental programs to anticipate forthcoming conditions. Epidermal cells of *Arabidopsis* roots are particularly responsive to nutritional signals. Cell fate decisions are altered in the absence of some essential but immobile minerals, such as Fe and P. The resulting phenotype is characteristic for the respective condition. In response to Fe deficiency, wild-type *Arabidopsis* plants form bifurcated root hairs, which results in a substantial increase in the absorptive surface area without a significant increase in the number root hairs formed. We have isolated a new mutant named IRONIC (iron deficiency root hair defective), which respond to Fe deficiency not only with the production of branched root hairs but also with a dramatic increase in the number of cells that form root hairs. Almost all of the epidermal cells in positions normally occupied by non-hair cells are reprogrammed to develop into hairs when subjected to Fe-deficient conditions. The phenotype seen is similar to that of the *werewolf* (*wer*) mutant, but in a nutrient specific manner. Deviations from the wild-type phenotype were neither observed under other nutrient stresses that affects root epidermal patterning nor under control conditions. The mutant carries a GAL4-GFP enhancer trap construct, enabling the visualization of the expression pattern and single cell-type specific gene expression analysis using fluorescence-activated cell sorting. The GFP expression in *ironic* is seen in epidermal cells independent of their position relative to underlying cortical cells and within the cortical cell layer. This expression pattern is not altered during Fe deficiency. Transcriptional profiling of the mutant has revealed genes with potential roles in cell fate changes in response to environmental signals.

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Contribution of KNAT2 and KNAT6 in the inflorescence. Laura Ragni¹, Marcus G nl¹, V ronique Pautot¹. ¹Laboratoire de Biologie Cellulaire, IJPB, Institut National de la Recherche Agronomique, Route de St Cyr, 78026 Versailles cedex, France

The aerial part of the plant is produced by the shoot apical meristem (SAM), a group of cells established during embryogenesis. The SAM has two main functions: the maintenance of a population of stem cells and the production of organs. The KNOTTED-like from *Arabidopsis thaliana* (KNAT) class I family of transcription factors plays a crucial role in controlling meristem activity. The KNAT proteins belong to the three amino acid loop extension (TALE) homeodomain superclass, and form heterodimers with other TALE proteins belonging to the BELL (BEL1-like) family. KNAT class I comprises four members: SHOOT MERISTEMLESS, is required for SAM initiation during embryogenesis and its maintenance during post-embryonic development, whereas BREVIPEDICELLUS/KNAT1 has a primary role in internode development. BP also interacts with PNY, a BELL member, to regulate inflorescence patterning. We previously showed that KNAT6 contributes with STM to SAM and organ boundary maintenance. KNAT2 does not have such a role.

To investigate the role of KNAT6 and KNAT2 in the inflorescence, we examined their interactions with BP/KNAT1 and PENNYWISE. L.

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E2F regulates FASCIATA1, a chromatin assembly gene whose loss switches on the endocycle and activates gene expression by changing the epigenetic status. Elena Ramirez-Parral¹, Crisanto Gutierrez¹. ¹Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Cantoblanco, 28049 Madrid, Spain

Chromatin reorganization is facilitated by histone chaperones, whose function is crucial for genome integrity. Deposition of histones H3/H4 can occur either in association with DNA replication during the S-phase of the cell cycle or independently of the replication process. These processes depend on the chromatin assembly factor 1 (CAF-1) or the HIRA chaperones, respectively. CAF-1 is a heterotrimeric complex which in *Arabidopsis* is composed of the products encoded by the FASCIATA1 (FAS1), FASCIATA2 (FAS2) and MULTICOPY SUPPRESSOR OF IRA1 (MSI1) genes. We have found that FAS1, which encodes the large subunit of CAF-1, is a target of the E2F family of transcription factors.

Depletion of CAF-1 in human cells leads to cell death whereas in *Arabidopsis*, where it is involved in heterochromatin compaction and homologous recombination, plants are viable. The mechanism that makes the lack of CAF-1 activity compatible with development is not known. Loss of FAS1 results in inhibition of mitotic progression, formation of organs with much fewer but larger cells, and a precocious and systemic switch to the endocycle program. A selective up-regulation of the expression of a subset of genes, including those involved in activation of the G2 DNA damage checkpoint, notably RAD51, BRCA1 and PARP1, also occurs upon FAS1 loss. This depends on the introduction of activation epigenetic marks just upstream of the corresponding ORFs. These data suggest that a correct chromatin assembly during S-phase is required to prevent unscheduled changes in the epigenetic marks of target genes. Interestingly, the same set of G2 checkpoint genes is detected upon treatment of wild type plants with DNA damaging treatments.

Together, our data support a model in which defects in chromatin assembly during S-phase and the DNA damage signaling share part of a pathway, which ultimately lead to mitotic arrest and triggers the endocycle program. In such way, this might be a bypass mechanism that makes development compatible with cell division arrest induced by DNA damage stress.

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A Subtilisin-like Serine Protease essential for Mucilage release from *Arabidopsis* Seed Coats. Carsten Rautengarten¹, Björn Usadel², Lutz Neumetzler², Thomas Altmann¹. ¹University of Potsdam, ²Max-Planck-Institute of Molecular Plant Physiology

During *Arabidopsis* seed development large quantities of pectinaceous mucilage are deposited into the apoplast underneath the outer cell wall. After imbibing mature seeds this mucilage expands and breaks the outer cell wall encapsulating the whole seed. Mutants carrying loss-of-function alleles of AtSBT1.7 are characterized by the lack of mucilage release upon hydration. AtSBT1.7 encodes one of 56 *Arabidopsis thaliana* subtilisin-like serine proteases (subtilases). Analysis of the mutant seed coat epidermis revealed no visible structural differences compared to Col-0 wild-type seeds. However, weakening of the outer primary cell wall using Ca²⁺ chelators triggered mucilage release from the mutant's seed coat. In contrast to mature wild-type seeds the mutant primary cell wall did not rupture at the radial walls but opened at the chalazal end of the seed and was released in one piece. This was exactly the same case in immature wild-type seeds collected from green siliques 6-8 DAP when treated with Ca²⁺ chelators. Extrusion and solubility but not the deposition of mucilage was affected in atsb1.7 mutants. In atsb1.7 mucilage the neutral monosaccharide content was increased by 25% compared to Col-0 seeds whereas galacturonic acid contents, representing the backbone of mucilage, remained unchanged. AtSBT1.7 is preferentially expressed in the seed coat of developing seeds as revealed by RT-PCR and promoter activity pattern analysis. This is consistent with its role in testa development. Moreover, atsb1.7 mutants were strongly impaired in germination under water limiting conditions. Therefore, it is evident that AtSBT1.7 is indirectly involved in the rapid rupture of the outer wall of seeds for mucilage release upon imbibition. AtSBT1.7 may act via 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 outer mucilage.

P-309

Non-cell autonomous KNAT1 function for control of plant architecture. Yeonggil Rim¹, Jin-Hee Jung¹, Raju Datla², David Jackson³, Jae-Yean Kim¹. ¹Division of Applied Life Science, PMB-BRC/EB-NCRC, Gyeongsang National University, Jinju 660-701, Korea, ²Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, SK, Canada S7N 0W9, ³Cold Spring Harbor Laboratory, 1 bungtown Rd, Cold Spring Harbor NY11724, USA

How plant shape/architecture is regulated is a central question in plant biology. *Arabidopsis brevipedicellus* (bp) mutants provide a wonderful system to study the control of plant architecture, especially related to the patterning of stems. bp mutants show a phenotype with shorter internodes and pedicel lengths, downward pointing siliques, a compact inflorescence architecture, bends at nodes and an epidermal stripe of disorganized cells along the stem. BP encodes the KNAT1 homeodomain protein, a member of the KNOTTED1-related HOMEOBOX (KNOX) proteins, which is the strongest *Arabidopsis* homolog of maize KNOTTED1 that can traffic cell-to-cell. KNAT1 is specifically expressed in the cortical cell layers of the inflorescence stem and pedicel, but its knock-out alleles also display disruption in epidermal cell differentiation. This suggested that KNAT1 functions non-cell autonomously either by its direct cell-to-cell trafficking or by the movement of some downstream factors. We carried out genetic complementation assay of bp mutations using a series of fusion constructs that express either cell-autonomous or non-cell autonomous KNAT1 fusion proteins. We here show the ability of cell-to-cell trafficking of KNAT1 is required for its biological function involved in the establishment of plant architecture.

P-310

The ACR4 receptor kinase is required for giant cell development in *Arabidopsis* sepals. Adrienne Roeder¹, Vijay Chickarmane¹, Carolyn Ohno¹, Elliot Meyerowitz^{1,2}. ¹ California Institute of Technology, Pasadena, CA, USA

One of the key questions in developmental biology is how cell division and cell expansion are regulated in conjunction with cell fate specification to produce specialized cells of the correct size and shape. The outer epidermis of the *Arabidopsis* sepal contains highly elongated giant cells interspersed between smaller pavement cells, guard cells, and trichomes, making it a useful system for addressing this question. Giant cells average 364 μm (145 s.d.) in length, but the longest ones reach over half the length of the sepal. Giant cells also have enlarged nuclei suggesting that they have undergone endoreduplication, a specialized cell cycle in which the DNA is replicated, but cell division does not occur. To identify genes that are required for giant-cell development, we have conducted an EMS mutant screen and we have isolated a number of mutants that lack giant cells as well as one mutant with almost entirely large cells. We have focused on two allelic mutants, in which fully elongated giant cells are absent in the sepals. We have observed, however, that smaller giant cells appear to develop in the sepals of these mutants. The nuclei in the smaller giant cells of the mutant are not as large as wild-type giant-cell nuclei suggesting that they have undergone fewer endocycles. On the molecular level, a giant cell-specific marker is expressed in the smaller giant cells present in the mutant. A second marker expressed specifically in the non-giant pavement cells of wild-type sepals is similarly excluded from the smaller giant cells of the mutant. These results suggest that giant-cell fate is specified, at least partially, in the mutant, but that the full expansion of the giant cells fails to occur. Through positional cloning we have found that these mutations are in the *Arabidopsis* CRINKLY4 gene, which encodes a receptor kinase involved in epidermal and ovule development (Gifford et al., 2003; Watanabe et al., 2004; Cao et al., 2005), suggesting that a signaling pathway is involved in giant cell development.

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HD9 homeobox gene is involved in *Arabidopsis* fruit development. Maida Romera-Branchat¹, Soraya Pelaz^{1,2}. ¹ Laboratori de Genetica Molecular Vegetal, CSIC-IRTA. Barcelona, Spain, ² ICREA. Barcelona, Spain

Arabidopsis fruits consist of a seedpod that encloses and protects the seeds as they mature and disperse them at maturity. The process that facilitates the dispersal of the fruit is known as dehiscence and depends on three different tissues: valve, replum and valve margin. The valves, or seed pod walls, encircle the developing seeds and join to the replum through a specialized stripe of cells that define the valve margin (VM). Dehiscence occurs at the VM where the valves will separate from the replum. VM are divided into two layers, the separation layer at the replum side that permits the detachment of the valve from the replum through cell-cell separation, and the lignified layer at the valve side of the margin that provides together with the lignified valve cells the necessary tension that helps the valves to detach. In the recent years, many factors involved in the pod-shatter have been identified, but still some gaps exist in the complex genetic network that controls fruit development.

In this line of work, we are studying a new homeobox transcription factor, HD9. The characterization of mutant lines and overexpressing plants suggests a role of HD9 in the lignification pattern of the valve margin and a putative function in valve development. The 35S::HD9 fruits are small and show weak lignification and a wide replum whereas hd9 mutant fruits display a narrow replum, thin valves and a not well defined valve margin. It will be interesting to determine the exact location of HD9 within the genetic network of fruit patterning, and for that, different crosses of hd9 mutants and 35S::HD9 plants with mutants in known genes involved in fruit development are being performed. The unraveling of HD9 function will give us further insight in the understanding of the complex process of fruit development, and should provide useful tools to improve important crop plants.

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Overlapping and antagonistic roles of BELL TALE homeodomain proteins in shoot apical meristem phase identity. Bas Rutjens¹, Evelien van Eck-Stouten¹, Marco Brand¹, Sjef Smeekens¹, Marcel Proveniers¹. ¹ Utrecht University, Utrecht, The Netherlands

Floral induction is controlled by a plethora of genes acting in different pathways that either repress or promote floral transition at the shoot apical meristem (SAM). During vegetative development high levels of floral repressors maintain the *Arabidopsis* SAM incompetent to respond to promoting factors. Among these repressors, *FLOWERING LOCUS C* (*FLC*) is the most prominent. The processes underlying down-regulation of *FLC* in response to environmental and developmental signals have been elucidated in considerable detail. However, the basal induction of *FLC* and its up-regulation by *FRIGIDA* (*FRI*) are less understood. Here we demonstrate that the BELL-class proteins ARABIDOPSIS THALIANA HOMEOBOX1 (*ATH1*) and PENNYWISE (*PNY*) act redundantly as positive regulators of *FLC* expression. Plants that constitutively express *ATH1* display a vernalization-sensitive late flowering habit. Analysis of lines that differ in *FRI* and/or *FLC* allele strength show that this late flowering is caused by up-regulation of *FLC* as a result of synergy between *ATH1* and *FRI*. Consistently, *ath1* mutants flower early in short days (SD) and display attenuated *FLC* levels. Moreover, *ath1* mutations partially suppress *FLC*-mediated late flowering of both a *FRI*-expressing line and that of *fca-1* and *fve-1* autonomous pathway mutants. Like *ath1*, *PNY* mutants are characterized by strongly reduced basal *FLC* levels and an early flowering phenotype in SD. Also similar to *ath1*, *PNY* partially suppresses late flowering in the presence of dominant *FRI* alleles. In contrast to the relatively weak effects of either single mutant, absence of both *ATH1* and *PNY* almost fully impairs *FRI*-mediated late flowering. Intriguingly, no role for the BELL protein POUND-FOOLISH (*PNF*), an established redundant partner of *PNY* required for the competence of the SAM to respond properly to floral inductive signals, was found in *FLC*-mediated flowering time control. Taken together, this suggests that throughout plant development different BELL combinations dictate developmental phase identity by controlling key components of phase identity.

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Constitutive CKI1 activity modulates cytokinin signaling and regulates vascular tissue development in *Arabidopsis*. Hojin Ryu¹, Jan Hejátko², Gyung-Tae Kim³, Romana Dobe?ová⁴, P?emysl Soukup⁴, Petra Borkovcová⁵, Klaus Palme⁶, B?etislav Brzobohatý⁷, Ilwoo Hwang⁸. ¹ POSTECH, Pohang, South Korea, ² Masaryk University, Brno, Czech Republic, ³ Dong-A University, Busan, Korea, ⁴ Masaryk University, Brno, Czech Republic, ⁵ Masaryk University, Brno, Czech Republic, ⁶ Universität Freiburg, Freiburg, Germany, ⁷ Masaryk University, Brno, Czech Republic, ⁸ POSTECH, Pohang, South Korea

Cytokinins play essential roles in cell division and vascular tissue development. While cytokinin signals are perceived by the histidine kinase receptors AHK2, AHK3, and AHK4/CRE1/WOL, recent genetic analyses indicate the existence of additional cytokinin signaling pathway in *Arabidopsis*. Here we show that histidine kinase CKI1 constitutively activates the cytokinin signaling pathway. CKI1 induces expression of cytokinin-responsive type-A ARR_s and ARR₂ phosphorylation in cytokinin-deficient and wild-type protoplasts and dominant negative CKI1 allele suppresses AHKs-dependent activation of ARR₆. Hormone-regulated CKI1 expression was found in vascular tissues, root tip and shoot apex. Ectopic expression of CKI1 causes abnormal cell division and differentiation in the vascular bundles of inflorescence stems and in axillary meristems. CKI1 RNA interference and dominant negative CKI1 plants reveal cytokinin-dependent aberrations in the inflorescence vascular tissue development. These results suggest that the hormone-dependent transcriptional regulation of constitutive CKI1 activity modulates cytokinin signaling and regulates vascular tissue development in *Arabidopsis*.

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Analyses of CLV3 molecular mechanisms, and gain-of-function phenotypes of dodeca CLE peptides. Shinichiro Sawa¹, ¹University of Tokyo, Tokyo, Japan

12 amino acid MCLV3 peptide function in meristem size maintenance in *Arabidopsis*. MCLV3 treatment with wild type plants reduced SAM and RAM size. Reporter gene activities of CLV3::GUS, CLV3::GFP, and WUS::GUS were decreased when these reporter lines were treated with MCLV3 peptide. MCLV3 did not function in the *clv1* and *clv2* mutants, MCLV3 function in the CLE signaling pathway. We isolated mutants, which are resistant against to the MCLV3 peptides. These mutants can grow even on the agar medium including MCLV3 peptides. Here we will discuss about these mutant and causal genes.

On the other hand, dodeca CLE peptides are known to have a function in RAM size regulation. Using 26 chemically synthetic CLE peptides, which correspond to the predicted products of the 31 *Arabidopsis* CLE genes, we investigated the CLE peptide function in *Arabidopsis* SAM. Furthermore, we examined the CLE peptides in rice and moss, *Physcomitrella patens*. Some CLE peptides reduced colony size, cell elongation rate, and gametophore formation. Interestingly they did not affect meristematic nature in moss. Moss may not utilize CLE peptides in the regulation of meristematic apical cell activity. Moss does not have multicellular meristem, and the moss might not need to utilize CLE peptides as intercellular signaling molecule to organize the meristematic apical cell architecture. Instead, CLE peptides may function in the cell elongation and gametophore formation in the moss. CLE9/10 was most effective to reduce colony size, and the peptide sequence of CLE9/10 and PpCLE1 has only one amino acid difference. These results may suggest that the CLE function between bryophyte and angiosperm is highly diverged, and the CLE function may be varied from as an intracellular signaling molecule in bryophyte to as an intercellular signaling molecule in angiosperm. This idea may be supported by the result that the putative CLE protein sequences of *Arabidopsis* and rice have typical signal sequence for secretion, but PpCLE1 of moss does not have. CLE protein may be evolved as a secreted intercellular signaling molecule when plant acquired organized multicellular meristem during its evolution.

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The use of *Arabidopsis* class 1 KNOX gene, BP/KNAT1 in *Agrobacterium*-mediated transformation of the wild orchid species *Phalaenopsis amabilis*. Endang Semiarti^{1,2}, Ari Indranto¹, Azis Purwantoro³, Sulastri Isminingsih², Nilo Suseno¹, Takaaki Ishikawa^{4,5}, Yasushi Yoshioka⁴, Yasunori Machida⁴, Chiyo Machida^{5,6}. ¹Faculty of Biology, Gadjah Mada University, Yogyakarta, Indonesia, ²Center Study of Biotechnology, Gadjah Mada University, Yogyakarta, Indonesia, ³Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia, ⁴Graduate School of Science, Nagoya University, Nagoya, Japan, ⁵Plant Biology Research Center, Chubu University, Kasugai, Japan, ⁶College of Bioscience and Biotechnology, Chubu University, Kasugai, Japan

Phalaenopsis hybrids constitute a major ornamental crop. An important parent species for many of these hybrids is *Phalaenopsis amabilis*. We developed a convenient method for the genetic modification of *P. amabilis* using *Agrobacterium tumefaciens*. The transformed intact protocorms, which are young orchid seedlings of *P. amabilis*, regenerated plants under the same conditions that showed the highest frequency of shooting. A kanamycin resistance gene under the control of the 35S promoter can be used as a selective marker. In addition, T-DNA vectors containing the *Arabidopsis* class 1 KNOX gene, BP/KNAT1, were successfully introduced into protocorms. Shoots were generated with an abnormal leaf shape that was easily distinguished from that of normal shoots, indicating that BP/KNAT1 can be used as a visible marker gene. Furthermore, the protocorms transformed with BP/KNAT1 produced multiple shoots. Both the presence and expression of the transgene in transformed plants were confirmed by molecular analysis.

Key words: Agrobacterium, KNOX, multiple shoots, *Phalaenopsis amabilis*, transgenic orchid.

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WEREWOLF, a Regulator of Root Hair Pattern Formation, Controls Flowering Time through the Regulation of FT. Eunjoo Seo¹, Jihyeon Yu¹, Kook Hui Ryu², Myeong Min Lee², Ilha Lee^{1,1}
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In *Arabidopsis*, *FT* integrates floral stimuli from the photoperiod, the vernalization, and autonomous pathways to trigger the floral induction. Since *FT* performs a great important role for flowering, *FT* is strictly controlled by many genes: *CO*, *FLC*, *TFL2*, and *EBS*. This study demonstrates that the additional factor, *WEREWOLF* (*WER*) known as a regulator of root hair patterning, is involved in *FT* regulation. *wer-1* mutants flower late in long days but not in short days, and they show weak sensitivity to vernalization in long days. It suggests that *WER* controls flowering time through the photoperiod pathway. Real-time PCR and double mutant analysis show that *WER* modulates *FT* expression levels independent of *CO* and *FLC*; besides, *WER* affects on not transcription but stability of *FT* mRNA. However, *WER* transcripts were observed in epidermis in which *FT* mRNA does not exist. In conclusion, we insist that *WER* regulates *FT* mRNA stability in a non-cell autonomous manner.

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Comprehensive functional analyses of *Arabidopsis* transcription factors using the chimeric repressor gene silencing technology (CRES-T). Masahito Shikata^{1,2}, Tomotsugu Koyama^{1,2}, Nobutaka Mitsuda^{1,2}, Kyoko Matsui¹, Yoshimi Umemura^{1,2}, Akira Iwase¹, Miho Ikeda¹, Masaru Ohme-Takagi^{1,2}. ¹Research Institute of Genome-based Biofactory, Advanced Industrial Science and Technology, Central 4, Tsukuba 305-8562, Japan, ²Core Research for Evolutionary Science and Technology (CREST), Japan Science and Technology Agency (JST), Saitama 332-0012, Japan.

It becomes evident that transcription regulation play an important role for the control of growth and development especially in plants. Therefore, identification of the function of transcription factors is an important subject for the control or improvement of plant traits. However, plant genes are frequently duplicated and many transcription factors form large family. This structural and functional redundancy often interferes with efforts to identify the functions of these factors. Even when gene-knockout or antisense lines specific for a particular transcription factor can be isolated, such lines often fail to exhibit an informative phenotype that might provide some direct clue to the factor's function, for example, a loss-of-function phenotype. To overcome such difficulties, we developed a novel gene silencing system, called Chimeric REpressor Gene-SilencingTechnology (CRES-T), in which a chimeric repressor is produced by fusion of a transcription factor to the plant-specific ERF associated Amphiphilic Repression (EAR) motif. We have shown that the chimeric repressors derived from various transcription factors dominantly suppress the expression of the respective target genes, and that the resultant transgenic plants exhibit loss-of-function phenotypes specific for the target genes. With the CRES-T system, we are comprehensively analyzing function of *Arabidopsis* transcription factors. We describe the results of the functional analysis of the member of NAC, TCP, MADS and MYB family transcription factors on the regulation of plant growth and development, namely secondary wall synthesis, control of meristem development, flower formation and flavonoid biosynthesis. We also present that the CRES-T system is a powerful tool for the manipulation of plant traits.

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Genetic Dissection of Parent of Origin Effects in Seed Development. Reza Shirzadi¹, Moritz K. Nowack², Reidunn B. Aalen¹, Arp Schnittger², Paul E. Grini¹. ¹ Department of Molecular Biosciences, University of Oslo, Oslo, Norway, ²University Group at the Max-Planck-Institute for Plant Breeding Research, Department of Botany III, University of Cologne, Cologne, Germany

In the developing offspring of mammals the two parental genomes are differentially expressed due to epigenetic marks. A parent of origin dependent expression has also been observed in the offspring of flowering plants and mutations in the imprinting machinery lead to embryonic lethality, primarily affecting the development of the endosperm, a structure in the seed that nourishes the embryo analogous to the mammalian placenta. Seed development is the product of the double fertilization of the egg cell and the central cell by two sperm cells from the pollen and requires a coordinated interplay of the embryo, the endosperm and the maternal seed coat. We have used a mutant in the *Arabidopsis Cdc2/CDC28* homologue, *CDKA*; 1 as a tool to dissect the involvement of parental gene programs in seed development. *cdka*; 1 mutant pollen fails to undergo the second pollen mitosis, resulting in pollen with a single sperm cell that exclusively fertilize the egg cell. Although not fertilized, the central cell in *cdka*; 1 ovules with fertilized egg cells is triggered to initiate endosperm proliferation. Surprisingly, viable seedlings can develop from seeds lacking a paternal contribution to the endosperm, given the mother is mutant for any of the FIS genes that encode Polycomb group (PcG) chromatin remodeling factors, thus suggesting that the action of the PcG FIS-complex balances the contribution of the paternal genome. In order to identify further factors involved in crosstalk between the components of the seed and parent of origin specific regulation of seed development, we have generated microarray transcriptional profiles of seeds with uniparental endosperm. Our preliminary data identifies several regulated genes that could represent novel paternal specific transcripts (downregulated) or maternal targets of paternal repressors (upregulated).

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"AtBLH6" Homeobox gene affects flowering time and floral organ development. Cristina Silva¹, Soraya Pelaz^{1,2}. ¹Laboratori de Genetica Molecular Vegetal, CSIC-IRTA. Barcelona, Spain, ²ICREA. Barcelona, Spain

The plant hormone auxin plays a crucial role in a wide variety of plant morphogenetic and physiological responses. Proper cellular responses to auxin gradients established by polar transport underlie organ patterning. Several studies established that apical-basal patterning of the gynoecium, the female reproductive organ of flowering plants, involves both auxin responses and polar auxin transport.

The genes "PINOID" ("PID") and "PIN-FORMED1" ("PIN1") are both regulators of polar auxin transport involved in the establishment of an auxin gradient along the apical-basal axis of gynoecium. This auxin gradient is recognised by different transcription factors, forming a genetic network that carry out the correct gynoecium development. Among these transcription factors, "ETTIN" ("ETT") seems to be crucial recognising the auxin gradient. "ETT" encodes an auxin response factor (ARF) that binds to auxin response elements in the promoters of early auxin response genes and regulates their expression. Mutations in the "PID", "PIN1", and "ETT" genes cause the development of abnormal gynoecia, in which the boundary between ovary and gynophore is more distal than in the wild type.

In this work, we focused on the study of "AIBLH6", a member of the of the BELL family. This family was defined as a sub-group of the Homeobox family when the first gene was identified, "BELL1" ("BEL1") gene. "BEL1" is required for the normal ovule development since mutations in this gene affect the ovule identity. The constitutive expression of "AIBLH6" results in a delay in flowering time suggesting a role of this gene in the process of floral induction. Moreover, fruits of 35S::BLH6 plants presented several developmental defects that resemble those observed in the plants carrying mutations in the "PID", "PIN1", and "ETT" genes. This suggests a role for "AIBLH6" in the control of gynoecium patterning through a putative involvement in the processes of auxin signalling.

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Components of CLE signalling pathways. Rüdiger Simon¹, Andrea Bleckmann¹, Ralf Müller¹. ¹Institut für Genetik, Heinrich-Heine Universität, Düsseldorf, Germany

Stem cells are required to support the indeterminate growth style of plants. In shoot meristems, stem cell fate is decided at the populational level by an exchange of signals between the stem cell domain at the meristem tip and a small group of cells (the organizing centre or OC) underneath. Cells in the OC express the homeodomain transcription factor WUS, which is required for the induction and maintenance of stem cells at the tip. How WUS performs this function has not been characterized so far, but one of the (direct or indirect) target genes that depends on WUS is the stem cell specific CLV3 gene. CLV3 encodes a signaling molecule that belongs to the CLE peptide family. CLV3 acts via the CLV1 receptor kinase to downregulate WUS transcription. This negative feedback regulation shall ensure a constant stem cell population during meristem growth and development.

We have studied this regulatory pathway in more detail, and identified new components that are essential for perception of CLE signals.

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Regulation of BDL during early embryo development. Ive De Smet¹, Steffen Lau¹, Jasmin S. Ehrismann¹, Marika Kientz¹, Gerd Jrgens¹. ¹ZMBP Entwicklungsgenetik - Universitat Tübingen, Tuebingen, Germany

The *Arabidopsis* zygote divides asymmetrically to give an apical and a basal daughter cell. Subsequently, auxin is transported from the basal into the apical daughter cell of the zygote [1]. The auxin signal is converted into a specific response by auxin-promoted degradation of the Aux/IAA protein BDL/IAA12 that prevents auxin response factor (ARF) transcription factor MP/ARF5 from regulating auxin-responsive target genes [2, 3, 4]. The MP/BDL-dependent response in the apical cell lineage results in PIN1 expression and auxin efflux into the basally adjacent future hypophysis, thus initiating embryonic root formation [4].

To learn more about mechanisms underlying the initial difference between apical and basal lineages, we are focusing on the regulation of the BDL gene, which is expressed in the apical daughter cell of the zygote and its progeny. A yeast one-hybrid screen has yielded candidate transcriptional regulators (Regulator Of BDL or ROB), which are being characterized functionally. First, we validate the interaction with the BDL promoter using various approaches, such as yeast one-hybrid assay, transactivation assay and DNA-binding assay. Second, we describe the expression pattern in the developing embryo using mRNA *in situ* hybridization, PROB::GFP and PROB::ROB:GFP fusions. Third, we analyze the embryo phenotype upon altered expression levels of the ROBs in the wild-type and bdl mutant background. Fourth, we investigate the BDL expression in knock-out mutants of the ROBs. Taken together, these results provide a starting point to dissect the transcriptional control of BDL.

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The Arabidopsis MYB26/MALE STERILITY35 gene controls anther dehiscence by regulating secondary thickening in the anther endothecium. Jie Song¹, Caiyun Yang¹, Zoe A Wilson^{1,1}. Plant Sciences Division, University of Nottingham, Loughborough, LE12 5RD, UK

Male sterility typically occurs due to the lack of viable pollen production, but can also be due to the failure of pollen release. Anther dehiscence is a two-phase process involving lytic opening of the stomium and then retraction of the anther wall. During microspore maturation, cellulose and lignified thickenings are deposited in the anther endothecium (Dawson et al., 1999). This cell layer is important in generating the forces required for dehiscence, initially by swelling and causing the stomium to rupture, then by desiccation causing differential shrinkage of thickened and unthickened parts of the cell wall, resulting in an outward bending force, retraction of the anther wall and anther opening.

MYB26/MS35 acts as a key regulator of lignified secondary thickening in the anther endothecium and subsequent dehiscence. The ms35 mutant fails to produce secondary thickening in the endothecium (Dawson et al., 1999). MYB26/MS35 expression occurs early during endothelial development and is maximal during pollen mitosis I and the bicellular stage, suggesting that it may have a regulatory role in specifying early endothelial cell development.

Over-expression of MYB26 results in ectopic secondary thickening and changes in expression in several genes linked to secondary thickening, including IRREGULAR XYLEM 1 (IRX1), IRX3, IRX8, IRX12, NAC SECONDARY WALL PROMOTING FACTOR1 (NST1) and NST2. This indicates that MYB26 functions in a regulatory role in determining endothelial cell development and acts upstream of the lignin biosynthesis pathway (Yang et al., 2007). Affymetrix microarray analysis using isolated anthers suggests that a number of additional genes are also involved in this pathway. Immunolocalisation of pectin and xylan has provided further evidence for the role of MYB26 in the regulation of secondary wall development in the anther. These data and the potential role for MYB26 will be discussed.

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The homeotic protein AGAMOUS controls late stamen development by regulation of jasmonic acid biosynthesis. Serena Lim Tze Soo¹, Yu Hao^{1,2}, Elliot M. Meyerowitz³, Toshiro Ito^{1,2,1}. Temasek Life Sciences Laboratory, Singapore 117604, Singapore,² National University of Singapore, Singapore 117543, Singapore,³ California Institute of Technology, Pasadena CA 91125, USA

The Arabidopsis floral homeotic gene AGAMOUS (AG) plays a central role in reproductive organ development. Although AG is the most studied example of a plant homeotic gene, the mechanisms by which a floral primordium responds to the genetic activities initiated by AG, and thereby develops stamens and carpels, are largely unknown. AG RNA is expressed in the organ primordia of floral 3rd and 4th whorls (the positions of stamens and carpels, respectively) from floral stage 3 until late in flower development, after all of the organs are fully formed. While early AG expression acts in specification of stamens and carpels, the role, if any, of continued AG expression in later flower development is unknown.

In order to examine the timing of AG action and its possible late stage functions, we performed a series of time course experiments using a transgenic line with inducible AG activity, in an ag homozygous mutant background. We show that ectopic AG activity can transform organ primordia of as late as stage 6 floral buds into normal-looking stamens. In contrast, activation of AG in floral buds after stage 7 results in 2nd and 3rd whorl organs that were stamen-like filamentous petals. This suggests that AG can induce the gene activities necessary for filament elongation, but not for microsporogenesis, at late floral stages. We also show by using timed activation of AG that AG controls late-stage stamen development, including anther morphogenesis and dehiscence, as well as filament formation and elongation. Furthermore, we show that AG controls maturation of stamens by regulating the transcription of a catalytic enzyme for the phytohormone jasmonic acid biosynthesis in late stamen development. Our results show that stamen identity and differentiation control by AG is achieved by the regulation of different transcriptional cascades in different floral stages, with organ specification cascades induced early, followed by activities necessary for filament formation and anther maturation.

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The C-terminus of INFLORESCENCE DEFICIENT IN ABSCISSION is sufficient to control floral abscission. Grethe-Elisabeth Stenvik¹, Nora M. Tandstad¹, Wenche Kristiansen¹, Melinka A. Butenko¹, Reidunn B. Aalen^{1,1} Department of Molecular Biosciences, University of Oslo, Norway

Abscission is a physiologically determined program of cell separation that enables plants to shed unwanted organs. The *Arabidopsis* floral organ abscission mutant *inflorescence deficient in abscission* (*ida*) (Butenko et al. 2003, Plant Cell 15) is completely deficient in abscission. The 77 amino acid IDA protein, which controls the final step of floral organ abscission, is a member of a novel group of putative ligands in plants. Both IDA and the IDA-LIKE (*IDL*) proteins contain a predicted N-terminal signal sequence and a conserved 12 aa motif (PIP) at the C-terminus. Promoter: GUS expression studies of the *IDL* genes indicate that these genes may be involved in regulating various cell separation events such as root cap sloughing, stomata development and formation of hydathodes. A 35S;IDA line constitutively overexpressing IDA exhibits earlier abscission of floral organs and ectopic abscission at the bases of the pedicel, branches of the inflorescence, and cauline leaves (Stenvik et al. 2006, Plant Cell 18). Overexpression of *IDL* genes results in similar phenotypes, suggesting functional redundancy of the PIP domain. We have found the C-terminal portion of the IDA protein, which contains the PIP domain, to be sufficient for IDA function. In addition the N-terminal signal sequence of IDA must be present to induce the overexpression phenotypes seen in 35S;IDA plants. Exogenous application of a synthetic peptide covering the C-terminal portion of IDA will be used to see if early floral abscission can be induced in wild-type plants. In addition, constructs where parts of the IDA and IDL proteins were swapped have been used to determine the level of complementation of the *ida* phenotype.

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Overexpression of INFLORESCENCE DEFICIENT IN ABSCISSION Activates Ectopic Abscission. Grethe-Elisabeth Stenvik¹, Melinka A. Butenko¹, Sarah M. McKim², George W. Haughn², Reidunn B. Aalen¹. ¹Department of Molecular Biosciences, University of Oslo, Norway, ²Department of Botany, University of British Columbia, Canada

Abscission is an active process that enables plants to shed unwanted organs. Because the purpose of the flower is to facilitate pollination, it often is abscised after fertilization. We have identified a floral organ abscission mutant in *Arabidopsis*, *inflorescence deficient in abscission* (*ida*) which shows a complete lack of floral organ abscission. The *IDA* gene encodes a small protein with an N-terminal signal peptide, suggesting that the *IDA* protein is a ligand of an unknown receptor involved in controlling the final step of cell separation of floral organs (Butenko et al., 2003 Plant Cell 15). *Arabidopsis* 35S:IDA lines constitutively overexpressing *IDA* exhibit earlier abscission of floral organs, which shows that the abscission zones are responsive to *IDA* soon after the opening of the flowers (Stenvik et al., 2006 Plant Cell 18). In addition, ectopic abscission was observed at the bases of the pedicel, branches of the inflorescence, and cauline leaves. In regions at the bases of the organs lost in the 35S:IDA lines differentiated abscission zone (AZ) cells were present in wild-type plants. By transforming the floral abscission deficient mutant *bop1/bop2*, which does not develop a floral or cauline leaf AZ, with the 35S:IDA construct we show that differentiation of AZs is crucial for *IDA* function. Scanning electron microscopy of 35S:IDA indicated a spread of middle lamella degradation from preformed abscission zone cells to neighboring cells. In addition, a white substance, identified as arabinogalactan, was secreted in large amounts at the sites of abscission and a transcript encoding an arabinogalactan protein (AGP24) was upregulated in the 35S:IDA lines. We suggest that the restricted expression pattern of *IDA* to the floral AZ precludes abscission of non-floral organs in *Arabidopsis* and that plant species that shed organs other than petals, sepals, and anthers have a different expression pattern of putative *IDA* orthologs.

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How Homeotic Protein AGAMOUS Controls Floral Meristem Determinacy in Arabidopsis. Bo Sun¹, Toshiro Ito¹. ¹Temasek Life Sciences Laboratory, Singapore, Singapore

In *Arabidopsis*, floral meristem activity ceases after formation of four different types of floral organs including sepals, petals, stamens and carpels. It is known that *AGAMOUS* (*AG*), which encodes a MADS-domain transcription factor, controls stamen and carpel development and also functions in terminating meristematic activity in flower buds. *WUSCHEL* (*WUS*), which encodes a homeodomain transcription factor, is essential for maintenance of stem cells. Even though *WUS* is suggested to be suppressed in the downstream of *AG*, it is still a mystery how *AG* represses *WUS*. Thus, we started working on the transcriptional cascades from *AG* to *WUS* to understand how and in what timing meristematic activity is terminated.

KNUCKLES (*KNU*), mutation of which leads to ectopic stamens and carpels inside developing gynoecia, is involved in floral meristem determinacy. *KNU*, a C2H2 zinc finger protein, has the C-terminal EAR-like repression motif, suggesting that *KNU* could be a transcriptional repressor. However, the relationships among *KNU*, *AG* and *WUS* are unclear.

We first analyzed *KNU* expression in transgenic plants with inducible *AG* activity and found that *AG* induces *KNU* within 48 hours. Our genetic data also showed that *ag* is epistatic to *knu*. Through GUS reporter analysis of *KNU* promoter, we confirmed that *KNU* expression is fully dependent on *AG*. All of these data suggest that *KNU* is a downstream gene of *AG*. Next, to test whether *KNU* represses *WUS*, we constructed the transgenic line with inducible *KNU* activity. After the continuous induction, we observed *wus*-like flowers that lack gynoecium. We also observed prolonged *WUS* expression in *knu* mutant. These suggest *KNU* functions as a repressor of *WUS*. Furthermore, we observed that over-expression of *KNU* could rescue the indeterminate phenotype of *ag-1*, showing that *KNU*, a downstream gene of *AG*, plays a major function to terminate *WUS* expression in the floral meristem.

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A novel function of ORC1 in transcriptional regulation. María de la Paz Sánchez¹, Crisanto Gutierrez¹. ¹Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Cantoblanco, 28049 Madrid, Spain

Initiation of DNA replication is tightly regulated in eukaryotic cells. During this process, the origin recognition complex (ORC) is required for initiation of DNA replication at replication origins in all eukaryotes. The ORC complex is formed by six proteins that bind to DNA as part of the pre-replicative complex (preRC), the first step to assemble the DNA replication machinery. Some DNA binding proteins frequently play multiple roles in different cellular functions, including transcriptional regulation, one example being ORC1, the large subunit of the origin recognition complex. ORC1 has been involved in gene silencing in yeast and mammalian cells, process where ORC1 is required to recruit Sir proteins to silencers; in addition, in mammalian cells ORC1 is able to bind a histone acetyl transferase (HBO1).

One of our interests is to define the roles of *Arabidopsis* ORC1 in DNA replication and transcriptional regulation. *Arabidopsis* have two ORC1 genes (A and B), both of which show high amino acid homology, and conserve the typical domains of ORC proteins that participate in ORC complex formation and interact with DNA. By pull down assays we found that ORC1 interacts with histone proteins and with a Histone Acetyl Transferase (HAT) homologue of human HBO1. Here we will focus in discussing the physiological function of those associations and its implication in transcriptional regulation.

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Expression studies and overexpression phenotypes indicate a role in cell separation processes for the "IDL" genes in "Arabidopsis thaliana". Nora M. Tandstad¹, Grethe-Elisabeth Stenvik¹, Melinka A. Butenko¹, Asbjørn Holmgren¹, Reidunn B. Aalen¹. ¹Department of Molecular Biosciences, University of Oslo, Oslo, Norway

Small peptides can act as signaling molecules that coordinate development, growth and differentiation. Upon interaction with a receptor the ligand can trigger downstream pathways which induce cellular responses or regulation of gene expression. Recently, a novel group of putative ligands in plants, the IDA-LIKE (IDL) proteins, were identified based on their similarities to *IDA*, a putative ligand involved in floral organ abscission (Butenko et al. 2003, Plant Cell, 15: 2296-307). Localization in the extracellular space is a necessity for being a ligand. The IDL proteins contain an N-terminal signal peptide predicted to guide the proteins out of the cell. In consistence with this, our preliminary results indicated that IDL3-GFP fusion protein in transgenic *Arabidopsis* was localized to the periphery of the cell. Histochemical analyses of promoter-GUS constructs for the five "IDL" genes in transgenic *Arabidopsis* have been performed. Since *IDA* is involved in the cell separation process that induces the floral organs to be shed, it was interesting to find that the GUS expression of the the "IDL" genes in many cases was associated with sites of cell separation, such as AZs, valves of developing carpels and at the base of pedicels. Overexpression of the "IDL" genes resulted in similar phenotypes featured by early senescence in rosette and cauline leaves, premature floral organ abscission, and shedding of organs that are normally not shed in *Arabidopsis*; pedicels and cauline leaves. In contrast, plants overexpressing IDL1 lacking the signal peptide, were not phenotypically affected, confirming that the extracellular localization of these proteins is necessary in order to be functional. Similar overexpression phenotypes were also observed in plants overexpressing *IDA* (Stenvik et al., 2006, Plant Cell, 18: 1467-76). These data suggest some degree of functional redundancy between "IDA" and the "IDL" genes, and may indicate a possible role for the "IDL" genes in cell separation processes.

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Two SET-domain proteins involved in *Arabidopsis* reproductive development. Tage Thorstensen¹, Vibeke Alm¹, Paul E. Grini¹, Reidunn B. Aalen¹. ¹Department of Molecular Biosciences, University of Oslo, Oslo, Norway

The pattern of methylation of lysine residues on histone tails is a part of the "histone code" involved in the regulation of eukaryotic gene expression and chromatin structure. Histone methyltransferases (HMTases) contain a SET domain, and in *Arabidopsis* more than 30 genes encoding SET-domain proteins have been identified that can be divided into the evolutionarily conserved subclasses Su(VAR)3-9, E(Z), TRX and ASH1 (Baumbusch et al., 2001, Nucleic Acids Res 29, 4319-4333). Here we focus on two genes in the ASH1 subclass, ASHH2 and ASHR3. Promoter:GUS constructs and *in situ* hybridization studies show that ASHR3 is expressed in developing anthers, especially in the tapetum layer. Overexpression of ASHR3 results in anther degeneration and complete male sterility. Protein-protein interaction studies revealed an interaction between ASHR3 and the bHLH transcription factor AMS, affecting pollen development. The ASHR3 protein is localized to euchromatin, and we suggest that AMS tether ASHR3 to target genes important for microspore and stamen development. ASHH2 is known to be involved in the control of flowering time. However, ashh2 mutants show pleiotropic developmental phenotypes, e. g. loss of apical dominance, dwarfism, occasional floral homeotic transformations and severely reduced fertility. Homozygous ashh2/ashh2 plants are defective in both male and female meiosis. In addition, reciprocal crosses of heterozygous ashh2 to wild type plants indicate a requirement for ASHH2 in both male and female gamogenesis. Using *in vitro* assays we have demonstrated histone 3 lysine 36 HMTase activity for ASHH2. Moreover, in microarray transcriptional profiles, the expression levels of a number of transcription factors are affected in the ashh2 mutant, and we are currently verifying these as putative targets for ASHH2.

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The CAPRICE-like Myb3 (CPL3) is involved in the determination of epidermal cell fate in *Arabidopsis*. Rumi Tominaga¹, Mineko Iwata¹, Ryosuke Sano¹, Kiyotaka Okada¹, Takuji Wada¹. ¹RIKEN, Yokohama, Kanagawa, Japan

CAPRICE (CPC) encodes a small protein with a R3 MYB motif and promotes root-hair cell differentiation in *Arabidopsis* (Wada et al., 1997). We showed that the CPC protein moves from hairless cells to neighboring hair-forming cells and represses the expression of the homeodomain-leucine zipper gene GLABRA2 (GL2) (Wada et al., 2002). We found four additional CPC-like Myb sequences including TRIPTYCHON (Schellmann et al., 2002) in *Arabidopsis*. Plant lines that overexpress these four genes had a reduction in the number of trichomes and an increase in the number of root-hairs, which is similar to a plant with an additional copy of CPC (35S:CPC). We also made double and triple mutants to clarify the function of each gene. These results suggest that the CPC-like R3 MYB genes cooperatively regulate epidermal cell differentiation, particularly in structures that arise from epidermal cell proliferation. Unlike CPC, CPC-like Myb GFP fusion proteins did not move from cell to cell. Promoter-GUS analyses indicated that these CPC homologs were specifically expressed in epidermal cells, including stomate guard cells. Notably, the CAPRICE-like Myb3 (CPL3) gene alone has pleiotropic effects on stomate guard cell distribution, flowering development and epidermal cell size through the regulation of endoreduplication.

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LACY, an *Arabidopsis* Receptor-like Cytoplasmic Kinase is Required for Proper Organ Growth. Carl-Erik Tornqvist¹, Ronan O'Malley¹, Anthony B. Bleeker¹, Sara E. Patterson¹. ¹University of Wisconsin, Madison, USA

In an effort to elucidate the functions of *Arabidopsis* receptor-like kinases (RLKs), we have isolated homozygous T-DNA lines with insertions in 150 different RLKs with unknown function. Of particular interest is a mutant we call *lacy*, with a single T-DNA insertion, which displays asymmetric leaf morphology, small stature, increased axillary branching, and irregular trichome growth. *lacy* is a recessive mutation caused by a disruption in a Receptor-like Cytoplasmic Kinase (RLCK). RLCKs belong to the RLK family, but have neither a transmembrane nor an extracellular domain. To uncover the function of LACY, we have carried out phenotypic observations, analyzed gene expression, and looked at possible genetic interactions. We have conducted extensive phenotypic characterizations of *lacy*, including basic plant morphology, vasculature, and trichome formation. RT-PCR analysis allowed us to determine if the *lacy* phenotype is associated with altered expression of hormone response genes and/or other genes involved in growth and development. To link LACY's function to current developmental pathway models, several marker lines have been crossed to *lacy*, harboring hormone, homeobox, meristem maintenance, and cell division gene promoter-reporter constructs. Studies of genetic interactions between LACY and genes critical to growth and development will also be presented. Since RLKs have been implicated in plant functions other than growth and development, such as disease resistance and the self-incompatibility response, the homozygous T-DNA collection will also serve as a valuable resource for researchers interested in many aspects of plant science. Distribution to the ABRC of all homozygous lines is being completed, making this collection available to the entire *Arabidopsis* community.

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Organ size, cell size and ploidy: how an increase in ploidy promotes cell and organ growth? Hirokazu Tsukaya¹, Christian Breuer², Keiko Sugimoto-Shirasu². ¹The University of Tokyo, Tokyo, Japan, ²John Innes Centre, Norwich, UK

Tetraploid plants generally have larger cells than diploid plants. Similarly, increasing genome size by endoreduplication has positive impacts on cell size in plants. To investigate these functional links between ploidy, cell size and organ size, we have generated series of tetraploid plants in several *Arabidopsis* mutant backgrounds. Mutations in the DNAtopoisomerase VI complex arrest endoreduplication cycle prematurely, leading to the development of severely dwarf plants. In these mutants, many large cell types that normally endoreduplicate up to 32C are replaced with smaller cells (Sugimoto-Shirasu et al. 2002). We found that tetraploidisation in the mutant backgrounds doubles the nuclear DNA content and this partially rescues their cell and organ size phenotypes, suggesting that the mechanism that allows ploidy-dependent cell enlargement is common between tetraploidisation and endoreduplication. To explore the genetic mechanism of this ploidy-dependent cell enlargement, we are now in the process of making tetraploid plants from several other dwarf mutants that have specific defects in cell expansion. In addition, we have recently isolated several new mutants that have defects in the compensatory mechanism (Fujikura et al. 2007) in which the reduction of cell number in leaf primordia triggers abnormal cell expansion (Tsukaya 2006). We are now using these mutants to address whether this process involves ploidy-dependent cell enlargement. Based on the results obtained from these experiments, we will discuss how ploidy may influence cell and organ size in plants.

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P-333

Analysis on the mutants with increased cell number and decreased cell size in leaves. Takeshi Usami¹, Gorou Horiguchi², Hirokazu Tsukaya^{1,2}. ¹National Institute for Basic Biology, ²Graduate School of Science, The University of Tokyo

During leaf development, cell proliferation at the base of leaf primordia and cell expansion at the tip takes place simultaneously and in a coordinated manner. Coordination of cell proliferation and cell expansion is essential for leaf development. It is known that a defect in cell proliferation in leaf primordia induces an increase in cell size in many mutants such as *angustifolia3* (*an3*) and *aintegumenta* (*ant*). This phenomenon, called compensation syndrome, is thought to reflect the intrinsic mechanism that coordinates cell proliferation and cell expansion, and has been extensively studied in recent years. On the other hand, an increase in cell number caused by overexpression of *AN3* or *ANT* does not reduce cell size. To gain further insight into the coordination of cell proliferation and expansion, we screened for mutants that have increased cell number and decreased cell size in leaves. We isolated four such mutants and investigated them. Time course analysis of leaf development showed that decreased cell size in these mutants might not be the consequences of a prolonged cell proliferation period at the expense of cell expansion period. Genetic analysis showed that two of them were allelic to *squint* and *paused*, which showed accelerated heteroblasty. Another one also showed significantly accelerated heteroblasty. We then observed cellular changes associated with heteroblasty in wild type and found that the leaves at higher nodes contained larger number of smaller-sized cells than those at lower nodes. To our knowledge, this is the novel cellular phenotype of heteroblasty in *Arabidopsis* leaves. The leaves of the three mutants with accelerated heteroblasty must have characteristics of those at higher node of wild type. Unknown factor(s) that changes with heteroblasty might have an important role in regulating both the number and the size of leaf cells. Further investigation about this factor and the fourth mutant, which showed no significant acceleration in heteroblasty, will give us a clue to unravel the mechanism that coordinates cell proliferation and cell expansion during leaf development.

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Endoreduplication during leaf and root development is differentially regulated by the Anaphase-Promoting Complex Activators CCS52A1 and CCS52A2 in *Arabidopsis thaliana*. Marleen Vanstraelen¹, Sylvie Tarayre¹, Mikhail Baloban¹, Eva Kondorosi¹. ¹Plant Science Institute (ISV)/CNRS, Gif-sur-Yvette, France

Plant organs develop mostly post-embryonically from persistent or newly formed meristems. After cell division arrests, cell differentiation starts which frequently involves endoreduplication and cell enlargement. CCS52A was previously shown to function in the transition from mitosis-to-endocycles during nodule development in *M. truncatula*. *Arabidopsis* has two CCS52A genes, CCS52A1 and CCS52A2. They share a high degree of homology and are expressed from late M-phase to S-phase in cell cultures. In plants, we found that the CCS52A genes are expressed in young differentiating tissues. CCS52A promoter-driven β -glucuronidase activity showed coloration in young developing leafs and in case of CCS52A1 staining was also observed near the root tip. In these tissues, we studied DNA ploidy levels in plants mutant for the CCS52A genes. Deletion of either CCS52A genes led to less endoreduplication cycles and small cell sizes in rosette leafs. In case of *ccs52a1*, normal leaf size was obtained by elevated cell numbers. Constitutive overexpression of the CCS52A genes triggered the opposite effect with higher ploidy levels and increased cell sizes. Reduced ploidy levels were also observed in roots of *ccs52a1* plants. These roots grew faster than wild type roots due to bigger meristems with more dividing cells. Deletion of the CCS52A2 gene did not affect endoreduplication cycles in roots. However, roots of these plants were smaller with smaller meristems and less dividing cells. Together the data show that both CCS52A1 and CCS52A2 function in the transition from mitosis-to-endocycle during leaf development, whereas only CCS52A1 contributes to endoreduplication during root development.

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Identification and functional characterization of BIGPETALp and BIP, two interacting protein involved in the control of petal size in *Arabidopsis thaliana*. Emilie Varaud¹, Florian Brioudes¹, Judit Szcsi¹, Karim Bordji², Caroline Joly¹, Philippe Vergne¹, Mohammed Bendahmane¹. ¹Ecole Normale Supérieure Lyon France, ²Université Caen France

Organ final size can be influenced by cell number or cell expansion or both. We identified a novel petal-expressed bHLH encoding gene (BIGPETAL or BPE) which limits petal size by influencing post-mitotic cell expansion in *Arabidopsis thaliana*. BPE is expressed via two proteins: a petal expressed protein (BPEp) and a ubiquitously expressed protein (BPEub). Both proteins have identical N-terminal domains (containing the bHLH domain) but have different C-terminal domains. A yeast-two hybrid screen was performed and permitted to identify a putative BPEp interacting protein we named BIP (for BPEp Interacting Protein). Plants knockout for BIP have larger petals as a result of increased cell size, showing that like for BPEp, BIP interferes with post-mitotic cell expansion. Our data suggests that BPEp and BIP must be part of a protein complex involved in the control of petal size by limiting cell expansion. The significance of this interaction in modulating and/or participating to BPEp biological functions during petal morphogenesis will be discussed.

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MIKC * MADS-protein complexes regulate pollen maturation in *Arabidopsis*. Wim Vreliet¹, David Twells², Heinz Saedler¹, Thomas Muenster¹. ¹Max Planck Institute for Plant Breeding Research, Cologne, Germany, ²University of Leicester, Leicester, United Kingdom

Pollen grains represent the haploid male gametophyte in flowering plants, and play an essential role in sexual reproduction. However, the pathways that regulate pollen development remain largely uncharacterized. We found that five pollen-specific MIKC * MADS transcription factor complexes play a major role in regulating gene expression during the last stages of pollen development in *Arabidopsis thaliana*. The absence of these MADS-complexes results in a partial arrest of the normal, developmentally regulated changes in the pollen transcriptome. Microarray experiments on mutant pollen, lacking all MIKC * complexes, revealed that the expression of nearly 500 immature-pollen-specific genes fails to be downregulated before anthesis, while at least 250 mature-pollen-specific genes are not properly activated. Overall, the expression of more than 1000 genes is directly or indirectly regulated by the MIKC * complexes in *Arabidopsis* pollen grains. These include important components of hormone pathways, the secretory pathway, cell wall synthesis, carbohydrate metabolism and the ubiquitin-mediated proteolysis system, as well as 27 non-MADS transcription factors. In addition, comparison of the pollen transcriptome of various single, double and triple MIKC * mutants enabled us to visualize the functional redundancy between the five different MIKC * complexes on the level of their target genes. Our data thus indicate that the MIKC * complexes rank high in a regulatory network that controls pollen maturation. These datasets form a solid experimental basis for the systematic unravelling of the regulatory pathways that control pollen development in *Arabidopsis*, and provide a unique insight into the complexity of a pollen-specific transcription factor network.

P-337

Structure and function of cell wall pectic arabinan and galactan during "Arabidopsis thaliana" seedling growth and development. Yves Verhertbruggen¹, Susan E. Marcus¹, J. Paul Knox¹. ¹University of Leeds, LS2 9JT, United Kingdom

Plant cell walls are key cellular components underpinning plant growth and development. They are also highly complex structures with a great diversity of components and architectures across species. Cell wall polysaccharides occur in three major classes: cellulose, cross-linking glycans (or hemicelluloses) and pectins. Pectins are a highly structurally diverse class that includes polymer domains such as homogalacturonans (HG), rhamnogalacturonans (RG), xylogalacturonans (XGs) and arabinogalactans. Although studies have suggested roles for pectic rhamnogalacturonan-I (RGI) and its associated arabinan and galactan domains, in cell division, cell expansion, cell differentiation and in modifying the mechanical properties of plant cell walls, it is still unclear how the structures of these polymers is regulated "in vivo" and how they function during cell development. We have developed a series of monoclonal antibody probes to (1→4)-beta-galactan and (1→5)-alpha-arabinans that are structural features of RGI and have used these to track polymer occurrence in shoot organs and at the root surface of "A. thaliana" where they show dynamics in relation to root growth. Arabinans are shown to be a structurally complex class of polymers which displays extensive regulation in relation to cell development. At the surface of "A. thaliana" seedling roots, the occurrences of RGI galactan and arabinan epitopes are differently modulated in response to environmental, hormonal and other factors that reduce root growth including light, auxin and inhibitors of reactive oxygen species (ROS). These antibody probes are being used for the analysis of RGI structures in response to mutations that impact upon cell walls and development in "Arabidopsis".

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In silico identification of co-transcribed core cell cycle regulators and transcription factors in Arabidopsis. Yixing Wang¹, Ming Yang¹. ¹Oklahoma State University, Stillwater, OK, USA

Regulatory networks involving transcription factors and core cell cycle regulators are expected to play crucial roles in plant growth and development. In this report, we describe the identification of a putative network involving five core cell cycle regulators and six transcription factors via a two-step in silico screening strategy. We first screened the Arabidopsis microarray data (AtGeneExpress: Expression Atlas of Arabidopsis Development) in <http://www.arabidopsis.org/> for core cell cycle regulators and transcription factors detected in young leaves and stage-15 carpels but not in mature and senesced leaves and sepals, petals and stamens at floral stage 15. We then explored the correlations in transcriptional profile among the cell cycle genes and the transcription factor genes identified in the first screening using the AtGenExpress Visualization Tool. The identified core cell cycle regulators and transcription factors fall into two groups with matching transcriptional profiles. One such group consists of TARDY ASYNTROCHONOUS MEIOSIS (CYCA1;2), CURLY LEAF and a Forkhead-associated domain transcription factor gene, and the other group consists of CYCB1;1, CYCB2;1, CDKB1;2, CDKB2;2, AINTEGUMENTA, a MYB gene, another Forkhead-associated domain transcription factor gene, and a SCARECROW family gene. Results from Pearson's correlation coefficient analysis are also consistent with the two groups of genes being co-transcribed, respectively. Promoter analysis revealed a possible web of cross- and self-regulations among the identified genes. Because some of the genes are known regulators of cell proliferation and all of them are only predominantly transcribed in young organs, we predict that these genes are specially involved in organ growth in Arabidopsis.

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'ALCATRAZ-INTERACTING PROTEIN1' encodes a lysine rich nuclear protein and is expressed ubiquitously in Arabidopsis
Fang Wang¹, Jie Liu¹, Weicai Yang¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

bHLH transcription factors often bind to specific DNA target sites as either homodimer or heterodimer. ALCATRAZ (ALC) is a member of the bHLH family which is critical for cell separation during fruit dehiscence (Rajani and Sundaresan, 2001, Current Biology 11: 1914-1922). To investigate the molecular mechanism of ALC in regulating pod shattering and other developmental processes, a yeast two-hybrid screen was performed using ALC as bait for Arabidopsis whole plant cDNA library. A gene named as AIP1 (ALC Interacting Protein1) was identified. AIP1 encodes a novel nuclear protein with a lysine rich domain.

Deletion experiments showed that both the Lys-rich domain and the C-terminal of AIP1 are able to interact with ALC in yeast cells, but the latter has lower affinity, indicating that AIP1 interacts with ALC preferentially via its Lys-rich domain. The in vivo interaction of ALC and AIP1 was further confirmed by bimolecular fluorescence complementation (BiFC). RT-PCR analysis and PAIP1::GUS reporter assay indicated that AIP1 and ALC have overlapping expression pattern, suggesting they likely function together in planta. RNA in situ hybridization analysis showed AIP1 transcripts were detected strongly in the mesocarp of the valve, not in epidermis and the separation zones. Later in development, AIP1 transcripts were mainly detected in vasculature. The investigation on the function of AIP1 in plant development is underway.

P-340

Control of flowering time by the SPL3/miR156 pathway.
Jia-Wei Wang¹, Benjamin Czech¹, Detlef Weigel¹. ¹Max Planck Institute for Developmental Biology, Tuebingen, Germany

A drastic developmental transition is the switch from vegetative to reproductive development. The initiation of flowering requires an endogenous developmental program to specify the floral identity of the new structures that arise at the shoot apex. Genetic analysis has identified four main signals and corresponding pathways that control flowering time in Arabidopsis: the photoperiod, autonomous, vernalization, and gibberellin pathways. Overexpressing of one Arabidopsis microRNA (miRNA), miR156, leads to a plant with delayed flowering and shortened plastochrons (the interval between formation of primordia at the shoot apex) (Schwab et al., 2005). miR156 targets a group of genes encoding plant-specific transcription factors, named squamosa promoter binding protein-like (SPL) proteins. Overexpression of a miR156-resistant form of SPL3 (rSPL3) accelerates flowering in both long day and short day condition. Using mis-expression, promoter GUS (β -glucuronidase) reporter fusions, and genetic analyses, we show here that miR156 regulates SPL3 levels in leaves and that SPL3 promotes flowering in a FT (FLOWERING LOCUS T)-dependent manner. We will discuss the position of SPL/miR156 in the current framework of flower signal integration.

P-341

An Arabidopsis PEL may play a role in male gametophyte development. Si-Qi Wang¹, Wei-Cai Yang¹. ¹INSTITUTE OF GENETICS AND DEVELOPMENTAL BIOLOGY, CHINESE ACADEMY OF SCIENCES, Beijing, China

Pectin is a main component of plant cell wall and is partly responsible for the maintenance of the structural integrity of cell walls. Pectate lyases (PELs) catalysis the cleavage of the de-esterified pectin and cause its degradation. Plant PELs presumably participate in the developmental progress such as in pollen tube initial germination and penetration into the style, fruit-ripening, and lateral roots emergence. Here we report an Arabidopsis transposon mutant line kd468, in which a gene coding for a putative PEL is disrupted. This line displayed non-Mendelian segregation of KanR marker gene. The ratio of KanR to KanS is 1:16, instead of the Mendelian 3:1 segregation. Reciprocal crosses indicate that Ds transmission through the male gametophyte is severely affected, while the transmission through the female gametophyte is almost normal. Pollen development seems normal when stained with Alexander and DAPI compared with that of wild type. Molecular analyses show that a single Ds is inserted at the ORF of the PEL gene. Complementation experiment and detailed phenotypic study are ongoing.

P-343

Molecular mechanism in the control of floral dorsoventral and organ internal asymmetries during zygomorphic flower development in *Lotus japonicus*. Jiechen Wang¹, Zhigang Cai¹, Yonghai Luo¹, Shilei Xu¹, Lin Weng¹, Jun Yang¹, Luo Da¹. ¹National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Graduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China

In a model legume *Lotus japonicus* the zygomorphic flower consists of three types of petals: a dorsal bilateral-symmetric petal, two asymmetric lateral petals and two asymmetric ventral petals. Two types of asymmetry in the flower could be identified: the floral dorsoventral asymmetry along the plan of the flower and the organ asymmetry in the plan of petals. This raises the questions about the genetic factors in the control of these two types of asymmetry and the mechanism coupling them to form handedness symmetry between a pair of lateral or ventral petals. It has been showed that in a distinct related model plant, *A. majus*, development of zygomorphy is controlled by two TCP genes, CYC and DICH. Our previous work showed that a CYC homologue, LjCYC2, plays a key role in the development of zygomorphy in *Lotus japonicus*, although the evolution of zygomorphy should be independent among different species. Furthermore, a legume specific factor, KEW1, was identified, which plays a key role in the control of lateral petal identity.

This work has been investigating the function of LjCYC1, a close homologue of LjCYC2 in *Lotus japonicus*, and the interaction between LjCYC1/LjCYC2 and KEW1. We demonstrate that LjCYC1 and LjCYC2 play a redundant role in the control of the dorsal identity and regulate cell differentiation during petal development, and the function of KEW1 was involved in the coupling of floral dorsoventral asymmetry with the organic asymmetry during zygomorphic development. Furthermore, knock-down of the two LjCYC genes in kew1 background revealed a default stage when the dorsal and lateral activities being abolished, indicating that there exit other genetic factors in the control of organic asymmetry during floral zygomorphic development in *Lotus japonicus*.

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LFR(text in italics) point mutant affects leaf and flower development in Arabidopsis. Zhijuan Wang¹, Sujuan Cui¹. ¹Laboratory of Molecular and Cell Biology, Hebei Normal University, Shijiazhuang, China

Leaf and flower, as major aerial organs of plant, play important roles in plant individual development. During screening SALK T-DNA insertion mutant lines of Arabidopsis, one novel mutant line was named 'lfr' (text in italics), which shows both leaf and flower mutant phenotype. 'lfr' (text in italics) has curling-up young leaves. When the leaf matures, it can expand. The number of petal in 'lfr' (text in italics) flower increases and stamen reduces. The early flowers are infertility, for the filament is too short to pollinate. The latter flowers can produce seeds but less than wild type. Besides these mutant phenotypes, the cotyledon vein pattern, bolting time and angle of secondary bolts are also different from those of wild type. 'LFR' (text in italics) gene may function in many processes in plant aerial organ development.

'lfr' (text in italics) mutant phenotype was shown not linked with inserted T-DNA by TAIL-PCR and Southern blot. Genetics cross analysis indicated the mutant phenotype may be controlled by one recessive locus. To clone the locus, we used the map-based cloning strategy using SSLP and CAPS markers. Among 2012 recombinant chromosomes, we mapped the locus between two markers in chromosome (3). Sequencing of DNA and RT-PCR product showed a G to A transversion in the first base of the third intron of gene 'LFR' (text in italics). The transversion causes a 67 bp deletion of the 'LFR' (text in italics) mRNA, which results in an early stop codon. To further identify the linkage between LFR and mutant phenotype, SALK T-DNA inserted line was screened but we didn't get null mutant, but '35S::LFR' (text in italics) cDNA and 'LFR::LFR' (text in italics) transgenic plants can rescue the phenotype completely.

As annotation, 'LFR' (text in italics) is one unknown gene, and LFR protein contains 3 putative protein-protein interaction domains which involved in cell signaling or cellular architecture in many eukaryotic. We suppose that LFR may interact with other proteins or might be an integrator to involve in so many processes. Ongoing or further works will be done to explain the mechanism of LFR functions.

P-344

At5PTase13 is involved in root gravitropism through modulating vesicle trafficking. Yuan Wang¹, Wen-Hui Lin¹, Xu Chen¹. ¹Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Inositol polyphosphate 5-phosphatases (5PTases) is involved in phosphatidylinositol metabolism and affect various aspects of plant growth and development. Through molecular and genetic approaches, we are trying to study the physiological functions of the At5PTase13, which has been proved to regulate the homeostasis of auxin. The knockout mutant At5pt13 showed elevated sensitivity to gravistimulation in the root gravitropic response. Treatment with polar auxin transport inhibitor 1-N-naphthylphthalimide (NPA) indicated that At5PTase13 also altered auxin transport, which is closely related to gravitropism. Observations of the membrane-selective dye FM4-64 revealed that At5PTase13 negatively regulated vesicle trafficking and At5pt13 was less sensitive, comparing with wild type, to the inhibition of brefeldin A (BFA) on vesicle cycling. Further studies showed that BFA had less effect on seedling growth in At5pt13. These results suggest that At5PTase13 may have an influence on auxin transport through regulating vesicle trafficking and subsequently involve in root gravitropism.

P-345

The effect of flowering on vegetative phase change in *Arabidopsis*. Matthew R. Willmann¹, R. Scott Poethig¹. ¹Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

Shoot development in most flowering plants is characterized by three primary postembryonic phases: juvenile vegetative, adult vegetative, and reproductive. These phases are distinguished by differences in vegetative characteristics, reproductive competence, phytochemical production, and disease and insect resistance. It is believed that a series of temporally overlapping pathways regulate the transitions between phases. Under long day conditions, *Arabidopsis* flower primordia were already visible in most young Columbia wild-type seedlings when the fifth leaf was only 1 mm long. Therefore, cell division, differentiation, and expansion within most leaves on a shoot are occurring under the context of floral induction and are likely regulated by this process. In attempt to distinguish the effects of the juvenile-to-adult transition on vegetative growth and development from those of the reproductive onset, we characterized vegetative phase change under different photoperiods and in early ("FRI; flc-3") and late-flowering ("FRI; FLC") genotypes of the flowering time repressors FRI and FLC. Short-day conditions, which inhibit flowering, also delayed vegetative phase change in all genotypes. Under both short and long-day light regimes, the timing of the onset of vegetative phase change in these genotypes was statistically the same as wild-type Columbia ("fri; FLC"). The length of the transition zone, however, was dependent on flowering time. The early flowering genotype had a shorter transition zone than wild type. Plants with delayed flowering ("FRI; FLC" plants, as well as all genotypes grown under short days) had a much longer transition zone. Preliminary data suggests that the FLC targets SOC1 and FT may mediate these effects of flowering on vegetative development. This work indicates a role for flowering in the acceleration of adult vegetative characteristics in *Arabidopsis*.

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NUCLEAR-PORE ANCHOR, the *Arabidopsis* Homolog of Tpr/Mlp1/Mlp2/Megator, Is Involved in mRNA Export, SUMO Homeostasis and Affects Diverse Aspects of Plant Development

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Vertebrate Tpr and its yeast homologs Mlp1/Mlp2 are long coiled-coil proteins associated with the inner basket filaments of the nuclear pore. They are involved in mRNA export, telomere organization, spindle pole assembly, and unspliced RNA retention. We have identified a single gene in *Arabidopsis thaliana*, NUCLEAR PORE ANCHOR (NUA), which encodes a protein of 237 kD with similarity to Tpr. Immunolocalization in *Arabidopsis* root cells demonstrates that NUA is located at the inner surface of the nuclear envelope in interphase and in the vicinity of the spindle in prometaphase. Four T-DNA insertion lines were characterized in detail. They comprise an allelic series of increasing severity for several correlating phenotypes, such as early flowering under short days and long days, increased abundance of SUMO conjugates, altered expression of several flowering regulators, and nuclear accumulation of poly(A) + RNA. Nua mutants phenocopy mutants of EARLY IN SHORT DAYS 4 (ESD4), an *Arabidopsis* SUMO protease concentrated at the nuclear periphery. Nua esd4 double mutants resemble nua and esd4 single mutants, suggesting that the two proteins act in the same pathway or complex, supported by yeast two-hybrid interaction. Together, our data indicate that NUA is a component of nuclear pore-associated steps of sumoylation and mRNA export in plants, and that defects in these processes affect the signaling events of flowering time regulation and additional developmental processes.

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ASYMMETRIC LEAVES1/2 and JAGGED are required for sepal and petal organogenesis by defining organs from their boundaries. Ben Xu¹, Ziyu Li², Yan Zhu², Hua Wang¹, Hong Ma³, Aiwu Dong², Hai Huang¹. ¹National Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China., ²State Key Laboratory of Genetic Engineering, Department of Biochemistry, School of Life Sciences, Fudan University, Shanghai 200433, China., ³Department of Biology and the Huck Institute for the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA

Boundary formation is crucial for organ development in multicellular eukaryotes. In higher plants, boundaries between meristem and organ or between organs are regions that are slower in cell proliferation than their adjacent tissues. In addition, boundaries coincide with the action domains of a set of boundary-specifying genes. Here we show that the *Arabidopsis* genes ASYMMETRIC LEAVES1, 2 (AS1 and AS2) and JAGGED (JAG) are critical for sepal and petal organogenesis by suppressing genes involved in boundary formation in the initiated perianth. Loss-of-function as1/as2 jag double mutants produced extremely tiny sepals and petals due to a disrupted cell proliferation. These abnormal phenotypes are accompanied by ectopic expression of the boundary-specifying genes PETAL LOSS (PTL), CUP-SHAPED COTYLEDONS1 and 2 (CUC1 and CUC2) in the organs. Together, our results reveal a previously unrecognized fundamental regulation, by which AS1/AS2/JAG act to define organs from their surrounding boundaries through suppressing boundary genes for normal organogenesis.

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***Arabidopsis* eIF3e is regulated by the COP9 signalosome and impacts development and protein translation.** Avital Yahalom¹, Tae-Houn Kim², Albrecht G. von Arnim², Daniel A. Chamovitz¹. ¹Tel Aviv University, ²University of Tennessee

The roles of individual eIF3 subunits are largely unclear, though some are essential, while others are thought to have regulatory roles. The "e" subunit, also known as Int-6, is a candidate for a regulatory subunit as it is not essential for translation initiation in yeasts. eIF3e associates with the COP9 signalosome and localizes in certain tissues to the nucleus. To further elucidate the roles of eIF3e, we have taken a genetic approach using *Arabidopsis* as a model system. Overexpression of eIF3e results in defects similar to mutations in the COP9 signalosome. eIF3e protein, but not transcript, over-accumulates in csn mutants, and eIF3e is degraded in a proteasome-dependent fashion. Over-expression of eIF3e leads to accumulation of ubiquitylated proteins. In vitro and in vivo assays suggest that excess eIF3e inhibits translation. We conclude that CSN maintains a precise regulation of eIF3e levels which is necessary for normal development in *Arabidopsis*.

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The MALE STERILITY1 protein regulates tapetal gene expression and pollen wall development. Caiyun Yang¹, Jianping Lu¹, Gema Vizcay Barrena¹, Zoe A Wilson¹. ¹Plant Sciences Division, University of Nottingham, Loughborough, LE12 5RD, UK

Pollen development is a fundamental stage in the higher plant life cycle that is pivotal for breeding and seed production. The control of pollen viability and release is of major commercial importance in the development of crops for hybrid seed production and selective breeding. One of the key tissues involved in pollen development is a specialised cell layer in the anther, the tapetum. The tapetum is metabolically very active with major secretory roles, including nutrition for the developing microspores, regulating callose breakdown and controlling pollen wall development. It has a highly regulated life-cycle with a close, reciprocal interaction with the developing microspores. Disruption of the tapetum is frequently seen in many male sterile plants.

We have shown that the *Arabidopsis* MS1 gene is critical to tapetal development/tapetal programmed cell death (Vizcay-Barrena and Wilson, 2006) and pollen wall formation. Orthologues of MS1 and the related genes linked to the MS1 complex have been identified in oil-seed rape and rice indicating the conservation of this molecular pathway. The MS1 protein contains a leucine zipper followed by a C-terminal PHD-finger motif (Wilson et al., 2001). PHD-fingers are highly conserved motifs which have been associated with chromatin remodelling and gene regulation (Asaland et al., 1995). We have shown that MS1 is nuclear localised and has significant effects on transcription during anther development. Microarray analysis of ms1 mutant buds indicates major gene changes compared to wt, particularly in genes associated with pollen wall development. RT-PCR has shown that some of these are reciprocally regulated in MS1 over-expressing lines (35S::MS1). These data support the role of MS1 as a key transcription regulator of tapetal gene expression, particularly those associated with pollen wall development. The role of MS1 in tapetal development and pollen formation will be discussed.

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THE IMPACT OF PUTATIVE UBIQUITIN PROTEINS ON FLOWERING TIME IN ARABIDOPSIS. Shai Yerushalmi¹, Rachel Green^{1,1}
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A vast range of organisms from cyanobacteria to plants and mammals use their endogenous circadian rhythms to anticipate the changes in daily and annual environmental conditions and adapt their physiological and developmental states accordingly. In many plants, reproductive development is regulated by a photoperiodic pathway that integrates circadian rhythms and light signals. This research aims at identifying genes that are involved in the circadian-regulation of flowering. Using a bioinformatics-based approach we have identified a circadian-regulated gene that encodes a putative ubiquitin fusion degradation (UFD1) protein. In yeast and animals, UFD1 is known to form a ternary complex together with NPL4 and CDC48/P97 in the cytosol. Yeast UFD1 protein is required for ubiquitin-dependent protein degradation in the endoplasmic reticulum associated degradation (ERAD) process. The function of UFD1 protein in plants is not yet known. In *Arabidopsis*, this gene is part of a family that contains three other UFD1-like genes that share high homology and marked here as UFL1, UFL2 and UFL3. Mutations in UFD1, UFL1 and UFL2 significantly alter flowering time in light regime of long days but have only subtle effects on flowering time in short days, suggesting that this gene may have a role in photoperiodic regulation of flowering. Double mutants of ufd1 x ufl1 flower earlier than the wild type in long days but not earlier than ufd1 and ufl1, suggesting a functional redundancy with other proteins in the UFD1 family. Northern blot analysis shows that the expression of circadian-regulated genes is not altered in ufd1 or ufl1 plants, suggesting that the protein is active downstream of the circadian oscillator. Our hypothesis is that UFD1 and the UFD1-like proteins may participate in the degradation of a component that promotes flowering in long days but not in short days.

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A subfamily of transcription factors control root hair development downstream of RHD6/RSL1. Keke Yi¹, Beno t Menand¹, Liam Dolan¹. ¹Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK

The development of root hairs involves the establishment of the root hair cell identity, followed by the initiation of the root hair and its elongation by tip growth. Although the established root hair cell identity is well characterized at the genetic level, the mechanism controlling the initiation and tip growth remains to be elucidated. Previously, in our lab, we isolated the RHD6 (ROOT HAIR DEFECTIVE 6) and RSL1 (RHD SIX-LIKE1) genes (a pair of duplicated genes), which participate in the root hair initiation (Menand et al., accepted). These two genes are specifically expressed in the trichoblast in the meristem and elongation zone. We performed microarray transcription profiling analysis for the rhd6/rsl1 double mutants to identify target of RHD6/RSL1, and found a subfamily of transcription factors, which might function downstream of RHD6 and RSL1. The expression of this subfamily of genes is significantly repressed in the rhd6/rsl1 double mutant background. Using whole gene GFP fusions we showed that each of these genes are specifically expressed in the trichoblast at the stage where root hairs initiate. Therefore we named these genes RHS (ROOT HAIR SPECIFIC) 1 to 4. When RHS1, RHS2 or RHS3 was expressed ectopically in the rhd6/rsl1 double mutant background, the root hair deficient phenotype of rhd6/rsl1 was partially rescued. This suggests that this subfamily of genes are also involved in root hair development. rhs1 loss of function mutants have a short root hair phenotype compared with wild type. While root hair density is decreased in mutants that lack RHS3 function. No distinct root hair phenotype can be detected in mutants that lack either RHS2 or RHS4 function. The double, triple and quadruple mutants are being generated. These genetic resources will be used for future microarray transcription profiling analysis, which will help us to define a transcriptional regulating network for the process of root hair initiation and tip growth.

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INCREASED LEVEL OF POLYPLOIDY 1(ILP1) encodes a novel transcriptional repressor that controls endoreduplication level in Arabidopsis. Takeshi Yoshizumi¹, Yuko Tsumoto¹, Tomoko Takiguchi¹, Noriko Nagata², Yoshiharu Yamamoto³, Mika Kawashima¹, Takanari Ichikawa¹, Miki Nakazawa¹, Naoki Yamamoto⁴, Minami Matsui¹. ¹Plant Functional Genomics Research Team, PSC, RIKEN, Yokohama, Japan, ²Department of Chemical Biological Sciences, Japan Women's University, Tokyo, Japan, ³Center for Gene Research, Nagoya University, Nagoya, Japan, ⁴Graduate School of Human Environmental Science, Ochanomizu University, Tokyo, Japan

In plants most organs are composed of cells of various ploidy levels and endoreduplication is a key process in the plant's developmental plan. We have isolated 'ilp1-1D' as a dominant mutant in which polyploidy level is increased in the seedlings. 'ilp1-1D' showed correlation between increased cell volume with polyploidy. ILP1 gene encodes for a novel nuclear protein and this protein functions as a transcriptional repressor in vivo. The expression of all the member of 'CYCA2' family was reduced in an 'ILP1' over-expressing line. This indicates that ILP1 is a repressor that control 'CYCA2' expression. T-DNA insertion mutants of 'CYCA2;1' has increased ploidy level. Here we demonstrate that ILP1 regulates endoreduplication through control of 'CYCA2' expression in 'Arabidopsis'. ILP1 has homologs not only in plants but also in insects and mammals, and the mouse ortholog of the ILP1 also repressed 'cyclin A2' expression in mouse NIH3T3 cells.

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Characterization of embryo and endosperm mutants caused by Ds insertion into genes encoding putative leucine-rich repeat-containing receptor kinases. Tian-Ying Yu¹, Jie Liu¹, Wei-Cai Yang¹. ¹The Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Datun Road, Beijing 100101, China

In flowering plant, double fertilization gives rise to a diploid zygote and a triploid primary endosperm cell that develop into embryo and endosperm respectively. To investigate the genetic control of embryo and endosperm development, we screened a Ds gene trap collection for mutants defective in embryo and endosperm development. One of the mutants was isolated that showed 27.82% seed abortion. Embryo development was blocked at either the zygotic stage (97/1136 = 8.54%) or one-celled embryo stage (180/1136 = 15.85%). Molecular analysis showed that the Ds element was inserted into a gene encoding for a putative leucine-rich repeat receptor-like kinase (LRR-RLK). GFP fusion experiment showed that the gene product is localized in plasma membrane. Expression analysis revealed by RT-PCR indicated that the gene is expressed highly in siliques between fertilization and 4-8 cell embryos. The expression level is very low in inflorescence, and no expression was detected in other tissues. These data suggested that the gene is expressed in embryo and/or endosperm. In situ experiment further confirmed that the gene is expressed specific in embryo and endosperm. Further molecular study on its function in embryo and endosperm development is being carried out.

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Molecular mechanism of PSII biogenesis. Lixin Zhang¹, Lianwei Peng¹, Jinfang Ma¹, Jinkui Guo¹, Wei Chi¹, Qingtao Lu¹, Congming Lu¹. ¹Institute of Botany, Beijing, CHINA

Chloroplast development is largely dependent on the nuclear encoded proteins. To further unravel the regulation of chloroplast biogenesis, we screened 60000 T-DNA tagged lines and isolated 90 *Arabidopsis* mutants exhibiting the phenotype with high chlorophyll fluorescence. A mutant with reduced PSII accumulation was subjected to further investigation. The leaves of the mutant were paler than those of the wild type, and growth was significantly reduced in the mutant. In addition, the wild-type chloroplasts displayed well-developed membrane systems composed of grana connected by the stroma lamellae, but the thylakoid membrane systems in lpa1 chloroplasts were disturbed, and the membrane spacing was not as clear. To identify the precise site of the lpa1 mutation, noninvasive fluorometric analyses were performed. In the lpa1 mutant, Fv/Fm appeared to be significantly lower (0.53) than in wild-type plants (0.82), implying that the mutants have defects in energy transfer within PSII or a partial loss of PSII capacity. In vivo protein labeling experiments showed that synthesis of the D1 and D2 proteins was greatly reduced in the lpa1 mutant, while other plastid-encoded proteins were translated at rates similar to the wild type. In addition, turnover rates of the PSII core proteins CP47, CP43, D1, and D2 were higher in lpa1 than in wild-type plants. The newly synthesized PSII proteins were assembled into functional protein complexes, but the assembly was less efficient in the mutant. LPA1 encodes a chloroplast protein that contains two tetratricopeptide repeat domains and is an intrinsic membrane protein but not an integral subunit of PSII. Yeast two-hybrid studies revealed that LPA1 interacts with D1 but not with D2, Cyt. b6, or Alb3. Thus, LPA1 appears to be an integral membrane chaperone that is required for efficient PSII assembly, probably through direct interaction with the PSII reaction center protein D1.

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The *Arabidopsis* gene REVOLUTA is required to function together with the 26S proteasome subunit genes for floral organ initiation and floral meristem maintenance. Zhenzhen Zhang¹, Qihua Ling¹, Limin Pi¹, Hua Wang¹, Hai Huang¹. ¹Shanghai Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences

During flower development, cells in the central part of a flower primordium initially constitute the floral meristem, from which four whorls of floral organs develop sequentially. Here we report that the *REVOLUTA* (*REV*) and the 26S proteasome subunit genes play important roles in floral organ initiation. *REV* belongs to the plant-specific HD-ZIPIII gene family, participating in a variety of developmental processes. We previously demonstrated that the double mutant combination of *rev* and *ae3*, which carries a defective 26S proteasome subunit RPN8a, resulted in plants with a complete arrest of meristematic growth. We show in this study that plants carrying *rev* and *ae4*, which harbors another disrupted 26S proteasome subunit RPN2a, displayed some similar phenotypes to those of *rev ae3*, but its meristem defects appeared weaker. The reduced severity of the *rev ae4* double mutant allowed us to characterize its abnormalities in the reproductive growth in more detail. *rev ae4* has an abnormal inflorescence structure, with a slowly developing manner. While a few early flowers had their seeds set, the later flowers lacked the inner whorl floral organs, stamens and gynoecia, resembling those of the loss of *WUSCHEL* (*WUS*) function plants. During later reproductive stages, the inflorescence meristem in the double mutant produced filamentous structures, instead of flowers, around the reproductive apex. In situ hybridization experiments revealed that *WUS* expression was repressed in floral primordia of the *rev ae4* double mutant, whereas *SHOOTMERISTEMLESS* and *APETALA1* appeared normally expressed despite the lack of any apparent floral organ development. These results suggest that *REV* and the 26S proteasome play a critical role in flower development by promoting floral organ initiation and sustaining meristematic capacity of the floral primordia.

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DAU encodes a membrane protein that is critical to pollen maturation in *Arabidopsis*. Li Yuan¹, Jie Liu¹, Weicai Yang¹. ¹The Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Datun Road, Beijing 100101, China

In plants, pollens go through a dehydration process after pollen mitosis II to protect the male gametes after pollen release from the anther. Proper timing and initiation of the dehydration processes are critical to pollen viability and fertility. Here we report the characterization of an *Arabidopsis* mutant, dayu (dau) which displayed premature pollen germination before anther dehiscence, leading to male sterility. Compared to wild-type, mutant pollen grains displayed ultrastructural changes typical of germinating pollen and germinated within anthers prior to dehiscence if water was supplied. DAU was cloned by transposon tagging and encodes a putative membrane protein. DAU-DsRed2 fusion experiment showed that DAU indeed is localized in the endomembrane system of male gametophytes. RNA in situ hybridization showed that DAU is transiently expressed in male gametophytes from pollen mitosis I on till anther dehiscence, no expression could be detected in pollen grains after dispersed onto the stigma. These data imply that DAU plays a critical role in controlling the initiation of pollen maturation process in plants.

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Identifying potential target proteins for degradation regulated by the Hawaiian Skirt gene (HWS). Xuebin Zhang¹, Zinnia H Gonzalez-Carranza¹, Jeremy A Roberts¹. ¹School of Biological Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom.

The ubiquitin (Ub)/ 26S proteasome pathway is probably the most studied process to degrade proteins in the cell. A hierarchical cascade of three types of enzymes are involved in the turnover of targets which include; ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin ligase (E3) enzyme. Cells identify the proteins that must be degraded by attaching an ubiquitin chain. The canonical E3 is the complex Skp1-cullin-F-box protein (SCF) and is composed of four subunits: Cullin, SKP1, RBX1 and an F-box protein.

Recently a novel F-box protein from Arabidopsis has been identified; the *Hawaiian skirt* gene (*HWS*, alias *HS*) by the mapping of its mutant *hws-1*; which was identified because its floral organs do not shed; the sepals remain fused at their base retaining the rest of the floral organs inside. The characterization of the mutant and comparison with over expressor lines suggest a role of the *HWS* gene in plant growth (Gonzalez Carranza et al., in press)

The protein of *HWS* contains an F-box domain located 40-85 amino acids downstream of the N-terminus, and a Kelch-2 domain in its C-terminus. To test the hypothesis that *HWS* is the component from an SCF complex and is involved in the ubiquitin proteasome pathway; an anther specific Y-2-H library using both a full transcript and a truncated version without the F-box domain from the *HWS* protein as baits has been screened. Current work, including an analysis of the proteome in wild type and *hws* material, is focused on identifying both potential SCF_{*hws*} components and potential substrate(s) for *HWS* degradation.

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Expression and function analyses of abscission related gene At3g14380. Li zhang¹. ¹University of Nottingham

Abscission is an important process during plant development. The shedding of plant organs, such as leaves, flowers and fruits, takes place at a predetermined position, called an abscission zone (AZ). Studies about abscission have been focusing on identifying abscission specific genes. Arabidopsis gene AT3G14380 is predicted to be up-regulated during abscission based on microarray experiment. RT-PCR showed that this gene was expressed in the abscising flower tissues. The expression of GUS was seen at the AZ and stigmatic surface in At3g14380; GUS transgenic plants. To identify the function of this gene, the construct with down regulated of At3g14380 expression was generated and transformed to wild type (Arabidopsis Columbia). Delayed abscission was observed in those transgenic plants compared with wild type. Petal break strength was measured for those plants and results showed that the mutant displayed delayed abscission in the agreement with phenotypic analysis. At3g14380 over-expression construct driving by 35S promoter has been transformed to Arabidopsis. No aberrant phenotype was found in F5 transgenic plants. Analyses of homozygous lines are in the progress.

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Identification and Characterization of the Arabidopsis Orthologs of Nuclear Transport Factor 2, the Nuclear Import Factor of Ran. Qiao Zhao¹, Sara Leung², Anita H. Corbett², Iris Meier¹. ¹Plant Cellular and Molecular Biology and Plant Biotechnology Center, The Ohio State University, Columbus, OH, USA, ²Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, USA

Ran is a multifunctional small GTPase that is involved in nucleocytoplasmic transport, mitotic spindle assembly, and nuclear envelope formation. Nuclear import of Ran relies on a small RanGDP-binding protein, Nuclear Transport Factor 2 (NTF2). Three proteins are expressed in Arabidopsis (*Arabidopsis thaliana*) that show significant sequence similarity to human and yeast (*Saccharomyces cerevisiae*) NTF2. Here, we demonstrate that two of them, AtNTF2a and AtNTF2b, can functionally replace the essential NTF2 gene in yeast. Consistent with this finding, both AtNTF2a and AtNTF2b interact with yeast and Arabidopsis Ran. The third NTF2-related protein, AtNTL, does not functionally replace NTF2 in yeast. Similar to yeast NTF2-green fluorescent protein (GFP), AtNTF2a-GFP and AtNTF2b-GFP accumulate at the nuclear rim. We find that a putative Arabidopsis FG-repeat nucleoporin (NUP-FG) can interact with NTF2a as shown by co-IP in *Nicotiana benthamiana*. A GFP-NUP-FG fusion protein is located at the nuclear rim in transgenic Arabidopsis. We are exploring the function of NUP-FG in targeting NTF2 to the Arabidopsis nuclear pore.

AtNTF2a E38K and E91K mutants, which fail to bind Ran, are not functional in yeast, indicating conservation of the requirement for these key amino acids in plants and yeast. AtNTF2a overexpression, but not AtNTF2aE38K overexpression, blocks nuclear import of a plant transcription factor in *Nicotiana benthamiana* leaves, indicating that excess AtNTF2a disrupts nuclear import in a Ran-binding dependent manner. On the basis of these results, we propose that AtNTF2a and AtNTF2b function in Ran import in Arabidopsis and that nuclear import of Ran is functionally conserved in plants.

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Signaling of Anther Cell Fate Determination by the EMS1 Receptor-Like Kinase In Arabidopsis. Dazhong Zhao¹, Xiaodong Liu¹, Jian Huang¹, Amy Rymaszewski¹. ¹Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA

Sexual reproduction requires specification of cells with distinct fates in plants and animals. In flowering plants, anthers are the male parts of flowers, which bear pollen for producing sperm. A mature anther is usually a four-lobed structure. Each lobe contains five types of highly specialized cell layers, which are epidermis, endothecium, middle layer, tapetum and microsporocytes (pollen mother cells). Among them, microsporocytes are reproductive cells that generate pollen via meiosis. The remaining somatic cells, particularly tapetum, are required for normal development and the release of pollen. However, very little is known about the molecular mechanisms governing the determination of somatic and reproductive cell fates. The Arabidopsis mutant, excess microsporocytes1 (ems1), produces excess microsporocytes and lacks tapetal cells. The fact that the number of excess microsporocytes in the mutant is close to the sum of wild-type microsporocytes and tapetal cells suggests that there is a trade off between somatic and reproductive cells. The EMS1 gene encodes a leucine-rich repeat receptor-like protein kinase (LRR-RLK), and its expression is associated with the differentiation of the microsporocytes and tapetal cells, indicating that EMS1 mediates signals that control cell fate determination during anther development. Identification of other signaling molecules in the EMS1 signal pathway is in progress.

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Biochemical Characterization of SPA proteins and Their Role In the Assembly of COP1 Complexes and Repression of Photomorphogenesis. Danmeng Zhu^{1,2,3}, Ute Hoecker⁴, Jae-Hoon Lee², Haiyang Wang², Lijia Qu¹, Xing Wang Deng^{1,2,3,1} Peking-Yale Joint Center of Plant Molecular Genetics and Agrobiotechnology, Peking University, Beijing, China,² Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven,³ National Institute of Biological Sciences, Beijing, China,⁴ Department of Plant Developmental and Molecular Biology, Institute fur Entwicklung-sund, Molekularbiologie der Pflanzen, Heinrich-heine-Universitaet, D-40225 Duesseldorf, Germany

COP1 (constitutive photomorphogenesis 1) is one of the most important negative regulators in repressing photomorphogenesis, which functions as an E3 ligase to target the light promoting transcription factors for degradation via 26 proteosome. Unlike COP9 signalosome (CSN) and CDD complex (COP10, DDB1 and DET1), the configuration of the COP1 complex has not been addressed so far, although COP1 was believed to act in concert with SPA1 in a 700KD complex. Here, we report a family of four partially redundant SPA proteins work in concert with COP1 in vivo and raise the possibility that SPA proteins are likely to be components of a family of heterogeneous COP1-SPA complexes. Furthermore, We characterized the biochemical property and expression patterns of the four endogenous SPA proteins by their individual epitope-tagged stable transgenic lines and member-specific antibodies. Our data indicated that SPA proteins are capable of both self association and heteromeric interactions in vitro and in vivo, which possibly results in their functional redundancy in the regulation of photomorphogenesis. While their distinct function was shown on the effect of COP1 accumulation by analysis of various triple mutants of SPA proteins. Moreover, like SPA1, SPA2 to SPA4 also contribute to promote COP1 mediated HY5 degradation. Additionally, other COP/DET/FUS proteins are also involved in the proper accumulation of the four SPA proteins. Thus, this study gave a complete view of the four SPA proteins expression and regulatory behavior in the light control of development at the protein level.

Genomics and Genetics**P-362**

The pam74 mutant is caused by an insertion in a receptor-like kinase gene. Faisal Saeed Awan¹, Anja Schneider², Dario Leister², Iftikhar Ahmad Khan¹. ¹Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad, Pakistan,²Ludwig-Maximilians Universitat, Department Biologie I: Botanik, Munich. Germany

Eight photosynthetic *Arabidopsis* mutants were screened for co-segregation of a photosynthetic phenotype with the T-DNA insertion. These mutants were selected from out of around 80 photosynthetic *Arabidopsis* mutants, which were previously identified by the Leister group. These mutants showed a difference in the quantum efficiency of PSII ($PSII = ((Fm' - Ft)/Fm')$), therefore they were called pam (photosynthesis affected mutant). One of these mutants, pam74 was used for further studies, because the T-DNA insertion co-segregated with the mutant phenotype. The homozygous mutant plants either died or turned pale green and showed a stunted growth (loss of function) on MS plates. The mutants could only grow under greenhouse conditions in the heterozygous state. The affected gene in pam74 was identified. Sequence analysis of the flanking regions indicated that a gene has been disrupted coding for a secretory pathway protein and with a tentative function as a receptor-like kinase (RLK/Pelle), belonging to the LRR-1a subfamily. We confirmed that the mutant phenotype is caused by disruption of the receptor-like kinase gene by complementing the mutant phenotype with the wild-type gene.

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There and back again: From Genomics to Proteomics in *Arabidopsis*. Katja Baerenfaller¹, Jonas Grossmann¹, Franz Roos¹, Wilhelm Gruissem¹, Sacha Baginsky¹. ¹ETH Zurich

Mass spectrometry based proteomics has become widely accessible due to recent technical and conceptual advancements and the availability of gene and genome sequence databases. As a result, efforts of identifying and quantifying full proteomes are underway, which will complement the genomics approaches. Furthermore, the exploitation of the proteome data will improve the annotation of the genomes and their gene models. One such effort is the *Arabidopsis thaliana* Model Organism Proteomics (AtMOP) project. AtMOP forms a part of the Model Organism Proteomics (MOP) project, in which the first comprehensive maps of the proteomes of *Drosophila*, *C. elegans* and *Arabidopsis* are currently being generated.

Up to date, we have identified more than 10'000 expressed proteins in *Arabidopsis* cell culture cells and differentiated tissues using standard database searches. In order to increase proteome coverage and to identify proteins, which are not represented in protein databases we developed a specialised analysis pipeline for high-throughput mass spectrometry data (reviewed in Baginsky and Gruissem, 2006). Subjecting our data to this pipeline has already lead to the identification of several alternatively spliced proteins, proteins containing SNPs or posttranslational modifications, or proteins that are not correctly predicted by current gene models.

The data produced in AtMOP are integrated into SBEAMS and PeptideAtlas (Desiere F, et al. 2005), in which all the identified and validated peptide sequences are mapped to the *Arabidopsis* genome and such allows the integration of protein data with genomic data. Through collaborations both with PPAP and TAIR, the data will be incorporated into protein and genome databases, and such disseminated to the scientific community.

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QTL ANALYSES OF GROWTH RELATED TRAITS UNDER POTASSIUM & PHOSPHATE STARVATION REGIMES Department of Plant Breeding and Genetics, Max Planck Institute for Plant Breeding. Hugues Barbier¹, Maarten Koornneef¹, Barbara Eilts¹, Matthieu Reymond¹. ¹Max Planck Institute

Plants in natural environment experience abiotic starvation at each step of their development, which can have an important effect on overall plant performance and fitness (growth and reproduction). The purpose of this study is to detect the chromosomal regions (Quantitative Trait Loci - QTL) involved in the responses of plant performance under phosphate and potassium starvation regimes.

Growth-related traits (seed size, germination, rosette expansion rate, shoot and root weight, root length) were studied during vegetative phase. Natural variation of growth was analyzed using a set of 145 Recombinant Inbred Lines (RIL) of *Arabidopsis thaliana* derived from *Landsberg erecta* (Ler, Poland) and *Kashmir* (Kas-2, Kashmir) ecotypes. This RIL population was grown in a hydroponic system in order to monitor the amount of nutrients available for the plants. 3 different regimes were used in this study: Control ("C"), Phosphate starvation ("‐P": -4.7 Fold) and Potassium starvation ("‐K": -3.4 Fold). In order to detect QTLs, we used a mixed model developed under SAS software ($y = \text{Markers} + \text{Blocks} + \text{Traits} + \text{Treatments} + \text{Markers} * \text{Treatments} + \text{Markers} * \text{Traits} + \text{Markers} * \text{Traits} * \text{Treatments}$). This model takes into account the variance between replicates (Blocks) and allows us to analyze jointly the different traits and treatments. For each of the growth-related traits, common QTL were detected whatever the level of nutrients. These QTL have been considered as constitutive QTLs for this trait. Another set of QTL were specific to phosphate and potassium starvation and have been considered as starvations QTLs. Finally, QTL were detected specifically to a particular nutrient level and been considered as adaptive QTLs. We found also some growth QTL common whatever the traits and the starvations and have been considered as Master QTLs.

The results obtained describe the genetic architecture of growth in response to different nutrient levels. Molecular characterization of the major QTL described will be performed, allowing us to better understanding GxE interactions in plant performance.

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Arabidopsis QTLs affecting RNA editing in mitochondria Stephane Bentolila¹, Leah Elliott¹, Maureen Hanson¹. ¹Cornell University, Ithaca, NY, USA

Transcripts of both the *Arabidopsis* chloroplast and mitochondrial genomes undergo C-to-U editing. Editing usually results in changes in the encoded amino acid and can also create start and stop codons. Editing is likely to be essential for transcripts to encode functional mitochondrial proteins. Editing of mitochondrial Cs occurs at an astonishing level: more than 1 in every 15 mitochondrial Cs are edited in *Arabidopsis*, resulting in about 450 C targets of editing. How can so many different targets of editing be recognized by the editing apparatus, which must remain selective, avoiding undesirable C-to-U changes? Features of the cis-acting elements required for mitochondrial editing have been discerned by introducing exogenous transcripts carrying C editing targets into electroporated mitochondria or into mitochondrial extracts. However, no trans-factor involved in plant mitochondrial RNA editing has yet been identified.

We have taken a genetic approach in order to identify trans-acting components of the mitochondrial editing apparatus. A pilot study with the 39 C targets of editing in *ccb206* transcripts revealed an editing efficiency polymorphism between *'Arabidopsis thaliana'* accessions Ler and Col. The C at position 406 in *'ccb206'* is edited in Ler at an average of 23% and in Col at 51%; this polymorphism is controlled by a major QTL on chromosome 4 (Bentolila, Chateigner-Boutin, Hanson, 2005, Pl. Phys. 139:2006). We now report that 362 C-to-U editing sites in 33 mitochondrial genes have been surveyed with RNA extracted from rosette leaves. We detected 67 new editing events in leaves that were not observed in a prior report of mitochondrial editing in *Arabidopsis* suspension cultures. Furthermore, 37 of the 441 C-to-U editing events reported in *Arabidopsis* suspension cultures were not observed in leaves. Forty editing sites that are polymorphic in extent of editing were detected between Col and Ler. QTL mapping with recombinant inbred lines has detected 12 major QTLs for 11 of the 13 editing traits analyzed, indicating that map-based cloning of major mitochondrial editing factors should be possible. (Supported by NSF MCB 0344007 to MRH, and an NSF BTI/Cornell PGRP summer internship to LEE.)

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Regulation of the *Arabidopsis TRANSPARENT TESTA 10* gene involved in seed coat flavonoid oxidation. Przemyslaw Bidzinski¹, Lucille Pourcel¹, Jean-Marc Routaboul¹, Loïc Lepiniec¹, Isabelle Debeaujon¹. ¹Seed Biology Laboratory (UMR 204 INRA-AgroParisTech) ; INRA, Jean-Pierre Bourgin Institute, route de Sait-Cyr, 78026 Versailles, France, e-mail: isabelle.debeaujon@versailles.inra.fr

The *TRANSPARENT TESTA 10* (*TT10*) gene is involved in developmental seed coat browning by triggering flavonoid oxidation. *TT10* (At5g48100) encodes a laccase-type polyphenoloxidase (AtLAC15). Mature *tt10* mutant seeds contained more soluble proanthocyanidins (PAs) than the wild type (WT), and more epicatechin monomers. Flavonol analysis revealed that quercetin-3-O-rhamnoside (QR) monomers were more abundant and QR dimers were reduced in *tt10* compared with WT. The *TT10* gene is expressed mainly in the testa during seed development. Detailed analysis with GUS and GFP fusions revealed that promoter activity co-localized with two seed coat layers where flavonols and tannins are accumulated, respectively (Pourcel et al., 2005, Plant Cell 17 : 2966-2980).

5'-end promoter dissections have been realized and the corresponding *in planta* patterns of GUS expression are being analyzed. Promoter regions for siliques and anther expression have been identified. Currently, detailed analysis is being made on seed sections. To progress further in promoter activity understanding, site-directed mutagenesis and gain-of-function experiments will be used to determine which cis-elements are involved in gene expression and particularly in the two-cell layer pattern. Promoter activity is also studied in three regulatory mutant backgrounds *tt1* (a zinc finger protein), *tt16/abs* (MADS) and *ttg2* (WRKY). Apart from developmental regulation, *TT10* can also be induced by various stress conditions. For instance, we found out that it is highly induced by drought stress and in response to jasmonic acid. The *TT10* level of expression varies according to the accession and is influenced by UTRs (Pourcel et al., 2005). Both aspects are currently the subject of detailed analysis.

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Divergent evolution of recent duplicate gene revealed by genetic incompatibility between *Arabidopsis* natural populations. David Bikard¹, Dhaval Patel², Claire Le Mett¹, Christine Camilleri¹, Malcolm Bennett², Olivier Loudet^{1,1} INRA, SGAP, Versailles, France, ²Univ. of Nottingham, UK

While generating sets of Recombinant Inbred Lines (RILs), many researchers have witnessed that genetically unlinked loci may not always segregate independently: it is actually quite frequent among *Arabidopsis thaliana* RIL sets that one homozygous allelic combination at two independent locus is rare or totally absent in the descendants of a specific cross (which, by the way, explains an important part of the segregation distortion almost inevitably inherited in such material). We have fine-mapped such a two-locus interaction for which a combination of the Col-0 allele at the bottom of chrom. 1 and the Cvi allele at the top of chrom. 5 causes arrested embryo development at the globular stage. In one intermediate heterozygous combination, the seed developed normally but primary root growth is significantly reduced. We have identified that the mechanism for such a genetic conflict results from the divergent evolution of a duplicate gene pair: the histidinol-phosphate amino-transferase gene is essential for histidine biosynthesis and is present in two almost-identical copies in the reference genome of Col-0. We show that Col-0 and Cvi retain alternate efficient copies of the amino-transferase: the chrom. 1 gene is not expressed in Col-0 while it is in Cvi; the chrom. 5 gene is completely deleted in Cvi, while it is present and expressed in Col-0. Quantitative complementation tests by crossing to different mutants clearly show how the different alleles at the two duplicate genes interact to qualitatively control embryo development and quantitatively limit primary root growth. We are now analysing the extent of natural variation at those duplicate genes to characterize further intra-specific evolution of essential recent duplicate genes. Interestingly, the same genetic incompatibility is seen between the same loci when crossing Cvi to Ler. Although both genes are expressed in Ler, the chrom. 1 gene contains a premature stop codon. Our study has revealed that there is strong selective pressure to retain the function of only one copy of the histidinol-phosphate amino-transferase gene.

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TBR and TBLs-a Novel Gene Family required for Secondary Cellulose Biosynthesis. Volker Bischoff¹, Ana-Silvia Nita¹, Wolfgang Scheible¹. ¹Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany

The EMS-induced trichome birefringence (*tbr*) mutant of *Arabidopsis* is deficient in secondary cellulose deposition in trichomes and to some extent in the vasculature, causing a lack of trichome birefringence under polarized light (Potikhia et al., 1995).

Additional phenotypes of the mutant include (i) reduced trichome density, (ii) callose deposition in subsidiary cells at the base of trichomes and in the vasculature system, and (iii) a variable but general reduction in growth. We identified TBR by map-based cloning and found this gene encoding a protein of unknown function with the predicted topology of a type II membrane protein. TBR has several homologs in the *Arabidopsis* genome, and several of these TBR-like (TBL) genes, show co-expression with secondary cellulose synthases, e.g. CESA7. Consistently, T-DNA insertion and RNAi lines of TBL2 show reduced levels of cellulose in inflorescence stems, and GC cell wall sugar patterns typical for secondary cell wall mutants. We present a novel and non-described family of genes, involved in secondary cell wall synthesis and will give direct evidence of these genes being situated near the plasma membrane, confirming their function in cellulose synthesis.

P-369

Data-mining from 600 oligomer arrays. Atle M. Bones¹, Tommy J rstad¹, Per Winge¹. ¹Norwegian University of Science and Technology

We have performed some 600 microarray experiments using printed oligomer arrays covering probes for 25000 *Arabidopsis* genes. Samples from various mutants, treatments, conditions and developmental stages have been used. This set of data has been used for characterization of the transcriptome of *Arabidopsis*. In this presentation we will present results which we think will have general interest for the large number of scientists presently performing microarray studies.

P-371

Predicting functional modules root hairs that drive tip growth. Gordon Breen¹, Claire Grierson¹. ¹University of Bristol

Root hairs are an ideal cell type to investigate polarized cell growth in eukaryotes. Unidimensional plant cell development requires the coordination of an array of cellular processes that need to work in unison to create a straight, precisely dimensioned cellular outgrowth to facilitate a variety of functions. By using a range of microarray datasets and applying Bayesian statistics it has been possible to confidently identify over 150 *Arabidopsis thaliana* genes involved in root hair morphogenesis. These include cell wall modifiers, G protein inhibitors, activators and effectors, cytoskeleton assemblers, vesicle transporters and modifiers, and many genes yet to be analysed. A network analysis using the coexpression profile of these genes has identified two highly linked modules connected by two separate pathways. Both modules contain genes with different associated functions suggesting each module (or molecular machine) has a separate role in tip growth. Integrating protein interaction data and phenotype mapping into this network will enable a detailed look at how these genes could interact to bring about tip growth.

P-370

A high resolution root expression map predicts cellular function and reveals novel expression patterns. Siobhan Brady¹, David Orlando¹, Ji-Young Lee¹, Jean Wang¹, Daniel Mace¹, Uwe Ohler¹, Philip Benfey¹. ¹Duke University, Durham, NC, USA

Transcriptional programs that regulate the development of multicellular organs are exquisitely controlled in both space and time. Elucidating the regulatory networks that underlie development is essential to understand the acquisition of cell and tissue identity within an organ. A previous root expression map profiled 5 radial tissues and 3 developmental stages. We present a more detailed root expression map with profiles of nearly all cell types (15) in the root and 13 developmental stages.

First, we identified transcriptional signatures of individual cell types and their associated biological processes. In many cases, these processes predict novel cellular function. To deal with this magnitude of high-resolution data, we then developed an algorithm that identifies dominant expression patterns and asked how expression defines root development. 51 groups of co-regulated genes were identified that correlate with individual cell-types, spatially related cell types, and intriguingly, to cell types which are spatially and ontologically unrelated. Analysis of these groups suggests that there are transcriptional and biological connections between disparate cell types. The 40 dominant developmental patterns demonstrate that at the transcriptional level, transcriptional programs occur in a gradient along the longitudinal axis, and do not strictly correlate to previously defined meristematic, elongation and maturation zones. Furthermore, many of these programs show expression oscillations along the root longitudinal axis. We also examined the extent of between-root variation and found that while some groups exhibit reproducible expression, others exhibit potential dynamic responses. To combine these cell type and developmental expression profiles, a method was developed that proved accurate in identifying relative peaks of high expression in both space and time.

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Genes encoding defense signaling proteins in plants are more conserved than those encoding recognition proteins. Katherine Caldwell¹. ¹University of California, Davis

The close interplay between pathogen effectors, their host targets, and the guard proteins that monitor these targets provides various opportunities for opposing cycles of selection acting on plant and pathogen to achieve or abrogate resistance, respectively. Selection had previously been shown to be acting to maintain diversity in both plant proteins involved in pathogen recognition and some of the pathogen effectors they guard against. Here we provide the first analysis of the signatures of selection at genes encoding defense signal transduction proteins in plants, which are putative targets of pathogens. We show that in contrast to recognition genes, the majority of signaling components appear to be either evolving neutrally or under purifying selection. This is consistent with these proteins not being targets of effectors and/or indicative of functional constraints on their evolution. However, there was significant evidence of selection maintaining diversity at the *NPR1*, *PAD4*, and *EDS1* loci. Differences in the signatures of selection observed may reflect the numbers of effectors that target a particular protein, presence or absence of a cognate guard protein, as well as interactions with other plant proteins.

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PlantTFDB: The Plant Transcription Factor Database. Xin Chen¹, An-Yuan Guo¹, Ge Gao¹, Qi-Hui Zhu¹, Ying-Fu Zhong¹, He Zhang¹, Xiao-Cuan Liu¹, Xiaocheng Gu¹, Kun He¹, Jingchu Luo¹. ¹Center for Bioinformatics, Peking University, Beijing 100871, China

PlantTFDB: The Plant Transcription Factor Database Xin Chen, An-Yuan Guo, Ge Gao, Qi-Hui Zhu, Ying-Fu Zhong, He Zhang, Xiao-Cuan Liu, Xiaocheng Gu, Kun He, Jingchu Luo. Center for Bioinformatics, Peking University, Beijing 100871, China We implemented the following approach to make genome-wide identification of transcription factors (TFs) and constructed the plant transcription factor database PlantTFDB (<http://plantfdb.cbi.pku.edu.cn>). First, we collected a list of plant TF families from literature and compiled HMM profiles of the DNA binding domains for all these families with the information retrieved from Pfam and obtained from our own analysis. Second, we made HMMER search against the protein sequence of plant genomes to identify putative TFs. We chose seed sequences to make BLASTP search for some families without HMM profiles. Finally, we checked the predicted results manually to reduce false positives and redundant hits.

To provide comprehensive information for the putative TFs, we made extensive annotations at both family and gene levels. A brief introduction and key references were presented for each family. Structure and functional information as well as the target sequence were also provided if available. For each identified TF, general description as well as gene structure, putative domains and PDB hits were provided. Cross-references to various databases such as GenBank, UniGene, UniProt, Prosite, Pfam and TransFac were linked to each entry. In addition, PlantTFDB has a simple interface to allow users to search the database by IDs or free texts, to make sequence similarity search using BLAST, and to download all TF sequences.

Currently, PlantTFDB contains TFs identified from the genome sequences of three model organisms, Arabidopsis, rice and poplar. We are adding two other genomes, the green alga Chlamydomonas reinhardtii and the moss Physcomitrella patens. We are also working on the available EST sequences from 17 plant species including crops (maize, barley, wheat, etc), fruits (apple, orange, grape, etc), trees (pine, spruce, etc) and other economically important plants (cotton, potato, soybean, etc).

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OSRs, a family of MscS-like Proteins, involve in Arabidopsis responses to osmotic stress. Yi-Fang Chen¹, Hong Li¹, Xin-Jie Han¹, Jie Pang¹, You-Han Kong¹, Wei-Hua Wu¹. ¹State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, National Plant Gene Research Centre (Beijing), China Agricultural University, Beijing 100094, China

Mechanosensitive (MS) ion channels were originally identified as essential components as the osmolyte efflux system activated under hyperosmotic shock in bacteria, and were found to also exist eukaryotes, including both the plants and animals. However, their functions remain unclear so far in higher plants. We isolated a low phosphate tolerant mutant lpt1, and it turned out that LPT1 gene encoding a protein containing the same motif with MscS and named as AtOSR1 (Osmotic Stress Responsive). Blast with all noted genes in Arabidopsis genome, other 9 genes were founded to encode the same motif with MscS, and named as AtOSR2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively. Transcription of most AtOSRs in Arabidopsis plants was increased by osmotic stress induction. Furthermore, tolerance of a MscS-deficient bacterial mutant line to osmotic-shock can be partially rescued by 9 AtOSRs respectively.

AtOSR1 overexpression Arabidopsis lines showed greater tolerance to low Pi stress, increased Pi uptake rate and Pi content compared with wild-type plants. More interestingly, tolerance to low Pi stress for wild-type plants was induced by an osmotic stress treatment, which suggests that osmotic stress-induced expression of AtOSR1 may increase plant tolerance to low Pi stress. The AtOSR2 knockout mutant (atosr2) was more sensitive to drought stress and exhibited an increased water loss rate and decreased stomatal aperture, indicating that AtOSR2 may play an important role in plant response to drought. Additional experimental results further showed that function of AtOSR2 may somehow relate to mitochondria. The AtOSR9 knockout mutant (atosr9) had the similar phenotype with atosr2. In addition, AtOSR4 and AtOSR7 may involve in plant responses to water stress, and AtOSR8 may function in embryonic development.

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Parental imprinting, dosage compensation and dosage effects are observed at a gene expression level in response to changes in genome dosage in *Arabidopsis thaliana*. Rachel Clifton¹, Olivier Garnier¹, Pjotr Prins², Charles Spillane¹. ¹University College Cork, Cork, Ireland, ²Wageningen University, Wageningen, The Netherlands

A range of epigenetic phenomena appear to be involved in regulating gene expression in plants, derived from observations of dosage compensation, dosage effects and parent-of-origin effects following genome dosage increases. Using the model plant Arabidopsis, this study investigates the transcriptomic consequences of increasing genome dosage by examining gene expression in diploid, maternal and paternal excess triploid and tetraploid plants. Whilst the majority of the Arabidopsis seedling transcriptome is unaffected by changes in the quantity of each parental genome contribution or by whole genome dosage increases, analysis of the fraction of genes differentially expressed in response to altered dosage provides insight into the mechanisms that may be involved. A series of expression responses are observed implying multiple mechanisms may be involved in reprogramming gene expression following an increase in whole genome dose. The function, chromosomal location and expression response of the dosage responsive genome complement of *Arabidopsis thaliana* is explored and its implications discussed.

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PRX34 GENE IS INVOLVED IN LIGNIFICATION AT THE YOUNG STEMS OF ARABIDOPSIS. Mohammad MIR DERIKVAND¹, Nathalie CAULET¹, Brigitte POLLET², Valérie MECHIN², Dominique BUFFARD¹, Catherine LAPIERRE², Lise JOUANIN¹.

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The plant peroxidases have generally the various roles in the plants, but it is supposed that each particular gene has the specific activity (ies). Dehydrogenative polymerization of lignin precursors (monolignols) is one of the proposed roles for plant peroxidases and laccases. Indeed, Lignins as the major phenolic components of the secondary cell wall are indispensable to plants.

In this study, four peroxidases were identified among N-glycosylated proteins isolated from *Arabidopsis* mature stems using affinity chromatography on Concanavalin A Sepharose and 2D electrophoresis. Two of the corresponding genes (*PRX33*: At3g49110 and *PRX34*: At3g49120) are located in tandem on chromosome 3. By RT-PCR analysis, the *PRX34* gene was shown to be highly expressed in stems whereas *PRX33* expression was low.

Null and over-expressing mutants of each gene were identified in the Versailles and SALK collections and characterized. The total peroxidase activity was reduced by 20% in the knockout *prx34* mutant whereas no reduction was observed in the knockout *prx33* mutant. Chromatographic purification of peroxidase activity on CM-sepharose led to two peroxidase-containing peaks. One peak was greatly reduced in *prx34* and not in *prx33*. In addition, this peak was increased in a *PRX34* overexpressing line and in the *prx34* line complemented with the *PRX34* cDNA under the control of the CaMV35S promoter. In the *prx34* null mutant, lignin deposition was reduced in fibers of young stems as shown by Wiesner staining. Klason and thioacidolysis analysis showed decreased lignin content in young stems of this mutant but not in mature stems. Purified *PRX34* was able to oxidize coniferyl alcohol *in vitro* and produce oligomers.

Taken together, these results suggest that *PRX34* is involved in lignin polymerization whereas *PRX33* is not involved in this process. However, *PRX34* is only one of the oxidases responsible for the polymerization of monolignols, and other peroxidases need to be identified.

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Transcriptional regulation of proanthocyanidin biosynthesis in *Arabidopsis* seed. Christian DUBOS¹, Jos LE GOURRIEREC¹, Antoine BAUDRY¹, Jean-Marc ROUTABOUL¹, Isabelle DEBEAUJON¹, Loïc LEPINIEC¹. ¹INRA Versailles

In *Arabidopsis thaliana*, proanthocyanidins (PAs; *syn.* condensed tannins) accumulate specifically in the innermost integumentary layer of the seed coat (endothelium), giving the mature seed its brown colour after oxidation. Metabolomic analysis has shown that PA accumulation is also tightly regulated at the spatio-temporal level. The biosynthesis of PAs is controlled through the specific expression of structural genes such as BAN (anthocyanidin reductase). We have previously shown that TT2 (MYB), TT8 (bHLH), and TTG1 (WDR) form a ternary protein complex that directly controls BAN and TT8 expression at the transcriptional level, in a self-activated feedback loop. In this complex, TT2 and TT8 are required for DNA binding *in vivo*, whereas TTG1 appears to act as a co-factor.

To progress further in our understanding of the transcriptional regulation of PA biosynthesis, we are now focusing on the identification of the cis-elements on the BAN and TT8 promoters (or other putative target genes), that are necessary for the binding of the TT2-TT8-TTG1 complex *in vitro* (EMSA) and *in vivo* (yeast and *in planta*). The second aspect of this work deals with the negative regulation of TT2-TT8-TTG1 complex activity and the possible involvement of other MYBs in this mechanism, like it is the case in some other known MYB-bHLH-WDR regulatory complexes. Finally, the TT2 and TT8 promoters are studied in order to identify the cis-elements involved in their spatial and temporal activation specificity (promoter dissection and site-directed mutagenesis). This study should allow us to determine which cis-elements are of particular interest to trigger and control the self-activated loop, and to identify new regulators involved in the transcriptional regulation of TT2 and TT8 (yeast one-hybrid screening).

The importance of these regulatory mechanisms in the strong and specific induction of proanthocyanidin biosynthesis in *Arabidopsis* seed coat will be discussed.

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Evolution of miRNAs from random sequences. Felipe Fenselau de Felippe¹, Tobias Dezulian², Korbinian Schneeberger¹, Michael Schröder², Daniel H. Huson², Detlef Weigel¹. ¹Max Planck Institute for Developmental Biology, Tbingen, Germany, ²Center for Bioinformatics, Tbingen University, Tbingen, Germany

Plant microRNAs (miRNAs) are produced from precursors that contain self-complementary foldbacks. These precursors are processed by DICER-LIKE1 (DCL1), generating the mature miRNAs that are incorporated into the RISC, a protein complex that regulates miRNA target genes. In plants, some young miRNA genes arose by inverted duplication of what later might become target genes of the miRNAs. However, analysis of the evolutionary origin of young miRNAs suggests that this is not the only path to the emergence of new miRNAs. *In silico* folding of a typical plant genome results in hundreds of thousands of potential foldback sequences. To test if such random foldbacks could give origin to new miRNAs genes, we previously used data from massively parallel signature sequencing (MPSS) to identify new potential *Arabidopsis thaliana* miRNA genes that are not conserved in other genomes. These potential miRNA precursors were assayed for both their ability to generate a miRNA and potential effects on target mRNAs. Analysis of these potential miRNAs together with database entries of miRNAs that are private to *A. thaliana* (*i.e.*, not found in poplar or rice) suggest that while some arose from a sequence that either has self-complementarity by chance or that represents a highly degenerate inverted duplication. We propose that miRNAs can arise spontaneously from foldback sequences captured by transcriptional regulatory sequences. Subsequent stabilization through co-evolution with potential targets may lead to subsequent fixation of a small subset.

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Cosima-The Community Semantic Interrogation Machine. Andreas Groscurth¹, Heiko Schoof^{1,2}. ¹ Max-Planck-Institute for Plant Breeding Research

In the present internet one can find biological knowledge mostly stored in relational databases, accessible through web services and presented in a human readable form. The idea of the semantic web is to bring structure to the meaning of information and thereby to enable the integration of the huge amount of distributed biological data.

The key feature of the semantic web are ontologies, which allow to capture existing knowledge in a systematic and standardized way and describe terms and their relationships. The usual way to create such ontologies is to hire highly trained experts. This approach faces the issues of shortage of available person-hours and the either not deep or not broad enough knowledge of the experts to cover the full scope of information. This makes ontology creation expensive and not flexible enough to keep track of the fast changing knowledge in biology. Also text mining approaches failed due to the impossibility to extract the full semantic meaning of publications without a preexisting ontology.

One emerging approach is engaging a wider community. The Cosima application provides a mechanism for rapid and inexpensive knowledge mining by learning an ontology from the interaction with the community. It represents an open, parallel and mainly decentralized system, which builds ontologies from the knowledge of many, who do not need to be specially trained in knowledge or ontology engineering. The system provides an intuitive web front end based on so-called "chatterbots" in which the biologist can easily interact with the system and contribute his or her knowledge into the ontology.

In an open platform where anyone can contribute, a validation systems needs to check the consistency of the ontology. But also this can be addressed by a community approach. Every statement which the system has learned will be checked again by asking others to confirm it. In this way, disputed statements can be highlighted for expert review or contradicting statements removed from the ontology.

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From Chlamydomonas to Arabidopsis: a bZIP history. Luiz Gustavo Guedes Correa¹, Renato Vicentini², Diego Ria o-Pachon¹, Bernd Mueller-Roeber^{1,3}. ¹Universität Potsdam, ²Universidade Estadual de Campinas, ³MPI Molecular Plant Physiology

Growth and development of all organisms depend on proper regulation of gene expression. Differential gene expression is the result of the control of transcription initiation rates by transcription regulation factors. bZIP transcription factors were described in all eukaryotes. Their conserved domain is constituted by a basic amino acid rich region that binds to DNA bases, and a leucine zipper, responsible for protein dimerization. Genetic, molecular and biochemical analysis indicate that plant bZIP factors are important regulators of specific processes such as light sensing, sugar signaling, osmotic control and the control of the carbon/nitrogen balance. Identification of Possible Groups of Orthologous genes (PoGOs) from different evolutionary lineages allows rationalizing functional studies. Within this context, the present work aims at a better understanding of the evolution and function of bZIP transcription factors in plants. We identified a non-redundant set of 77 bZIP factors in *Arabidopsis thaliana*, 93 in *Oryza sativa*, 89 in *Populus trichocarpa*, 7 in *Chlamydomonas reinhardtii* and 8 in *Ostreococcus tauri* genomes, respectively. The phylogenetic analysis of *Oryza*, *Arabidopsis* and *Populus* sets of bZIP transcription factors allowed the identification of 13 groups of homologous genes. A more detailed analysis led to the identification of 36 PoGOs, that possibly correspond to 36 ancestral functions in angiosperms, two exclusive monocot PoGOs and a possible Group of Paralogous genes in *Arabidopsis*. Inclusion of Algae, Bryophyte and Gymnosperm bZIP sequences in the analysis shed new light on the origin of the different PoGOs. Based on the results presented here, we propose an updated bZIP classification and a model of their evolution. This approach is revealing new perspectives on bZIP functional studies.

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ChIP on chip-based identification of target genes for key transcription factors involved in seed development of *Arabidopsis*. Urs Haehnel¹, Gudrun Moenke¹, Astrid Vorwiegner¹, Michaela Mohr¹, Prisca Viehoever², Linh My Tran¹, Jens Tiedemann¹, Ansgret Tewes¹, Andreas Czihal¹, Bernd Weisshaar², Ivo Grosse¹, Helmut Baeumlein¹, Udo Conrad¹, Lothar Altschmied¹. ¹Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, ² Institute of Genome Research, University Bielefeld, Bielefeld, Germany

Seed formation is of utmost importance for human nutrition and animal feed and will provide the basic compounds for a bio-based economy and CO₂-neutral energy production in the future. The development of seeds is a complex process including the storage of reserve compounds, the acquisition of desiccation tolerance, and the development of dormancy. Several transcription factors (TF), namely LEC1, LEC2, FUS3, ABI3, MYB44, MYB77, ET1, and ET2, have been identified as key regulators in seed development. The underlying regulatory network is only partially understood, because many of the primary target genes for these TF are still unknown. Using chromatin immunoprecipitation (ChIP) coupled with the hybridization to promoter arrays (chip) we aim to identify TF target genes *in vivo* at a genome wide scale.

Towards that goal transgenic *Arabidopsis* lines with estradiol- (Zuo et al. 2000, Plant J 24: 265-273), resp. dexamethasone-inducible (Aoyama and Chua 1997, Plant J 11: 605-612) expression have been created for most of the TF mentioned above and antisera were raised against TF domains expressed in *E. coli*. Protocols for chromatin immunoprecipitation were adapted to developing seeds of *Arabidopsis* and an array containing >10.000 promoter fragments of *Arabidopsis* was constructed in cooperation with the SAP project (www.psb.ugent.be/SAP). Novel motif-finding algorithms based on variable order Markov models and variable order Bayesian trees were developed (Grau et al. 2006, Nucl. Acids Res. 34: W529-533) to identify potential TF binding sites in non-aligned target promoters. Initial results for ChIP on chip experiments with ABI3 and LEC1 will be discussed.

P-382

Poly (A) -dependent RNA degradation in plants. Sarah Holec¹, Heike Lange¹, Jean Canaday¹, Dominique Gagliardi¹. ¹IBMP, Strasbourg, France

Eukaryotic mRNAs require 3' polyadenylation for their stability and translation. In contrast, addition of short poly(A) tails mark bacterial transcripts for degradation. Polyadenylation also triggers RNA degradation in chloroplasts and in mitochondria of several organisms including plants. Indeed, we have shown that in *Arabidopsis thaliana* mitochondria, polyadenylated mRNAs and rRNAs are degraded by the 3'-5' exoribonuclease mtPNPase (PolyNucleotide Phosphorylase). In absence of mtPNPase, polyadenylated maturation by-products of tRNA and rRNA synthesis, and aberrant transcripts generated from intergenic regions accumulate to sometimes extraordinary high levels. These results suggest that the mitochondrial transcriptome is mostly defined by posttranscriptional RNA surveillance involving polyadenylation and PNPase (1).

In the course of this study, we noticed that some nuclear non-coding RNAs were also polyadenylated, suggesting that a degradative mechanism involving oligoadenylation may also occur in the nucleus of plants. In yeast, it was recently shown that polyadenylation could trigger degradation of nuclear non-coding RNAs (2-4). This novel pathway of degradation involves the TRAMP polyadenylation complex and Rrp6p, a RNase associated with the exosome. The exosome is a multiprotein complex that participates in RNA maturation, degradation and quality control (5).

We identified three genes exhibiting significant homology to Rrp6p in the genome of *Arabidopsis thaliana*. We determined the subcellular localization of these Rrp6p-like proteins using GFP fusion proteins and found that AtRRP6A and B are targeted to the nucleus whereas AtRRP6C is cytoplasmic. Only Atrrp6b mutant plants accumulate a maturation by-product of nuclear rRNA synthesis suggesting different substrate specificity between AtRRP6B and A. Interestingly, this rRNA maturation by-product accumulates as polyadenylated species. These preliminary results suggest that AtRRP6B is indeed involved in the degradation of polyadenylated transcripts in the plant nucleus.

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P-383

Pollen development and function; integration of transcriptomic and proteomic data. David Honys^{1,2}, David Twiss³. ¹Institute of Experimental Botany ASCR vvi, Prague, Czech Republic, ²Charles University in Prague, Prague, Czech Republic, ³University of Leicester, Leicester, United Kingdom

Male gametophyte development in higher plants is a complex process that requires the coordinated participation of various cell and tissue types and their associated specific gene expression patterns. The male gametophytic life cycle can be divided into a developmental phase leading to the formation of mature pollen grains, and a functional or progamic phase, beginning with the impact of the grains on the stigma surface and ending at double fertilisation. Pollen ontogeny is also an excellent model in which to dissect the cellular networks that control cell growth, polarity, cellular differentiation and cell signaling. Pollen transcriptomic studies have provided an extensive genome-wide view of gene expression during male reproductive cell development in *Arabidopsis*. These studies have revealed at least two successive global gene expression programs and the identity of a large number of male gametophyte-specific genes and putative transcriptional regulators. Transcriptome analysis has also revealed the striking overrepresentation of expressed genes associated with cell wall metabolism, cytoskeleton and signaling in preparation for pollen germination and tube growth during the progamic phase. Although transcriptomic analyses provide valuable information about global and specific gene expression patterns it does not measure the influence of posttranscriptional control of gene expression. In male gametophyte, posttranscriptional and translational control mechanisms have already been demonstrated to be of great importance during pollen maturation and the progamic phase. Recently, the first studies characterizing the pollen proteome are emerging. Although currently available proteomic data are incomplete these data provides the first opportunity to overcome some of the limitations of transcriptomic data and already offers some interesting insights.

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Arabidopsis Genome Annotation - Challenges and Opportunities. Eva Huala¹, David Swarbreck¹, Chris Wilks¹, Robert Muller¹, Philippe Lamesch¹, Tanya Berardini¹, Hartmut Foerster¹, Donghui Li¹, Vanessa Swing¹, Christophe Tissier¹, Peifen Zhang¹, Seung Rhee¹. ¹Carnegie Institution, Stanford, CA, USA

In March 2007 TAIR released a new version of the *Arabidopsis* genome annotation, TAIR7, incorporating community submissions directly to TAIR and new cDNAs and ESTs submitted to GenBank since the previous TAIR6 release. The new release includes 681 new genes bringing the total gene set to 32,041 genes, of which 26,819 are protein-coding, 3889 are pseudogenes or transposable elements and 434 are ncRNAs. The release also contains updates to 9755 genes, including 784 updates to protein sequences and addition of 1003 new splice variants as well approximately 10,700 updates to UTRs. A total of 34 gene merges and 41 gene splits were also carried out. TAIR7 has been released to GenBank and can be accessed from the NCBI Plant Genomes section as well as through TAIR. Current progress and future plans for maintaining and updating the *Arabidopsis* genome annotation will be discussed.

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Constructing a complex RIL population derived from inter-crossing 8 *Arabidopsis* accessions. Xueqing Huang¹, Maria-Joao Paulo¹, Sigi Effgen¹, Maarten Koornneef^{1,†} Max Planck Institute for Plant Breeding Research, D-50829, Cologne, Germany

The genetic control of the majority of biological traits differing between natural accessions is complex. Analysis of quantitative trait loci (QTL) affecting these complex traits is often pursued in single-cross experiments which can only reveal genomic regions that are polymorphic between the parents of the population. Considering several populations derived from diverse parental materials increases the probability that a QTL will be polymorphic in at least one population and consequently increases the power of QTL detection and the discovery of epistatically interacting QTL. Here, we reported that a complex cross population was created from a set of 8 accessions of *Arabidopsis thaliana* (Col, Kyo, Cvi, Sha, Eri, Ler and C24). The population consists of 12 subpopulations each of 100 F5 lines each, having per sub population four different parents. A total of 108 microsatellite markers with 3-7 different alleles among the 8 parents and SNP based markers for 206 loci were used to construct a framework linkage map for the complex population. This map provides a starting point for genetic dissection of complex traits. The current effort is towards to use this complex population to map multiple developmental traits such as flowering time, branching pattern, leaf shape for which phenotypic data were collected in the F5 generation. The population as a whole will provide a novel resource for QTL mapping in *Arabidopsis*, which will allow a higher detection power and detection of QTL with complex epistatic interactions that can result from interactions between specific alleles derived from more than two parents.

P-387

PlantAFAWE: Automatic Functional Annotation in a distributed Web Service Environment. Anika Joecker¹, Moritz Schoen¹, Heiko Schoof¹. ¹Max Planck Institute for plant breeding research

A huge amount of high throughput functional genomics data is (becoming) available. This provides a data basis for intelligent filters and an automatic functional annotation. Currently, several methods for automatic protein function prediction are in use, for example homology detection, structure prediction and comparison and phylogenetic tree prediction. However, each of these methods has limited prediction accuracy and the comparison of the results and decisions about significance of hits are tasks of the manual annotator.

In the last years a few hybrid approaches were published, which use machine learning algorithms for automatic prediction of ontology terms. They all have an increased accuracy, but in all cases only one feature is assigned and furthermore the tools can only handle the functional prediction of data from one organism and this data is not always up to date. Another problem is that in some cases the automatic functional prediction is not able to give a significant result. In this case combination of individually insignificant data could give clues to the function of a protein. But at the moment there is no program able to display these data.

These problems motivate to implement an automatic functional annotation system with an intuitive web interface. This will integrate multiple inputs and evaluate correlations also in available functional genomics data to improve prediction. All available analysis results can be graphically and tabularly displayed and are iteratively combined by a rule-based system. Terms from well-known nomenclatures for protein function description and a human readable description are assigned to each protein. The program runs analysis tools for function prediction through web services in a distributed system. This improves the scalability, accessibility, maintainability, efficiency and simplifies the process.

Another advantage is that the used data is always up to date. This also means that, even in cases where the automatic machine learning algorithm does not output significant results, the tool will still be highly useful for manual annotation by summarizing in one user interface all available data on a given protein.

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Genome-wide expression profiling of the *Arabidopsis* female gametophyte identifies families of small, secreted proteins
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The female gametophyte of flowering plants, the embryo sac, develops within the sporophytic tissue of the ovule. While embryo sac expressed genes are known to be required at multiple stages of the fertilization process, the set of embryo sac expressed genes has remained poorly defined. In particular, the genes responsible for mediating intracellular communication between the embryo sac and the male gametophyte, the pollen grain, is unknown.

We used high throughput cDNA sequencing and whole genome tiling arrays to compare gene expression in wild type ovules to that in *dif1* ovules, which entirely lack embryo sacs, and *myb98* ovules, which are impaired in pollen tube attraction. We identified over 400 genes that are down regulated in *dif1* ovules. 75% of these embryo sac dependent genes were predicted to encode for secreted proteins, and over 60 % belonged to multigenic families. Our results define a large number of candidate intracellular signaling molecules that may act during embryo sac development or during fertilization, less than half of which are represented on the widely used ATH1 expression array. In particular, 39 out of 40 genes encoding Domain of Unknown Function 784 (DUF784) domains require the synergid specific transcription factor MYB98 for expression in the embryo sac, implicating the DUF784 gene family as mediators of late stages of embryo sac development or interactions with pollen tubes.

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THE FOX HUNTING SYSTEM: AN ALTERNATIVE GAIN-OF-FUNCTION GENE HUNTING TECHNIQUE. Takanari Ichikawa¹, Miki Nakazawa¹, Mika Kawashima¹, Izumi Haruko¹, Kuroda Hirofumi¹, Kondou Youichi¹, Akie Ishikawa¹, Seki Motoaki¹, Miki Fujita², Mieko Higuchi¹, Kazuo Shinozaki¹, Minami Matsui¹. ¹RIKEN PSC, ²CREST JST

We have developed a novel gain-of-function system that we have named the FOX hunting system (Full-length cDNA over-expressing gene hunting system). We used normalized full-length cDNA and introduced each cDNA into *Arabidopsis* by *in planta* transformation. About 10,000 independent *Arabidopsis* full-length cDNAs were expressed independently under the CaMV 35S promoter in *Arabidopsis*. Each transgenic *Arabidopsis* contained on average 2.6 cDNA clones and was monitored under various categories such as morphological changes, fertility and leaf color. We found 1,487 possible morphological mutants from 15,547 transformants. When 115 pale green T1 mutants were analysed, 59 lines represented the mutant phenotypes in more than 50 % of the T2 progeny. Characterization of two leaf color mutants revealed the significance of this approach. We will also introduce 6 other mutant lines that showed the visible phenotypes upon retransformation of the wild type plants with the rescued full-length cDNAs. Applications of this system for functional genomics will be discussed.

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"RBR1" and "MSI1" activate maternal expression of methylation-dependent imprinted genes in "Arabidopsis". Pauline E Julian¹, Assaf Mosquna², Nir Ohad², Frédéric Berger¹. ¹Temasek Lifesciences Laboratory, Singapore, Singapore, ²Tel Aviv University, Tel Aviv, Israel

Genomic imprinting involves the epigenetic inactivation of one allele of a gene and the activation of the other one depending on their parental origin, resulting in monoallelic expression of this gene. In "Arabidopsis", "FIS2" and "FWA" genes are known to be expressed from their maternal allele only. Their paternal allele is silenced by the DNA methyltransferase MET1, which maintains methylation on CpG site through cell division. The activation of their maternal allele requires the action of "DEMETER" responsible for removal of DNA methylation on the maternal allele causing its expression. Here we show that in addition to "DEMETER", "RBR1" ("RETINOBLASTOMA RELATED 1") and "MSI1" ("MULTICOPY SUPPRESSOR OF IRA1") are required for the maternal expression of FIS2 and FWA. However they are not required for the maternal expression of "MEA", imprinted in a DNA methylation independent manner. MSI1 and RBR1 are known to interact together in other organisms and to play a role in regulation of cell cycle progression. This interaction is also conserved in "Arabidopsis". We further show that "MSI1" is required to repress "MET1" expression. We propose that "RBR1" and "MSI1" activate "FIS2" and "FWA" maternal allele through repression of "MET1" expression therefore linking cell cycle regulation to DNA methylation and genomic imprinting.

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VirtualPlant: A Software Platform to Support Systems Biology Research in the Post-Genomic Era. Manpreet Katari¹, Steve Nowicki¹, Chris Poulton², Varuni Pravhakar¹, Theresa Colombo¹, Dennis Shasha², Gloria Coruzzi¹, Rodrigo Gutierrez^{1,3}. ¹Department of Biology, New York University, New York, USA, ²Courant Institute of Mathematical Sciences, New York University, New York, USA, ³Department of Molecular Genetics & Microbiology. P. Universidad Católica de Chile, Santiago, Chile

Our long term goal is to understand how internal and external perturbations affect processes and networks controlling plant growth and development. In this project, we start with data integration of the known relationships among genes, proteins and molecules (extracted from public databases and/or generated with predictive algorithms) as well as experimental measurements under many different treatments. We go beyond data integration to conceptual integration by using novel visualization techniques to render the multivariate information in visual formats that facilitate extraction of biological concepts.

We also use mathematical and statistical methods to help summarize the data. We implement and combine these approaches in a system we term "VirtualPlant". Whereas our project relates specifically to Arabidopsis, the data structures, algorithms, and visualization tools are designed in a species-independent way. Thus the informatic, math, statistic and visualization tools that we develop can be used to model the cellular and physiological responses of any organism for which genomic data is available. We have implemented a proto-type that is already being actively and effectively used (<http://www.virtualplant.org>). This tool is being used by biologists and computer scientist alike for the purpose it was designed for - to support the analysis of original genomic data generated by the researchers themselves. We have found that working with experimental biologists, even from very early stages of software development, to be the most effective way to generate real solutions to the problems encountered by researchers in the laboratory.

<http://www.virtualplant.org/>

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Molecular and Functional Characterization of VPS23-3 (ELCH homolog) in *Arabidopsis*. Channa Keshavaiah¹, Mojgan Shahriari¹, Aneta Sabovljevic¹, Martin Huelskamp¹, Swen Schellmann¹. ¹University of Cologne, Cologne, Germany

Recently the *Arabidopsis* ELCH gene, a homolog of Vps23/TSG101 and the key component of plant ESCRT-I complex, has been functionally characterized (Spitzer et al, Development, 2006). The elch mutant shows multiple nuclei in various cell types, indicating a role in cytokinesis. VPS28 and VPS37 are the other known components of ESCRT-I. In addition to the ELCH homolog VPS23-2 we have identified another, *Arabidopsis*-specific homolog VPS23-3 encoded by At2g38830. Interestingly VPS23-3 showed a lesser degree of homology (47%) to *Arabidopsis* ELCH compared to the homology of ELCH to *Oryza sativa* ELOH (66%) and VPS23-2 (72%). Similar to ELCH, VPS23-3 is ubiquitously expressed, localized on endosomes and binds to ubiquitin with its N-terminal UEV domain. However, VPS23-3 interacts only with VPS28 whereas ELCH interacts with VPS37 in yeast-two hybrid and bi-molecular fluorescence complementation (Bi-FC) assays. The vps23-3 mutant shows an embryonically lethal phenotype. This indicates that it is not simply redundant to ELCH. We are currently performing a genetic and functional characterization of this novel gene in the context of the ESCRT pathway in plants.

P-392

Mitotic Epigenetic inheritance by meristematic DNA demethylation in *Arabidopsis*. Minhee Kim¹, Hyonhwa Ohr¹, Yeonhee Choi¹. ¹Department of Biological Sciences, Seoul National University

DEMETER, a DNA glycosylase domain-containing protein which functions as a DNA demethylase, shows highly specific expression during plant development. In plant reproductive stage, DME is temporarily expressed in central cell within the embryo sac. In vegetative stage, DME expression is restricted to the region including shoot apical meristem and root apical meristem. Based on this specific DME expression, DNA hypomethylation has been induced to the region where DME is expressed by generating antisense MET1 under the DME promoter. Since the expression of antisense MET1 is dependent on DME promoter, MET1 is expected to be knocked-down in the restricted area including meristematic region and central cell of the female gametophyte, but not in elsewhere. However, global DNA hypomethylation and expression of silent genes and transposons have been detected where DME promoter is inactive such as leaf tissues and pollen. These phenomena imply DNA methylation/demethylation is epigenetically inherited from meristem to the whole plant tissue throughout mitosis.

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Current status of plant resource project in RIKEN BRC. Masatomo Kobayashi¹, Hiroshi Abe¹, Satoshi Iuchi¹, Toshihiro Kobayashi¹, Takuro Tamura², Satoshi Oota¹, Kaoru Fukami-Kobayashi¹. ¹RIKEN BRC, Tsukuba, Japan, ²BITS Co., Ltd., Tokyo, Japan

RIKEN BioResource Center (BRC) was established in 2001 to preserve and distribute biological materials produced in Japan. Experimental Plant Division of RIKEN BRC conducts a project on the resources of model plants including *Arabidopsis thaliana*. We have distributed approximately 23,000 materials to the world research community during these five years. The number of laboratories that have received our materials is nearly 1,000. The major resources are as follows:

(1) RIKEN Arabidopsis Transposon-tagged Mutant (RATM) lines ("KO"-plants, ca. 16,000 lines)

(2) RIKEN Arabidopsis Full-Length cDNA (RAFL) clones (ca. 240,000 clones)

Now we are preparing homozygous seeds of RATM lines. Up to now, homozygous seed stock has been prepared for 1,100 lines. We also preparing a new cDNA database, "SABRE" (Systematic consolidation of *Arabidopsis* and other Botanical REsource). With this system, you can search not only RAFL clones but also homologous cDNA clones of non-*Arabidopsis* plants such as *Physcomitrella patens* and *Populus nigra* by using AGI code. If you have an interest, please visit our website

(<http://www.brc.riken.jp/lab/epd/Eng/>). Comments and questions are welcome (plant@brc.riken.jp).

P-395

Evolutionary Dynamics in Glucosinolate Biosynthesis. Juergen Kroymann¹. ¹Max Planck Institute for Chemical Ecology

Plants synthesize an immense number of secondary metabolites, so called because their significance for processes of basic growth and development is not immediately evident. More than 200,000 known secondary metabolites provide an increasingly exploited reservoir for the generation of pharmaceutically active agents, and many more await discovery. Classic hypotheses that seek to explain the generation of this vast metabolic diversity propose a stepwise and reciprocal process of adaptation and counteradaptation between plants and their natural enemies, moulded by mutual selection.

Glucosinolates are a major component in the range of secondary metabolites in cruciferous plants, and they contribute to resistance against herbivorous insects and other enemies. More than 120 glucosinolates are known which share a chemical core structure, but differ in their amino acid-derived side chain. Methylthioalkylmalate synthases (MAM) encoded by a small gene family in *Arabidopsis thaliana* and related plants control an early step in glucosinolate biosynthesis and, therefore, are central to the diversification of glucosinolate metabolism. In the MAM gene cluster, gene duplication, neofunctionalization and positive selection provide the evolutionary basis for biochemical alteration. These processes occur repeatedly in the history of this gene family, indicating their fundamental importance for the generation of plant metabolic diversity both within and among species. However, different processes, including multiple gene deletion events, genetic interchange between tandemly arranged MAM gene family members, and balancing selection, explain how glucosinolate diversity is maintained within *Arabidopsis thaliana*.

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Transcriptional regulation of the *Arabidopsis thaliana* diacylglycerol kinase gene "AtDGK7" in the root. Jonas Krebs¹, Fernando Arana-Ceballos¹, Miroslaw Kwasniewski^{1,2,3}, Bernd Mueller-Roeber^{1,2}. ¹University of Potsdam, Institute of Biochemistry and Biology, Karl-Liebknecht-Str. 24-25, Haus 20, 14476 Potsdam-Golm, Germany, ²Max-Planck Institute of Molecular Plant Physiology, Am Muehlenberg 1,, ³ Department of Genetics, University of Silesia, Jagiellonska 28, 40-032 Katowice, Poland

Plants employ various types of signalling molecules to coordinate developmental and physiological processes. Recently, phosphatidic acid (PA) has been identified to play an important role as one of such factors. PA is produced through two different pathways, one including the enzyme diacylglycerol kinase (DGK). The "AtDGK7" gene, one of seven members of the "*Arabidopsis thaliana*" DGK family, exhibits a highly specific expression pattern in roots, where it is activated early during root development. Here we report the investigation of the transcriptional regulation of "AtDGK7" including the identification of functionally relevant cis and trans regulatory elements. To identify cis elements of the "AtDGK7" promoter contributing to the highly specific and strong expression in roots, promoter deletion studies were performed, using -glucuronidase as reporter. One of the deletion constructs - containing a motif known to be recognized by Zn-finger transcription factors - was shown to be necessary for "AtDGK7" expression. By using public available databases of "*A. thaliana*" gene expression data, we identified one Zn-finger transcription factor gene exhibiting a highly overlapping pattern of expression in the root. This expression was confirmed in transgenic plants transformed with a promoter-GFP fusion. The function of the candidate transcription factor was analysed further by analysing over-expression and loss-of-function mutant lines. Details will be presented.

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Positional cloning of the TUMOROUS SHOOT DEVELOPMENT2 gene reveals a mutation in a putative methyltransferase that is necessary for cell adhesion and co-ordinated plant development. Eva Krupková¹, Thomas Schmitting¹. ¹Institute of Biology/Applied Genetics, Free University of Berlin, Berlin, Germany

Mutations in the TUMOROUS SHOOT DEVELOPMENT2 (TSD2) gene reduce cell adhesion and cause non-coordinated shoot development and tumor-like growth in vitro. *tsd2* mutants exhibited increased activity of axial meristems, reduced root growth and enhanced de-etiolation. Expression analysis of shoot meristem marker genes KNAT1:GUS and KNAT2:GUS indicated enlarged expression domains in the mutant background. Soil-grown *tsd2* mutants were dwarfed but showed an overall morphology similar to wild type. The TSD2 gene was identified by map-based cloning. It encodes a novel 684 amino acid long polypeptide containing a single membrane-spanning domain in the N-terminal part, and S-adenosyl-L-methionine binding and methyltransferase domains in the C-terminal part. Expression of a TSD2:GUS reporter gene was detected mainly in meristems and young tissues. A GFP-tagged TSD2 protein localized to the Golgi apparatus. The cell adhesion defects indicated altered pectin properties and we hypothesize that TSD2 acts as a pectin methyltransferase.

P-397

Chromosomal context of transgenes affects RNA-directed transcriptional gene silencing and DNA methylation. Markus Kuhlmann¹, Katja Richert-Poeggeler¹, Ute Fischer¹, Ales Pecinka², Renate Schmidt¹, Michael Florian Mette¹. ¹Leibniz Institute of Plant Genetics and Crop Plant Research, 06466 Gatersleben, Germany,²present address: Gregor Mendel Institute of Molecular Plant Biology, 1030 Vienna, Austria

RNA-directed transcriptional gene silencing of transgenes in plants provides a versatile experimental system for the study of epigenetic gene regulation. In such a setup, transcription of a promoter inverted repeat provides an RNA signal that can trigger transcriptional gene silencing and methylation of unlinked homologous promoters in trans. In order to determine the contribution of chromosomal localisation to gene inactivation, 26 single copy target transgenes integrated at defined positions of the *Arabidopsis thaliana* genome were combined by crosses with a silencer transgene containing a transcribed pNOS inverted repeat. Indeed, the activity and the degree of silencing of a pNOS-NPTII reporter gene present in the target transgenes was found to vary for the different chromosomal integration sites. In absence of the silencer, NPTII transcript levels deviated at maximum 10-fold among the target transgenes. When challenged by the silencer transgene providing the pNOS RNA signal, reduction of the NPTII expression in the F1 generation varied more than 100-fold ranging from no reduction to reduction to less than 1 per cent of the non-silenced level. Generally, silencing was correlated with proportional levels of DNA methylation in the pNOS region, but for one target transgene, substantial DNA methylation without adequate silencing was observed. Silencing and methylation were progressive through sexual reproduction, but differences between target transgenes were maintained to the F3 generation. The efficiency of silencing of target transgenes appeared to be mainly determined by their flanking sequences, with repeats promoting and functional genes diminishing the response to the silencing signal. Current results from further analysis will be presented. The work is supported by German Research Foundation grant SFB 648 C4.

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A mutant-based phenome analysis and collection of mutant-phenotype information. Takashi Kuromori¹, Asako Kamiya¹, Takuya Ito², Takuji Wada¹, Takashi Hirayama^{2,3}, Kazuo Shinozaki¹. ¹RIKEN Plant Science Center, Yokohama, Japan, ²RIKEN Discovery Research Institute, Tsukuba, Japan, ³Yokohama City University, Yokohama, Japan

By the availability of a variety of research resources, it is now able to investigate mutant lines for almost every gene in *Arabidopsis*. *Arabidopsis* is, then, not only a model plant for plant research, but also a model species in which it is possible to carry out saturation mutagenesis, and totally analyze each gene and mutant in an organism. One of the future goals of phenome project is to collect knockout-mutant phenotypes for each *Arabidopsis* gene. To make a total phenome database, we are collecting phenotype information by two activities, a mutant research activity and a publication research activity.

In mutant research activity, we are setting three categories of measurement to see various phenotypes, such as physical, chemical or biological method. We selected 4,000 transposon-tagged lines with a transposon insertion in gene-coding regions and systematically observed the visible phenotype as a first step of phenome analysis. Totally about 200 clear visible phenotypes were classified into 43 categories of morphological phenotypes. Phenotypic images have been entered into a searchable database (<http://rarge.gsc.riken.jp/phenome/>) (1, 2). In parallel, we have been selecting homozygous transposon-tagged plants, which would be useful resources to see other phenotypes, such as chemically or biologically measured phenotypes.

In publication research activity, we are collecting mutant phenotype information in published reports of journals. We are focusing the information of single-gene mutants, since we referred to Dr. Meinke's work (3). To date we obtained the information about 1,400 genes. Two activities will be combined to make a comprehensive phenotype database.

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P-400

Phenome Analysis of the *Arabidopsis* GRAS Genes. Mi-Hyun Lee¹, Sang-Kee Song², Bohye Kim¹, Jeong-Ok Heo¹, Miran Kim¹, Dong Giwan Kim¹, Sung Ho Sohn¹, Shin Ae Lee¹, Nan-je Yu¹, Chae Eun Lim¹, Myeong Min Lee², Jun Lim¹. ¹Department of Bioscience and Biotechnology, Konkuk University, ²Department of Biology, Yonsei University

GRAS proteins belong to a plant-specific transcription factor family. Currently, 33 GRAS members including an expressed pseudogene have been identified in the *Arabidopsis* genome. To provide a comprehensive evaluation of the *Arabidopsis* GRAS family, we conducted a large-scale analysis of the GRAS genes. With a reverse genetic approach, we constructed a "phenome-ready unimutant collection". Of this collection, we focused on loss-of-function mutations in 23 novel GRAS members. Under standard conditions, homozygous mutants have no obvious morphological phenotypes as compared to those of wild-type plants. Expression analysis of the members using quantitative real-time and semi-quantitative RT-PCR, microarray data mining, and promoter::GUS analysis revealed their tissue-specific expression patterns. Our analysis on protein-protein interaction and subcellular localization of individual members indicated their physiological roles as transcription regulators. Our phenome-ready unimutant collection of the GRAS genes will be a useful resource to better understand individual GRAS members that play diverse roles in plant growth and development.

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Screening of upstream regulator of DEMETER, a DNA glycosylase domain-containing protein, in *Arabidopsis* by Activation tagging mutagenesis. Jee-woong LEE¹, Geuntae Park¹, Yeonhee Choi¹. ¹Department of Biological Sciences, Seoul National University, Seoul, South KOREA

DEMETER (DME) is a DNA glycosylase domain protein that controls maternal expression of imprinted genes such as MEDEA (MEA), Fertilization-Independent Seed 2 (FIS2) and FWA in the central cell of the female gametophyte. DME is required for seed viability. Restricted DME expression in the central cell before fertilization is essential for the activation of maternally expressed-imprinted genes, thus provides the establishment of the imprinting in the endosperm after fertilization. An active DNA glycosylase domain of DME, normally associated with DNA repair, replaces methylated cytosine to unmethylated cytosine. Ectopic DME expression in pollen or endosperm activates expression of the normally silenced paternal MEA and FWA allele.

To detect the upstream regulators of DME, activation tagging mutagenesis has been performed by inserting T-DNA of pSKI015 vector into DME::GUS homozygous plants. Activation tagging can induces insertional mutation like other conventional T-DNA as well. In DME::GUS control plants, GUS gene is expressed only in the central cell nucleus within the ovules, but not in pollen grain or seeds. If T-DNA causes recessive insertional mutation and is inserted in the DME activator, transgenic plants will show the decreased 50% T2 ovule expression compared to the 100% T2 ovule in T1 DME::GUS homozygous control plants. If T-DNA is inserted into DME repressor and causes recessive mutation, we expect to see ectopic 50% T2 pollen expression in T1 plants. About 3,000 lines have been screened so far. Five lines of which showed the 50% T2 ovule expression have been selected for the further research. This screen will tell us how DME-restricted expression and genomic imprinting are regulated during gametogenesis.

P-402

Cloning and characterization of a TIR-binding protein in maize Mutator transposon. Yew Lee¹, Jin-Woo Jung¹, Soo-Hwan Kim¹. ¹Department of Life Science, Yonsei University, Wonju-Si 220-710, Korea

The Mutator family (Mu) of maize transposable elements is the most efficient gene-tagging systems in plants. All Mu elements share highly conserved ~210 bp of terminal inverted regions (TIRs) where Mutator transposase, MURA, binds a 32 bp region in the TIR of Mu1. The internal DNA of different members of the Mu system is non-homologous both in the size and sequence. Mutator shows "cut & paste" excision activity in the somatic cells while it switches to replicative transposition activity in the germinal cells and gametophytes. Another characteristic activity of the Mutator system is the fact that it is tightly regulated in the maize life cycle so that events are restricted to terminally differentiating cells. In an effort to understand host contribution to the regulation of Mutator activities, we cloned two TIR-binding proteins that bind to 13 bp Mu1 TIR sequence (CGGGAACGGTAAA, previously designated as Site I by Zhao and Sundaresan). Sequence analysis reveals that one of the binding proteins, St1-65, contains a nuclear targeting signal and Zn-Cys signature which is a Cys-rich motif that is involved in zinc-dependent DNA binding activity. Moreover, a gel shift analysis using yeast-expressed protein as a probe shows that St1-65 specifically binds to Site I in vitro. More biochemical analyses of binding activities and their *in vivo* meaning in regulation of Mutator activities will be discussed.

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Genetic Diversity and Population Structure of *Arabidopsis thaliana* in the U.S. and World-wide Collections. Yan Li¹, Luz Rivero², Joy Bergelson³, Magnus Nordborg⁴, Diane Byers⁵, Eric Holub⁶, Randy Scholl², Justin Borevitz^{7,8}. ¹Department of Ecology and Evolution, University of Chicago, ²Arabidopsis Biological Resource Center, Ohio State University, ³Department of Ecology and Evolution, Committee on Genetics, University of Chicago, ⁴Molecular and Computational Biology, University of Southern California, ⁵Department of Biological Sciences, Illinois State University, ⁶Warwick-HRI, University of Warwick, United Kingdom, ⁷Department of Ecology and Evolution, ⁸Committee on Genetics, University of Chicago

Natural variation in *Arabidopsis thaliana* can be exploited to identify new alleles of known genes, new quantitative trait loci (QTLs), perform association mapping studies, and discover the ecological and evolutionary forces driving local adaptation and shaping the genetic diversity. We genotyped over 5000 lines for 149 common SNPs to determine the relatedness and population structure, including ~800 world-wide samples from the Arabidopsis Biological Resource Center (ABRC), ~ 3500 lines from Europe, and over 1600 lines collected from different locations in the U.S. Midwest across different times of year. We observed different levels of genetic diversity and population structure in both global and regional samples. Certain common genotypes were identified across multiple collection locations. Clones with identical genotypes at 149 common SNPs have been identified and will be removed to reduce the redundancy in these world-wide collections including stock center lines. Genetic distance between regions will be compared with each other and against the geographic distance to test for the presence of isolation-by-distance. The possible ancestral origins of the U.S. Midwest populations will be inferred via model-based structure analysis with world-wide collections. A HapMap panel of 384 strains with maximal genetic diversity and controlled population structure is being selected for high density genotyping with a custom 250kSNP/tiling array (AtSNPtile1), which will be used for whole genome association mapping. The high marker density, large population size, and species wide diversity will allow powerful LD mapping studies to reveal common variation important for adaptation.

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The GABI-Kat *Arabidopsis thaliana* T-DNA Mutagenised Population for Flanking Sequence Tag-Based Reverse Genetics.
 Yong Li¹, Mario G. Rosso¹, Prisca Viehöver¹, Heinz Saedler²,
 Bernd Weisshaar^{1,2}. ¹Bielefeld University, Institute for Genome Research, Bielefeld, Germany,²Max Planck Institute for Plant Breeding Research, Koeln, Germany

The GABI-Kat population of T-DNA mutagenised *Arabidopsis thaliana* lines with sequence-characterised insertion sites is used extensively for efficient progress in plant functional genomics. Here we provide details about the establishment of the material, discuss results from quality control studies, and inform about the progress of donation of confirmed lines to NASC as well as the projected end of GABI-Kat services in 2008. T-DNA insertion mutants were created in the accession Columbia (Col-0). DNA from leaves of T1 plants was extracted and used for the production of flanking sequence tags (FSTs). About 109,700 FSTs that "hit" regions on the *A. th* pseudochromosomes were submitted to EMBL/GenBank/DDBJ and are available from the GABI-Kat website (<http://www.gabi-kat.de>). This website also gives access to information about which insertion is present in which line, and about the predicted location of insertions relative to a given gene. The GABI-Kat database has recently been updated to contain additional information such as segregation data, gene-specific primers and confirmation sequences. This information not only helps users to evaluate the usefulness of the mutant lines, but also covers a big part of the molecular characterisation of the insertion alleles. In addition to directly serving the community with confirmed insertion mutants, we have started in 2005 to transfer confirmed GABI-Kat lines to the Nottingham *Arabidopsis* Stock Centre (NASC). As of February 2007, 4,293 confirmed lines represented by sets of T3 seed have been delivered to NASC. We will continue to transfer confirmed lines to NASC until the projected end of the project in December 2008. The last user order will be accepted in June 2008 to be able to deliver within the duration of the project. In parallel to user orders, we will confirm the "most valuable GABI-Kat insertions", i.e. insertions that are unique to GABI-Kat and those in which we have an CDSi-hit while other populations have alleles with a much lower chance of causing a null mutation.

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Genetic Redundancy and Recessive Antimorphism: Insights From Genetic Analyses Of The Ribonucleotide Reductase Small Subunit Mutants *tso2*. Zhongchi Liu¹, Chunxin Wang¹, Connie Yoon¹, Boyana Grigorova¹. ¹University of Maryland

Genetic redundancy often hinders our ability to decipher gene function. For example, T-DNA insertional mutations in *Arabidopsis* often fail to cause a mutant phenotype due to multiple copies of similar genes in the genome. We observed and characterized a phenomenon coined "recessive antimorphism", which is currently poorly understood and may provide novel insights in the study of functionally redundant genes.

Ribonucleotide reductase (RNR) converts NDPs into dNDPs for DNA synthesis and repair and thus is essential for growth and development. RNR is a tetrameric protein complex consisting of two large subunits (R1) and two small subunits (R2). In *Arabidopsis*, the R2 small subunit is encoded by three functionally redundant genes: TSO2, RNR2A and RNR2B (Wang and Liu, 2006 *Plant Cell*). While *tso2-5*, a T-DNA insertion near the N-terminus of TSO2 protein, exhibited no abnormality, four independently isolated *tso2* missense mutations (*tso2-1*, 2, 3, 4) caused severe developmental defects and a reduction of dNTP level, suggesting that the *tso2* missense mutations might be antimorphic. While previously reported antimorphic alleles are always dominant, the four *tso2* missense alleles are completely recessive, raising the possibility that these *tso2* alleles are recessive-antimorphic. We hypothesize that the antimorphic (poisonous) effect of TSO2 mutant proteins depends on their ratio to the wild type R2 proteins encoded by TSO2, RNR2A and RNR2B. To test this hypothesis, the missense *TSO2-1* mutant gene under 35S promoter as well as *TSO2* native promoter was introduced into wild type *Arabidopsis* to test if the *TSO2-1* mutant gene could induce *tso2*-like mutant phenotype. Our data support that the *TSO2-1* mutant gene exhibits antimorphic effect. However, the phenomenon is more complex and will be described in more detail.

Our study of recessive antimorphism provides novel insights in the study of functionally redundant genes. It is likely that missense-based recessive antimorphic alleles are more effective in eliminating gene function by interfering with wild type proteins produced by other gene family members.

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Characterization of Ubiquitin-Specific Proteases in Arabidopsis. Yanfen Liu¹, Feng Wang¹, Huiyong Zhang¹, Hang He¹, Xing Wang Deng¹. ¹National Institute of Biological Sciences, Beijing, China

Ubiquitin-Specific Proteases (UBPs) are highly conserved family of proteins in eukaryotes that play critical roles in cellular protein de-ubiquitination. A total of 27 UBP s are present in the *Arabidopsis thaliana* genome, which can be further grouped into 14 subfamilies based on their sequence similarity. Microarray data provide detailed expression profiles of those UBP s and an analysis of 38 available T-DNA insertion lines in 25 specific UBP s covering all 14 subfamilies result in that only mutants in 2 subfamilies exhibited observable phenotypes. One of the subfamilies, *AtUBP15* subfamily, whose members also possess a single zinc-finger domain, contains 5 individual genes, mutations in which leading to 3 distinct phenotype consequences. *AtUBP15* mutants display narrower, serrated and flat rosette leaves partially due to lack of cell proliferation, as well as other phenotype such as early flowering, apical dominance losing, short or sometimes unfertile siliques, while the overexpression lines result in opposite phenotype. Both *in vitro* and *in vivo* assays confirm it is a bona fide de-ubiquitinating enzyme and Cysteine447 is essential for this activity. Further transcriptome analysis show expression level of 804 genes are miss-regulated in *Atubp15-1*, including down regulation of a MADS box (*MAF5*) gene. Phenotype of double mutant *Atubp15 Atubp16* is more severe than that of *AtUBP15* mutant, whereas *Atubp16 Atubp17* is comparable with *AtUBP15* mutant, implying *AtUBP15* and *AtUBP16* but not *AtUBP17* involve in the same pathway. Intriguingly, mutation of another subfamily member *AtUBP19* leads to embryo lethality. Distinct AtUBPs genes, even within closely related subfamilies, function in different while sometimes overlapped developmental pathways to regulate protein de-ubiquitination.

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Molecular and functional characterization of a putative AtCLC Gene Family in Arabidopsis. Qundan Lv¹, Renjie Tang¹, Huiqiong Zheng¹, Hongxia Zhang¹. ¹Shanghai Institute of Plant Physiology and Ecology, SIBS, CAS, Shanghai , 200032, P. R. China

Chloride channels (CLC) are passive anion channels that play a variety of roles in turgor maintenance, stomatal movement, nutrient transport, metal tolerance as well as cellular signal perception and transduction. In *Arabidopsis thaliana*, seven candidate genes encoding putative chloride channels were cloned. The predicted protein of *AtCLC* genes after revision consists of 8 to 10 transmembrane spans and two conserved CBS domains at the carboxyl end, which are proposed to have regulatory roles. Among these seven genes, CLC-c and CLC-d could suppress, with different efficacy, the MnCl₂-sensitive phenotype of a yeast mutant that is deficient in *GEF1*, the sole chloride channel homologue in yeast *Saccharomyces cerevisiae*. RT-PCR analysis of each transcript during seed germination and in different organs indicates that this gene family was ubiquitously expressed in *Arabidopsis*, some of which, however, exhibit tissue preference. Promoter-GUS analysis in transgenic *Arabidopsis* showed that *AtCLC* was differentially distributed in young seedlings. *AtCLC-a, b, c, d, g* were strongly expressed in vascular tissues but not in root tip, while *AtCLC-e, f* expressions were dominantly localized in leaf. Recently *AtCLC-a* has been reported to be a nitrate/proton anionporter mediating nitrate accumulation in plant vacuoles (De Angeli A et al, 2006), which stimulate us to reconsider and dig out substrate specificity of the remaining members of this gene family. We are also isolating and characterizing clc knock-out mutants to address the functions of *AtCLC* family *in vivo*, coupled with genetic, physiological and biochemical investigations.

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Comprehensive analysis of the *Arabidopsis* GATA factors reveals light- and clock-regulation and organ-specific patterns of gene expression. Iain Manfield¹, Toby Joyce¹, Paul Devlin², Chih-hung Jen¹, David Westhead¹, Phil Gilmartin¹. ¹ University of Leeds, Leeds, U.K. ,² School of Biological Sciences, Royal Holloway University of London, U.K.

Comprehensive expression analysis of the 29 GATA genes in *Arabidopsis* across a range of organs has identified genes which are light up-regulated, clock-regulated and expressed only in green tissues and these genes are therefore candidates to play roles in light signalling. Other genes are light down-regulated and with evening peaks of expression. Therefore, there is a complex pattern of up and down-regulation by light and clock regulation of many GATA genes, representing diverse regulatory inputs. In contrast, some genes show organ-specific expression, in roots or siliques, but with no evidence of light regulation. Our results also reveal a small group of genes with expression patterns similar to "HAN", suggesting that there may be additional GATA genes with related roles. Correlation of expression between genes across many microarray experiments can suggest similar regulation; this analysis can be performed using the *Arabidopsis Co-expression Tool* (www.arabidopsis.leeds.ac.uk/ACT). Co-expression of certain GATA genes with well-characterised "guide genes" is being used for function prediction and aiding design of experiments for analysis of mutants.

Determination of gene structures and expression patterns for all GATA genes has provided information additional to comparison of coding sequences. Together with analysis of gene pairs using the ACT Scatter Plot tool, these results reveal subtle differences in expression for duplicated genes, suggesting redundancy and divergence of functions.

Microarray analysis of two GATA mutants which did not show a visible defect has identified mis-regulation of a number of salt- and cold-responsive genes. In addition, promoter:GUS reporter constructs for these genes show expression in guard cells and in vascular tissue. We will present results demonstrating the salt-sensitivity of mutants and exploring the positions of GATA genes in abiotic stress-response pathways.

Manfield et al. , 2007 Plant Physiology 143:941-958

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Arabidopsis Co-expression Tool (ACT); Web-based tools to identify co-regulated sets of genes and predict gene function.
 Iain Manfield¹, Chris Needham², James Bradford³, Chih-hung Jen³, John Pinney³, Ioannis Michalopoulos³, Andy Bulpitt², David Westhead³, Phil Gilmartin¹. ¹ Centre for Plant Sciences, University of Leeds, Leeds, UK., ² School of Computing, University of Leeds, Leeds, U.K., ³Institute of Molecular and Cellular Biology, University of Leeds, Leeds, U.K.

Large amounts of microarray data from diverse experiments contain information beyond the original purpose of each experiment and analysis of this data en masse can identify genes regulated in the same manner. This analysis is provided by the Arabidopsis Co-expression Tool (www.arabidopsis.leeds.ac.uk/ACT) which ranks 22000 genes across a large microarray dataset according to how closely their expression follows a gene of interest. We will illustrate how ACT elucidates gene regulatory mechanisms with examples of genes encoding components of the circadian clock and by predicting functions for poorly-characterised transcription factor genes.

Corroboration of pre-calculated co-expression lists can be performed by calculating user-defined lists using only the arrays or experiments deemed relevant. GO and word-counting tools measuring the over-representation of themes in the annotations of co-expressed genes can suggest biological significance. Novel tools dissect co-expression lists into groups of genes likely to be coherently regulated.

CLIQUE FINDER identifies the genes consistently co-expressed with each other affording objectivity to determination of cut-offs, giving sets of genes for further characterisation (e.g. promoter motif prediction).

SCATTER PLOT displays the correlation r-values for nearly 22 000 genes versus two query genes and can reveal clusters of outlying genes. Highlighting well-characterised genes on these plots can be used to infer common properties for co-expressed genes.

Beyond co-expression analysis, we are using Bayesian inference methods to predict genetic regulatory networks from microarray data, without specific perturbation experiments. This reproduces aspects of the regulation of well-characterised genes and predicts positions of less well-characterised genes within experimentally-identified regulatory networks.

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REM18 and REM53: two direct targets of the ovule identity complex of Arabidopsis Luis Matias-Hernandez¹, Raffaella Battaglia¹, Vittoria Brambilla¹, Marco Rubes¹, Martin M. Kater², and Lucia Colombo¹ ¹Dipartimento di Biologia, Università Luis Matias-Hernandez¹, Lucia Colombo¹. ¹University of Milano

Three genes responsible for ovule identity determination in *Arabidopsis thaliana* have been identified which are SEEDSTICK (STK), SHATTERPROOF1 (SHP1) and SHATTERPROOF2 (SHP2) (Pin-yopich et al., 2003), all of them belonging to MADS-box transcription factors family. STK is the only one specifically expressed in the ovule, while SHP1 and SHP2 are also involved in carpel formation. STK, SHP1, and SHP2 form a multimeric complex together with SEPALLATA (SEP) MADS-box proteins to regulate ovule development (Favarò et al., 2003).

To identify targets of this ovule identity complex we have done microarray analysis using RNA isolated through Laser Capture Microdissection (LCM) from ovules primordia. Once a small subset of transcription factor genes were identified, based on their expression profile and having in their regulatory regions multiple MADS-box binding sites, Chromatin Immunoprecipitation (ChIP) experiments were performed to confirm the *in vivo* targets of the ovule identity complex.

Two novel genes REM18 (At5g18000) and REM53 (At3g53310) have been demonstrated to be targets using ChIP techniques, and we have further confirmed these results through *in situ* hybridization analyses. REM18 and REM53 belongs to REproductive Meristem (REM) family (Franco-Zorrilla et al., 2002), an almost unknown family of transcription factors unique in plants. Both genes are developmentally regulated, firstly expression is localized into few cells in the shoot apical meristem, secondly REM18 and REM53 expression expand to the whole reproductive meristem, and finally is restricted to the ovule in later stages of flower development. In the triple mutant *stk-shp1-shp2* expression of REM18 and REM53 is not detected inside the ovule, confirming that these two novel genes are the first targets of the ovule identity complex.

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GREENPHYL: A comparative genomic platform for Rice and Arabidopsis. Conte MG¹, Gaillard S¹, Guideroni E¹, Périn C¹. ¹CIRAD

Gene ortholog identification has become a major objective for mining the increasing amount of sequence data generated by full or partial genome sequencing projects. Comparative and functional genomics urgently need reliable ways to identify orthologs to shorten gene function inference and conservation/divergence of genetic pathways between species.

We developed a two-step strategy for ortholog prediction between *Oryza Sativa* and *Arabidopsis thaliana* using transcription factors genes as a golden test case. We first evaluated the feasibility of accurate gene family clustering of *Arabidopsis thaliana* and *Oryza sativa* genes using TribeMCL software. We then developed GreenPhyl, an optimized phylogenomic pipeline which can predict ortholog and paralog relationships based on gene family clustering. The outcomes of our studies show that GreenPhyl achieved high accuracy level in predicting ortholog/paralog relationships. We then demonstrated that a phylogenomics approach is suitable for plants and outperform the similarity methods for ortholog prediction.

GreenPhyIDB (<http://greenphyl.cines.fr>), a database dedicated for *Oryza sativa* and *Arabidopsis thaliana* gene family classification and ortholog predictions, has been developed in order to store the results of the GreenPhyl pipeline. Until now, GreenPhyIDB contains 5800 manually annotated plant gene families with cross references (Uniprot, KEGG, Interpro, TAIR, TIGR, OrygenesDB). Currently 4000 of these plant families were phylogenetically analysed. In addition, we have developed a set of tools around GreenPhyIDB to retrieve as exhaustively as possible information related to queries with several starting points: TAIR or TIGR ID, sequence, keywords, Interpro, KEGG, family name, gene name.

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Characterization of interaction of ARIADNE E3 ligases with the unusual cap binding protein nCBP. Christina Mladek¹, Suzanne Cathrin Burey¹, Juan-Antonio Torres Acosta¹, Marie-Theres Hauser¹. ¹BOKU - University of Natural Resources and Applied Life Sciences Vienna

ARIADNE (ARI) proteins belong to the class of "RING between RING fingers" (RBR) domain proteins with E3 ligase activity (Eisenhaber et al., 2007). The Arabidopsis genome codes for 16 ARI genes that are subdivided into three subclasses. E3 ligase activity of AtARI8 (subclass B) has been shown to depend on the ubiquitin-conjugating enzymes (UBC) 8, 10 and 11 (Mladek et al. 2003). Here we present protein-protein interaction results for AtARI representatives of each subclass showing that they are able to form homo- and heteromers in yeast to hybrid assays. Whereas homomerization was only detected for AtARI12 (subclass B), AtARI1 (subclass A) forms heteromers with both AtARI12 and AtARI15 (subclass C).

Furthermore, the novel RNA cap binding protein, nCBP, has been identified in yeast two hybrid assays as strong interactor of AtARI12 and AtARI15, but also weakly interacts with AtARI2 (subclass A). The complex formation of AtARI12 with nCBP was further confirmed by coimmuno-precipitation and -localization. AtARI12 and nCBP can be detected in similar speckles in the cytoplasm and nucleus in Arabidopsis cell cultures. We will present the results of our analyses to test the hypothesis that nCBP is a candidate substrate of the AtARI E3 ligases and propose a model on the role of AtARIs as post-translational regulators of nCBP during plant development and stress responses.

Eisenhaber et al. (2007) *Genome Biol* 8, 209

Mladek et al. (2003). *Plant Physiol* 131, 27-40

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P-413

PolIV, Central proteins in sRNA production. Rebecca A. Mosher¹, David Studholme¹, Frank Schwach¹, David C. Baulcombe¹. ¹Sainsbury Laboratory, Norwich, UK

Most endogenous sRNAs in plant cells are derived through DNA-dependent RNA polymerase IVa (PolIVa), RNA-dependent RNA polymerase 2 (RDR2), and Dicer-like 3 (DCL3). At some loci, these small RNAs act via Argonaute 4 (AGO4) and a second isoform of PolIV (PolIVb) to direct DNA methylation. Many targets of RNA-directed DNA methylation (RdDM) are transposable elements or repetitive DNA, though developmental genes such as *SUPERMAN*, *FWA*, and *MEDEA* are also regulated by RdDM.

In order to define and characterize endogenous sRNA-generating loci, we sequenced sRNA libraries from wildtype and PolIV mutants (*nrdp1a-4* and *nrdp1b-1*). 3838 bona fide sRNA loci (containing ≥ 4 sRNA matches with ≥ 1 unique match) were identified, and 94% require PolIVa. PolIVa-dependent loci are highly enriched for all classes of transposable elements and concentrate in the pericentromere. All tested loci requiring PolIVa also require *RDR2* and *DCL3*, but show varying degrees of dependence on PolIVb. Non-LTR retrotransposons require both PolIV isoforms, while LTR-retrotransposons only require PolIVa. The inherent ability of LTRs to sustain siRNA production indicates that PolIVb may play a role in maintaining siRNA production at loci without such repeats. RdDM of PolIVa-dependent loci was assessed with bisulfite sequencing. In addition to the expected loss of asymmetric methylation, gain of methylation was detected in *nrdp1a*. Both changes required PolIVb, though sRNA accumulation at these loci does not.

This work highlights the extensive and diverse role of PolIV. PolIVa-dependent loci comprise more than 1% of the Arabidopsis genome, including 550 unique regions. PolIVb likely functions at each of these loci, directing various DNA modifications and/or maintaining sRNA production.

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Characterization of Ds-tagged mutants for nuclear-encoded chloroplast proteins involved in plastid development and oxidative stress responses. Fumiyo Miyouga¹, Haruko Izumi¹, Takashi Kuromori¹, Reiko Motohashi², Kazuo Shinozaki¹. ¹RIKEN Plant Science Center, ²Shizuoka University

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 Ds or T-DNA tagged mutant lines in Arabidopsis. Putative 2,090 chloroplast protein genes were identified by using 4 prediction softwares. Base on databases of tagged mutant lines, such as RIKEN, NASC, Wisconsin, CSHL and SALK, we collected 3,416 tagged-lines disrupted by Ds transposon or T-DNA that encode 1,374 chloroplast proteins. From these mutant resources, we have collected 702 of RIKEN Ds tagged-lines and 88 of CSHL Ds tagged-lines. By screening the 790 Ds tagged-lines, we identified 652 homozygotes with no obvious phenotype, 67 mutants showing abnormal seedling and 66 mutants of which homozygotes were not obtained. To determine whether the mutations with visible phenotypes are caused by the Ds insertions, we examined mutant alleles existed in other tagged-lines. Among 48 genes of 67 mutants showing abnormal phenotypes, 21 genes existed several alleles with similar phenotypes, suggesting that their important roles in chloroplast development.

The chloroplasts play important roles not only in photosynthesis but also in abiotic stress responses such as drought, salinity, freezing. Our collection of homozygous mutants is a powerful tool for the screening of chloroplast mutants with abnormal stress responses. By screening of 577 homozygous mutants of RIKEN Ds tag-lines, we isolated several mutant candidates for stress-related chloroplast proteins. We obtained 42 lines with different capabilities to survive under severe oxidative or osmotic stress conditions on agar plate medium. Among them, a lot of mutants showed abnormal responses to Paraquat and Alloxan, producers of reactive oxygen species (ROS). We are analyzing these mutants to identify nuclear-encoded chloroplast proteins involved in anti-oxidative defense responses.

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Arabidopsis Pseudo Response Regulators PRR9, PRR7, and PRR5 are Involved in the Circadian Phase Transition of the Transcriptome. Norihito Nakamichi¹, Miyako Kusano², Atsushi Fukushima², Masanori Kita³, Shogo Ito³, Takayuki Thoge², Takaumi Yamashino³, Kazuki Saito^{2,4}, Hitoshi Sakakibara², Takeshi Mizuno³. ¹Graduate School of Science, Nagoya University, ²RIKEN Plant Science Center, ³School of Agriculture, Nagoya University, ⁴Graduate School of Pharmaceutical Sciences, Chiba University

Arabidopsis Pseudo Response Regulator (PRR) genes are involved in the circadian clock mechanism. A prr9-10 prr7-11 prr5-11 triple mutant was arrhythmic and showed pleiotropic phenotypes. It was consistent with the evidence that a myriad of genes are regulated under the circadian clock. But it has remained largely unknown how PRRs regulate output events of the circadian clock. To address this issue, we performed GeneChip analysis of prr9-10 prr7-11 prr5-11 under light and dark (LD) cycles and under 18 days of constant light conditions (18dLL) as steady state of the circadian rhythm. The comparative GeneChip analyses suggested two consequences. (1) prr9-10 prr7-11 prr5-11 mutants stall circadian phase of many genes at late day phase, and (2) the expression levels of these genes were highly associated with PRR expression levels. In addition, novel physiological relevance, which was induced by the stalled circadian rhythm during day phase, was observed in prr9-10 prr7-11 prr5-11. For example, GeneChip and GC-TOF/MS analyses revealed that target genes and metabolites of DREB1A (the day-phased gene) were substantially upregulated in prr9-10 prr7-11 prr5-11, even under conditions free from cold or drought stress, and prr9-10 prr7-11 prr5-11 exhibited a phenotype of drought tolerance.

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Genome-wide analysis of the Tetraspanin gene family in *Arabidopsis*: phylogeny, gene structure and contribution of gene duplication events. Oscar Oliveira¹, Cátia Barbeta¹, Teresa Lino-Neto¹, Rui Tavares¹, Klaus Palme². ¹Departament of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal,² Faculty of Biology II, University of Freiburg, Schanzlestr. 1, Freiburg, Germany

Tetraspanin genes were first described in animals and are common to all multicellular organisms. They encode membrane glycoproteins with four conserved trans-membrane domains. Specific, highly conserved amino acid residues influence the molecular interactions crucial for the proteins function (Levy and Shoham, 2005). Tetraspanins are necessary for correct cellular signalling, as well as cell migration, adhesion, proliferation, recognition and fusion (Martin et al., 2005).

Although the majority of animal tetraspanins have been studied, only two tetraspanin genes have been characterized in *Arabidopsis*. These studies focus have focused on the mutant phenotypes for the TORNADO2 and the TOM2A genes.

Here, we report a genome-wide analysis of tetraspanins in *Arabidopsis*. We have identified 21 tetraspanin genes in publicly-available databases. Subsequent phylogenetic analysis reveals a complex evolution of tetraspanins in *Arabidopsis*, inferred from the complex arrangement of the subfamilies. A comparison among individual gene structures also supports a complex model for tetraspanin evolution, with several introns and exons forming conserved structural patterns.

We also evaluated the contribution of different duplication events in the *Arabidopsis* genome to the expansion of the tetraspanin family. The results from genome distribution of *Arabidopsis* tetraspanin genes support the existence of segmental and tandem duplication events for some genes. Despite this, our analysis suggests that after duplication some extensive rearrangement, and probable divergent evolution, might have occurred.

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AGRIS and ReIN: A systems biology framework to establish and link cis-regulatory networks and to identify direct targets for transcription factors in *Arabidopsis thaliana* using service oriented architecture. Saranyan K. Palaniswamy¹, Rebecca S. Lamb¹, Ramana V. Davuluri¹, Erich Grotewold¹. ¹The Ohio State University, Ohio, USA

Genome-wide gene regulatory networks govern the phenotypic states of different cell-types, tissues and developmental stages in eukaryotic organisms. The gene regulatory networks converge at the level of transcription, where the DNA-binding transcription factors recognize cis-regulatory elements in the promoter regions of target genes. This fact underscores that it is vital to establish the architecture of plant promoters to understand gene expression. The identification of direct target genes for selected TFs is serving as a platform in establishing genome-wide regulatory networks to explain the expression of all *Arabidopsis* genes, simultaneously validating an *Arabidopsis* cis-regulatory element map developed in parallel. In order to integrate this information into a web-based knowledgebase, we have developed AGRIS (Arabidopsis Gene Regulatory Information Server), which provides the scientific community with a platform to establish regulatory networks. AGRIS currently houses three linked databases: AtcisDB, AtTFDB and AtRegNet. AtTFDB contains more than 1,750 + *Arabidopsis* TFs and their sequences (protein and DNA) grouped into 50 + families with information on available mutants for the corresponding genes. AtcisDB consists of 25,000 + promoter sequences of annotated *Arabidopsis* genes with a description of putative cis-regulatory elements, both predicted and experimentally validated. AtRegNet links in direct interactions between several hundred genes and the TFs that control their expression. The current release of AtRegNet contains a total of 350 + direct TF/target gene interactions. ReIN is a web-based software tool for creating, visualizing and identifying regulatory networks with a multi-tier architecture based on J2EE technology. AGRIS has also been implemented in Service Oriented Architecture (SOA). SOA provides a standard way of publishing applications and data sources over the internet, enabling mass dissemination of knowledge. AGRIS can be accessed at <http://arabidopsis.med.ohio-state.edu>.

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Transposon-Induced Genome Rearrangements in Maize and Arabidopsis. Thomas Peterson¹, Chuanhe Yu¹, Lakshminarasimhan Krishnaswamy¹, Jianbo Zhang¹. ¹Iowa State University, Ames, Iowa, USA

Since their discovery by McClintock, transposable elements have been associated with the generation of a variety of genome rearrangements, including deletions, direct and inverted duplications, and translocations. In addition to providing dispersed sequence homologies for ectopic recombination, transposons can induce genome rearrangements through alternative transposition reactions that utilize the termini of different elements. Transposition reactions involving transposon termini in direct orientation can generate deletions and inverted duplications. In addition, pairs of Ac termini in reversed orientation can undergo transposition reactions resulting in inversions, deletions, and translocations. In each of these cases, the rearrangement breakpoints are bounded by the characteristic footprint or target site duplications typical of Ac transposition reactions. These results show how alternative transposition reactions could contribute significantly to genome evolution by generating chromosome rearrangements, and by creating new genes through shuffling of coding and regulatory sequences (Zhang, Zhang and Peterson, 2006). These alternative transposition reactions were first observed in natural maize variants, and have now been reproduced in transgenic maize and *Arabidopsis* plants. The system utilizes transgene constructs containing maize Ac termini in direct or reversed orientation. The action of Ac transposase on the Ac termini generates a variety of rearrangements, including deletions, inversions and translocations. In these rearrangements, one endpoint is at the Ac termini, and the other endpoint is at another genomic site. This transposition-based system provides an alternative to the cre-lox system for genome modifications. The current state of the project will be presented. To view an animation of the alternative transposition model, see <http://jzhang.public.iastate.edu/Transposition.html>.

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P-419

Formation of apomeiotic embryosacs in the "dyad" mutant of *Arabidopsis*. Maruthachalam Ravi¹, Mohan Prem Anand Mari-muthu¹, Imran Siddiqi¹. ¹Centre for Cellular and Molecular Biology (CCMB), Hyderabad, Andhra Pradesh, India 500 007

The "dyad" mutant was previously isolated in an EMS mutagenesis screen for genes that affect reproductive development in "Arabidopsis" (Siddiqi et al., 2000). DYAD is required for reductional chromosomal segregation and for female meiotic progression (Agashe et al., 2002). The megasporocyte mother cell in "dyad/sw1" undergoes an equational meiosis I and arrests at a two celled stage giving rise to two diploid cells leading to female sterility. However, a variable low frequency of seed set was observed in "dyad" plants. Chromosome analysis of those progenies revealed that majority of them were triploid. Through various genetic approaches we show that the triploids arise by the fusion of a diploid female gamete with a haploid male gamete. SSR marker analysis indicates that the "dyad" diploid female gametes lack recombination during their formation. Mounting evidence suggests that apomixis involves temporal and spatial deregulation of genes involved in the sexual pathway. Our results provide a proof of principle demonstration of the formation of unreduced embryo sacs resembling apomeiosis due to a mutation of a single gene involved in the sexual pathway. We also provide evidence for sexual polyploidization mediated by "dyad" diploid gametes and obtained plants upto the heptaploid level.

P-420

Antagonists of RNA-directed transcriptional gene silencing (TGS) and DNA methylation in *Arabidopsis*. Katja Richert-Poeggeler¹, Quanan Hu², Markus Kuhlmann¹, Renate Schmidt¹, M. Florian Mette¹. ¹Leibniz Institute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, Germany, ²Botanical Institute, University of Basel, CH-4056 Basel, Switzerland

RNA-directed TGS and DNA methylation, a branch of RNAi operating in the cell nucleus, has been extensively studied in plants. Work has mainly focused on the analysis of molecular features of the silenced state such as DNA methylation patterns as well as on the identification of RNAs and proteins involved in its establishment and propagation. A two component transgene system in *Arabidopsis* has proven particularly powerful in the genetic analysis of RNA-directed TGS and DNA methylation. The "silencer" transgene provides dsRNA homologous to a particular gene promoter, whereas the "target" transgene contains a reporter gene under the control of the same promoter which undergoes RNA-directed TGS and DNA methylation in the presence of the "silencer". Recent studies (Zhu et al., 2007 and references therein) have revealed that DNA methylation in *Arabidopsis* can be actively removed by plant enzymes like the DNA glycosylase/lyase ROS1. We are introgressing a ros1 "loss of function" mutation into our transgene system to analyse its possible involvement. Two different "targets" are being used. At a "target" resistant to RNA-directed DNA methylation that acquires only little promoter methylation in presence of the "silencer", we will test whether the ros1 mutation will lead to increased methylation levels. This would indicate a role in counteracting de novo DNA methylation. At a "target" acquiring high promoter methylation in presence of the silencer, we will analyse whether the inheritance of DNA methylation after segregation of the "silencer" will be increased by the ros1 mutation. This would indicate a role in counteracting maintenance DNA methylation. Furthermore we are setting up a new transgene system involving a conditional negative selectable marker for the acquisition of novel mutations enhancing RNA-directed transcriptional gene silencing.

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P-421

The Arabidopsis Biological Resource Center Current Collections and Activities. Randy Scholl¹, Luz Rivero¹, Deborah Crist¹, Emma Knee¹, James Mann¹, Natalie Case¹, Zhen Zhang¹, Garret Posey¹, Pamela Vivian¹, Christopher Calhoun¹, Sarah Pfingsten¹, Kanika Johar¹. ¹The Ohio State University, Columbus, (OH), USA

The Arabidopsis Biological Resource Center (ABRC) collects, preserves and distributes seed and DNA stocks of Arabidopsis. ABRC stock information is accessible through TAIR (<http://arabidopsis.org>).

The seed stock collection includes: A) 10,000 purified T-DNA lines from J. Ecker, B) 1,018 purified T-DNA insertion lines from different researchers, C) 53,084 SAIL T-DNA lines from Syngenta, D) 918 homozygous SAIL T-DNA lines, E) 2,800 lines from GABI-Kat representing 404 T-DNA insertions, F) 4,574 insertion lines from John Innes Centre, G) 626 RNAi lines from AGRIKOLA representing 66 genes, H) 2,500 mutant lines, I) 15 recombinant inbred populations, J) 1,500 natural accessions (ecotypes), K) 95 other species, and L) miscellaneous transgenic lines.

The T-DNA lines of the SALK, SAIL and Wisconsin collections provide insertions in 25,000+ different Arabidopsis genes. Diverse sets of RI populations have been received from groups associated with the following researchers: A. Lloyd, J. Borevitz, C. Schwartz and L. Comai.

The collection of DNA stocks includes: A) 15,146 sequence-validated open Reading Frame (ORF) clones from SSP/J. Ecker, B) 2,950 ORF clones from C. Town, C) 2,086 Gateway™ expression clones from S. P. Dinesh Kumar, D) 61 multifunctional vectors that utilize the Gateway® system, E) 1,000 expression clones from S. Clouse, and F) 20,000 GST entry clones from Agrikola. The present ORF and cDNA collection represents 13,500+ genes. The SSP ORF collection was formatted to plates at ABRC, transferred to Gateway™ vectors, and validated by end sequencing at SALK; 1,899 of these clones have been received.

During the past year, ABRC distributed 85,000+ 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 50% of the DNA stocks being shipped.

ABRC is supported by the National Science Foundation.

P-422

Acquisition and Distribution of Stocks for Genomics and Phenomics by ABRC. Randy Scholl¹, Emma Knee¹, Luz Rivero¹, Deborah Crist¹, Zhen Zhang¹, Natalie Case¹, James Mann¹, Garret Posey¹, Pamela Vivian¹, Christopher Calhoun¹, Sarah Pfingsten¹, Kanika Johar¹. ¹The Ohio State University, Columbus, (OH), USA

The Arabidopsis Biological Resource Center (ABRC) acquires, preserves, maintains, and distributes many stocks relevant to genome exploration and functional genomics. Several flank-tagged insertion collections are available from ABRC, specifically: the T-DNA lines from a) Salk Institute, J. Ecker lab, b) Syngenta Biotechnology, including the lines originally associated with MTAs, c) the Wisconsin Ds-Lox population, d) GABI-Kat lines. RNAi lines from the AGRIKOLA consortium are also distributed.

Developing initiatives related to insertion lines and their application to phenomics include: a) receipt of 10,000 lines to date of the confirmed, homozygous collection from J. Ecker; b) incorporation of 2,000 homozygous T-DNA insertion lines from misc. donors, and c) efforts to make these lines available for large phenotypic studies.

21,222 full-length open reading frame (ORF) cDNA clones from SSP and Salk (J. Ecker), TIGR (C. Town), Peking/Yale and J. Callis are available. These represent ca. 13,500 loci. GST entry clones from the AGRIKOLA project are available and RNAi constructs will be received shortly. We have formatted 11,000 of the SSP/SALK and 1,153 of the Peking/Yale clones into plates. TIGR and AGRIKOLA entry clones were also received in plate format. We plan to distribute similar large collections in this format in addition to providing individual clones.

Clones of the Salk and SSP collections are presently being received in Gateway® Entry vectors. Eventually, ca. 11,000 clones from these collections will be available as sequence-confirmed entry clones. We are receiving many ORFs cloned into Gateway® expression vectors, 3,243 of these (from S. P. Dinesh Kumar and other donors) are currently in house. Versatile Gateway™ and Uni-vector destination vectors for various expression applications in plants, bacteria, insects, mammals and yeast are also available.

ABRC is supported by the National Science Foundation.

P-423

Arabidopsis Whole-Genome Transcriptome Analysis under Drought, Cold, High-Salinity, and ABA Treatment Conditions using Tiling Array and 454 Sequencing Technology. Motoaki Seki¹, Akihiro Matsui¹, Jong-Myong Kim¹, Junko Ishida¹, Maiko Nakajima¹, Taeko Morosawa¹, Makiko Kawashima¹, Masakazu Satou¹, Taiko Kim To¹, Yukio Kurihara¹, Eli Kaminuma², Takaho Endo², Yoshiaki Mochizuki², Norio Kobayashi², Tetsuro Toyoda², Kazuo Shinozaki¹. ¹RIKEN Plant Science Center, ²RIKEN Genomic Sciences Center

Plants respond and adapt to drought, cold and high-salinity stresses in order to survive. Many stress-regulated genes have been identified by the expression profiling studies using the DNA microarrays. However, we think that novel antisense RNAs, non-coding RNAs, small RNAs and chromatin remodeling mechanisms have additional functions in the regulation of plant responses to the stresses.

Recently, whole-genome tiling array and 454 sequencing technology have become powerful tools for the analysis of the whole transcriptome, including analyses of sense-antisense transcripts, non-coding RNAs, small RNAs and chromatin remodeling. Now we are also applying Arabidopsis Affymetrix tiling arrays and the 454 sequencing technology to study the whole transcriptome under drought, cold, high-salinity stress, and ABA treatment conditions. The tiling array experiments showed that many novel stress-responsive transcriptional units and antisense RNAs exist in the "Arabidopsis" genome. Large-scale analyses of the small RNAs by the 454 sequencing technology showed that the expression of several novel small RNAs are regulated by the stress. Functional analyses of the stress-responsive functional RNAs are in progress.

P-425

A novel gene, ("SEN10"), regulates senescence in *Arabidopsis thaliana*. Hamad Siddiqui¹, Salma Balazadeh², Barbara Klier¹, Bernd Mueller-Roeber³. ¹ University of Potsdam, Potsdam, Germany, ² MPI of Molecular Plant Physiology, Potsdam, Germany, ³ University of Potsdam & MPI of Molecular Plant Physiology, Potsdam, Germany

Leaf senescence is a unique developmental process which involves a transition from a functional photosynthetic organ to an actively degenerating and nutrient-recycling tissue. Senescence in plants is a highly regulated process and depends on the expression of many genes. To identify transcription factors (TFs) induced during natural leaf senescence in *Arabidopsis thaliana* we performed quantitative real-time PCR (qRT-PCR), allowing to analyse the transcript abundance of more than 1600 TF genes. Several TF genes were found to exhibit enhanced or reduced expression, respectively, in senescent *Arabidopsis* leaves. One of the TF genes selected for further studies was ("SEN10"). The SEN10 protein was observed to be localised in the cell nucleus when fused to green fluorescence protein (GFP) reporter in a transient expression assay in *Arabidopsis thaliana* protoplast. Using qRT-PCR, ("SEN10") was found to be strongly expressed in fully expanded and 20 % senescent leaves, whereas expression was low to moderate in juvenile leaves.

Promoter-reporter (GUS) studies confirmed senescence-dependent expression of the ("SEN10") gene. Constitutive over-expression of ("SEN10") under the control of the Cauliflower Mosaic Virus 35S promoter caused precocious senescence while RNAi lines exhibited a significant delay in leaf senescence. ("SEN10") transcript abundance was not significantly altered in WRKY53 knock-out lines, indicating that it functions independent of the WRKY53-mediated senescence signalling pathway. Promoter-GUS studies revealed a strong induction of ("SEN10") upon wounding of rosette leaves. Wound-induced ("SEN10") expression was confirmed by qRT-PCR, showing that expression was highest two hours after applying the wound stimulus. Recent experimental data will be presented.

P-424

Evolutionary Genomics of self-incompatibility in *Arabidopsis*. Kentaro Shimizu¹, Michael Purugganan². ¹University of Zurich, Institute of Plant Biology, ²North Carolina State University

While millions species are living on the earth with astonishing diversity, little is known about two fundamental questions regarding molecular basis of biodiversity. Which genes are responsible for adaptation and for speciation? What evolutionary processes affect allele frequency of these genes? The integration of molecular genetics, ecology and evolutionary genomics are critical to answer the questions. In particular, genomics bridges field ecology and laboratory molecular genetics in two ways. First, the isolation of ecologically- and evolutionary-relevant genes is facilitated by genomic tools. Second, genome-wide polymorphism is essential to deduce natural selection and population history affecting allele frequency of these genes.

As a case study using a model species *Arabidopsis thaliana*, we will talk about the evolution of selfing, which is one of the most prevalent evolutionary trends in flowering plants. In 1876, Charles Darwin proposed the reproductive assurance model to explain the prevalence of self-pollination in plants, suggesting that selfing can be evolutionarily advantageous when pollinators or mates are scarce in spite of in-breeding depression. In the selfing plant *A. thaliana*, pseudogenes at the SCR/SP11 and SRK self-incompatibility loci are shown to underlie the evolution of selfing. We found lowest nucleotide diversity at SCR in the genomic region. This suggests the positive selection on selfing and supports the Darwin's hypothesis. Parallel disruption of those genes and the timing will be discussed in conjunction with its population structure.

P-426

The Arabidopsis AtbZIP9 protein fused to the VP16 transcriptional activation domain alters leaf and vascular development. Amanda Bortolini Silveira¹, Luiz Gustavo Guedes Corrêa², Luciane Gauer¹, Juarez Tomaz¹, Michel Vincentz¹. ¹Centro de Biologia Molecular e Engenharia Genética-UNICAMP, Campinas (SP), Brazil, ²Institut für Biochemie und Biologie, Universität Potsdam, Potsdam, Germany

Transcriptional regulatory factors play an important role in controlling growth and development. bZIPs transcriptional factors have been reported to act in several different plant-specific processes such as organ development, cell elongation, defense mechanism, hormones and sucrose signalization, light response, control of nitrogen/carbon balance, flowering and the unfolded protein response. The Arabidopsis Group C bZIP transcriptional regulatory factors includes four members that are homologous to the maize *Opaque-2* regulatory locus. These four Arabidopsis bZIP were organized into three groups of orthologues each of which possibly representing an ancestral angiosperm function. To better define the evolution of group C functions we initiated the characterization of *AtbZIP9*, a single Arabidopsis gene corresponding to one of the three group C ancestral functions. We showed that *AtbZIP9* expression is restricted to phloem cells and that its mRNA accumulation is repressed by glucose and induced by abscisic acid and cytokinin. Reverse genetic approaches such as RNAi, knockout and superexpression suggest that post-transcriptional regulation and/or functional redundancy may act on *AtbZIP9*. To overcome the redundancy aspect, Arabidopsis transgenic lines expressing a fusion between the *AtbZIP9* cDNA and the VP16 transcriptional activation domain were produced. Transformants displayed leaf developmental defects, as well as metabolic and physiologic modifications, such as phenolic compound accumulation in mesophyl cells, early cell death and senescence symptoms. *AtbZIP9* can also be involved in the control of leaf and root vascular system development. We suspect that the observed alteration of leaf morphology and physiology possibly reflects consequences of changes in phloem transport properties, due to defects in vascular cylinder cell differentiation and organization.

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P-427

TILLING strategy to identify change of function mutations in Arabidopsis. Georg Strompen¹, Nadja H drich², John Lunn², Thomas Altmann¹. ¹University of Potsdam, Institute of Biochemistry and Biology, Department of Genetics, Potsdam, Germany, ²Max-Planck-Institute of Molecular Plant Physiology, Golm, Germany

The GABI-TILL project Arabidopsis use the TILLING (Targeting Induced Local Lesions IN Genomes) technology for the analysis of gene functions. The utility of TILLING will be enhanced by technical improvements and exploration of novel applications first tested in the Arabidopsis reference species. The value of mutants identified by TILLING will be demonstrated by the identification of mutant alleles of lead genes in the crop species like sugar beet and barley. These alleles will allow researchers to determine the importance of the analysed genes for traits of agronomic and commercial importance. Supported by international co-operation, we have implemented the TILLING technology and applies it now on a routine basis. An Arabidopsis EMS mutant collection (40.500 M1 plants) composed of approx. 7,500 M2 plants has been established, DNA has been extracted and pooled. A novel 2D pooling strategy was implemented that improved the mutant detection efficiency and reduced the number of necessary reactions. TILLING screens were performed for several genes of interest in "proof of concept studies" and hitherto 134 mutations were found in 11 genes. The 134 mutations were detected and 80 mutations were checked and confirmed by sequencing. This includes SPS2 (major isoform of sucrose phosphate synthase in leaves) and APS1 (catalytic subunit of ADP glucose pyrophosphorylase) genes which encode key regulatory enzymes of starch and sucrose synthesis, respectively. The aim is to generate a set of novel alleles of APS1 and SPS2. We are particularly interested in new forms of the enzymes with altered kinetic and regulatory properties substrate affinity, sensitivity to allosteric regulators or altered post-translational regulation.

To demonstrate the power of TILLING further "proof of concept" studies have been initiated. The mutant collections will be increased up to 10.000 M2 plants serving as the basis for a TILLING service carried out for interested users. For further information about the TILLING Service Arabidopsis visit our homepage: <http://www.gabi-till.de>.

P-428

Arabidopsis gateway compatible full-length cDNA library. Su Jeoung Suh¹, Sunghwa Choe¹. ¹Seoul National University, Seoul, Republic of Korea

A term plant hormone refers to signal molecules that regulate various processes such as growth, cell division, and stress responses. Thus exogenous application of hormones to plants should accompany induction of rarely-expressed genes. Concerning hormone metabolism and growth regulatory processes, great deal of metabolic and signal transduction pathways is already studied, but there are steps still waiting to be elucidated. To systematically study the function of the "unknown" genes in growth and development, we are isolating full length cDNA clones from Gateway-compatible library. RNAs were isolated separately from each different condition, such as auxin, gibberellin, cytokinin, salicylic acid, brassinolide, and callus, and mixed together to make cDNA. Adapter-ligated cDNAs were size fractionated using column to remove small fragment. Gateway system was used for cloning the cDNA for the purpose of sub-cloning into various expression vectors. As pioneering screening, we sequenced 100 clones and isolated more than 50 % full length cDNAs. Genome annotation analysis of the 100 sequences shows a similar distribution pattern to Arabidopsis whole genome analysis, indicating that the library represent the normalized cDNA clones reflecting the whole genome complexity.

P-429

An RT-PCR-based knockout screening system for rapid identification of transcription factors controlling nutrient uptake in *Arabidopsis*. Akinori Suzuki¹, Takashi Kuromori¹, Kazuo Shinozaki¹, Kazuki Saito^{1,2}, Hideki Takahashi¹. ¹RIKEN Plant Science Center, Yokohama, Japan, ²Chiba University, Chiba, Japan

Uptake of nutrients from the soil environment requires active ion transport systems in roots. During the past decade, numbers of transporters facilitating acquisition of major nutrients such as nitrate, ammonium, sulfate and phosphate have been identified from *Arabidopsis*. Among the isoforms of these functional nutrient uptake facilitators, high-affinity-type transporters in particular draw attention as they are regulated responding to nutrient stress and ideally localized in cell-types such as epidermis and root hairs having direct contact to the soil environment.

In general, uptake of nutrients correlates with the transcript levels of transporters in roots, emphasizing significance of their transcriptional regulation. However, only few transcription factors are known to participate in adjustment of this fundamental biological process. This perhaps originates from the difficulties to find clear visible phenotypes of plant nutrient responses or to measure observable changes of nutrient uptake activities in a large-scale experiment of mutant screening. In this study, we focused on transcription factors and established an RT-PCR-based knockout screening system to isolate mutants defective in regulating nitrate and sulfate transporters in *Arabidopsis* roots. All available candidates of mutants containing transposon insertions in transcription factors were collected from RIKEN collection (www.rage.gsc.riken.jp). Using RNAs from this mutant collection, we performed quantitative real-time RT-PCR of NRT2;1 nitrate transporter and SULTR1;2 sulfate transporter. Up to the present, approximately 300 homozygous insertion lines were tested, allowing us to identify a knockout of MADS-box transcription factor controlling the sucrose deficiency response of NRT2;1. In addition, we isolated a candidate of sulfur limitation response-less mutant from the screening using SULTR1;2 as a profiler gene. This screening system may further be utilized as a versatile tool to isolate mutants of transcription factors by applying any of the transcriptionally regulated genes as profilers.

P-430

Transcriptional analysis of the differentiated ovule and female gametophyte of *Arabidopsis thaliana* by Massively Parallel Signature Sequencing (MPSS). Nidia Sánchez-León¹, Mario Arteaga-Vázquez¹, Vianey Olmedo-Montil¹, Marcelina García-Aguilar¹, Vicenta García-Campayo¹, Víctor Pérez-España¹, Javier Menidiola-Soto¹, Ana Dorantes-Acosta¹, Mario Arteaga-Sánchez¹, Octavio Martínez-de la Vega¹, Kan Nobuta², Kalyan Vemaraju², Blake Meyers², Jean-Philippe Vielle-Calzada^{1,1} Laboratory of Reproductive Development and Apomixis, Langebio and Unidad Irapuato, Cinvestav, Campus Guanajuato, Irapuato M xico, ²Delaware Biotechnology Institute, University of Delaware Newark, DE, USA.

The female gametophyte of *Arabidopsis thaliana* is contained within the ovule and composed of seven cells: 3 antipodal, 2 synergids, the egg cell, and a binucleated central cell. We have developed a microaspirator-based strategy for ovule harvesting that allows the isolation of ~12 µg of total RNA in 2 hours. The technology was used to generate large collections of 21 nt mRNA-derived or non-coding RNA (ncRNA)-derived Massively Parallel Signature Sequencing (MPSS) tags. In wild-type ovules, a total of 9,649 genes were detected at significant and reliable expression levels; 1,373 are candidate to be preferentially expressed in the differentiated female gametophyte. To validate these results, we genetically screened 450 T-DNA lines corresponding to insertions in the same number of gene candidate to be specifically expressed in the female gametophyte. The identification of 20 new mutations (~4.4% of lines screened) suggests that our bioinformatic strategy was successful on identifying genes acting at the gametophytic level. Additional reverse-transcription PCR experiments (RT-PCR) and large-scale GUS translational fusions confirmed that many of these genes are specifically expressed in cells of the female gametophyte. The overall results are important to globally analyze the transcriptional universe present in the ovule prior to double fertilization.

P-431

Web services for *Arabidopsis* data integration. Chris Town¹, Dirk Haase², Hank Wu¹, Heiko Schoof². ¹The Institute for Genomic Research, Rockville MD USA, ²Max Planck Institute for Plant Breeding Research, Cologne, Germany

The ever increasing amount and complexity of *Arabidopsis* genomic data presents a growing problem that should be of concern to both the user and the bioinformatic community. Exploiting the full potential of these large and diverse datasets is currently hindered by the limited mechanisms of availability and the lack of integration. Web services provide a means whereby data residing at many different locations can be seamlessly integrated to provide the user with richer data sets. Unlike web pages that are idiosyncratic in their layout and content, and must be visited by a researcher one at a time, web services (in this case BioMOBY) provide data in well structured and agreed formats, document their availability through a central registry and can be combined to provide richer views of the data. Under a demonstration project encouraged by the Multinational *Arabidopsis* Steering Committee (MASC), our goal was to facilitate the deployment of web services from 16 data providers (eight each in Germany and in the USA) that vary both in the kinds of data that they host, and in the level of expertise at the sites. Training workshops for developers have been held both at the Max Planck Institute for Plant Breeding Research in Cologne and The Institute for Genomic Research in Rockville MD. These have both provided the attendees with the knowledge needed to deploy web services and have resulted in new services. In addition, the extensive "how to" documentation developed in conjunction with these workshops is freely available to the community (<http://bioinfo.mpi-zkoeln.mpg.de/araws>). Besides the new individual services, project bioinformaticians have developed "aggregator" web clients that can simultaneously invoke services from multiple locations in search of a particular data type (e.g. literature or images). The growing number of web services can also be combined into custom workflows using clients such as Taverna. Some example applications and tools will be highlighted and mechanisms to encourage a much more widespread use of web services discussed.

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P-432

The Arabidopsis 1-Aminocyclopropane-1-Carboxylic Acid Synthase (ACS) Gene Family. Atsunari Tsuchisaka¹, Takeshi Yamagami¹, Jose M. Alonso², Joseph R. Ecker³, Athanasios Theologis¹. ¹Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710, U.S.A. ²Department of Genetics, North Carolina State University, Raleigh, NC 27695, U.S.A. ³Salk Institute for Biological Studies, La Jolla, CA 92037, U.S.A.

1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE (ACS) catalyzes the rate-limiting step in the ethylene biosynthetic pathway in plants. The Arabidopsis genome encodes nine ACS polypeptides that form eight functional and one non-functional homodimers. Why are there so many ACS isozymes in Arabidopsis? We hypothesize that each member of the ACS gene family may have a distinct biological function. Here, we report various approaches for elucidating the role of each isozyme. Firstly, biochemical characterization of the various ACS polypeptides revealed that they are biochemically diverse. We think that the biochemical diversity defines a distinct biological function of each isozyme, which in turn defines its tissue specific expression. Second, functional intermolecular complementation experiments in *E. coli* show that all isozymes can form heterodimers. However, functional heterodimers are detected only among members that belong to the same phylogenetic branch. We propose that functional heterodimerization enhances the biochemical diversity of the ACS gene family; the non-functional heterodimers may have a regulatory role. Thirdly, analysis of promoter-GUS fusions reveals unique and overlapping expression patterns during plant development. This raises the prospect that functional ACS heterodimers may be formed in planta. Lastly, we have identified T-DNA insertion lines for seven (ACS1, ACS2, ACS4, ACS5, ACS6, ACS7 and ACS9) among the nine ACS genes; double and higher order mutations were constructed. The analysis reveals that ethylene produced by specific ACS isozymes play a central role in determining flowering time. The data suggest that isozyme specific ethylene production regulates the relative ratio of repressors/activators responsible for flowering time. These observations provide molecular insight into the unique and overlapping functions of the ACS gene family members in *Arabidopsis*.

P-433

Sorting sequences into the MapMan ontology: Using an automated pipeline to facilitate genome wide comparisons. Bjoern Usadel¹, Axel Nagel², Yves Gibon¹, Doerte Lodka¹, Oliver Thimm¹, Wolfgang Lein¹, Mark Stitt¹. ¹Max-Planck Institute of Molecular Plant Physiology, Golm, Germany, ²RZPD Deutsches Ressourcenzentrum für Genomforschung, Berlin, Germany

The recent advances in high-throughput sequencing and genomic technologies have made it possible to sequence full genomes or to obtain near full genome-scale EST clusters and compare them to the sequenced model plants *Arabidopsis* and rice. Furthermore, massively parallel sequencing of ESTs can be used to get an insight into the expression of genes within different tissues or under different conditions.

However, often when dealing with such high throughput data, or with EST sequencing projects it is difficult to classify these ESTs for tasks such as enrichment analysis or genome wide comparison of metabolic pathways. Here, we present a pipeline for automatic classification of sequences using the MapMan ontology. The pipeline uses a combination of machine learning and expert based rules for classification into the more than 1000 different MapMan classes and subclasses. It is currently available as a web-based tool, and will be equipped with a BioMOBY interface for integration into bioinformatics toolkits.

The resulting classes can then be used for over representation analysis and/or comparisons using *Arabidopsis* as a background model.

P-434

Analysis of gene expression modified by compatible virus infection: A comparative study between *Arabidopsis thaliana* and *Vitis vinifera*. Andrea Vega¹, Carmen Espinoza¹, Consuelo Medina¹, Patricio Arce-Johnson¹. ¹Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile. Santiago de Chile, Chile

In viral compatible interactions, pathogens spread through all plant tissues causing disease without resistance response associated. However, susceptible hosts are not passive against viruses and they can set up a defense response. Systemic viral infection causes several changes in plant gene expression, underlying the symptom development and also controlling virus levels. Molecular, cellular and physiological changes induced by viruses are part of the host defense response, but are not enough to stop viral replication and dissemination. With the aim of study the changes in gene expression triggered by compatible virus infection in diverse host plants, we compared the transcript profiles of two different models: *Arabidopsis thaliana* infected with TMV-Cg and *Vitis vinifera* infected with GLRaV-3. Changes in gene expression were evaluated using cDNA microarrays slides from the Arabidopsis Functional Genomics Consortium (AFGC). Complementary, transcript profiling of virus-infected grapevine was studied with the *Vitis vinifera* GeneChip from Affymetrix, validating the use of a heterologous microarrays system. We describe the identification of genes that are differentially expressed between infected plants and their corresponding healthy state in both models. Analysis of these genes permits to visualize the metabolic and cellular changes occurring during viral infection in *Arabidopsis* and *Vitis vinifera*, comparing the main processes affected. The most relevant changes in gene expression are associated with various biological functions including processes of translation, proteolysis, as well as modifications, targeting and folding of proteins. On the other hand, genes associated with leaf senescence and endomembrane system are significantly affected during infection in both models. Thus, the main biological and molecular processes triggered by viral compatible disease could be similar in different host plants.

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P-435

Molecular Analyses of the Plant WNK Gene Family and Evidence for their Regulation of Flowering Time in Arabidopsis. Yingxiang Wang¹, Huiyan Wan¹, Hong Liao¹, Chuxiong Zhuang², Hong Ma³, Xiaolong Yan¹. ¹Lab of Plant Nutritional Genetics, Root Biology Center, South China Agricultural University, Guangzhou 510642, China, ² Lab of Genetic Engineering, College of Life Science, South China Agricultural University, Guangzhou 510642, China, ³Department of Biology and Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA 16802, USA

The WNK (with no lysine kinase) protein kinase gene family was recently found as a novel gene family possibly involved in regulating the ion permeability of epithelia in mammals. However, WNK-like genes in plants have not been well characterized and their functions are largely unknown. We have previously identified a novel gene encoding a WNK homolog from soybean root through suppression subtraction hybridization (SSH). By searching the NCBI and TAIR databases, we found that there are 10 WNK genes in the *Arabidopsis* genome, designated as AtWNK1-10, respectively. The predicted sizes *Arabidopsis* WNK proteins range from 492 to 701 amino acids and they share 59% to 91% amino acid identities in the conserved kinase domain.

Phylogenetic analysis of *Arabidopsis* and rice WNK genes suggests that the most recent common ancestor of monocots and eudicots had 4 to 8 WNK genes, and that the WNK gene family may have experienced some duplication events, as well as possible gene losses. Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) experiments revealed that all *Arabidopsis* WNK genes except AtWNK6 are expressed in various organs from the seedling to flowering plant. T-DNA knockout mutations in the AtWNK2, AtWNK5 and AtWNK8 genes in different phylogenetic clades cause early flowering under long day conditions, suggesting that these genes may regulate flowering time. In contrast, a T-DNA knockout *wnk1* mutant exhibited a distinct phenotype, including severe growth retardation, rounded leaf shape, much delayed flowering time and somewhat shorter and thicker siliques compared with the wild type. Our results suggest that the *Arabidopsis* WNK gene family may be involved in regulating flowering time and other processes.

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The leucine-rich repeat receptor like protein family in *Arabidopsis*. Guodong Wang¹, Ursula Ellendorff², Chun-Ming Liu¹, Pierre J. G. M. de Wit², Gerco C. Angenent¹, Bart P. H. J. Thomma², Martijn Fiers¹. ¹Plant Research International, B. V., Business Unit of Bioscience, P. O. Box 16, 6700 AA Wageningen, The Netherlands, ²Laboratory of Phytopathology, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands

The "Clavata2" ("CLV2") gene in *Arabidopsis* encodes a leucine-rich repeat receptor-like protein (LRR-RLP) that assembles into a functional CLV1/CLV2 complex and is involved in restricting the number of stem cells in shoot apical meristem. The complex perceives the CLV3 signal and transduces it to the stem cell organizing centre to repress the expression of the stem cell-promoting *WUS* transcription factor. LRR-RLPs contain distinct domains: a predicted signal peptide, a variable number of extracellular leucine-rich repeats, a transmembrane domain and a short cytoplasmic tail that lacks any apparent signalling domain. Based on sequence and structural characteristics of CLV2 and TOO MANY MOUTHS (TMM), 57 LRR receptor-like proteins can be identified in the *Arabidopsis* genome. To investigate the function of these genes, we have identified homozygous T-DNA insertion lines for each gene. For most of the mutant lines, no obvious phenotype was found with respect to plant growth and development, susceptibility to pathogens, and sensitivity to various stress responses, suggesting that most of them are functionally redundant. However, knockout of "AtRLP41" leads to early leaf senescence with exogenous ABA. Loss-of-function mutation in "AtRLP10" ("CLV2") had pleiotropic effects with subtle changes in carpel number, more rosette leaves, shorter plants and failed to respond to the CLV3p/CLE19p peptide. "tmm-1" and T-DNA insertion mutant "AtRLP17" ("TMM") both display enhanced sensitivity to ABA. In addition, to determine the functional domains of CLV2, we have made several constructs in which different domains of CLV2 were deleted. Our data shows that the deletion of the island region does not affect the CLV2 function.

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The plant specific BPC/BBR family of GAGA-repeat Binding proteins. Dierk Wanke¹, Mareike Hohenstatt¹, Kenneth W. Barendzen¹, Sabine Hummel¹, Joachim Kilian¹, Üner Kolukisaoglu², Klaus Harter¹, ¹ZMBP-Pflanzenphysiologie, Universit t T bingen, Auf der Morgenstelle 1, 72076 T bingen, ²CELISCA (Center for Life Science Automation), Friedrich-Barnewitz-Strasse 8, D-18119 Rostock

BPC/BBR proteins comprise a novel class of transcription factors that are confined to the plant kingdom. They have recently been identified as essential key-regulators of homeotic gene expression in barley and *Arabidopsis*.

BPC/BBR-proteins have been identified due to their specific binding to a conserved element with its simple sequence repeat consensus of GA/TC dinucleotides. These proteins have properties of animal GAGA-binding factors, but they exhibit no sequence homologies to Trl and Psq of *Drosophila*, which encode functionally analogous proteins.

So far three distinct regions could be identified common to most BPC/BBR proteins: An N-terminal putative activation domain, a NLS and a highly conserved domain at its C-terminus, which mediates DNA-binding.

By structural means, the protein family can be subdivided into two groups which differ by their N-terminal domain. Similarly, phylogenetic analysis based solely on the DNA-binding domain sequence strongly supports the division into two distinct groups.

The basic DNA-binding domain, a 90 amino acid region, is structured as a typical zinc-finger-like motif putatively comprising two -sheets followed by an a-helix. A full genome target site analysis of putative binding motifs suggest a role in regulating other transcriptional regulators or auxin signalling related genes.

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Comparative Proteomics of Circadian and Flowering Proteins Between *Arabidopsis* and Rice. Duangdao Wichadakul¹, Prasit Palittapongarnpim^{1,2}, ¹BIOTEC Central Research Unit, National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand, ²Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand

Comparative genomics and proteomics reveal the conservation and diversification between genes and proteins among organisms. They shed light on their evolution and diversity. With the available *Arabidopsis* and rice proteomes, we deploy comparative proteomics to investigate the conservation and differentiation of circadian and flowering proteins between *Arabidopsis* and rice, the models organisms for the long day and short day plants. Steps in comparative proteomics includes: (1) compile all protein sequences of *Arabidopsis* and rice from TAIR1 and TIGR Rice Genome Annotation Database2 [1], (2) compile *Arabidopsis* gene ontology [2] from TAIR and GO terms and IDs from Gene Ontology3[3], (3) extract GO IDs and terms that are related to circadian rhythms, flowering (including flowering pathways such as vernalization), photoperiodism, and some other biological processes such as phosphorylation and response to cold, (4) extract all protein sequences in *Arabidopsis* that correspond to these GO IDs and terms, (5) PSI-BLAST [4] the extracted protein sequences to all proteins in rice. We analyzed and categorized the hit results based on the lowest e-values with the cutoff lower than 1e-40, the conserved domains and domain organizations using InterPro [5] and IntroProScan [6], and Gene Ontology. Our results confirm most results of comparative genomics studies in [7, 8] with several additional findings including the ortholog of *Arabidopsis* FRIGIDA protein in rice. Interestingly, InterProScan finds no hits domains for GI, ELF3, and EMF1.

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Completing the Expression Catalog of the Arabidopsis Transcriptome by Quantitative Real Time PCR. YONGLI XIAO¹, Beverly Underwood¹, Julia Redman¹, Wei Wang¹, Hui Quan¹, Hank Wu¹, William Moskal¹, Erin Monaghan¹, CHRIS TOWN¹. ¹The Institute for Genomic Research, A Division of the J. Craig Venter Institute, 9712 Medical Center Drive, Rockville, MD 20850, USA

Five sequential rounds of whole genome annotation of Arabidopsis at TIGR and one at TAIR produced a dataset that contained structural and functional annotation for 26,751 protein coding genes of which over 8,000 have both molecular function and biological process annotated as "unknown". Potential roles for many of these genes can be inferred by examining the correlation of their expression patterns with known genes and pathways. Publicly available datasets from Affymetrix ATH1 expression array combined with massively parallel signature sequencing have provided statistically significant expression values over diverse tissues for just over 22,000 distinct protein-coding genes. Because many of the genes of unknown function are expressed at levels that are too low to be effectively profiled by hybridization-based methods, our current NSF 2010 project is to generate expression profiles by quantitative real time PCR for all of the 4,000 + Arabidopsis genes for which expression data are unavailable. To date, we have performed quantitative real time PCR on over 1,000 genes that either lack reliable expression data from or are not represented on the ATH1 array using cDNAs from leaf, root and T87 cell culture and seedlings treated with IAA, SA and salt. Over 90% of the genes were expressed in one of our current cDNA populations and ~40% of them showed differential expressions in at least 2 out of 6 conditions. In addition, we have developed a high throughput pipeline to generate promoter-reporter constructs and transgenic Arabidopsis plants for 1,000 low-expressing genes of unknown function. So far, promoters from 812 genes have been cloned into a GFP reporter construct, 184 have been transferred into Agrobacterium and 82 have been transformed into Arabidopsis. Most of the transgenic plants examined to date show GFP expression localized to small regions of tissues and cell types. Both the qPCR and reporter construct data can be found at <http://www.tigr.org/tdb/e2k1/ath1/qpcr/index.shtml>

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Dual Autonomous Elements for the MITE mPing. Guojun Yang¹, Feng Zhang¹, C. Nathan Hancock¹, Susan Wessler¹. ¹University of Georgia

The first active miniature inverted repeat transposable element (MITE), mPing, was discovered by computer-assisted analysis of rice genome sequence. The mPing element is mobile in rice cell culture and in a few rice strains where it has amplified to over 1000 copies during recent domestication. However, determination of the transposase source and characterization of the mechanism of transposition have been hampered by the high copy number of mPing and the presence of several candidate autonomous elements in the rice genome. Here we report that mPing is active in *Arabidopsis thaliana* where its transposition is catalyzed by three sources of transposase from rice: the autonomous Ping and Pong elements and by a cDNA derived from a Ping transcript. In addition to transposase, the product of a second element-encoded ORF of unknown function is also required for mPing transposition. Excision of mPing in *A. thaliana* is usually precise and transposed copies usually insert into unlinked sites in the genome that are preferentially in or near genes. As such, this will be a valuable assay system for the dissection of MITE transposition and a potentially powerful new tagging system for gene discovery in eukaryotes.

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Small RNA-Directed Epigenetic Natural Variation Between *Arabidopsis* Ecotypes. Jixian Zhai^{1,2}, Jun Liu³, Pingchuan Li^{1,2}, Blake Meyers^{4,5}, Xuemei Chen⁶, Xiaofeng Cao¹, ¹State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P.R. China, ²Graduate University of the Chinese Academy of Sciences, Yuquan Road, Beijing 100039, P.R. China, ³School of Life Science, Shanghai University, Shangda Road 99, Shanghai 200436, P.R. China, ⁴Delaware Biotechnology Institute, ⁵Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19711, USA, ⁶Department of Botany and Plant Sciences, Institute of Integrative Genome Research, University of California, Riverside, Riverside, California 92508, USA.

Progress in epigenetics has revealed mechanisms which can heritably regulate gene function independent of genetic alterations¹. Nevertheless, little is known about the role of epigenetics in evolution. This is due in part to scant data on epigenetic variation among natural populations². In plants, small interfering RNA (siRNA) is involved in both the initiation and maintenance of gene silencing by directing heterochromatic modification and/or DNA methylation which could further influence gene expression³. Here we report that, in the model plant *Arabidopsis thaliana*, a cluster of ~24 nt siRNAs found at high levels in the ecotype *Landsberg erecta* (Ler) could direct DNA methylation and heterochromatinization at the promoter of *FLOWERING LOCUS C* (*FLC*), a major repressor of flowering⁴, whereas the same region in ecotype *Columbia* (Col) with almost identical DNA sequence, generates a set of low abundance siRNAs that do not direct these activities. DNA methylation of the Ler allele is hypersensitive to mutants in silencing pathway. A comparison of Ler and Col small RNAs identified at least 70 loci matched by significant level of ~24 nt siRNAs found specifically in Ler but not Col. These data reveal that there could be substantial epigenetic differences between two closely related *Arabidopsis* ecotypes, suggesting epigenetic variation may play an important role in the evolution of these and other lineages.

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Analysis of the pollen transcriptome changes during pollen germination and pollen tube growth in *Arabidopsis*. Wen-Zheng Zhang¹, Yi Wang¹, Lian-Fen Song¹, Jun-Jie Zou¹, Zhen Su¹, Wei-Hua Wu¹. ¹State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, National Plant Gene Research Centre (Beijing), China Agricultural University, Beijing 100094, China

Pollen germination as well as following pollen tube growth is an essential process for the reproduction of flowering plants. In addition, a germinating pollen with tip-growth characteristics, provides an ideal model system for the study of cell growth and morphogenesis. As an essential step towards a detailed understanding of this important process, the objective of this study was to comprehensively analyze the transcriptome changes during pollen germination and tube growth. This study, for the first time, analyzed the changes in transcriptome from desiccated mature pollen grains to hydrated pollen grains and to pollen tubes of *Arabidopsis thaliana* by using Affymetrix *Arabidopsis ATH1 Genome Arrays*. The number of expressed genes increased significantly from the desiccated mature pollen to the hydrated pollen and to the growing pollen tubes, which is consistent with the result that pollen germination and tube growth *in vitro* was significantly inhibited by transcriptional inhibitor. The results showed that expression of the genes related to signal transduction, cellular transport, and subcellular localization were significantly changed and the genes of several families, such as CaM/CML, CHX and Hsp, showed the most significant changes during pollen germination and tube growth. The presented results demonstrate that the overall transcription of genes, in both the number of expressed genes and the levels of transcription, was increased. Furthermore, the expression of numerous novel transcripts may be indicative of roles for these newly expressed genes in pollen germination and pollen tube growth.

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The Arabidopsis Chromodomain-containing Protein LIKE HETEROCHROMATIN PROTEIN 1 Colocalizes with Histone H3 Lysine27 Trimethylation. Xiaoyu Zhang¹, Sophie Germann², Bartlomiej J. Blus³, Sepideh Khorasanizadeh³, Valérie Gaudin², Steven E. Jacobsen^{1,4}. ¹ Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, Los Angeles, CA 90095, USA, ²Laboratoire de Biologie Cellulaire, IJPB, INRA, route de St Cyr, 78026 Versailles, France, ³ Department of Biochemistry and Molecular Genetics, University of Virginia Health System, Charlottesville, VA 22908, USA, ⁴Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA

The HP1 and POLYCOMB families of proteins are required for the establishment and maintenance of silent chromatin states mediated by histone H3K9 and H3K27 methylation in animals; however, little is known about these proteins in plants. By DamID-chip, we found that the Arabidopsis chromodomain-containing protein LHP1 localizes to chromatin associated with H3K27me3 genome-wide, and its chromodomain binds H3K27me3 with high affinity. These results suggest that LHP1 shares similar functions with POLYCOMB.

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EasyGO: Gene Ontology-based annotation and functional enrichment analysis system for agronomical species. Xin Zhou¹, Zhen Su¹. ¹College of Biological Sciences, China Agricultural University

Background: Interpretation of microarray experimental results has always been a difficult task. Recently a handful of tools have been developed for this need, but nearly none of them is designed to support agronomical species.

Results: We present EasyGO, a web server to perform Gene Ontology-based functional interpretation on groups of genes or microarray probe sets. EasyGO makes special contribution to agronomical research community by supporting Affymetrix GeneChips of both crops and farm animals, and is strengthened in result visualization and user interaction. Currently it supports 11 crops, 3 farm animals, and model plant *Arabidopsis*. We demonstrated EasyGO's ability to uncover hidden knowledge by analyzing a group of probe sets sharing common expression profile.

Conclusion: EasyGO is a good tool to help biologists to discover enriched biological facts that can provide solution or hints to original problem. It is freely available for all users at <http://bioinformatics.cau.edu.cn/easygo/>.

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Chemical genetic interrogation of natural variation. Yang Zhao¹, Tszfung Chow¹, Rachel Puckrin¹, Simon Alfred¹, Sean Cutler². ¹University of Toronto, ²University of California, Riverside

Natural sequence variation in drug metabolism and target genes can cause inter-individual or pharmacogenetic variation. Given the extensive metabolic variation documented between *Arabidopsis* accessions we reasoned that *Arabidopsis* could be used to identify natural drug sensitivity alleles, which in turn could facilitate the characterization of compounds discovered in chemical genetic screens. To test this, we subjected several *Arabidopsis* accessions to chemical genetic screens and discovered 12 polymorphic-hit molecules that induce accession-selective effects. The natural variation approach revealed that hypostatin, a new inhibitor of cell expansion, requires glycosylation for its bioactivity. Map-based cloning and population scans identified a prevalent SNP in HYR1, a UDP-glycosyl transferase (UGT), which explains most cases of natural resistance to hypostatin. Additionally, we show that HYR1 potentiates sensitivity to multiple xenobiotics. HYR1 is a member of the UGT-superfamily, which are pharmacogenetic factors in humans; thus natural UGT sequence-variation can modulate xenobiotic sensitivity across biological kingdoms. Our results demonstrate that natural variation that can be exploited to inform the biology of new small molecules. In addition to describing our natural variation work, in our workshop talk we will provide an update on community-targeted chemical genomic resources developed in our lab.

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Molecular and functional characterization of Nucleosome Assembly Protein 1(NAP1) family proteins in plant. Yan ZHU¹, Ziqiang LIU¹, Juan GAO¹, Yu YU¹, Wen-Hui SHEN², Aiwu DONG¹. ¹Department of Biochemistry, School of Life Sciences, Fudan University, Shanghai, China, ²Institut de Biologie Moleculaire des Plantes, Centre National de la Recherche Scientifique, Strasbourg, France

Plant growth is an iterative process of cell proliferation and differentiation in response to intrinsic genetic program and environmental signals; therefore, accurate duplication of genome in cell cycle as well as dynamic regulation on chromatin conformation plays critical biological roles. Current knowledge considers nucleosome as the fundamental unit of chromatin in structure and function, and several factors involved in nucleosome assembly have been identified, such as Nucleosome Assembly Protein 1 (NAP1). NAP1 is highly conserved in eukaryotes, while there is only one copy of NAP1 gene in yeast, plant genomes evolve out NAP1 family of multiple genes encoding NAP1 homologues and NAP1-Related Proteins (NRP). Our studies show that plant NAP1 and NRP proteins bind histones *in vitro* and *in vivo*, and present expression patterns related to proliferating state, consistent with their biological roles in nucleosome assembly. Microarray analysis shows that both NAP1 and NRP modulate gene expression in genome scale, however, with overlap in low level. Furthermore, *Arabidopsis* *nrp* double mutant is defective in root growth with cell cycle arrest in root tip after germination; additionally, its genome is more sensitive to genotoxic stress compared with that of wild-type, indicating NRP functions in chromatic duplication and structural maintenance. Our recent work shows that *nap1* triple mutant is insensitive to ABA treatment during seed germination, implying plant NAP1 proteins are involved in phytohormone regulation.

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Global analysis of natural target genes of NMD in *Arabidopsis thaliana*. Masato Yoine¹, Maya Akahori¹, Koichi Hori^{2,3}, Yuichiro Watanabe², Kenzo Nakamura¹. ¹Grad. Schl. Bioagric. Sci., Nagoya Univ., ²Dep. of Life Sci., Tokyo Univ., ³Life Sci., College Sci., Rikkyo Univ.

Nonsense-mediated mRNA decay (NMD) is an mRNA surveillance mechanism that recognizes mRNAs containing premature termination codons (PTCs) and targets them for degradation. In addition to mutation-derived PTCs, various forms of natural transcripts, either with or without PTC, are targets of NMD, and 4% to 10% of total genes in yeast, *Drosophila*, and human are estimated to be subjected to post-transcriptional regulation by NMD. Although major factors of NMD, namely UPF1, UPF2, and UPF3, are highly conserved among eukaryotes, the mechanisms to discriminate PTCs from natural termination codons are not evolutionarily conserved, and target genes of NMD are different among various eukaryotes. In *Arabidopsis*, missense mutant of *UPF1*, *Iba1/atupf1-1*, exhibits pleiotropic phenotypes such as anomalies in growth, flowering time, sugar signaling, and seed size (1,2), and null mutant of *UPF1* is seedling lethal (2). The T-DNA insertion line of *UPF3*, *atupf3-1*, also exhibits morphological aberration (3). These observations indicate that NMD plays important roles in plant growth and development. We identified putative natural target genes of NMD in *Arabidopsis* by comparing transcript levels in Col-0, *Iba1* and *atupf3-1* using Affymetrix ATH1 microarray. Approximately 2% of total genes analyzed showed more than 1.5-fold enhancement of transcript levels in both *Iba1* and *atupf3-1* compared to Col-0. These putative targets of NMD did not show significant bias towards genes with particular function, while transcripts that are up-regulated in both mutants were enriched with specific structural features. For example, 23% of these genes contained PTC-containing transcript that is generated by alternative splicing. Nearly 40% of genes contained intron in the 5'-UTR region of the gene, and 50% of genes contained uORF for longer than 10 amino acids in the 5'-UTR. Experiments with cordycepin suggested that increased levels of target transcripts in *Iba1* was due to enhanced mRNA stability. (1) Plant J. 47; 49 (2006), (2) Plant Cell Physiol. 47; 572 (2006); (3) Plant J. 43; 530 (2005).

Hormonal Responses**P-448**

Comparative investigation of the glucosinolate-myrosinase defense system in *Arabidopsis* hypocotyls and cell suspensions cultures. Sophie Alvarez¹, Yan He¹, Emily Wang¹, Xiu-Feng Yan², Sixue Chen^{1,2}. ¹University of Florida, Gainesville, FL32605, USA, ²Northeast Forestry University, Harbin 150080, China

Glucosinolates are secondary metabolites, derived from amino acids, found in many plants of the order Capparales in which *Arabidopsis thaliana* belongs. Upon hydrolysis by myrosinases, they produce a variety of biologically active compounds, such as isothiocyanates which showed fungicidal, nematocidal and herbicidal effects. In this study, the Glucosinolate-Myrosinase Defense System (GMDS) was studied in *Arabidopsis* suspensions cells and intact tissues treated with methyl jasmonate (MeJA), a signaling compound induced by herbivore attack and wounding to initiate plant defense mechanisms. In order to test whether the GMDS in suspension cells works similarly to that in planta, hypocotyls from 10 days old seedlings, where the suspension cells were derived, were used for the study at the same time. Among the 16 different glucosinolates identified in hypocotyls, the levels of indole glucosinolates were induced by MeJA as expected. In addition, we found that the levels of aliphatic glucosinolates, especially, 4-methylsulfinylbutyl and 8-methylsulfinyloctyl-glucosinolates, were significantly increased by MeJA treatment. We identified 8 glucosinolates in suspension cells. They showed different profiles and responses when treated with MeJA. The transcripts of several genes involved in the biosynthesis of different glucosinolates were analyzed and found to be induced in both suspension cells and hypocotyls after MeJA treatment. Myrosinase levels and activities were also monitored in the two systems. To systematically investigate changes in metabolic pathways and connect MeJA signaling and the GMDS, we have initiated proteomic analysis of MeJA signaling and metabolic network in the two systems. Recent results will be presented and discussed.

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THE IMPACT OF TEMPORAL AND SPATIAL REGULATION OF THE IPT GENE EXPRESSION BY VARIOUS PROMOTERS ON LEAF SENESCENCE AND WATER STRESS TOLERANCE. Idit Banner¹, Shimon Geprstein¹. ¹Techion-Israel Institute of Technology, Haifa, ISRAEL

A most dramatic transgenic intervention leading to a delay senescence phenotype concerns the manipulation of levels of endogenous cytokinins. An effective current approach is the autoregulation of cytokinins synthesis, based on a specific promoter of a senescence gene - SAG12. Surprisingly, this autoregulatory system did not show delay of leaf senescence in *Arabidopsis*. Two promising strategies for delaying senescence in tobacco and *Arabidopsis* plants are presented. The autoregulated biosynthesis of cytokinins, mediated by the expression of a isopentyltransferase (IPT) gene driven by an inducible early-senescence/late maturation promoter (SARK, senescence-associated receptor kinase), resulted in the remarkable delay of senescence of tobacco plants. Additional approach is the estrogen-inducible XVE system of the IPT gene for the production cytokinins in *Arabidopsis* transgenic plants. These transgenic plants displayed altered seedlings development and delayed leaf senescence.

Most surprisingly, the tobacco transgenic plants also displayed water stress tolerance as reflected by vigorous growth after a severe drought (18 days without watering). The transgenic plants retained photosynthetic activity during drought and did not show chlorophyll degradation. After rewetting, the plants recovered photosynthetic activity and active growth. Our results showed that plants expressing SARK::IPT, cytokinins were synthesized in the whole plant and transported to the bottom.

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AtPGP4 Facilitates Auxin Efflux in Arabidopsis Root Hair Cells and Tobacco Cells. Misuk Cho¹, Sang Ho Lee¹, Hyung-Taeg Cho¹. ¹Department of Biology, Chungnam National University, Daejeon 305-764, Korea

Formation of local auxin maxima through polar auxin movement has been implicated in plant growth and development. The polar transport of auxin is achieved by cooperation of polarly localized influx and efflux carriers particularly such as AUX1 (and LAXs) and PINs. On the other hand, albeit less polarized in their subcellular localization, some ABC transporters of multidrug resistance P-glycoprotein (MDR/PGP) subfamily, are involved in cellular auxin transport as well. AtPGP1 and AtPGP19 showed auxin efflux activities in plant cells whereas AtPGP4 was reported to catalyze auxin influx in animal and yeast cells. However, the phenotype of root hairs, whose elongation is enhanced by elevated intracellular auxin levels, implied an efflux activity of AtPGP4. We expressed AtPGP4-YFP (PGP4ox) using the root hair cell-specific Arabidopsis expansin7 promoter. PGP4ox developed shorter root hairs than control, which was expected from lowered auxin levels in the root hair cell by the auxin efflux activity of PGP4. The root hair phenotype of PGP4ox was similar to that of PGP1ox but opposite to that of AUX1ox. The efflux activity of AtPGP4 was further confirmed by using the tobacco BY-2 suspension cell system. AtPGP4 in tobacco cells showed a NPA-sensitive auxin efflux activity. The localization of PGP4-YFP in the plasma membrane is non-polar in Arabidopsis root hair cells and tobacco cells. A brefeldin-A (BFA) treatment caused internalization of PGP4-YFP as did PINs. These results suggest that AtPGP4 mediates auxin efflux in plant cells, differently from heterologous systems, and its endocytotic cycling could be mediated by BFA-sensitive trafficking machinery.

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A nuclear protein interaction cascade mediating coordinated regulation of Arabidopsis seedling development by plant hormone gibberellins and light signal. Suhua Feng¹, Cristina Martinez¹, Yu Wang², Liying Chen³, Giuliana Gusmaroli¹, Juan M. Iglesias-Pedraz⁴, Feng Wang³, Lu Yu³, Xiangdong Fu⁵, Liu-Min Fan², Xing Wang Deng¹. ¹Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, USA, ²Pei-king-Yale Joint Center for Plant Molecular Genetics and Agrobiotechnology, and National Laboratory for Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing, China, ³National Institute of Biological Sciences, Zhongguancun Life Science Park, Beijing China, ⁴Centro Nacional de Biotecnología-CSIC, Madrid, Spain, ⁵Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Gibberellins (GAs) are plant hormones implicated in different aspects of plant growth and development. DELLA proteins are repressors of GA responses and their stability is controlled by the ubiquitin-26S proteasome pathway. However, it is not well understood how GA triggers the degradation of DELLA proteins within a cell, and how the GA signal is transduced into DELLA's regulation of gene expression. Here, we characterize a nuclear protein-protein interaction cascade mediating transduction of GA signals to the activity regulation of a light-responsive transcription factor in Arabidopsis. In the absence of GA, nuclear localized DELLA proteins accumulate to higher level, interact with phytochrome-interacting factor 3 (PIF3, a bHLH-type transcription factor), and prevent PIF3 from binding to its target promoters, therefore abrogating PIF3-mediated light control of hypocotyl elongation of Arabidopsis seedlings. In the presence of GA, three largely redundant GA receptors (GID1 proteins) elevate their direct interaction with DELLA proteins in the nucleus, trigger DELLA's ubiquitination and proteasome-mediated degradation, and thus release the negative effect of DELLA proteins on PIF3. Therefore, DELLA proteins integrate the regulatory effect of GA and light on gene expression and plant development through modulating the activity of some light-responsive transcription factors like PIF3.

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Multiple phosphate starvation responses are modulated by GA signaling in Arabidopsis. Xiangdong Fu¹, Caifu Jiang¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P R China

Phosphate (Pi) is an essential macronutrient for plant growth, development and reproduction. Although the total amount of phosphorus in the soil maybe high, Pi is unavailable for plants uptake because of its low mobility. Plants have developed a number of adaptive physiological and biochemical strategies to cope with low Pi availability. Here we report that phosphate starvation induced responses are in part dependent on a family of nuclear growth-repressing DELLA proteins, the central components in GA signaling in Arabidopsis. We show that Pi deficiency promotes the GFP-tagged DELLA (GFP-RGA) accumulation in root nuclei but does not delay GA-mediated 26S proteasome-dependent DELLAs destruction. We show that Pi starvation induced multiple responses including root architecture, plants growth, anthocyanin induction and late flowering can be repressed by exogenous GA treatment. Lacking quadruple DELLA proteins in Arabidopsis gai-t6 rga-t2 rgl1-1 rgl2-1 mutant, also results in Pi starvation insensitivity. Whereas, enhanced DELLA functions, such as ga1-3 mutant, cause the hypersensitive responses under Pi starvation, suggesting that DELLA-mediated signaling is essential for Pi starvation responses in plant. Furthermore, these changes during phosphate starvation are paralleled by alterations of bioactive GA level by increases of GA-2ox2 transcripts and decreases of both GA-20ox1 and GA3ox1 transcripts. In addition, we investigate the role of GA in formation of root hairs. GA-deficient ga1-3 mutant was significant decreases in the length of root hairs but not frequency. However, it can be restored by exogenous GA treatment or lacking DELLA functions, suggesting that appropriate GA level is essential for root hairs development. These results demonstrate that down-regulation of active GA by low Pi availability is one of adaptively significant mechanism via which DELLAs modulating multiple phosphate starvation responses.

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Impairment in the mRNA degradation causes abnormal response to both abscisic acid and salicylic acid in Arabidopsis. Takashi Hirayama^{1,2}, Yoshihiro Narusaka³, Michiko Yasuda¹, Mari Narusaka³, Shimpei Hayashi^{1,2}, Nobutaka Kitahata⁴, Takashi Kuromori⁵, Tadao Asami¹, Kazuo Shinozaki⁶. ¹RIKEN, Wako, Japan, ²Yokohama City Univ., Yokohama, Japan, ³RIBS Okayama, Okayama, Japan, ⁴Univ. of Tokyo, Tokyo, Japan, ⁵RIKEN PSC, Yokohama, Japan

In eukaryotic cells, the stability of each mRNA is tightly regulated. Once mRNA enters the degradation pathway, its polyA is firstly removed for further degradation. Eukaryotes usually have three polyA removal activities. At the moment, it is obscure that they have distinct functions or not. In plants, disruption mutation of polyA specific ribonuclease (PARN) causes lethality, indicating its importance in plant systems. However, its physiological role has not been elucidated. By analyzing the ABA hypersensitive germination2 mutants, we showed that the reduction of Arabidopsis PARN (AtPARN/AHG2) activity causes multiple effects on the plant physiology including dwarf phenotype, higher accumulation of endogenous ABA in seeds and stressed plants, and constitutive activation of the SA response pathway (1). We further confirmed the enhanced SA response in the ahg2-1 mutant by showing its higher endogenous SA level and enhanced resistance to pathogens. In addition, several SA defective mutations partially suppress the dwarf phenotype of ahg2-1. These results suggest that PARN in plant is deeply involved in both biotic and abiotic stress responses. To address the molecular basis for AtPARN/AHG2 in hormone response, we are trying to identify the target genes by microarray experiments and genetic approaches, eg. isolating suppressor mutants for ahg2-1. Interestingly, some putative suppressor mutants show normal root growth, ABA insensitivity, and normal SA levels, although any ABA insensitive mutations or SA insensitive mutations could not give such effect as far as we examined. The function encoded by these suppressor loci will be discussed.

(1) Nishimura et al., Plant J., 44, 972-984, 2005.

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The FORKED1 gene is expressed in preprocambium and responds to auxin. Hongwei Hou¹, Elizabeth Schultz¹. ¹Department of Biology, University of Lethbridge, Alberta, Canada

We have previously described plants mutant for FORKED1 (FKD1), which have reduced leaf response to auxin and show lack of distal vein meeting in leaves. Our analysis of fkd1-1 has allowed us to propose a model whereby FKD1 is necessary for the auxin response that directs vein patterning. We have now identified FKD1 through map-based cloning. Several SALK lines, all corresponding to insertions in a single gene, had fkd1-1-like phenotypes and did not complement fkd1-1. Transformation of fkd1-1 plants with the wild type FKD1 gene resulted in a reversion to the wild type phenotype, indicating that we have indeed identified the FKD1 gene. FKD1 encodes a hypothetical protein with a pleckstrin homology (PH) domain, suggesting the involvement of FKD1 in IP3 signal transduction. We fused the region 1.8kb upstream of FKD1 to the reporter gene β -glucuronidase (PFKD1::GUS) and compared expression to PAtB8::GUS, a pre-procambial marker and to PDR5::GUS, a marker of auxin response. In leaves, PFKD1::GUS and PAtB8::GUS are expressed at the same time in the tip. Whereas AtB8::GUS expression in secondary veins progresses primarily acropetally, PFKD1::GUS progresses basipetally. To test if FKD1 is responsive to changes in auxin, we treated seedlings with auxin transport inhibitors NPA or NOA or with the synthetic auxin 2, 4-D. In all cases, PFKD1::GUS expands coincident with PDR5::GUS in leaves. During embryogenesis, PFKD1::GUS expression is first evident in late torpedo stage at the cotyledon tip and progresses basipetally, and then in secondary vein loops. This expression is similar to that of DR5::GUS, although unlike PDR5::GUS, PFKD1::GUS is not expressed in the embryonic root. In contrast, PAtB8::GUS begins earlier, in heart stage embryos, in cells anticipating veins in root and shoot. In roots of seedlings, PFKD1::GUS is expressed in vascular tissue and in vascular tissue precursors. As in leaves, treatment with NPA, NOA or 2, 4-D alters PFKD1::GUS root expression. The results indicate that FKD1 is a pre-procambial cell marker, whose expression is related to auxin response.

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Role of farnesylation of adenylate Isopentenyltransferase (AtIPT3) in control of enzyme targeting and cytokinin levels in Arabidopsis. Klára Hoyerová¹, Arnaud Galichet², Miroslav Kamínek¹, Wilhelm Gruissem². ¹Institute of Experimental Botany AS-CR, Prague, Czech Republic, ²Institute of Plant Sciences, ETH Zurich, Switzerland

Posttranslational prenylation of proteins facilitates their interactions with some other proteins and cell membranes. A number of prenylated plant proteins are involved in regulation of processes controlling plant development, signaling and responses to environmental stimuli. We report that plant isopentenyl transferase (AtIPT3) that catalyzes biosynthesis of isoprenoid cytokinins, is a farnesylated protein as it was confirmed by "in vitro" and "in vivo" farnesylation assays. Green fluorescent protein fused to AtIPT3 and expressed in Arabidopsis plants was localized in nucleus while it was accumulated in plastids when expressed in Arabidopsis "era1-2" mutant lacking functional protein farnesyl transferase (PFT). Ectopic expression of "AtIPT3" in Arabidopsis resulted in 4-fold increase in cytokinin levels. The increase was accompanied with preferential (11-fold) accumulation of isopentyl-type cytokinins namely of isopentenyladenine 7-glucoside. Expression of AtIPT3, in which acceptor cysteine was replaced with serine, significantly reduced cytokinin accumulation. This modification evidently cancelled the enzyme catalytic activity as indicates the lack of cytokinin accumulation in yeast and "E. coli" overexpressing AtIPT3 and modified AtIPT3 with serine. Evidently, the acceptor cysteine is essential not only for attachment of farnesyl moiety but also for AtIPT3 catalytic activity. Surprisingly, lack of PFT activity in the Arabidopsis "era1-2" mutant enhanced cytokinin levels indicating that farnesylation of AtIPT3 may slightly reduce the enzyme catalytic activity.

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Systemic and Intracellular Responses to High Light-Induced Oxidative Stress. Dawar Hussain¹, Pip Wilson¹, Jan Bart Rosell¹, Osman Mewett¹, Kate Howell², Jim Whelan², Kemal Kazan³, Barry Pogson¹. ¹ARC Centre of Excellence in Plant Energy Biology, Australian National University, ²ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, ³CSIRO Plant Industry, Australia

Since plants are unable to avoid oxidative damage caused by excess light they employ a repertoire of photoprotective measures which include minimizing light absorption, minimizing ROS over-accumulation, and repairing damaged proteins, lipids and photosystems. A large suite of genes are induced by excess light and many encode proteins that function in the aforementioned processes. We have been investigating the function of the transcription factor ZAT10 in modulating responses to oxidative stress. ZAT10 participates in the regulation of reactive oxygen species homeostasis, photosynthetic rates and multiple antioxidative processes in different organelles.

Additionally, we have used ZAT10 promoter:LUCIFERASE fusions to study systemic acquired acclimation (SAA). SAA is a novel systemic signal reported by Karpinski and colleagues (Karpinski et al., 1999) that resulted in the induction of ascorbate peroxidase 2 (APX2) gene expression in shaded leaves of a plant partially exposed to excess light. We have demonstrated that this signal can move substantial distances, namely from rosette to cauline leaves, and that several hundred genes are induced within 30 minutes of exposure to high light. Analysis of the arrays by clustering to stress and hormone responsive experiments and testing the induction of the systemic response in hormonal and other signalling mutants has provided insight into which hormones and processes may participate in the systemic signalling of oxidative stress.

Karpinski, S., Reynolds, H., Karpinska, B., Wingley, G., Creissen, G., and Mullineaux, P. (1999). Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. *Science* 284, 654-657.

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DHigh-sensitive and High-throughput Plant Hormone Analysis by LC-MS/MS. Yusuke Jikumaru¹, Atsushi Hanada¹, Eiji Nambara¹, Shinjiro Yamaguchi¹, Yuji Kamiya². ¹RIKEN Plant Science Center, Yokohama, Japan, ²RIKEN

Plant hormones interact each other and regulate many aspects of plant growth and development. Therefore it is important to analyze all the hormones from the same plant materials. We used to use GC-MS for hormone analysis. However, this method is not necessarily suitable for high-throughput and comprehensive hormone analysis. We have recently introduced a LC-ESI-MS/MS system, which does not require derivatization of hormones. Using both GC-MS and LC-MS we have performed comprehensive hormone analysis. We could identify 16 different GAs by a simple LC-MS from *Arabidopsis*. (Varbanova M. et al. *Plant Cell* 2007) ABA and PA, DPA and ABA-GE were analyzed from submerged rice shoots (Saika H. et al. *Plant Cell Physiol.* 2007). In a special case, we could quantify ABA even from a single seed of *Arabidopsis cyp707a1/a2/a3*.

Analysis of cytokinins by LC-MS is already established in RIKEN PSC. We are currently analyzing auxins, jasmonic acid and salicylic acid. Although brassinosteroids are still difficult to analyze with high-sensitivity, recently we could identify castasterone from tomato fruit by LC-MS (ca. 1g fresh weight). This method is at least 20 times more sensitive than a conventional GC-MS. We will also report the present situation of the hormone analysis platform of RIKEN PSC. For collaborative research on hormone analysis, contact Yuji Kamiya, ykamiya@postman.riken.jp

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Ethylene-Induced Opposite Redistributions of Calcium and Auxin are Essential Components in the Development of Tomato Petiolar Epinastic Curvature. Soo-Hwan Kim¹, Yew Lee¹, Jin-Woo Jung¹, June-Seung Lee², Seong-Ki Kim². ¹Department of Life Science, Yonsei University, Wonju-Si 220-710, Korea, ²Department of Life Science, Ewha Womans University, Seoul 120-750, Korea, ³Department of Life Science, Chung-Ang University, Seoul 156-756, Korea

Calcium has been suggested as an important mediator of gravity signaling transduction within the root cap statocyte. In a horizontally-placed root, it is redistributed in the direction of the gravity vector (i.e. it moves downward) and is closely correlated with auxin downward movement. However, the involvement of calcium in the regulation of ethylene-induced epinasty and auxin movement is not known. In this report, we examined the involvement of calcium in lateral auxin transport during ethylene-induced epinasty in an effort to understand the relationship among calcium, auxin, and ethylene. Ethylene-induced epinasty was further stimulated by exogenously applied Ca²⁺, the calcium effect being the strongest among divalent cations tested. Pretreatment with NPA, an auxin transport inhibitor, negated the promotive effect of calcium ions on the petiolar epinasty. Ethylene caused redistribution/ differential accumulation of 45Ca²⁺ toward the morphologically lower (abaxial) side of the leaf petioles, an effect opposite to that of 14C-IAA redistribution. Verapamil, a Ca²⁺ channel blocker, inhibited ethylene-induced epinasty, as well as the redistribution of 14C-IAA and 45Ca²⁺. When the petiole was inverted in the presence or absence of ethylene, the direction of 45Ca²⁺ differential accumulation was still toward the morphologically abaxial side of the petiole during epinastic movement regardless of gravitational direction. These results suggest that gravity-insensitive, ethylene-induced Ca²⁺ redistribution and accumulation toward the abaxial side are closely coupled to the adaxial auxin redistribution/ accumulation and, in turn, to the petiolar epinasty.

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Regulation of the Elongation and Gravitropic Responses of *Arabidopsis* Roots by Brassinolide and IAA. Tae-Wuk Kim¹, Seong-Ki Kim¹, Soo Chul Chang². ¹Department of Life Science, Chung-Ang University, Seoul, Korea, ²University College, Yonsei University, Seoul, Korea

Interactive effects of brassinolide (BL) with IAA on root gravitropic response and root elongation were investigated in "*Arabidopsis thaliana*". At various concentration exogenously applied BL increased gravitropic curvature but showed optimal curve in root elongation. The stimulatory effect of BL was evident at later stage of the gravitropic response. However, "BRI1-GFP" plants, which carry two copies of BRI1, exhibited a greater gravitropic curvature response and showed more root elongation. In contrast, the two parameters in "bri1-301" plants were less than those for wild-type (Col-0) plants.

When the mutant plants were treated with various concentrations of IAA, the gravitropic response of the "bri1-301" plants was increased in a dose-dependent manner, attaining the same curvature response as "BRI1-GFP" plants. In wild-type and "BRI1-GFP" plants, it changed only slightly. When the plants were treated with NPA or auxin-transport mutants were tested, the gravitropic responses to BL dose were greatly affected. The BL-related mutants exhibited differential gene expression of CYP79B2, a IAA biosynthetic enzyme. Taken together, BL seems to affect the gravitropic response at least in part by modulating endogenous IAA level. The patterns of root elongation for the BL-related mutant plants were not appreciably changed by treatment with different concentrations of IAA. Also, a result that ethylene reduced the elongation indicates that BL effect on the elongation is mediated by ethylene. Taken together, our results indicate that BL interacts with IAA in interdependent and partially additive ways in the processes of root gravitropic response and root elongation, respectively.

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Cross-talk between SA- and JA-dependent defense pathways coincides with a cellular increase in glutathione. Annemart Koornneef¹, Tita Ritsema¹, Floor C. Den Otter¹, Adriaan Verhage¹, L.C. Van Loon¹, Corn M.J. Pieterse¹. ¹Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, P.O. Box 800. 84, 3508 TB Utrecht, the Netherlands

The signaling hormones salicylic acid (SA) and jasmonic acid (JA) are important molecules that mediate inducible defense mechanisms in *Arabidopsis*. They each activate a differential set of defense-related genes, which are effective against a variety of pathogens and/or insects.

SA has an antagonistic effect on JA signaling, a process called pathway cross-talk, which allows the plant to fine-tune its defense response. Analysis of mutant *npr1-1*, which is impaired in SA-dependent systemic acquired resistance (SAR), revealed that the down-regulation of JA-responsive gene expression requires the regulatory protein NPR1.

Cross-talk was studied by applying the chemicals SA and methyl jasmonate (MeJA). Simultaneous treatment with these chemicals within hours results in down-regulation of JA-responsive *PDF1.2* expression, and lasts up to several days.

However, in nature, *Arabidopsis* might trigger SA- and JA-dependent defenses at different time-points. Therefore, we applied SA at several time-intervals before application of MeJA, and monitored *PDF1.2* expression. SA appeared to have a window of opportunity to suppress *PDF1.2*, which ranges between 0 and 30 hours before MeJA application. SA treatment more than 30 hours before MeJA did not trigger cross-talk, even though SA-responsive PR-1 expression was still detectable. Thus, SA triggers a transient response that mediates suppression *PDF1.2* gene expression.

Since induction of SAR and activation of NPR1 require a change in the redox state of the cell, we hypothesized that the redox state might be involved in this transient response. Therefore, we monitored the total levels of glutathione, a major redox buffer in plant tissue, upon application of SA. SA triggered a transient increase in glutathione levels, which returned to baseline after approximately 30 hours. Interestingly, this transient glutathione peak correlated with the time-frame in which SA was capable of suppressing *PDF1.2*.

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First insights into the functions of CUL3-based ubiquitin ligases in Arabidopsis. Esther Lechner¹, Alexis Thomann¹, Eva Dumbliauskas¹, Maureen Hansen², Joseph Kieber², Pascal Genschik¹. ¹IBMP, Strasbourg, France, ²University of North Carolina, department of Biology, Chapel Hill, NC, USA

Regulation of protein stability through the ubiquitin proteasome system (UPS) is now considered as a major mechanism underlying many cellular and organismal processes, such as cell division, DNA repair, developmental pathways, important parts of immune defence and in plants, light and phytohormone signal transduction. Degradation via the UPS is a two-step process: the protein is first tagged by covalent attachment of ubiquitin and subsequently degraded by a multicatalytic protease complex called the 26S proteasome. Conjugation of ubiquitin to the protein is achieved through an enzymatic cascade involving the sequential action of three enzymes: E1, E2 and E3. The E3 enzymes play the most important role by bringing the specificity to the system. Several hundred different E3s have been identified; among them Cullin (CUL)-dependent ubiquitin ligases are the most intensively studied. The CUL3-BTB ligases belong to this class of enzymes. In contrast to budding and fission yeast, CUL3 is an essential gene in metazoans. *Arabidopsis thaliana* encodes two related CUL3 genes, called CUL3A and CUL3B. These two genes are expressed in reproductive tissues and shows largely overlapping expression patterns suggesting possible functional redundancy. The disruption of both the CUL3A and CUL3B genes reduces gametophytic transmission and causes embryo lethality. Arrest of embryogenesis occurs at multiple stages of embryo development, but predominantly at the heart stage. This result demonstrates that CUL3 genes play an essential role during embryogenesis like in other metazoans. In order to understand the role of CUL3 during postembryonic development, we engineered a hypomorphic *cul3a/cul3b* double mutant. We will present here the phenotypical characterization of the *cul3a/cul3b* hypomorphic double mutant and give the first insight about the role of CUL3 in the regulation of ethylene biosynthesis.

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Crosstalk between Auxin and Brassinosteroids is via AuxIAA and BES1/BZR1-mediated regulation of the *DWF4* gene. Ok Sun Lee¹, Su Youn Jang¹, Sungwha Choe¹. ¹Seoul National University

DWARF4 catalyzes a flux-determining step in the Brassinosteroid biosynthetic pathways and its expression is subjected to a feedback-downregulation by BR treatment. However the molecular mechanisms for this regulation has remained unclear. To elucidate this, *DWF4*-driven GUS expression was studied after various genetic and hormone treatments. *DWF4* : : GUS expression was up-regulated in the BR-deficient dwarf mutants, whereas the change was not noticeable or even weaker in *axr2* and *axr6* background. In addition, the *DWF4* : : GUS expression was down-regulated in the brassinazole-resistant mutant *bzr1-D*. In contrast, *DWF4* : : GUS expression was significantly up-regulated by 2,4-D treatment, especially in the *bzr1-D* mutant background, suggesting that auxin may have provided additional room for BES1 action. Interestingly, the auxin-mediated up-regulation of the *DWF4* : : GUS expression was independent of both *BRI1* and *BIN2* functions, suggesting that auxin directly acts on the *DWF4* promoter. Chromatin Immunoprecipitation (ChIP) analysis revealed that BES1 directly binds to the *DWF4* promoter regardless of BL and 2,4,-D treatments. However, BZR1 interaction to *DWF4* promoter was abolished by auxin, suggesting that auxin-mediated increase of *DWF4* be due to de-repression mechanisms. Collectively, it is obvious that *DWF4* promoter serves as a focal point in a cross talk between BR and auxin, and this regulation is mediated by transcriptional activator BES1.

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PINs reveal differential auxin efflux activities in *Arabidopsis* root hair cells. Sang Ho Lee¹, Misuk Cho¹, Ok Ran Lee¹, Hyung-Taeg Cho¹. ¹Department of Biology, Chungnam National University, Daejeon 305-764, Korea

Auxin is the unique phytohormone that moves cell-to-cell with polarity. Intercellular transport of auxin is mediated by influx and efflux carriers in the plasma membrane. We have previously demonstrated that the protein kinase PINOID (PID) and an auxin efflux carrier PIN3 positively regulate auxin efflux using the auxin-sensitive *Arabidopsis* root hair cell system (Plant Cell 18: 1604-1616). Here, we have adopted the root hair system to compare the auxin efflux activities among different *Arabidopsis* PINs. *Arabidopsis* has eight PIN members whose distinctive primary structures imply diversity in spatial structures and thus molecular activities. Our assay result of the PIN activities demonstrated that different PINs have differential activities in facilitating auxin efflux in *Arabidopsis* root hair cells. We will be presenting the data for changes in root parameters and subcellular PIN-GFP localizations. Pharmacological approaches, regarding auxin-transporting and protein kinase activities, also revealed distinctive responses of PINs to the chemicals. Our study suggests that the root hair cell system provides a quantitative tool to study auxin carrier protein molecules.

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SOR12 is an essential component of cytokinin-mediated delay of leaf senescence. In Chul Lee¹, Hyo Jung Kim¹, Seung Hee Choi¹, Hong Gil Nam¹. ¹POSTECH, Pohang, South Korea

Senescence is a sequence of biochemical and physiological events that constitute the final stage of development. Senescence is now clearly regarded as a genetically programmed and evolutionally acquired developmental process. However, in spite of the biological and practical importance, genetic mechanism of senescence has been very limited. Previously, we reported that *ore12-1* has increased leaf longevity due to a missense mutation in *AHK3*, a sensor histidine kinase cytokinin receptor, and suggested that cytokinins exert their anti-senescing effect specifically and positively through *AHK3* to control senescence (Kim et al., 2006). To identify signaling components downstream of *AHK3*, we have undertaken a systematic genetic screening in an *ore12-1* allele through ethyl methanesulfonate (EMS)-mutagenesis. One suppressor named *sor12* (suppressor of *ore12-1*) was identified and showed complete suppression of the *ore12-1* senescence phenotypes. *sor12 ore12-1* double mutants exhibited accelerated senescence symptoms in age-dependent leaf senescence as well as in dark-induced senescence. Furthermore, *sor12 ore12-1* dramatically reduced the sensitivity of the plant to cytokinins in delaying leaf senescence and in inducing cytokinin-responsive genes, although these mutants still showed normal sensitivity to cytokinins in other responses, such as shoot induction and hypocotyl elongation inhibition.

Therefore, we suggest that SOR12 plays a major role in controlling cytokinin-mediated leaf senescence as a downstream component of *AHK3*. The identification of the mutated genes is underway and will be reported soon.

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Bestatin, an Inhibitor of Aminopeptidases, Provides a Chemical Genetics Approach to Dissect Jasmonate Signaling in Arabidopsis. Chuanyou Li¹, Wenguang Zheng¹. ¹State Key Laboratory of Plant Genomics and Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Bestatin, a potent inhibitor of some aminopeptidases, was shown previously to be a powerful inducer of wound response genes in tomato. Here, we present several lines of evidence showing that bestatin specifically activates jasmonic acid (JA) signaling in plants. First, bestatin specifically activates the expression of JA-inducible genes in tomato (*Lycopersicon esculentum*) and Arabidopsis (*Arabidopsis thaliana*). Second, the induction of JA responsive genes by bestatin requires the COI1-dependent JA signaling pathway, but does not depend strictly on JA biosynthesis. Third, microarray analysis using Arabidopsis whole genome chip demonstrates that the gene expression profile of bestatin-treated plants is similar to that of JA-treated plants. Fourth, bestatin promotes a series of JA-related developmental phenotypes. Taken together, the unique action mode of bestatin in regulating JA-signaled processes leads us to the hypothesis that bestatin exerts its effects through the modulation of some key regulators in JA signaling. We have employed bestatin as an experimental tool to dissect JA signaling through a chemical genetic screening, which yielded a collection of Arabidopsis bestatin-resistant (ber) mutants that are insensitive to the inhibitory effects of bestatin on root elongation. Further characterization efforts demonstrate that some ber mutants are defective in various JA-induced responses, which allowed us to classify the ber mutants into three phenotypic groups: JA-insensitive ber mutants, JA-hypersensitive ber mutants, and mutants insensitive to bestatin but showing normal response to JA. Genetic and phenotypic analyses of the ber mutants with altered JA responses indicate that we have identified several novel loci involved in JA signaling.

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Roles of RAV2 and ERF11 Arabidopsis Transcription Factors in Response to ABA and Glucose. Luis De Luna¹, Patricia León¹, Angel Guevara-García¹. ¹Departamento de Biología Molecular de Plantas, Instituto de

"Arabidopsis" dedicates over 6% of its genome to code for more than 1700 transcription factors, but the elucidation of the specific roles that each of them perform in different plant processes is incipient. RAV2 is a singular transcription factor that contains both an AP2 and a B3 binding domains, involved in flower development and abscisic acid (ABA) responses, respectively. Although RAV2 could be considered to regulate a wider range of genes, neither its target genes nor physiological functions are currently known. ERF11 is member of a large family of transcriptional regulators with important functions in a variety of plant processes related to growth development and responses to environmental stimuli. Interestingly, ERF11 contains an ERF-associated repression motif (EAR), which functions as a repression domain. Recently, ERF4, another member of the small subfamily of ERF repressors, has been involved in modulate ABA responses. However, no information is available with respect to the physiological roles and downstream targets of ERF repressors in plants. Both "RAV2" and "ERF11" genes were identified to be induced in a stress/defense transcriptome of "Arabidopsis".

ABA is a plant hormone that mediates myriad physiological responses to the environment such as drought, chilling, salinity, etc., and plays fundamental roles in seed and vegetative development. Glucose plays an important role as carbon source or plant growth and development, but also as signal to regulate metabolism, differentiation and stress responses. The co-regulation of several plant genes by both glucose and ABA has been reported, but the information about the molecular basis of that interaction is fragmentary.

In this study, the participation of "RAV2" and "ERF11" in ABA and glucose responses is investigated. To reach that goal, we compare the phenotype of "*A. thaliana*" transgenic lines with high (over-express) and low (RNAi) levels of expression of both genes, growing in ABA y glucose. Moreover, our study is complemented with an analysis of expression of "RAV2" and "ERF11" genes in response to ABA and glucose on wild-type plants in different tissues and developmental stages.

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The Arabidopsis Salt Tolerance 2 (AtSAT2) gene encode homologous GA MYB-binding protein involved in abscisic acid signal transduction. Gah-Hyun Lim¹, Cheol Soo Kim¹. ¹Department of Plant Biotechnology and Agricultural Plant Stress Research Center, Chonnam National University, Kwangju 500-757, Korea

We conducted a genetic yeast screen to identify salt tolerance (SAT) genes in maize kernel cDNA library. During the screening, we identified a maize clone (SAT2) that seemed to confer elevated salt tolerance in comparison to control cells. SAT2 cDNA encodes a 70-kDa protein which is 67 % identical to the Arabidopsis GA MYB-binding protein (AtSAT2). To further examine salinity tolerance in Arabidopsis, we functionally characterized the AtSAT2 gene and found that dehydration as well as abscisic acid (ABA) induced AtSAT2 expression. Constitutive expression of AtSAT2 in Arabidopsis led to elevated salt tolerance in transgenic lines. Interestingly, plants overexpressing AtSAT2 exhibited insensitivity to ABA. Our results suggest that AtSAT2 is involved in osmotic stress tolerance as well as in ABA signaling in Arabidopsis.

P-468**Functional characterisation of PIN5 auxin transport protein.**

Jozef Mravec¹, Markus Geisler², Petr Skupa³, Vassilena Gaykova¹, Christian Luschnig⁴, Jan Petrasek³, Eva Zazimalova³, Jiri Friml¹. ¹Center for Plant Molecular Biology (ZMBP), Auf der Morgenstelle 3, University Tbingen, D-72076 Tbingen, Germany, ²Zrich-Basel Plant Science Center, University of Zurich, Institute of Plant Biology, Molecular Plant Physiology, CH-8008 Zurich, Switzerland, ³Institute of Experimental Botany, ASCR, Rozvojov 135, 165 02 Praha 6, Czech Republic, ⁴Institute for Applied Genetics and Cell Biology, University of Natural Resources and Applied Life Sciences BOKU, Muthgasse 18, A-1190 Wien, Austria

Development of plant body strongly depends on intercellular transport and distribution of hormone auxin. According to the chemiosmotic hypothesis auxin transport requires auxin influx and efflux carriers located on the plasma membrane of the transporting cells. In Arabidopsis, several proteins have already been shown to be directly involved in cellular auxin export or import. Best characterised is the plant-specific family of PIN auxin efflux components. PIN proteins have been shown to determine rate and direction of intracellular auxin flow. Arabidopsis PIN gene family consists of 8 members, of which PIN5, PIN6 and PIN8 have not been functionally characterised yet. PIN5 is the most distant member lacking the typical central hydrophilic loop and his role in auxin efflux has not been demonstrated. Here we present results of reverse genetic analysis aimed to characterise developmental role of PIN5. Full knock-out mutant in PIN5 gene is defective in cell elongation and partially resistant to exogenous auxins and auxin transport inhibitors. On the other hand, overexpression of PIN5 causes phenotypes resembling mutants with increased auxin content. Both the loss-of-function and gain-of-function data are consistent with the role of PIN5 in auxin transport-related process. This data along with localisation studies and functional characterisation of PIN5 in heterologous systems will be presented.

P-469**Functional characterisation of PIN5 auxin transport protein.**

Jozef Mravec¹, Markus Geisler², Petr Skupa³, Vassilena Gaykova¹, Christian Luschnig⁴, Jan Petrasek³, Eva Zazimalova³, Jiri Friml¹. ¹Center for Plant Molecular Biology (ZMBP), Auf der Morgenstelle 3, University Tbingen, D-72076 Tbingen, Germany, ²Zrich-Basel Plant Science Center, University of Zurich, Institute of Plant Biology, Molecular Plant Physiology, CH-8008 Zurich, Switzerland, ³Institute of Experimental Botany, ASCR, Rozvojov 135, 165 02 Praha 6, Czech Republic, ⁴Institute for Applied Genetics and Cell Biology, University of Natural Resources and Applied Life Sciences BOKU, Muthgasse 18, A-1190 Wien

Development of plant body strongly depends on intercellular transport and distribution of hormone auxin. According to the chemiosmotic hypothesis auxin transport requires auxin influx and efflux carriers located on the plasma membrane of the transporting cells. In Arabidopsis, several proteins have already been shown to be directly involved in cellular auxin export or import. Best characterised is the plant-specific family of PIN auxin efflux components. PIN proteins have been shown to determine rate and direction of intracellular auxin flow. Arabidopsis PIN gene family consists of 8 members, of which PIN5, PIN6 and PIN8 have not been functionally characterised yet. PIN5 is the most distant member lacking the typical central hydrophilic loop and his role in auxin efflux has not been demonstrated. Here we present results of reverse genetic analysis aimed to characterise developmental role of PIN5. Full knock-out mutant in PIN5 gene is defective in cell elongation and partially resistant to exogenous auxins and auxin transport inhibitors. On the other hand, overexpression of PIN5 causes phenotypes resembling mutants with increased auxin content. Both the loss-of-function and gain-of-function data are consistent with the role of PIN5 in auxin transport-related process. This data along with localisation studies and functional characterisation of PIN5 in heterologous systems will be presented.

P-470**Regulation of 'BRX' expression by hormone pathways: evidence for 'BRX' as a putative target of MP.** Karen Osmont¹, Nadja Krogan², Thomas Berleth², Christian Hardtke¹. ¹University of Lausanne, Switzerland, ²University of Toronto, Canada

The novel growth regulator 'BREVIS RADIX (BRX)' has been proposed to function in a feedback loop that connects the auxin and brassinosteroid pathways to modulate root system growth and architecture. Consistent with the idea of a feedback loop, 'BRX' expression is regulated by both hormones and particularly responsive to auxin. Prototypical auxin response elements are present in the 'BRX' promoter, suggesting that the involvement of auxin response factors (ARFs) in 'BRX' regulation. A candidate ARF for this is 'MONOPTEROS (MP)', because 'BRX' and 'MP' expression patterns are strikingly similar. Expression of both genes is observed very early in embryogenesis, throughout the embryo, and becomes gradually restricted to the incipient vasculature. Further, post-embryonically, the expression of both genes is specific to the vasculature. Double mutant analyses of 'brx' with the redundant ARFs 'MP' and 'NPH4' and provide preliminary molecular and biochemical evidence that MP may indeed regulate 'BRX'. Details about this interaction as well as 'BRX' auto-regulation are reported.

P-471**A novel Arabidopsis MAPK phosphatase, MKP5, regulates ABA-signaling and lipid mobilization in seedling establishment.** Montserrat Pages¹, Victoria Lumbreras¹. ¹CSIC. Barcelona, Spain

The phytohormone abscisic acid (ABA) plays an essential role in adaptive stress responses. Although genetic analyses have identified numerous ABA-response mutants, the regulators of ABA signal transduction remain to be fully elucidated. Here, we report the identification and characterization of a novel MAPK phosphatase (MKP5) from Arabidopsis involved in developmental and stress responses. The m kp5 gene is expressed at very low levels in vegetative tissues, but is markedly induced during seedling establishment. Seeds expressing an MKP5-GFP fusion protein show phenotypic alterations, including ABA and salt hypersensitivity and a severe growth arrest after germination. This developmental arrest is a result from inefficient lipid reserve mobilization and is rescued by providing a source of exogenous sugar in the growth medium. This phenotype is not observed after overexpression of the normal MKP5 protein, suggesting that it arises from dominant negative effects of the MKP5-GFP protein, confirmed by a hypomorphic m kp5 allele. We also find that the constitutive MKP5 overexpression results in ABA and salt hypersensitivity and slight enhanced drought tolerance. Molecular analyses reveal upregulation of ABA signaling genes in those plants. The spatial expression pattern and protein MKP5 accumulation is consistent with their functional role. MKP5-GFP shows specific accumulation in tissues involved in water homeostasis, hydathodes and guard cells. Taken together, these data suggest that MKP5 is a regulator of ABA signaling pathway and present new evidence implicating a link by MAPK activities in the control of plant development and survival under stress conditions.

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Characterization of a novel transcriptional regulator gene At-SAT32: involvement in salinity response and gibberellin hormonal regulation of *Arabidopsis* root length. Min Young Park¹, Hee-Seok Koh¹, Sung-Ju Ahn¹, Cheol Soo Kim¹. ¹ Department of Plant Biotechnology and Agricultural Plant Stress Research Center, Chonnam National University, Kwangju 500-757, Korea

We conducted a genetic yeast screen to identify salt tolerance (SAT) genes in maize kernel cDNA library. During the screening, we identified a maize clone (SAT32) that seemed to confer elevated salt tolerance in comparison to control cells. SAT32 cDNA encodes a 48-kDa protein which is 40 % identical to the *Arabidopsis* unknown protein (AtSAT32). To further examine salinity tolerance in *Arabidopsis*, we functionally characterized the AtSAT32 gene and found that dehydration as well as gibberellin (GA) induced AtSAT32 expression. Constitutive expression of AtSAT32 in *Arabidopsis* led to elevated salt tolerance in transgenic lines. Interestingly, the AtSAT32 null mutant exhibited insensitivity to GA. Our results suggest that AtSAT32 is involved in salt stress tolerance as well as in mediating the control of plant root length by GA hormone.

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Cross-talk between salicylate and jasmonate signaling in *Arabidopsis* does not require inhibition of octadecanoid biosynthesis. Antonio Léon Reyes¹, Elvira S. De Lange¹, Annemart Koornneef¹, Floor C. Den Otter¹, Ruth G. Joosten¹, Tita Ritsema¹, Corn M. J. Pieterse¹. ¹ Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, P. O. Box 800. 84, 3508 TB Utrecht, the Netherlands

Salicylic Acid (SA) and jasmonates (JA) are plant hormones implicated in disease resistance. Interaction between hormone signaling pathways plays a role in determining the final outcome of defense responses in plants. In *Arabidopsis thaliana*, endogenously accumulating SA antagonizes JA-dependent defenses by prioritizing SA-dependent resistance over JA-dependent defense. In this study, by looking at the expression of the JA-responsive marker genes *PDF1.2* and *VSP2*, and the SA-responsive marker gene *PR-1*, we show that SA is capable of suppressing the JA response that is triggered by an array of JA-inducing pathogens, insects and wounding. Furthermore, we tested whether SA-mediated inhibition of JA biosynthesis plays a role in cross-talk between these two hormones. SA antagonized the expression of *PDF1.2* in the same time frame as it suppressed genes encoding enzymes of the JA biosynthesis pathway, such as *AOS*, *OPR3*, *LOX2* and *AOC3*. If the antagonistic effect of SA on JA signaling functions through inhibition of JA biosynthesis, then JA-biosynthesis mutants should no longer display cross-talk. Mutant *aos/dde2* appeared to be a suitable mutant to test this hypothesis. Mutant *aos/dde2* was incapable of expressing JA-responsive genes such as *PDF1.2* upon infection by the JA-inducing pathogen *Alternaria brassicicola*, whereas exogenous application of methyl jasmonate (Me-JA) resulted in wild-type levels of *PDF1.2* transcripts. Pharmacological assays showed that cross-talk between SA and JA is still intact in the *aos/dde2* mutant. This demonstrates that down-regulation of JA biosynthesis is not necessary for SA-mediated suppression of JA responses.

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Evolution effect of ABA on antioxidant enzyme and lipid peroxidation in *Arabidopsis thaliana* seedling under salt stress
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An experiment was carried out to study *Arabidopsis thaliana* ABA-seed priming effects on antioxidant enzyme content and lipid peroxidation of seedlings under salt stress.

Twenty five (25) seeds were planted in each Petri dish (7cm in diameter). Salt stress was applied at four levels including: 0.3 (control) 8, 16 and 24ds/m-1. Sodium chloride (NaCl) was used to apply salt stress. Distilled water was used for control Petri dishes. In order to prime, seeds were soaked in 10µmol ABA solution for 12hrs. The experiment was carried out in terms of factorial – completely random design with 8 treatments at four replications. Salt stress condition significantly decreased catalase content and increase peroxidase and superoxid dismutase content of seedling tissues but this increase was higher in ABA-priming seedling. ABA application decreased lipid peroxidation of seedling tissue. Data of this study showed positive correlation between antioxidant enzyme content of *Arabidopsis* seedling and decrease of lipid peroxidation of *Arabidopsis* tissue.

Key word: *Arabidopsis thaliana*, salt stress, ABA, antioxidant enzyme.

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Phosphorylation dependent Nucleocytoplasmic Shuttling of BZR1 Is Essential In *Arabidopsis* Brassinosteroid Signaling
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Phytohormone brassinosteroids (BRs) play critical roles in plant growth and development. BR exerts its action by modulating the phosphorylation status of two key transcriptional factors, BES1 and BZR1, upon perception of BR by BRI1/BAK1 receptors at the plasma membrane. However, a regulatory mechanism mediating BR signals from the plasma membrane-associated BR perception to expression of BR target genes in the nucleus remains unknown. We here show that phosphorylation by BIN2 directly induces the nuclear export of BZR1; in the cytoplasm, BR-activated BSU1 mediates their dephosphorylation and subsequent nuclear translocation. Two distinct potential phosphorylation domains in BZR1 were required for the nuclear export induced by BIN2. Transgenic *Arabidopsis* plants overexpressing BZR1 with mutations in these phosphorylation sites, displays constitutive BR response phenotypes. Taken together, we propose that the phosphorylation-dependent spatial redistribution of BZR1 is essential for proper BR signaling.

P-476

AtGL5, a cell wall protein in *Arabidopsis*, may regulates flowering and cell growth. Zhang Shengchun¹, Yang Chengwei¹, Wang Xiaojing¹. ¹Institute of Life Sciences, South China Normal University, Guangdong Key Lab of Biotechnology for Plant Development, Guangzhou 510631, P.R. China

AtGL5 gene is a GA regulated gene in *Arabidopsis*. The analysis of gl5 mutant showed an earlier flowering phenotype. Spatial localization of the β-glucuronidase (GUS) gene product driven by AtGL5 promoter was examined and GUS expression was tested by histochemical staining in T2 progeny of the transgenic plants. In 8-day old seedlings GUS expression was first detected in cotyledons, elongation part of the root, lateral root and root hairs. Stronger GUS expression was appeared in the shoot meristem. During reproductive development of the plants, stronger GUS expression was found in primary and secondary inflorescence meristems emerging at the base of the cauline leaf.

Branching sites of the flower from the inflorescence stem and rosette leaves had also a strong expression. Moreover, GUS expression could be detected in the ovary and in the vascular of the siliques. To determine the subcellular localization of AtGL5 protein, we constructed AtGL5-EGFP fusion protein that was expressed under the regulation of the cauliflower mosaic virus (CaMV) 35S promoter. When it was transiently expressed in onion epidermal cells, the transformed cells showed fluorescence of green fluorescent protein (GFP) in cell wall and no change under hypotonic solution. Whereas the control GFP protein was uniformly localized in the nucleus and cytoplasm and moves from the cell periphery to the central part of the cell volume in plasmolysis cells. Permanent expression of fusion protein in the cells of root hair in *Arabidopsis* obtained the same result, demonstrating that AtGL5 is localized in cell wall or the apoplasm of the cell. In addition, the expression of AtGL5 was up-regulated by NAA, SA and PAC and down-regulated by GA3. Further investigation of the AtGL5 function will be still elucidative. This work was supported by grants from National Natural Science Foundation of China 30570165.

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KEG, a Novel RING E3/Kinase/Ankyrin Protein Essential for *Arabidopsis* Growth and Development is Involved in Abscisic Acid Signaling. Sophia Stone¹, Luis Williams², Lisa Farmer³, Richard Vierstra³, Judy Callis². ¹Dalhousie University, Halifax, Nova Scotia, Canada, ²University of California-Davis, Davis, California, USA, ³University of Wisconsin, Madison, Wisconsin, USA

Ubiquitin-dependent protein degradation regulates numerous eukaryotic cellular processes by selectively targeting proteins for degradation by the 26S proteasome. Recent studies have highlighted the role of ubiquitination, specifically E3 ligases, in plant growth and development, hormone signaling and photomorphogenesis. The *Arabidopsis* proteome contains 469 RING-type E3 ligases, which are further classified into 30 different sub-groups based on domain organization. Of particular interest is the KEG protein, which belongs to the RING-ankyrin sub-group. In addition to the RING-HC domain and nine ankyrin repeats, KEG also contains a serine/threonine kinase domain as well as a twelve previously unidentified HERC2-like repeats. Both the RING and kinase domains were functional in *in vitro* ubiquitination and phosphorylation assays, respectively. To determine the function of KEG we analyzed four independent T-DNA insertional lines. keg seedlings exhibit characteristics of post-germinative growth arrest, such as inhibited cotyledon greening and expansion, indicative of elevated abscisic acid (ABA) signaling, a major phytohormone that plays a key role in plant development and survival under unfavorable conditions. As expected, keg seedlings are hypersensitive to exogenous sugars and keg roots are extremely sensitive to the inhibitory effects of ABA on root growth. The observations that KEG accumulates high levels of ABSCISIC ACID-INSENSITIVE 5 (ABI5) without exogenous ABA and that loss of ABI5 rescues the growth-arrest phenotype of keg seedlings point to a central role for KEG in ABA signaling. Future studies include functional characterization of the remaining RING-ankyrin proteins and other novel plant specific RING E3 ligases.

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Light Mediates Brassinosteroid Sensitivity via Activation of MSBP1 by HY5 and HYH. Qiu-Ming Shi¹, Song Li¹, Hong-Wei Xue¹. ¹Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Membrane Steroid Binding Protein 1 (MSBP1) which can bind brassinolide in vitro is known as a negative regulator of brassinosteroid (BR) signal transduction and cell expansion. Interestingly, the promoter-GUS assay showed the expression of MSBP1 in hypocotyls is induced by various lights. To further study the role of MSBP1 in light signaling, relationships of MSBP1 to COP1 and HY5/HYH were analyzed. Results showed that HY5 and HYH could directly bind to the promoter region of MSBP1. Further Western blot assay shows that the expression of MSBP1 in HY5, HYH and double mutant was repressed. The groundwork show that MSBP1 may play as a bridge between Light and brassinosteroid signal, the activation of MSBP1 by HY5 and HYH deals a great impact on the alternation of BR sensitivity and photomorphogenesis in dark-light transition.

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The sua mutant is an allele specific suppressor of abi3-5. Matteo Sugliani¹, Maarten Koornneef¹, Wim Soppe¹. ¹Max Planck Institute for Plant Breeding Research

The ABSCISIC ACID INSENSITIVE 3 (ABI3) transcription factor has an essential role during seed maturation for the acquisition of desiccation tolerance and dormancy. *Arabidopsis* plants carrying *abi3* mutant alleles, including *abi3-5*, are disturbed in seed maturation processes and yield seeds with reduced longevity. In a suppressor mutagenesis screen of the *abi3-5* mutant we have obtained the suppressor of *abi3-5* (*sua*) mutant, which reverts all of the *abi3-5* mutant phenotypes. Map based cloning revealed that SUA encodes an RNA binding protein. In the wild type background the *sua* mutation causes a higher sensitivity to abscisic acid during germination, which also correlates with higher dormancy. Interestingly, *sua* only reverts the phenotype of the *abi3-5* allele but does not affect other *abi3* alleles that we tested. We are currently analyzing the relationship between *sua* and *abi3-5* and are determining the role of *SUA* during seed maturation.

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Proteomic and genomic studies of the brassinosteroid signal transduction pathway. Wenqiang Tang¹, Yu Sun¹, Srinivas S. Gampala¹, Zhiping Deng¹, Joshua M. Gendron¹, Tae-Wuk Kim¹, Ming-Yi Bai², Sheng-Wei Zhu², Juan A Oses-Prieto³, Shenheng Guan³, Alma L. Burlingame³, Kang Chong², Zhi-Yong Wang¹. ¹Carnegie Institution, Stanford, CA, USA, ²Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, the Chinese Academy of Sciences, Beijing 100093, China, ³Mass Spectrometry Facility, Department of Pharmaceutical Chemistry, University of California, San Francisco CA 94143

The phytohormone Brassinosteroid (BR) plays essential roles in multiple developmental processes. BR is perceived by the receptor kinase BRI1 at the cell surface, and downstream BR signal transduction involves a receptor-like kinase (BAK1), a GSK3-like kinase (BIN2), a phosphatase (BSU1), and transcription factors (BZR1 and BZR2/BES1). BR binding to the extracellular domain of BRI1 induces BRI1-BAK1 dimerization and receptor kinase activation, leading to dephosphorylation of BZR1 and BZR2/BES1 possibly by inhibiting BIN2 or activating BSU1. When BR levels are low, BIN2 phosphorylates BZR1 and BZR2/BES1 to inhibit their nuclear accumulation and DNA binding. Our studies of BZR1-interacting proteins demonstrate that 14-3-3 proteins specifically bind to the BIN2-phosphorylated BZR1 to increase its cytoplasmic retention. Mutations of a BIN2-phosphorylation site in BZR1 that abolish 14-3-3 binding increase nuclear localization of BZR1 and BR responses. Genetic and genomic studies of target genes of BZR1 have identified new molecular links between BR signaling and developmental responses. Using proteomic approaches, we have identified over 70 BR-regulated proteins, including known BR signaling components as well as novel proteins potentially involved in BR signal transduction or downstream responses. Some of the BR-regulated proteins interact directly with known BR-signaling proteins.

Overexpression of a novel BR-induced protein suppresses the phenotypes of the BR-deficient *def2* mutant, demonstrating its function in BR responses. Our genomic and proteomic studies have begun to fill the gaps in the BR signal transduction pathway and elucidate the molecular networks that link BR signaling to various cellular and developmental processes. A model of the BR signaling pathway will be presented.

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Studies on the role of phytohormones and glucosinolates on sphinganine-analog mycotoxins-induced programmed cell death in *Arabidopsis* and tomato. Jiansheng Wang¹, Chengguo Jia¹, Qiaomei Wang¹. ¹Department of Horticulture, Zhejiang University, The State Agriculture Ministry, Laboratory of Horticultural Plant Growth, Development & Biotechnology, Hangzhou 310029, China

Fumonisin and AAL toxin are two common sphinganine-analog mycotoxins (SAMs) produced by *Fusarium moniliforme* in maize and *Asterina alternata* f. sp. *Lycopersici* in tomato, respectively. SAMs induce apoptosis-like programmed cell death (PCD) in plants. We have established model systems to study plant PCD in *Arabidopsis* and susceptible lines of tomato (asc/asc) using FB1 and AAL toxin. The effects of brassinosteroids (BRs), salicylic acid (SA), and jasmonate (JA) on SAM-induced PCD were investigated in *Arabidopsis* and tomato by hormone application and hormone biosynthesis and response mutant experiments. Our results showed that the role of SA in SAM-induced PCD is distinct between *Arabidopsis* and tomato, SA accelerated FB1-induced lesion formation in *Arabidopsis* leaves, while suppressed AAL toxin-induced PCD in tomato (asc/asc) leaves. Brassinolide (BL) could decrease the susceptibility of both *Arabidopsis* and tomato to SAM with an unknown mechanism; the cross-talk of JA/ETH and SA in SAM-induced PCD was also discussed.

Glucosinolates are a kind of sulfur- and nitrogen-containing secondary metabolites that have diverse biological functions. In *Arabidopsis thaliana*, 23 different glucosinolates have been identified, which makes *Arabidopsis* a model system to study the biosynthesis, metabolism and biological functions of glucosinolates. The hormone regulation of glucosinolates in *Arabidopsis* ecotypes (Col, Ler) and glucosinolate mutants (*Tu3*, *gcc8*) differing in glucosinolate composition, contents, and their roles in FB1-induced PCD in *Arabidopsis* were studied, and the results indicated that the biosynthesis of individual glucosinolates was under fine regulation of different phytohormones, the cross-talk of phytohormone and glucosinolate occurred at the level of biosynthesis and functions in the resistance of *Arabidopsis* to FB1.

P-482**Functional study of ETO1 gene family in *Arabidopsis thaliana*.**

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Phytohormones play an essential role in modulation of plant development and adaptation to the constantly changing environment. Ethylene is a gaseous phytohormone and has versatile function in plant physiology. Ethylene biosynthesis is highly regulated by developmental and environmental signals. The immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC), was converted by ACC synthase (ACS) from S-adenosylmethionine. ACS catalyzes the rate-limiting step of ethylene biosynthetic pathway and is encoded by gene family in all plant species. Previous studies showed that mutations in *ETO1* (*ETHYLENE OVERPRODUCER1*) resulted in a higher ethylene emanation, which was suppressed by a null mutation of ACS5, indicating *ETO1* is a negative regulator in ethylene biosynthesis.

There are two *ETO1* paralogs in *Arabidopsis thaliana*, *EOL1* and *EOL2* (*ETO1-LIKE*). *ETO1* and *EOL* proteins share 50 and 75 % of identity at the N terminal BTB (Broad-Complex, Tramtrack, Bric-brac) domain and the C terminal TPR (Tetratrico peptide Repeat) domain, respectively. While the TPR domain of *ETO1* is essential for the interaction with ACS5, the BTB domain has been shown to interact with CULLIN3 in the SCF (Skp1-Cul1-F box) ubiquitin E3 ligase. The working model is that *ETO1* negatively regulates ethylene biosynthesis by interacting with ACS, specifically ACS5 and ACS9, to promote protein degradation dependent on the 26S proteasome. The observation that recessive mutations in *ETO1* alone resulted in a constitutive triple response (ctr) phenotype in etiolated seedlings suggested distinct function of *EOL* genes.

We have identified homozygous T-DNA insertional mutants of *ETO1* and *EOL* genes for functional analysis. In addition to single mutants, we have also generated double and triple mutants. Null mutants of individual *EOL* did not show an obvious ctr phenotype or a higher ethylene emanation. However, double (*eto1-4 eol1-1* and *eto1-13 eol2-1*) and triple (*eto1-13 eol1-4 eol2-1*) mutants produced a higher level of ethylene than wild type did. Recent results of phenotypical analysis using these mutants will be presented.

P-483***Arabidopsis 1-aminocyclopropane-1-carboxylate Synthase Gene AtACS7 Is a System 2 ACS Gene***

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1-Aminocyclopropane-1-carboxylate synthase (ACC synthase, ACS) (S-adenosyl-L-methionine methylthiocadenosine-lyase; EC 4.4.1.14), the key enzyme for ethylene biosynthesis, is subjected to positive or negative feedback regulation by the hormone itself. Here we report, aided by promoter-GUS (β -glucuronidase) reporter approach, that among the multi-gene family of *Arabidopsis* ACC synthases, AtACS5 and AtACS7 exhibited active expression in similar localizations but quite different responses to ethylene. The AtACS5::GUS and AtACS7::GUS fusions were found to express in company during the entire life cycle of transgenic *Arabidopsis*. However, treating the 2-week-old light-grown seedlings with exogenous ethylene greatly increased the GUS activity driven by AtACS7 promoter but exhibited no effect on the expression level of AtACS5::GUS transgene. The highest expression level of AtACS5::GUS fusion was detected in the 3-d-old etiolated seedlings, and the developmental expression profile of AtACS5 was not affected by the *etr1-1* mutation. In contrast, the expression of AtACS7 during the entire life cycle of *Arabidopsis*, such as in 3-d-old etiolated seedlings, older rosette leaves, fully opened flowers, young and old-yellowing siliques, was dramatically suppressed in the ethylene-insensitive mutant *etr1-1*. These results strongly suggested that AtACS7 is subjected to the positive feedback regulation by ethylene and plays an important role in catalyzing the system 2 ethylene production while AtACS5 mainly responds to developmental stimuli.

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P-484***SBI1 regulates BRI1 level to control BR response.***

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Brassinosteroids (BRs) play important roles in plant growth and development. Over the past decade, studies have been focusing on finding new components in BR signaling pathway. As such, the several key components in BR signaling pathway have been identified, including receptor BRI1 and co-receptor BAK1, and downstream components BKI1, BIN2, BES1 and BZR1. BRs are perceived by BRI1, which inactivates a negative regulator, BIN2, a GSK3-like kinase, which then dephosphorylates and activates a family of plant-specific transcription factors (such as BES1 and BZR1) to regulate the expression of many BR responsive genes. The loss of function *bri1* mutant is similar to the strongest BR biosynthetic mutant, indicating that BRI1 is the master switch for BR function. However, we know little about how this master switch, BRI1, is regulated. We used a genetic approach to identify regulators of BRI1 using an unstable *bri1* mutant, a weak *bri1* mutant allele that accumulates less *bri1* protein. We screened for suppressors of this *bri1* mutant and found a BR signaling specific mutant (*sbi1*, suppressor of *bri1*) that accumulates a high level of BRI1 protein. *sbi1* does not suppress other *bri1* alleles in which the protein level is not altered. Nevertheless, the *sbi1* single mutant has an elevated BRI1 protein level and has an enhanced response to BL and reduced sensitivity to BR inhibitors. The increased accumulation of BRI1 protein may explain the basis for its suppression of *bri1* and the enhanced growth in *sbi1* single mutant. Interestingly, the expression of BRI1::*bri1* in the *bri1* mutant had similar phenotype as *sbi1/bri1* double mutant, suggesting that the *BRI1/bri1* protein accumulation in *sbi1* single mutant and *sbi1/bri1* double mutant is responsible for their respective enhanced response to BL compared to wild type and *bri1* respectively. Furthermore, the treatment of *bri1* mutant with a protease inhibitor leads to a similar accumulation of BRI1 protein as in the *sbi1/bri1* double mutant. Therefore, SBI1 plays an important role in BR signaling by regulating BRI1 levels.

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The Arabidopsis ROP9 Small GTPase positively regulate auxin responses. Fan Yang¹, Zhenbiao Yang¹. ¹Center for Plant Cell Biology, and Department of Botany and Plant Sciences, University of California, Riverside, California 92521, USA

Auxin is an important plant hormone that modulates a myriad of plant growth and developmental processes. Recent studies have established an auxin signaling pathway that involves auxin activated degradation of transcription-repressive IAA/AUX proteins directly through auxin stabilizing the interaction between IAA/AUX and the cyto-nucleoplasmic auxin receptors, which are the TIR1/AFBs F-box proteins that are a component of the SCF E3 ubiquitin ligase complex. However, it is not clear whether other signaling mechanisms are also functionally important in auxin signaling. In this reporter, we demonstrate that the plasma membrane-localized ROP9 small GTPase is an important positive regulator of auxin signaling using a combination of genetic, molecular, and biochemical analyses. *rop9-2* knockout mutant seedlings exhibit reduced lateral root formation, shorter hypocotyls and roots as well as reduced responses to auxin in primary root elongation and lateral root initiation. In contrast, transgenic lines expressing a constitutively active mutant of *ROP9* are enhanced in auxin responses. By using a promoter::GUS fusion, we show that *ROP9* expression is up-regulated by exogenous auxin in hypocotyls and root tips. We demonstrate that *ROP9* mediates auxin-responsive gene expression. DR5::GUS expression is reduced in *rop9-2* mutant but promoted by the constitutive active form of *ROP9*. In addition, *ROP9* modulates auxin-activated IAA degradation. The auxin sensitive turnover of IAA17-LUC is reduced in *rop9-2* mutant but enhanced by constitutively active form of *ROP9*. Importantly, exogenous auxin promotes *ROP9* activation within 10 minutes after auxin application. These results show that *ROP9* is a positive regulator of auxin signaling leading to IAA/AUX degradation. We are currently interested in whether and how *ROP9* signaling is connected to the TIR1-IAA-ARF auxin signaling pathway.

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Investigating the role of ETA2/CAND1 in regulating SCF complex activity. Wenjing Zhang¹, Hironori Ito¹, William M. Gray¹. ¹University of Minnesota, Twin Cities, MN, USA

The *eta2-1* mutant was identified in a genetic screen for enhancers of the *tir1-1* auxin (*eta*) response defect. *eta2-1* plants exhibit several phenotypes related to impaired auxin response. Molecular studies found that these phenotypes are the result of reduced SCF(TIR1) activity in *eta2-1* mutants. Isolation of the *ETA2* gene revealed that it encodes an Arabidopsis ortholog of human CAND1 (Cullin-Associated and Neddylation-Dissociated). Biochemical studies with mammalian cell lines suggest that CAND1 acts as a negative regulator of SCF function by sequestering unmodified CUL1 away from SKP1 and the F-box protein, thus preventing assembly of the SCF complex. In contrast, we find that the *eta2-1* mutation diminishes the ability of CAND1 to interact with CUL1, demonstrating that the interaction between these two proteins is required for SCF activity and that CAND1 positively regulates SCF function. These paradoxical findings have been explained by a model invoking CAND1 regulation of a dynamic cycle of assembly and disassembly of the SCF complex *in vivo*, through association and dissociation with CUL1. Double mutant analysis with the *cxr6-2* and *cxr6-3* alleles of CUL1 reveals additional insight into the interactions between CAND1 and CUL1. Whereas *eta2-1* and *cxr6-3* interact synergistically, *eta2-1 cxr6-2* double mutants show mutual suppression of *eta2-1* and *cxr6-2*. Although the *eta2-1* mutation itself is recessive, its suppression of *cxr6-2* is dominant, indicating a heightened sensitivity to CAND1 dosage level. This genetic study, together with co-immunoprecipitation experiments suggests that the *cxr6-2* mutation may inhibit dissociation of CUL1 from the CAND1-CUL1 complex. The result that the *eta2-1* mutation suppresses the rub-modification defect in *cxr6-2* implies that in order to be modified by rub CUL1 needs to be dissociated from CAND1 first. Gel-filtration experiments show some surprising differences in the fractionation patterns of SCF subunits between *eta2-1* mutants and wild-type, shedding new light on CAND1's function. Molecular studies examining the effects of these mutations on SCF homeostasis are underway.

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Bestatin, an Inhibitor of Aminopeptidases, Provides a Chemical Genetics Approach to Dissect Jasmonate Signaling in *Arabidopsis*. Wenguang Zheng¹, Chuanyou Li¹. ¹State Key Laboratory of Plant Genomics and Center for Plant Gene Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

Bestatin, a potent inhibitor of some aminopeptidases, was shown previously to be a powerful inducer of wound response genes in tomato. Here, we present several lines of evidence showing that bestatin specifically activates jasmonic acid (JA) signaling in plants. First, bestatin specifically activates the expression of JA-inducible genes in tomato (*Lycopersicon esculentum*) and *Arabidopsis* (*Arabidopsis thaliana*). Second, the induction of JA responsive genes by bestatin requires the COI1-dependent JA signaling pathway, but does not depend strictly on JA biosynthesis. Third, microarray analysis using *Arabidopsis* whole genome chip demonstrates that the gene expression profile of bestatin-treated plants is similar to that of JA-treated plants. Fourth, bestatin promotes a series of JA-related developmental phenotypes. Taken together, the unique action mode of bestatin in regulating JA-signaled processes leads us to the hypothesis that bestatin exerts its effects through the modulation of some key regulators in JA signaling. We have employed bestatin as an experimental tool to dissect JA signaling through a chemical genetic screening, which yielded a collection of *Arabidopsis* bestatin-resistant (*ber*) mutants that are insensitive to the inhibitory effects of bestatin on root elongation. Further characterization efforts demonstrate that some *ber* mutants are defective in various JA-induced responses, which allowed us to classify the *ber* mutants into three phenotypic groups: JA-insensitive *ber* mutants, JA-hypersensitive *ber* mutants, and mutants insensitive to bestatin but showing normal response to JA. Genetic and phenotypic analyses of the *ber* mutants with altered JA responses indicate that we have identified several novel loci involved in JA signaling.

Metabolism

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Classification of mutants by metabolite fingerprinting- the Hi-MeT project. Michael Beale¹, Jane Ward¹, Delia-Irina Corol¹, John Baker¹, Yudong Cai¹. ¹National Centre for Plant and Microbial Metabolomics, Rothamsted Research, Harpenden, Herts, UK

The HiMeT project was conceived to test the ability of metabolite fingerprinting (datasets generated from unpurified plant extracts) to phenotype plants. The aim was to utilise a collection of previously well characterised *Arabidopsis* mutants, selected to represent a spectrum of biochemical pathway knockouts, as a base set, to test the hypothesis that comparison of plants grown in controlled environment would allow focus on metabolite signatures specific for the mutation. In this project we used rapid fingerprinting techniques such as NMR and direct infusion ESI-MS, underpinned by classical metabolite analysis by hyphenated-MS to form a database of mutant specific chemical signatures arising from multivariate comparison of mutants with wild-type. The ability to use these signatures to predict phenotype was tested using unknown SM mutants selected from the ATIDB transposon insertion database.

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Metabolic engineering of terpene biosynthesis in *Arabidopsis* and consequences for plant-environment communication.

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Terpenoids are a class of secondary metabolites that play an important role in the communication of plants with their environment. In the past few years rapid progress was achieved in terpenoid metabolic engineering in plants, including *Arabidopsis*. These engineering experiments have demonstrated that the presence of terpenoid precursors in sub-cellular compartments is not as strictly separated as previously thought and that multi-step pathway engineering, even across cell compartments is feasible [1-4]. Hence it is now becoming feasible to create transgenic plants producing terpenoids on demand. With such engineered plants fascinating results have been obtained that show that insect behaviour is strongly influenced by terpenoids. The nature of these effects varies with terpenoid compound and insect species, and for example concerns repellency of aphids and thrips [1,2], but also attraction of predatory mites, the natural enemies of spider mites [3]. However, also rhizosphere communication with other types of organisms such as parasitic plants and arbuscular mycorrhizal (AM) fungi is mediated by terpenoids. Also here *Arabidopsis*, although not a host of AM fungi, turns out to be a suitable model. For the future, we foresee great progress in the engineering of terpenoid production in *Arabidopsis*. We expect that such transgenic plants will increase our understanding of the biological relevance of these secondary metabolites in the communication of plants with a multitude of other organisms but may also lead to commercial applications.

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P-489

Characterization of *Arabidopsis* acyltransferase mutants affected in cutin synthesis. Fred Beisson¹, Yonghua Li¹, Abraham Koo¹, John Ohlrogge¹, Mike Pollard¹. ¹Department of Plant Biology, Michigan State University, East Lansing, MI, USA 48824

Cutin and suberin are the two major cell wall-associated lipid polymers of plants. Cutin is a fatty acid- and glycerol-based polyester that constitutes the insoluble polymer matrix of the epidermal cuticle. The cutin polymer is embedded and covered with waxes.

Major issues of cutin research include the identification of biosynthetic enzymes (only 3 genes known so far), the intra- or extra-cellular site(s) of polymerization of monomers, the mechanism of transport of cutin monomers, oligomers or polymers through the cell wall.

We have obtained *Arabidopsis* cutin mutants by an approach combining microarray analysis of the transcriptome of *Arabidopsis* stem epidermis and reverse genetics. Cutin content was measured by a GC/MS method developed in our lab that allows routine analysis of *Arabidopsis* cutin. Single T-DNA insertional knock-outs for the acyltransferases GPAT4 and GPAT8 had no changes in cutin load and cuticle permeability. However, double KO's gpat4,gpat8 showed:

- a 60% -70% reduction in cutin in leaves and stems
- normal amounts of cuticular waxes and intracellular lipids
- the replacement of the normal electron-dense cuticle by a thicker but less compact structure
- a steep increase in water loss
- increase susceptibility to infection by the fungus *Alternaria brassicicola*.

These results demonstrate that the acyltransferases GPAT4 and GPAT8 are functionally redundant and essential for cutin synthesis.

P-491

Investigating putative functions of peroxisomal proteins through microarray analysis. John D Bussell¹, Itsara Pracharoenwattana¹, Andy AG Wiszniewski¹, Wenzu Zhou¹, Steven M Smith¹. ¹ ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia

Transcription of genes involved in specific pathways is often tightly regulated. For example using a time course microarray experiment, Smith et al. (Plant Physiol 136, 2687-2699, 2004) demonstrated coordinated expression of several starch degradation enzymes during the leaf diurnal cycle, leading to the discovery of a new enzyme required for starch metabolism.

We used this same data set as a tool to probe peroxisomal function during the diurnal cycle (12 h dark, 12 h light). Beginning with experimentally determined (SUBA, <http://www.plantenergy.uwa.edu.au/applications/suba/index.php>) and bioinformatically-predicted (ARAPEROX, <http://www.araperox.uni-goettingen.de/>) peroxisome proteins, we extracted microarray data for transcripts of genes involved in peroxisomal pathways.

Enzymes of photorespiration, including HPR, SHMT1 GGT1 and AGT are coordinately expressed. Their transcript levels vary approximately 2 fold with the maximum 4 h into the light period and the minimum 1 h into dark period. In contrast core beta-oxidation genes (LACS6, ACX4, MFP2, KAT2) and some apparently associated transcripts (CHY1) experience a maximum during the dark period and reduced expression in the light. This observation probably reflects changing metabolic requirement and cycling of resources during the diurnal cycle. This analysis allowed us to assign PMDH2 to the photorespiratory gene set and PMDH1 to the beta-oxidation set. Some transcripts encoding enzymes of unknown function have similar expression profiles to those of beta-oxidation, implying a role in fatty acid metabolism. Other genes potentially associated with beta-oxidation exhibit different expression patterns with maxima in the light period, including AIM1/MFP1 and KAT5, and those involved in JA biosynthesis (At4g05160, At5g63380, OPR3).

We have thus identified transcripts of peroxisomal proteins that are co-expressed with photorespiration, B-oxidation and other known peroxisomal pathways. This analysis provides new gene targets that are now the subject of genetic and functional studies.

P-492

Looking beyond *Arabidopsis* and angiosperms for biomass crop improvement genes. Clint Chapple¹. ¹ Purdue University, West Lafayette, Indiana, USA

One of the most significant barriers to the efficient conversion of biomass to biofuels is the lignin polymer. The biosynthesis of lignin is now relatively well understood in flowering plants, and can be readily manipulated. We have now begun investigations into lignin monomer (monolignols) biosynthesis in lycophytes to determine whether these modern relatives of the earliest tracheophytes might provide new tools for the modification of lignin in biomass crops. Fossil records show that the lycophyte clade arose 400 million years ago, 150-200 million years earlier than the angiosperms. Our interest in the genus *Selaginella* in particular was piqued by the observation that it deposits syringyl lignin, a type of lignin often regarded as being restricted to angiosperms. To gain insight into the evolution of syringyl lignin biosynthesis in *Selaginella*, we cloned candidates for the *S. moellendorffii* homologs of the three phenylpropanoid cytochrome P450-dependent monooxygenases that play essential roles in determining lignin composition in angiosperms. Although genetic approaches and transformation are not currently available in *S. moellendorffii*, complementation experiments in *Arabidopsis* and enzyme kinetic assays indicate that this *S. moellendorffii* P450 is a ferulate 5-hydroxylase (F5H) that can functionally replace its angiosperm counterpart in syringyl monolignol biosynthesis. Phylogenetic analysis suggests that the identified *Selaginella* F5H is very divergent from all known plant P450s in sequence and appears to have evolved independently from angiosperm F5Hs. This project is thus providing insight into the evolution of phenylpropanoid metabolism in vascular plants, while simultaneously augmenting our lignin modification toolbox.

P-493

Global Measurement of Plant Protein Turnover. Wen-Ping Chen¹, Xiaoyuan Yang², Jerry Cohen¹, William Gray². ¹Department of Horticultural Science, University of Minnesota, St. Paul, MN 55108, USA, ²Department of Plant Biological Sciences, University of Minnesota, St. Paul, MN 55108, USA

An increasing body of evidence has revealed that protein turnover is critical to the cellular regulatory processes that allow cells to not only rapidly respond to intracellular signal molecules but also to changing environmental conditions. In our lab, we are developing isotopic labeling strategies coupling with high-throughput mass spectrometry that will enable the measurement of dynamic protein turnover for plant proteins with the ultimate application for use as a proteome-wide fingerprinting technique. These procedures should result in sensitive measures of protein turnover profiles which would give us a powerful new insight into protein metabolism in plants and its importance on plant growth, development and adaptation to changing environmental conditions.

So far, we have been working on the construction of plant growth systems where labeling experiments using *Arabidopsis* with $^{13}\text{CO}_2$ and ^{15}N -nitrogen sources will be performed. As part of experiments to optimize the systems, once plants are grown in these systems in the presence of labeled primary nutrient sources (^{15}N nitrate or ammonia, $^{13}\text{CO}_2$), the enrichment in individual amino acids will be determined by GC-MS analysis of the free amino acids as well as protein hydrolysates. In addition, enrichment in a series of targeted proteins will be monitored by their isolation using immuno-affinity columns and analysis by MALDI-TOF or LC-MS-MS. The developed methods will be then used to investigate the kinetics of protein turnover for a set of proteins, for example those in the auxin biosynthetic and signaling pathways, by examining changes in label loss after a pre-labeling period. Labeling at high enrichments will be compared to subtle modifications to determine which methods provide the best overall biological and statistical significance. Such experiments with known proteins should greatly help define the suitability of each method for further protein turnover studies on larger scale. If successful, we will have established methods with good general applicability and, importantly, with limited artifacts.

P-494

New properties of the glucosinolate-myrosinase defense system discovered by vacuolar proteomics. Sixue Chen¹, Jean-Emmanuel Sarry², Richard Collum², Emily Wang¹, Chao-Xing Yuan², Philip A. Rea². ¹University of Florida, Gainesville, FL32605, USA, ²University of Pennsylvania, Philadelphia, PA19104, USA

Processes that converge on the vacuole, one of the largest organelles known, include pH and regulatory Ca²⁺ homeostasis, nutrient storage, xenobiotic sequestration and processing, volume regulation, and macromolecule turnover and salvage. If we are to understand how plant cells work at the systems level, and if the latter are to be engineered for enhanced nutritional quality and for the provision of environmentally friendly source of fine chemicals and pharmaceuticals, such as glucosinolates, it will be critical that researchers have ready access to a vacuolar proteomic toolbox. Knowing this, we have refined techniques for the purification of "proteomics-grade" intact vacuoles inclusive of their luminal protein complement from the model plant *Arabidopsis thaliana*. We have clearly resolved 270-300 polypeptides from the vacuolar lumen of *Arabidopsis* by 2-dimensional gel electrophoresis and identified relatively abundant proteins by MALDI-TOF-MS and ESI-LCQ-MS. Among the proteins are proteins which have been established to have a vacuolar localization, for instance aspartic proteinase, cysteine protease, leucine aminopeptidase, mannosidase, vegetative storage protein VSP1, and others, such as SOUL-like proteins which might be expected to have a vacuolar localization, but which were not known to be such, and several proteins of unknown functions.

It is interesting to note that myrosinase TGG1, TGG2, myrosinase associate proteins (MyAP) and myrosinase binding proteins (MBP) were all identified in the vacuoles isolated from rosette leaves. TGG1 are present as several glycosylated forms. At early developmental stage, TGG1 was more abundant than TGG2, which was only present as a small spot on 2D gels. In fully expanded leaves, both TGG1 and TGG2 levels increased. Concurrently, MyAP became a dominant spot on the 2D gel map. The co-localization of myrosinases with interacting proteins and the concurrent expression during development imply important regulatory mechanism of the myrosinase-glucosinolate system in plant development and interactions with environment factors.

P-495

A Systems Approach to Nitrogen Networks and the "Virtual-Plant". G Coruzzi¹, D Shasha², M Katari¹, M Gifford¹, K Birnbaum³, C Poultney², R Gutierrez^{4,1}, ¹NYU, Biology, NY, NY, ²NYU, Courant, NY, NY, ³ NYU, Comparative Functional Genomics, NY, NY, ⁴P. Universidad Catolica de Chile, Santiago, Chile

Our goal is to understand how internal and external perturbations affect gene networks linking plant metabolism with development. We seek to explain how changes in molecular networks evoke responses and to predict network states under untested conditions or in response to gene modifications. This systems approach should enable researchers to test biotechnological strategies for gene modification in silico, prior to testing in transgenic plants. Our systems approach starts with the integration of *Arabidopsis* genomic data into a "multi-network" 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, & literature-based interactions. This multi-network currently contains 7,000 nodes and 230,000 interactions. As proof-of-principle, we used this *Arabidopsis* multinetwork to identify gene networks transcriptionally controlled by light, carbon and nitrogen signals using microarray data from specific organs or cell-types. In selected cases, the networks identified in wild-type plants have been validated using microarray and other data from *Arabidopsis* mutants. To support this type of plant systems biology approach, we have implemented a set of data integration, analysis and visualization tools into a system called "VirtualPlant" (www.virtualplant.org). This platform 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 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. Funding: NIH, NSF and DOE.

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Recent Advances in Plant Cell Wall Biology. geoffrey fincher¹.¹
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Cell walls are central determinants of plant form, growth and development, and change in response to environmental and pathogen-induced stresses. Walls play important roles in the quality of plant-based foods for both human and animal consumption, and in the production of fibres during pulp and paper manufacture. In the future, wall remnants in crop residues could be used as a source of renewable fuel. Cellulose, a range of non-cellulosic polysaccharides, proteins and phenolic compounds are the major components of plant cell walls. They form covalently and non-covalently linked molecular networks that have the strength, flexibility and porosity necessary for cellular function. The fine structures and sizes of wall polysaccharides can be altered following deposition into the wall, to accommodate changing physicochemical requirements as cells grow and differentiate. The chemical structures of most wall polysaccharides have been defined in detail, but the enzymes involved in their synthesis and remodelling remain largely undefined. While there have been real advances in our understanding of cellulose biosynthesis in plants, with few exceptions the identities and modes of action of polysaccharide synthases and other glycosyltransferases that participate in the biosynthesis of the non-cellulosic wall polysaccharides are not known. Emerging functional genomics, molecular genetics and X-ray crystallographic technologies are allowing us to re-examine the central questions related to wall biosynthesis. For example, the availability of the rice, Populus and *Arabidopsis* genome sequences, various mutant populations, high density genetic maps for cereals, high throughput genome and transcript analysis systems, extensive publicly available genomics resources, and an increasing array of analytical systems for the definition of candidate gene function, permit a broad, non-biased approach to the description of wall biosynthesis in plants.

P-497

Vitamin B6 metabolism in plants: All is not as it had seemed
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Vitamin B6 is an essential metabolite in all organisms. It can act as a coenzyme for numerous metabolic enzymes and has recently been shown to be a potent antioxidant. Its de novo biosynthesis occurs only in bacteria, fungi and plants, making it an essential nutrient in the human diet. Despite its paramount importance, the pathway has classically only been investigated in *'Escherichia coli'* where it is synthesized from the condensation of 1-deoxy-D-xylulose-5-phosphate (a precursor in the non-mevalonate pathway of isoprenoid biosynthesis) and 4-hydroxy-threonine phosphate catalyzed by the concerted action of PdxA and PdxJ. It was tacitly assumed that the vitamin is synthesized in the same way in plants. However, recent studies have implicated that in the majority of organisms (including plants) that are capable of producing vitamin B6, a pathway distinctive and exclusive from *'E. coli'* exists. This pathway is characterized by the presence of two genes, namely 'PDX1' and 'PDX2'. We have identified the functional 'PDX1' and 'PDX2' homologs in '*Arabidopsis thaliana*' and will report on the biochemistry and physiology of vitamin B6 biosynthesis in plants. Specific topics that will be addressed will include the chemistry behind the synthesis of the vitamin, biochemical and structural aspects of the PDX1 and PDX2 proteins involved in the novel pathway, their essentiality for plant viability and the relationship of vitamin B6 biosynthesis to oxidative stress.

P-498

Induction of glucosinolates biosynthesis in *Arabidopsis* cell culture by carbon nanotubes (CNT) and thidiazuron (TDZ)
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Elicitors are molecules that stimulate defense or stress-induced responses in plants. They trigger signal cascades that activate several defense responses such as reinforcement of cell walls, induction of pathogenesis-related proteins or synthesis of phytochemicals. The enhancement of the content of phytochemicals as well as detection of novel ones upon elicitation suggests that elicitation may be a functional tool of technology for phytochemicals production. CNT and TDZ were found to act as abiotic elicitors when added to *Arabidopsis thaliana* callus culture. Quantitative estimation of total glucosinolates was achieved using microchip capillary electrophoresis and showed about 5.5 (0.082 mM) and 4 (0.06mM) folds increase in case of cultures containing CNT and TDZ respectively compared to normal cultures (0.015mM). Glucosinolates contents in cell suspension cultures, containing TDZ, were found to be low (\approx 0.007mM) compared to normal cultures.

This may be due to high activity of myrosinase enzyme in suspension culture compared to callus culture.

Glucosinolates qualitative analysis was achieved using microchip capillary electrophoresis and MALDI-TOF-MS. Seven different glucosinolates were identified in both normal cultures and cultures containing TDZ, while ten different glucosinolates were identified in cultures containing CNT. This study suggests CNT to be a valuable technology tool that can affect plant cell system biology and elicit the plant defense network leading to production of valuable phytochemicals.

P-499

Omics-based identification of R2R3-Myb transcription factors regulating aliphatic glucosinolate biosynthesis. Masami Yokota Hirai¹, Kenjiro Sugiyama², Yuji Sawada¹, Takayuki Tohge¹, Takeshi Obayashi^{3,4}, Akane Suzuki¹, Ryoichi Araki^{1,5}, Nozomu Sakurai², Hideyuki Suzuki², Koh Aoki², Hideki Goda¹, Osamu Ishizaki Nishizawa^{1,5}, Daisuke Shibata², Kazuki Saito^{1,3}. ¹RIKEN Plant Science Center, Yokohama, Japan, ²Kazusa DNA Research Institute, Kisarazu, Japan, ³Chiba University, Chiba, Japan, ⁴CREST, Japan Science and Technology Agency, Saitama, Japan, ⁵Kirin Brewery Co., Ltd., Yokohama, Japan

Understanding plant metabolism as an integrated system is essential for metabolic engineering aimed at the effective production of compounds useful to human life and the global environment. By integrating transcriptomics and metabolomics, we elucidated global regulation of the transcriptome and metabolome of Arabidopsis under nutritional stress conditions, and presented a strategy to identify novel gene functions with an example of sulfotransferase genes involved in the biosynthesis of glucosinolates (GSLs), bioactive secondary metabolites [1, 2]. In this presentation we report the discovery of two R2R3-Myb transcription factors that positively control the biosynthesis of aliphatic GSLs [3]. Combined transcriptome coexpression analysis of publicly-available condition-independent data, and the condition-specific (i.e., sulfur-deficiency) data identified *Myb28* and *Myb29* as candidate transcription factor genes specifically involved in the regulation of aliphatic GSL production. Analysis of a knockout mutant and ectopic expression of the gene demonstrated that *Myb28* is a positive regulator for basal-level production of aliphatic GSLs. *Myb29* presumably plays an accessory function for methyl jasmonate-mediated induction of a set of aliphatic GSL biosynthetic genes. Overexpression of *Myb28* in Arabidopsis cultured suspension cells, which don't normally synthesize GSLs, resulted in the production of large amounts GSLs, suggesting the possibility of efficient industrial production of GSLs by manipulation of these transcription factors. A working model for regulation of GSL production involving these genes, renamed *Production of Methionine-derived Glucosinolate (PMG)* 1 and 2, are discussed.

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- [2] Hirai et al. (2005) JBC 280:25590
- [3] Hirai et al. (2007) PNAS 104:6478

P-500**DIFFERENTIAL ROLES OF ARABIDOPSIS PIP2 AQUAPORINS**

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The Arabidopsis major intrinsic protein (MIP) family consists of 35 members. Several MIPs facilitate the flow of small uncharged molecules across the plasma membrane (PIP) or tonoplast (TIP). Due to the frequently observed water permeability they are named aquaporins and thought to be involved in plant water relations. PIPs are subdivided into 5 PIP1 and 8 PIP2, constituting highly homologous subfamilies. We focused on the PIP2 members asking whether they might exhibit differential functions. Promoter-GUS constructs showed gene-specific, discernable patterns. Most PIP genes were expressed in the region of the vasculature in roots and/or aerial parts, however, individual members were expressed in root exodermis (PIP2;4) or more uniformly in the leaves (PIP2;5, PIP2;7).

Except for PIP2;7 knock-outs for all PIP2 genes could be obtained. They did not show visible phenotypes under normal growth conditions. Using a custom-made DNA array (500 gene-specific targets) focusing on membrane proteins we discovered only minor, but distinct changes for the pip2 knock-outs. A PCA of the expression data corroborated the differential responses. Furthermore, the individual PIP2 genes were analyzed for co-expressing genes at [bbc.botany.utoronto.ca](http://bbc.botany.utoronto.ca/www.arabidopsis.leeds.ac.uk/act/index.php) and www.arabidopsis.leeds.ac.uk/act/index.php. Only PIP2;1 and PIP2;2 showed significant correlations with other PIPs, however most PIP2 genes were also correlated to TIP isoforms, which may indicate an interaction of plasma membrane and tonoplast permeabilities. Although there were a few functional categories enriched among the co-expressed genes in all cases (transport, cell wall), the affected genes were different. A third approach was based on the stress-responsive expression patterns of the individual PIP2 genes from publicly available array data. PCA analyses revealed three distinct groups of PIP2 members.

Based on these analyses, we propose a differential, and mostly non-redundant function for the closely related PIP2 genes.

P-501

A natural fusion gene encoding uridine kinase and uracil phosphoribosyltransferase in Arabidopsis and rice. M. R. Islam¹, Kang S. W.¹, Kim H.¹, Y. M. Jeong¹, B. W. Kim², D. K. Kim², J. S. Kim², S. G. Kim¹. ¹Seoul National University, Seoul, Republic of Korea, ²Cheju National University, Jeju, Republic of Korea

Uridine kinase (UK) and uracil phosphoribosyltransferase (UPRT) are enzymes catalyzing the formation of uridine 5'-monophosphate from uridine and adenosine 5'-triphosphate or uracil and 5-phosphoribosyl-1-pyrophosphate in pyrimidine salvage pathway, respectively. The genes for UK or UPRT were reported as separate genes in bacteria or yeasts. Here we report the analysis of a gene with dual domains for UK and UPRT from Arabidopsis (*AtUK/UPRT1*) and rice (*OsUK/UPRT1*). Sequence analysis was revealed that *AtUK/UPRT1* and *OsUK/UPRT1* are encoded as a molecular mass of approximately 53 kDa and 51 kDa, respectively. The predicted amino acid sequences of *AtUK/UPRT1* and *OsUK/UPRT1* are similar to the two proteins for UK and UPRT from bacteria and yeasts. Amino-terminal region is similar to UDK of *Escherichia coli* and many bacteria and contains an ATP/GTP-binding site motif A called P-loop whereas the carboxyl-terminal is similar to UPP of *E. coli* and contains signature-binding motif for a uracil and a PRPP. Expression of *AtUK/UPRT1* in a upp and udk mutant of *E. coli* led to growth inhibition effect with 5-fluorouracil (5-FU) or 5-fluorouridine (5-FD). Identical results were obtained when the UK and UPRT domains were separated into the upp-udk and upp mutants. We analyzed an Arabidopsis mutant of *AtUK/UPRT1* which its growth was not affected whereas wild-type Arabidopsis showed drastic growth retardation with treatment of 5-FU or 5-FD. These results suggest that the *AtUK/UPRT1* and *OsUK/UPRT1* products can use uracil or uridine as a substrate and would be natural fusion proteins of a UK and a UPRT enzyme.

P-502

Chloroplast NADPH thioredoxin reductase: a novel thiol redox regulator of amino acid biosynthesis in chloroplasts?
Saijaliisa Kangasjärvi¹, Anna Lepistö¹, Kati Hännikäinen¹, Riitta Ruokamo¹, Gert Brader², Nina Sipari³, Markku Keinänen³, Eevi Rintamäki¹.¹ Department of Biology, University of Turku, FI-20014 Turku Finland, ²Faculty of Biosciences, Department of Biological and Environmental Sciences, Genetics, FI-00014 University of Helsinki, Finland, ³ Faculty of Biosciences, University of Joensuu, FI-80101 Joensuu, Finland

A novel chloroplastic thiol redox regulator, an NADPH-dependent thioredoxin reductase (NTRC), was recently described in *Arabidopsis thaliana*. To gain insights into the physiological significance of NTRC, we have characterized T-DNA insertion mutant lines of the *NTRC* gene. Homozygous *ntrc* plants showed reduced growth and a pale green phenotype when grown under moderate light intensity. Biochemical analysis revealed that these characteristics were not connected with deficiencies in the accumulation or function of photosynthetic membrane protein complexes, or with impaired carbon assimilation capacity of Rubisco. Rather, *ntrc* plants showed distinct developmental disorders: mesophyll cells were small and possessed lowered amount of chloroplasts, the number of stomata was increased, and senescence was delayed. Interestingly, all these characteristics were more pronounced under short day conditions (8/16 hour light rhythm). The developmental deficiencies correlated with imbalances in basic metabolism: the profile of plastid-derived amino acids was altered, and the auxin content of ten-day-old *ntrc* plants was diminished. Accordingly, growth of *ntrc* plants on a medium supplemented with auxin, aromatic amino acids or isoleucine restored the cell size and the number of chloroplasts per cell in *ntrc* leaves. Interestingly, *Arabidopsis* belongs to the family of Brassicas, the members of which possess an additional tryptophan-dependent pathway for auxin biosynthesis that takes place in chloroplasts. Altogether, our results suggest a novel function for NTRC in the photoperiod-dependent modulation of plant development via thiol redox regulation of amino acid biosynthesis in chloroplasts. Yeast two-hybrid analysis and thiol-redox labelling experiments to demonstrate the target proteins of NTRC are underway.

P-503

Functional Analysis of a Peroxisomal Acyl-Coenzyme A Synthetase Protein Family from *Arabidopsis thaliana* Uncovers Enzymes Participating in Jasmonic Acid Biosynthesis. Lucie Kienow¹, Katja Schneider¹, Michael Bartsch¹, Otto Miersch², Claus Wasternack², Erich Kombrink¹.¹ Max Planck Institute for Plant Breeding Research, Cologne, Germany, ²Leibniz-Institute of Plant Biochemistry, Halle, Germany

The *Arabidopsis* genome contains a large number of carboxylic acid-activating enzymes, including nine long-chain fatty acyl-CoA synthases (LACS), four 4-coumarate CoA ligases (4CL), and nine proteins closely related to 4CLs with unknown functions (4CLL). We systematically examined the function of the latter group by applying an extensive substrate screen to recombinant 4CLL enzymes. This analysis uncovered the activation of fatty acids of variable chain length as the common feature of all active members of this protein family, thereby defining a new group of fatty acyl-synthetases, which is distinct from the known LACS. Significantly, four family members also displayed high activity towards different biosynthetic precursors of jasmonic acid (JA), including 12-oxo-phytodienoic acid (OPDA), dinor-OPDA, 3-oxo-2-(2-[Z]-pentenyl) cyclopentane-1-octanoic acid (OPC-8) and OPC-6, but not OPC-4 and JA. Detailed analysis of the *in vitro* substrate specificity uncovered substantial differences for the individual enzymes acting on JA precursors, identifying one unique OPC-8:CoA ligase (At1g20510). To further understand the *in vivo* function of At1g20510, an *Arabidopsis* insertion mutant for the gene was analyzed by transcript and jasmonate profiling. We found that JA accumulation after wounding was reduced in the mutant whereas the precursors OPC-8, OPC-6 and OPC-4 accumulated to enhanced levels. The analysis of At1g20510-promoter::GUS reporter lines showed the induction of At1g20510 expression after wounding or treatment with *Pseudomonas syringae*. Collectively, the results demonstrate that OPC-8:CoA ligase catalyzes an essential step in JA biosynthesis by initiating the beta-oxidative chain shortening of its precursors.

P-504

Functional analysis of rice CYP85. BoKyung Kim¹, Sunghwa Choe¹.¹ School of biological sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Korea

Brassinosteroids (BRs) collectively refer to steroidal plant hormones that are essential for growth and development of plants. It has been proposed that BRs are synthesized via two parallel pathways, the early and the late C-6 oxidation pathways according to the order of C-6 oxidation status. One is the early C-6 oxidation pathway, in which oxidation at C-6 occurs before the introduction of vicinal hydroxyls at C-22 and C-23 of the side chain. The other is the late C-6 oxidation pathway in which C-6 is oxidized after the introduction of hydroxyls on the side chain. The C-6 oxidation of BR intermediates is catalyzed by the enzymes encoded by Cytochrome P450 85 (CYP85) genes. Arabidopsis CYP85 enzymes have been shown to catalyze C-6 oxidation of 6-deoxo intermediates. Interestingly, Arabidopsis CYP85A2 was also found to mediate the ultimate step which is Baeyer-Villiger type oxidation of C-6. However, the functions of rice CYP85 protein are still unknown. Therefore, we aimed to understand the function through feeding experiments with a yeast strain that is heterologously expressing the rice CYP85 gene. Feeding tests following GC-MS based analyses revealed that both Arabidopsis CYP85A2 and rice CYP85 metabolize 6-deoxo-BRs into Castasterone. Further experiments with Castasterone will confirm whether OsCYP85 mediates the ultimate step or not.

P-505

Isolation of rice cDNAs for higher accumulation of pigments from rice FOX lines. Youichi Kondou¹, Mika Kawashima¹, Takanari Ichikawa¹, Akie Ishikawa¹, Yukako Hasegawa¹, Yuko Makita¹, Hirohiko Hirochika², Minami Matsui¹. ¹RIKEN, Yokohama, Japan, ²NIAS, Tsukuba, Japan

FOX hunting system is novel gain-of-function technique. In this system, random over-expression of a normalized plants full-length cDNA library cause dominant gain-of-function mutations and it is expected that plant acquire useful phenotype. We are establishing rice FOX lines using 13,000 non-redundant rice full-length cDNAs to introduce them into 'Arabidopsis' plants. At present, we are screening these lines with various traits for exploration of useful rice genes. In this study, we introduce screening strategy for isolation of mutants of higher accumulated pigments in rosette leaves. We already isolated some mutants by screening in T1 generation from about 6,000-7,000 lines. Rice cDNAs isolated from these mutants encoded stress related genes, some enzymes, unknown proteins and so on. Some of these rice cDNAs have, upon overexpression in 'Arabidopsis', re-exhibited higher accumulation of pigments. At present, we introduce these cDNAs into rice (*Nipponbare*).

In addition, we analyzed microarray experiments by using these 'Arabidopsis' overexpressors to re-isolate useful genes for higher accumulation of pigments from 'Arabidopsis' genome, and some commonly increased genes in these overexpressor were isolated as candidates. And the categorization of gene ontology by using data sets of these microarray analyses should appear some pathway for regulation of pigments in 'Arabidopsis'. We would discuss transcription factors, which should regulate these pathways.

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P-506

A new class of membrane proteins as potential amino acid exporters/facilitators. Friederike Ladwig¹, Axel Hirner¹, Wolfgang Koch¹. ¹ZMBP Plant Physiology, Tuebingen, Germany

Nitrogen is one of the limiting nutrients in plants. In the phloem, nitrogen can be transported as amino acids from the site of biosynthesis to the sink. Concerning the cellular transport at the plasma membrane, all so far characterized amino acid transporters function as importers. The export mechanism out of the cell is still unclear. We suppose that a new class of membrane proteins, the N21-proteins, act as potential exporters/facilitators. These proteins are plasmamembrane localized. Initial experiments show that amino acids can be transported in both directions independently of pH and membrane potential, probably in a concentration-dependant manner. Some of the proteins tested localize to specific regions in roots and developing seeds which supports the idea of a function as selective amino acid filters. A knockout mutant for one of the N21 genes displays a visible phenotype in developing seeds. Analytical analysis of amino acids in knockout plants in comparison to wildtype exhibits an altered amino acid composition. Proteins of the large N21 gene family are good candidates for amino

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Monoacylglycerols are Components of Root Waxes and can be Produced in the Aerial Cuticle by Ectopic Expression of a Suberin-associated Acyltransferase. Yonghua Li¹, Fred Beisson¹, John Ohlrogge¹, Mike Pollard¹. ¹Department of Plant Biology, Michigan State University, East Lansing, MI, USA

The interface between plants and the environment is provided for aerial organs by epicuticular waxes that have been extensively studied. By contrast, little is known about the nature, biosynthesis and role of the waxes at the root-rhizosphere interface. Waxes isolated by rapid immersion of *Arabidopsis* roots in organic solvents were rich in saturated C18-C22 alkyl esters of p-hydroxycinnamic acids, but also contained significant amounts of both alpha- and beta-isomers of monoacylglycerols (MAGs) with C22 and C24 saturated acyl groups, and the corresponding free fatty acids (FFAs). The production of these compounds in root waxes was positively correlated to the expression of GPAT5, a gene encoding an acyltransferase previously shown to be involved in suberin synthesis. This suggests a direct metabolic relationship between suberin and some root waxes. Furthermore, when ectopically expressed in *Arabidopsis*, GPAT5 produced very long chain saturated MAGs and FFAs as novel components of cuticular waxes. The crystal morphology of stem waxes was altered and the load of total stem wax compounds was doubled while the major components of wild-type waxes decreased. These results indicate that GPAT5 functions *in vivo* as an acyltransferase to a glycerol-containing acceptor and has access to the same pool of acyl intermediates and/or may be targeted to the same membrane domain as that of wax synthesis in aerial organs.

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Engineering of "Arabidopsis thaliana" FMOs increases production of the cancer-preventive isothiocyanates. Jing Li¹, Bjarne Gram Hansen¹, Daniel J. Kliebenstein², Barbara Anne Halkier¹. ¹Copenhagen University, Copenhagen, Denmark, ²University of California-Davis, Davis, USA

A high dietary intake of cruciferous vegetables has been associated with a reduction in cancers, and isothiocyanates (ITCs) derived from the natural products glucosinolates (GSLs) are considered to be responsible for the cancer-preventive action. Epidemiological studies have shown that ITCs derived from methylsulfinylalkyl GSLs particularly sulforaphane (the isothiocyanate hydrolysis product from methionine-derived 4-methylsulfinylbutyl GSL) has been identified as a very potent cancer-preventive agent. Here we report identification of a cluster of 5 *Arabidopsis* flavin-monooxygenase (FMO) genes, FMO GS-OX1-5, encoding for enzymes responsible for the S-oxygenation of methylthioalkyl into methylsulfinylalkyl GSLs. This is evidenced by biochemical characterization of recombinant proteins and analyses of the GSL profile in FMO over-expression lines and FMO knock-out mutants. As an example, FMO "GS-OX1" over-expression lines show almost complete conversion of methylthioalkyl into methylsulfinylalkyl GSLs, with a ~ 5 fold increase in 4-methylsulfinylbutyl GSL in seeds. Our finding represents the first identification of S-oxygenating FMOs in plants and provides an important molecular tool for breeding of Brassica vegetable crops with increased levels of these important GSLs. This has implications for production of functional foods enriched with cancer-preventive ITCs, including the well-known sulfor-

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Analysis of S-Adenosyl-L-Homocysteine Hydrolase Gene Expression Reveals a Role of Cytokinin in Promoting Transmethylation Reactions. Chun-Hong Li¹, Shi-Min Jiang¹, Ling-Jian Wang¹, Xiao-Ya Chen¹. ¹National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, SIBS, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China

S-Adenosyl-L-homocysteine hydrolase (SAHH), catalyzing the conversion of S-adenosyl-L-homocysteine into adenosine and L-homocysteine, is a key enzyme for the maintenance of cellular transmethylation potent. SAHH has been shown to be a cytokinin-binding protein, however, the effect of cytokinin on transmethylation reactions has been unclear. In *Arabidopsis thaliana*, there are two genes encoding SAHH, *AtSAHH1* and *AtSAHH2*, which show non-overlapping expression patterns. A T-DNA insertion mutant of *AtSAHH1* (*sah1-1*) and the RNA interference (RNAi) plants (*dsAtSAHH2*) exhibited different degrees of phenotypic changes, including higher chlorophyll contents, delayed flowering and senescence, and bushy architecture. We found that expression of a cytokinin synthesis gene, *AtPT7*, was up-regulated. On the other hand, cytokinins positively regulate the transmethylation pathway genes, including *AtSAHH1* and *AtADK1* (for adenosine kinase), and this regulation involves the cytokinin signaling pathway, but not the purine structure. Unlike adenine and adenosine which are SAHH inhibitors, the adenine-type cytokinins have no effect on SAHH activity at protein level. Changing of endogenous cytokinin levels by overexpressing the cytokinin synthesis or degradation genes resulted in alterations of DNA methylation status in the *sah1-1* background, suggesting that cytokinins affect DNA methylation under metabolism-stress conditions. These data demonstrate a cross-talk between the SAM-dependent transmethylation cycle and the cytokinin signaling.

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Carbon availability and stress response. Do different DAHP synthase isoforms serve different demands? Sven Martin¹, Sabine Preiskowski¹, Anika Wiese¹, Ingar Janzik^{1,2}. ¹Institute Phytophysphere (ICG 3), Research Centre Jlich, 52425 Jlich, Germany, ²Heinrich Heine University Düsseldorf, Germany

The DAHP synthase (DAHPS) catalyses the first step of the shikimate pathway, a key pathway for the synthesis of aromatic primary and secondary plant metabolites. The substrates for the DAHPS are intermediates of the oxidative or reductive pentosephosphate cycle and glycolysis and hence depend directly on the availability of carbon in primary metabolism. On the other hand many plant reactions to environmental stress include the induction of aromatic secondary metabolites, derived from shikimate pathway products. Thus carbon flux through shikimate pathway depends on two main influences: the substrate availability and the drain into secondary metabolism. This led to the hypothesis that the DAHPS might be regulated by these two different signals independently.

To test this hypothesis, we first supplied *Arabidopsis* leaves with external glucose. Glucose turnover is known to act as a direct signal for many genes in response to increased carbon availability. By isoform specific Real-time-PCR we were able to show, that the supply of external glucose but not sorbitol - led to a strong increase in the DAHPS transcripts of two of the three existing isoforms. This was independent from the analysed ecotype. Further more we could demonstrate, that this induction was not regulated by the HXK1 mediated signalling cascade, what is in agreement with the regulation of other enzymes relevant for aromatic secondary metabolism, like PAL and CHS. But in contrast we could also demonstrate that the stress specific induction of aromatic secondary metabolites, resembling a drain of shikimate pathway products, only was preceded by the induction of one of the three isoforms. These results indicate that an increased carbon flux into primary aromatic metabolism, triggered by an increase in substrate availability might be mediated by another isoform, than a carbon efflux, which is forced by an increase in aromatic secondary metabolites. So that the different isoforms of the DAHPS are regulated by different signals and serve different demands, depending on the activation stimulus.

P-511

A single MYB domain protein, AtMYBL2, acts as a negative regulator in flavonoid biosynthesis. Kyoko Matsui^{1,2}, Masaru Ohme-Takagi¹. ¹Research Institute of Genome-based Biofactory, National Institute of Advanced Industrial Science and Technology (AIST), ²GreeenSogna, Inc. <http://greensogna.com>

A protein complex composed of MYB and bHLH transcription factors associated with a WD40 repeat protein has been shown to regulate flavonoid biosynthesis in plants. A similar regulatory complex specifies epidermal cell fate. In *Arabidopsis*, CPC, TRY and ETC1, which are single MYB domain transcription factors and lack transactivation domain, were found to be negative regulator for epidermal cell differentiation. However a single-MYB domain-type transcription factor that is involved in the flavonoid biosynthesis has not been identified. Recently we showed that the PAP1 chimeric repressor, in which the EAR-motif repression domain was fused to PAP1 (35S:PAP1SRDX), inhibited the accumulation of proanthocyanidin and anthocyanin. The seeds of 35S::PAP1SRDX plants exhibited light yellow due to suppression of the accumulation of proanthocyanidin. To isolate a single-MYB domain-type transcription factor that is involved in the regulation of flavonoid biosynthesis, we prepared transgenic plants that expressed the chimeric repressor for a single-MYB domain protein and found that the expression of the chimeric AtMYBL2 repressor induced pale-color in seeds. The ectopic expression of AtMYBL2 (35S:AtMYBL2) also suppressed accumulation of proanthocyanidin in the seeds. In addition, accumulation of anthocyanine in leaves of both 35S::AtMYBL2 and 35S::AtMYBL2SRDX plants were lower than those of wild type plants, indicating that AtMYBL2 acts as transcriptional repressor. Transient assay revealed that AtMYBL2 acted as a transcriptional repressor and the six amino acids at the carboxy-terminal region sufficiently confer the repression activity, which is a novel repression domain different from known EAR-motif. The 35S:AtMYBL2ΔC transgenic plants and AtMYBL2 knockout lines exhibited an increase of anthocyanin accumulation in rosette leaves. We will discuss a possible role of AtMYBL2 on the regulation of flavonoid biosynthesis.

P-512

Isolation and characterization of novel phosphatidic acid phosphatases in *Arabidopsis*. Yuki Nakamura¹, Mami Tsuchiya¹, Ryota Koizumi¹, Hiroyuki Ohta¹. ¹Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology

Phosphatidic acid phosphatase (PAP) hydrolyzes phosphatidic acid (PA) to yield diacylglycerol (DAG). Since PAP in chloroplast mediates crucial step for synthesizing indispensable galactolipids, its activity was well-characterized to date. In addition, recent identification of TGD1, a potent ER-to-chloroplast lipid transporter, emphasized the importance of PA as a central metabolite (Xu et al., 2003, 2005). Despite the importance of PAP, however, its phylogenetic origin was unknown.

Although *Arabidopsis* has four lipid phosphate phosphatases (LPP1~4) (Katagiri et al., 2005) as mammalian LPP homologues, they are not predicted to be localized at chloroplast. Since no mammalian LPP homologues are identified in cyanobacteria, a possible ancestor of chloroplast, we hypothesized that there is a different type of LPP in cyanobacteria and *Arabidopsis*. Based on a distinct strategy, we identified a strong candidate for cyanobacterial PAP and its five homologues (LPPbeta, LPPgamma, LPPdelta, LPPepsilon1 and LPPepsilon2) in *Arabidopsis* which were distinct from LPP1~4. Protein sequence analysis showed that these homologues are distant from known mammalian and Yeast LPP but close to cyanobacteria and other prokaryotes, suggesting that LPP may be categorized as either prokaryotic or eukaryotic subfamily. Localization analysis by specific antibodies showed that three of them (LPPgamma, LPPepsilon1 and LPPepsilon2) were localized to the chloroplast. They possessed significant PAP activity and LPPgamma showed enzymatic properties mostly similar to that of native PAP activity in *Arabidopsis* chloroplasts. Expression study by RT-PCR and GUS staining showed that LPPgamma was primarily expressed in shoot. Knock out mutants of LPPepsilon1, LPPepsilon2 and their double knock out, showed no detectable changes in lipid composition whereas single knock out mutant of LPPgamma was not isolated both by T-DNA insertion and RNAi gene suppression, suggesting that complete loss of LPPgamma might be lethal. Latest results regarding the novel PAP will be presented.

P-513**Using metabolomics to decipher functions of *Arabidopsis* genes in the context of metabolic and regulatory networks**

Basil Nikolau¹, Julie Dickerson¹, Philip Dixon¹, Oliver Fiehn², Bernd Markus Lange³, Seung Yon Rhee⁴, Vladimir Shulaev⁵, Lloyd Sumner⁶, Ruth Welti⁷, Eve Syrkina Wurtele¹. ¹Iowa State University, ²University of California, Davis, ³Washington State University, ⁴Carnegie Institution, Stanford, ⁵Virginia Bioinformatics Institute, ⁶The Samuel Roberts Noble Foundation, ⁷Kansas State University

A multi-institutional consortium of labs (www.plantmetabolomics.org) is developing metabolomics as a new functional genomics tool for elucidating the functions of *Arabidopsis* genes that are currently annotated as having an "unknown function". Approximately one third of the *Arabidopsis* genes are so annotated. The consortium utilizes five distinct analytical platforms that couple different separations methods (GC, LC, and CE) to mass-spectroscopic detection systems. These analytical platforms are used in both "non-targeted" and targeted metabolomics analyses, which in combination detect approximately 2,000 metabolites, of which 700 are chemically defined. The consortium is applying these platforms to reveal changes in the metabolome associated with knockout mutations in genes of unknown function and comparing these to similar mutants in genes of known functions. We will discuss initial data generated from a small set of exemplar mutants. These data indicate that metabolomics can reveal metabolic changes in mutants that are otherwise "silent" in phenotype. These data are being interpreted via two strategies: 1) as a "fingerprint" of the metabolic consequence of each mutation, which can be used to functionally cluster genes; and 2) by mapping metabolite changes on metabolic and regulatory network maps, such as AraCyc and MetNetDB, to identify specific functions that are affected by each mutation. Thus, metabolomics, in combination with other "-omics" technologies promises to be a new resource for determining the function of *Arabidopsis* genes.

P-515**Understanding the complex machinery responsible for cuticle assembly in plants.** David Panikashvili¹, Sigal Savaldi-Goldstein², Tali Mandel¹, Tamar Yifhar¹, Rochus Franke³, Ren Hfer³, Lukas Schreiber³, Joanne Chory², Asaph Aharoni¹. ¹Weizmann Institute of Science, Rehovot, Israel, ²The Salk Institute, La Jolla, CA, USA, ³Institute of Cellular and Molecular Botany, Bonn, Germany

The aerial organs of plants are covered with a continuous layer overlaying the outermost cell walls of the epidermis, termed the cuticle. Cutin, the main component of the cuticle, is composed of various interesterified hydroxy- and epoxy-C16-18 (un)saturated fatty acids. It is embedded with intracuticular and covered with epicuticular waxes consisting largely of derivatives of very long chain fatty acids. Metabolic routes involved in cuticle assembly may be divided into biosynthetic and transport pathways. The transport pathway comprises the extrusion of cutin monomers from their site of biosynthesis to the extracellular space through the plasma membrane and cell wall. The molecular mechanisms of this process are poorly understood. Here, we report on the isolation and characterization of the "desperado" mutant in "*Arabidopsis*", which exhibits an array of severe surface defects including postgenital organ fusion and epidermal ruptures suggesting a malfunctioning cuticle. Transmission Electron Microscopy revealed unusual cytoplasmatic inclusions in leaf and stem epidermal cells and disappearance of the cuticle in fusion areas. Loss of "DESPERADO" function resulted in a striking phenotype, characterized by altered morphology of stomatal cells. "DESPERADO" encodes a putative ABC transporter and is localized to the plasma membrane. Chemical analysis showed that total cutin monomers load was reduced in the *desperado* knockout line. "DESPERADO" expression is induced by abscisic acid (ABA), salt and mechanical wounding. Consistent with this observation, the "desperado" mutant is susceptible to salt and shows a reduction in root branching, a phenomenon associated with increased ABA and salty environment. Thus, we provide evidence that "DESPERADO" is required for the export of cutin monomers to the surface through the plasma membrane of epidermis cells. Yet, it is possible that "DES" might interact with a non-specific lipid transfer protein that could carry out cutin transport through the hydrophobic cell wall.

P-514**Co-expression of two sorghum desaturases in transgenic "*Arabidopsis*" plants results in the accumulation of unusual terminal double bond-containing fatty acids "in planta".** Zhiqiang Pan¹, Scott R. Baerson¹, Agnes M. Rimando¹. ¹USDA-ARS-NPURU, University of Mississippi, University, MS 38677

Polyunsaturated fatty acids play important roles as structural components of membrane glycerolipids, and as precursors of signalling molecules in eukaryotic cells. Previously, we isolated two cDNAs encoding fatty acid desaturases from "*Sorghum bicolor*", designated "SbDES2" and "SbDES3", which consecutively convert 16:1 to 16:3 FAs (16:3Δ9,12,15) possessing a terminal double bond when co-expressed in "*S. cerevisiae*" (Pan et al, 2007). To study the functionality of these enzymes when overexpressed "in planta", a binary vector (pCBDA1) was constructed for co-expression of the "SbDES2" and "SbDES3" under the direction of the CaMV 35S promoter. The mutant "fad7" line of "*Arabidopsis thaliana*" (L.) Heynh., deficient in hexadecatrienoic acid (16:3Δ7,10,13), was transformed with recombinant "*A. tumefaciens*" strains harboring pCBDA1, using the floral dip procedure. The expression of both the "SbDES2" and "SbDES3" in transgenic "*Arabidopsis*" lines was confirmed by RT-PCR. Significantly, the accumulation of 16:3Δ9,12,15 fatty acid was detected in multiple lines co-expressing the two desaturases. Molecular and biochemical characterization of these transformants will be presented.

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Jasmonates, fast-acting master switches between growth and defence responses in *Arabidopsis*. Laurens Pauwels¹, Kris Morreel¹, Wout Boerjan¹, Dirk Inzé¹, Alain Goossens¹. ¹VIB Department of Plant Systems Biology, UGent, Gent, Belgium

Jasmonates (JAs) are plant-specific signalling molecules that steer a diverse set of cellular processes, of which their ability to invoke defence responses is the most renown. To further unravel the pleiotropic functions of JAs, the transcriptome of an *Arabidopsis thaliana* cell suspension culture treated with methyl jasmonate (MeJA) was monitored over time with microarrays. Additionally, cycloheximide (CHX) was used to distinguish primary and secondary MeJA response genes. Early MeJA response genes were predominantly involved in transcriptional regulation and JA biosynthesis, and were superinduced in the presence of CHX. In a second transcriptional wave, MeJA coordinately upregulated the expression of genes involved in monolignol biosynthesis and several supporting or related pathways and processes. Simultaneously, MeJA repressed the expression of genes peaking in the M phase of the cell cycle. Targeted metabolite profiling and flow cytometric analysis confirmed the transcriptome data, showing a MeJA-mediated increased flux through the monolignol pathway and a G2 cell cycle arrest, respectively. When comparing the microarray dataset from MeJA elicited suspension cells with other, publicly-available datasets reflecting the responses of various *Arabidopsis* tissues to JA raises, a context dependency of the JA transcriptional response was observed.

The MeJA inducible oligolignol biosynthesis model system and the clearly defined gene platform that have been established here are now being exploited to characterize hitherto unknown biochemical and molecular aspects of *Arabidopsis* phenylpropanoid metabolism. Furthermore, the potential role of the primary MeJA response genes, for instance members of different families of putative transcription factors, in the steering of the dual MeJA effect on growth and defence is being investigated.

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Metabolomics-based functional genomics in *Arabidopsis thaliana*. Kazuki Saito^{1,2}. ¹RIKEN Plant Science Center, Yokohama, Japan, ²Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Metabolomics is a rapidly-emerging sector of post-genome research. The metabolome (a set of all metabolites of an organism) represents not only the ultimate phenotype of cells by the perturbation of gene expression and the modulation of protein functions caused by the environment or mutations, but the metabolome can also feed back on gene expression and protein function. Therefore, metabolomics plays a key role for understanding cellular systems. The completion of the whole genome sequence of *Arabidopsis thaliana* has made it possible to discover the genes involved in metabolism in a high throughput manner by determining gene-to-metabolite correlation through the comprehensive analysis of metabolite accumulation and gene expression. In this seminar I will present the aims, rationale and some examples of the identifying gene function of *Arabidopsis* by such analysis. *In silico* co-expression analysis of genes involved in flavonoid metabolism in *Arabidopsis* was performed using a publicly available transcriptome database of DNA microarrays. We inferred a co-expression framework model of the genes involved in the pathways of flavonoids, suggesting specific functions and co-regulation of the genes of pathway enzymes and transcription factors. Changes in flavonoid profiles of wild-type plants and T-DNA insertion mutants of the delimited genes led to the confirmation of gene function (Yonekura-Sakakibara et al., *J Biol Chem*, doi: 10.1074/jbc.M611498200 (2007)). We also applied this strategy to glucosinolate biosynthetic pathway for identification of MYB transcription factors crucial for aliphatic glucosinolate production (Hirai et al., *PNAS*, 104, 6478 (2007)). These results suggest that the functional genomics approach by integration of metabolome with transcriptome provides an efficient way of identifying novel gene functions involved in plant metabolism.

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Strategy for plant metabolic systems analysis: glucosinolate and amino acid biosynthesis in *Arabidopsis*. Yuji Sawada¹, Kazuki Saito^{1,2}, Masami Yokota-Hirai¹. ¹RIKEN Plant Science Center, Suehiro-cho 1-7-22, Tsurumi-ku, Yokohama, Kanagawa, Japan, ²Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Integration of transcriptomics and metabolomics enabled us to identify the co-expressed genes and co-accumulated metabolites in *Arabidopsis* under sulfur deficiency condition [1]. The remarkable co-expression patterns were very similar to the condition-independent co-expression patterns clarified by using publicly-available *Arabidopsis* DNA array data sets. We assumed that the co-expressed genes are involved in the same metabolic pathway. In this study, co-expression analysis was applied to a group of genes encoding Methionine-derived glucosinolate (MET-GSL) biosynthesis enzymes and their transcriptional factors. The candidate genes, which were thought to encode the enzymes catalyzing the sequential four steps in MET side-chain elongation [methylthioalkylmalate synthase (MAM), methylthioalkylmalate isomerase (MAM-I), methylthioalkylmalate dehydrogenase (MAM-D) and methionine-analog aminotransferase (MAAT)], were successfully predicted based on co-expression pattern with known MET-GLS biosynthesis genes. Interestingly, the candidate genes of MET side-chain elongation enzymes (MET-ELONG) are homologs of the genes coding for leucine biosynthesis enzymes (isopropylmalate synthase, isopropylmalate isomerase, isopropylmalate dehydrogenase, and branched chain aminotransferase). The predicted gene functions were confirmed by the changes in metabolite profiles, i.e., glucosinolate and amino acid profiles, of T-DNA insertion mutants of corresponding genes. That is, in the MET-ELONG gene knock-out mutants, the levels of MET-GSLs with elongated side-chain markedly decreased. Using a similar strategy, we have also identified the two genes encoding Myb transcription factors that positively control the biosynthesis of MET-GSL [2]. These results suggest that a metabolic system, MET-GSL biosynthesis and its regulation, is constituted of these and other candidate genes we found. Further experiments on these genes are being conducted to confirm our prediction.

[1] Hirai et al. (2005) *JBC* 280:25590

[2] Hirai et al. (2007) *PNAS* 104:6478

P-519

Metabolite profiling of the plant *RCD1-SRO* gene family: amino acids and TCA cycle intermediates. Nina Sipari¹, Tiina Blomster², Markku Keinänen¹, Jaakko Kangasjärvi². ¹University of Joensuu, Joensuu, Finland, ²University of Helsinki, Helsinki, Finland

The *rcd1-1* (*radical-induced cell death1*) mutant of *Arabidopsis* has been shown to be defective in the containment of programmed cell death and in the signalling of several plant hormones. The *RCD1* protein function is yet unknown, but according to a yeast two-hybrid analysis, it may include interactions with several stress-related transcription factors. *RCD1* belongs to a novel gene family with 5 unknown genes encoding proteins distinctively similar to *RCD1* (*SRO1-SRO5*; SIMILAR TO *RCD-ONE 1-5*). Interestingly, a conserved domain of ADP-ribosylation has been assigned to all the *RCD1-SRO* proteins. *RCD1* appears to have partially overlapping functions with at least *SRO1*, because *rcd1-sro1* double mutant plants have a severely stunted phenotype even in control conditions. The role of markedly increased *SRO3* expression in *rcd1* will also be further studied.

In addition to global gene expression with microarrays, we have accomplished metabolite profiling of the T-DNA insertion mutants of the "RCD1-SRO" gene family, *rcd1-1*, and *Col* wildtype, both after stress treatments and in control condition by HPLC-MSn and GC-MS. Recent HPLC-MSn and GC-MS analysis have revealed differences between *Col* and *rcd1-1* in the diurnal rhythm of some amino acids and their TCA cycle intermediates, respectively. The observed metabolic changes are discussed in respect to the latest results obtained from microarrays.

P-521

The *Arabidopsis AtOPT3* oligopeptide transporter plays a role in iron homeostasis. Minviluz G. Stacey¹, Ami Patel¹, Sharon Pike¹, Elizabeth E. Rogers¹, Walter Gassmann¹, Gary Stacey¹. ¹University of Missouri, Columbia, MO, USA

The *Arabidopsis AtOPT3* gene encodes a predicted transmembrane protein belonging to the oligopeptide transporter (OPT) family, a largely uncharacterized family of transporter proteins involved in the transport of short peptides or modified peptides. A null mutation in *AtOPT3* (*opt3-1*) resulted in embryo-lethality, indicating an essential role for *AtOPT3* in embryo development. A second *AtOPT3* mutant line, *opt3-2*, harboring a T-DNA insertion in the 5' UTR of *AtOPT3*, was recently identified. The T-DNA insertion in the *AtOPT3* promoter resulted in reduced but sufficient *AtOPT3* expression to allow embryo formation in *opt3-2* homozygous seeds. Phenotypic analyses of *opt3-2* plants revealed three interesting loss-of-function phenotypes associated with iron metabolism. First, reduced *AtOPT3* expression resulted in the constitutive expression of root iron-deficiency responses in *opt3-2* plants regardless of exogenous supply of iron. Second, deregulation of root iron uptake processes in *opt3-2* roots resulted in the accumulation of very high levels of iron in *opt3-2* tissues. Hyperaccumulation of iron in *opt3-2* resulted in the formation of brown necrotic areas in *opt3-2* leaves and was more pronounced during the seed filling stage. Third, reduced *AtOPT3* expression resulted in decreased accumulation of iron in *opt3-2* seeds. The reduced accumulation of iron in *opt3-2* seeds is especially noteworthy considering the excessively high levels of accumulated iron in other *opt3-2* tissues. *AtOPT3*, therefore, plays a critical role in two important aspects of iron metabolism, namely, maintenance of whole-plant iron homeostasis and iron nutrition of developing seeds. We propose that *AtOPT3* transports a peptide/modified peptide iron chelator or Fe-chelator complex to cells or organs that are involved in the mobilization of iron to seeds and in shoot-to-root signaling of iron-deficiency responses.

P-522

Seeing in the dark. Integrating metabolite, transcript and enzyme measurements during diurnal cycles and prolonged darkness. Bjoern Usadel¹, Yves Gibon¹, Axel Nagel², Oliver Blaesing¹, Jan Hannemann¹, Peter Krueger¹, Dirk Walther¹, Mark Stitt¹. ¹Max-Planck Institute of Molecular Plant Physiology, Golm, Germany, ²RZPD Deutsches Ressourcenzentrum für Genomforschung, Berlin, Germany

To investigate the changes of the metabolism in *Arabidopsis* plants during diurnal cycles and after transfer into darkness, metabolite and transcript levels as well as enzymatic activities were measured. The data were analyzed both globally as well as by focusing on potential key pathways within the data set.

The MapMan software family was used for data analysis in order to a) normalize microarray data and to statistically evaluate simple experiment designs; b) visualize data in the context of pre-existing biological knowledge using transcript, metabolite and protein/enzyme profiling experiments; c) compress large experiments down to a simple 1-2 page display by using summary statistics on functional categories using statistics such as pathway enrichment analysis; d) investigate into the data in the light of other experiments.

Using this approach, for several pathways such as nitrogen metabolism or phospholipid biosynthesis, an agreement at all three functional levels was found. However, for some pathways where only metabolites and transcripts were measured, discrepancies were sometimes evident, indicative of possible posttranscriptional modification of enzymes.

Furthermore, a global view of the experiments was obtained by using correlative approaches between metabolites and transcripts. Many metabolite-transcript correlations could be identified, and for several, these could be explained by investigating the respective pathway context. Interestingly, most metabolite-transcript correlations could be identified for sugars, indicating the pivotal role of sugars during the investigated conditions.

P-520

Characterization of two ammonium transporters in a salt-resistant green alga, *Dunaliella viridis*. Ting Song¹, Rentao Song², Zhengkai Xu^{1,2}. ¹Shanghai Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China, ²School of Life Science, Shanghai University, Shanghai, China

Ammonium and nitrate are primary nitrogen sources for plant. Nevertheless, ammonium is used preferentially over nitrate by most species. So ammonium transport is a key process in nitrogen metabolism. To elucidate the role of ammonium transporters in N nutrition of a salt-resistant green alga, *Dunaliella viridis*, two ammonium transporter genes were isolated from cDNA libraries of *D. viridis* treated by different NaCl concentrations. Each cDNA has a long 3'-UTR sequence, almost 1Kb. DvAMT1;1 has only 35.9% amino acid identity with DvAMT1;2, which also appears in Chlamydomonas. When expressed in yeast mutants defective in ammonium uptake, however, only the latter complemented the defective phenotype. To provide a direct evidence for plasma membrane location for the two transporters, subcellular localization in Chlamydomonas has been done. Real Time PCR showed similar expression patterns for *DvAMT1;1* and *DvAMT1;2*, both of which were induced by low nitrogen and inhibited by high nitrogen, especially NH4+. Different from ammonium transporters identified in higher plant, expression of these two ammonium transporter genes were not diurnal regulated.

P-523

Systems approach to understand integration of the isoprenoid pathway into the cellular gene network. Eva Vranová¹, Marta Closa², Anja Wille³, Albert Ferrer², Wilhelm Gruissem¹. ¹Institute of Plant Sciences, ETH Zurich, 8092 Zurich, Switzerland, ²Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain, ³Reverse Engineering Group, ETH Zurich, 8092 Zurich, Switzerland

Plant isoprenoids represent the largest group of biologically active metabolites with at least 30 000 chemical compounds. Plant isoprenoids have essential functions in physiological and biochemical processes (e.g., photosynthesis, respiration, membrane fluidity, pathogen defense, key plant hormones -cytokinins, brassinolides, gibberellic acid, abscisic acid; protein prenylation). Many plant isoprenoids also have substantial commercial, pharmacological and agricultural value (e.g., essential oils, anticancer drug taxol, antimalarial drug artemisinin, polymers in rubber, antibiotics, carotenoid and tocopherol antioxidants, repellents).

Understanding the regulation of the plant isoprenoid biosynthetic pathway and its integration into the cellular gene network is therefore not only of scientific but also of commercial interest.

In our laboratory, we are using systems approaches to understand the isoprenoid pathway regulation as well as pathway integration into the cellular genetic network. To this end, using variety of publicly available genome-wide gene expression data sets, we modeled the isoprenoid pathway network using sparse Gaussian graphical modeling (Wille et al., 2004). This analysis revealed some novel unexpected dependencies between the genes in the pathway that can be further investigated.

Another approach that we are undertaking to understand the pathway integration into the cellular network is the molecular analysis of responses upon systematic perturbation of the isoprenoid pathway using Arabidopsis loss-of-function mutants. This approach, as well as preliminary results will be discussed in more details at the meeting.

Wille A., Zimmermann P., Vranov E., Bleuler S., F rholz A., Henrig L., Laule O., Prelc A., von Rohr P., Thiele L., Zitzler E., Gruissem W. and B hmann P. (2004) Sparse graphical gaussian modeling for genetic regulatory network inference. *Genome Biology* 5: R92.1-R92.13

P-524

Structural Prediction and Study of Uridine Diphosphate Glycosyltransferases. Xiaoqiang Wang¹, Hui Shao¹, Zhenzhan Chang¹, Lenong Li¹. ¹Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA

Glycosylation plays very important roles in chemical diversity of plant secondary metabolites and control their bioactivities and functions. Plants contain a large number of uridine diphosphate glycosyltransferases (UGTs) involved in glycosylation of secondary metabolites, e.g. over 100 putative UGT genes have been identified in *Arabidopsis thaliana*. Its a challenging task to characterize the function of each enzyme. We are using structure-based approaches, including crystallography and computer prediction/modeling, to explore the structure-function relations of these complex and divergent enzymes.

We are developing a predictive server based on plant UGT structures determined recently, including structures of *Medicago truncatula* UGT71G1 and grape VvGT1. With this predictive tool, we may identify putative key residues involved in substrate binding for a new UGT, and generate a predicted three dimensional structural model, providing a structural basis to analyze the putative substrate binding pocket and predict the substrates and functions. We are utilizing this approach to study several *Arabidopsis* UGTs involved in flavonoid metabolism and hormone homeostasis. Crystallographic study of these *Arabidopsis* UGTs is also in progress. The cDNAs of the target UGTs were cloned into *E. coli* expression vectors pET28a or pGEX-2T containing an His-tag or GST-tag with a thrombin cleavage site. Proteins were expressed in *E. coli* BL21(DE3) and purified with Ni²⁺-NTA or glutathione-agaroses. The N-terminal His- or GST-tags were cleaved with biotinylated thrombin. The proteins were further purified on Resource Q and Superdex-200 gel filtration columns.

Crystallization screening towards structure determination was carried out. These studies help to identify and confirm the key residues for enzyme functions and substrate specificities, and reveal the detailed interactions between enzymes and substrates.

P-525

Identification and characterization of mono-ADP-ribosylation activity in *Arabidopsis thaliana*. Hai Wang¹, Kaiming Cao¹, Xiaochun Ge¹. ¹Department of Biochemistry and Molecular Biology, School of Life Sciences, Fudan University, Shanghai, 200433, China

ADP-ribosylation is a novel post-translational modification involved in diverse cellular processes such as signal transduction, cell differentiation and protein trafficking. It could be divided into two types: mono-ADP-ribosylation and poly-ADP-ribosylation. While mono-ADP-ribosylation is catalyzed by mono-ADP-ribosyltransferases (mADPRTs) which transfer only a single ADP-ribose residue from β NAD⁺ to a specific amino acid of the acceptor protein, poly-ADP-ribosylation is catalyzed by poly(ADP-ribose) polymerases (PARPs) which transfer multiple ADP-riboses to target proteins. Interestingly, both mono- and poly-ADP ribosylation could be reversed, either by enzyme activities which could hydrolyze the protein-ADP-ribose linkage or by those that degrade poly-ADP-ribose, suggesting the existence of ADP-ribosylation/de-ADP-ribosylation loops as a regulatory mechanism for protein substrates of these reactions.

Although PARPs have been extensively studied in various organisms (including plants) and have been shown to affect stress tolerance, cell death and energy homeostasis, the characterization of mono-ADP-ribosyltransferase activity has only been reported in bacteria, fungi, and the animal kingdom. However, in a trial to study mono-ADP-ribosylation activity in *Arabidopsis thaliana*, we identified a protein with molecular mass of 32-KDa which is likely the protein substrate of endogenous mono-ADP-ribosylation reaction. The modified amino acid of the protein was further investigated by means of characterizing the chemical stability of the ADP-ribose linkage. After treatment of the [³²P]ADP-ribosylated 32-KDa protein with NaOH or HCOOH for 2h, the 32-KDa band disappeared, as opposed to treatment with HgCl₂ (which act on ADP-ribosylated cysteine) or NH₂OH (specific to ADP-ribosylated arginine), both of which had no significant effect on the 32-KDa band. These results indicate that the ADP-ribose residue is most likely linked to serine or threonine residues. Further investigation is needed to characterize the identity of this protein as well as the enzyme which catalyzes this reaction.

P-526

Global analysis of lipid metabolism regulation in *Brassica napus* developing seeds. Guo-Zhang Wu¹, Ya Niu¹, Rui Ye¹, Wen-Hui Lin¹, Qiu-Ming Shi¹, Xiao-Dong Xu², Rao Li², Yu-Guang Du³, Hong-Wei Xue¹. ¹Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China, ²United Gene Holdings, LTD., Shanghai, China, ³The Nature Products and Glycoconjugate Research Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning, China

Multiple high-throughput genomic approaches were performed to study the gene expression profiles during *Brassica napus* seed development and fatty acid (FA) metabolism, as well as the relevant regulation. Serial Analysis of Gene Expression (SAGE) using seed materials obtained a total of 68,716 tags, of which 23,895 were unique and 503 tags were functionally identified, and further revealed the transcriptome of approximately 35,000 transcripts in *B. napus* developing seeds. Further, ~22,000 independent ESTs were obtained by large-scale sequencing using immature embryos at different stages, and 8462 uni-ESTs and 3355 full-length cDNAs were identified respectively, resulting in the systemic identification of *B. napus* FA biosynthesis-related genes. Gene expression profiles were further studied employing cDNA chip hybridization to reveal the global regulatory network of FA metabolism in developing seeds. Together with the analysis on FA amounts and composition, it was shown that 17-21 days after pollination (DAP) was a crucial stage for transition of seed to sink tissue. High expressions of FA biosynthesis-related genes and transition of FA components are mainly at stages 21 DAP or 21-25 DAP respectively. Compared to Arabidopsis, more critical roles of starch metabolism are detected for *B. napus* seed FA metabolism and storage components accumulation. Crucial effects of starch metabolism, oxidative pentose phosphate pathway (OPPP), photosynthesis, other regulators and different carbon flux model compared to Arabidopsis in FA metabolism were discussed.

P-527

Arabidopsis acyl-CoA binding proteins ACBP4 and ACBP5 are localized in the cytosol. Shi Xiao¹, Hong-Ye Li¹, Mee-Len Chye¹. ¹Department of Botany, The University of Hong Kong, Pokfulam, Hong Kong

Arabidopsis acyl-CoA binding proteins (ACBPs) are encoded by a gene family consisting of six members. They range in size from 10.4 kDa to 73.1 kDa and show conservation at the acyl-CoA binding domain. We have demonstrated that they display varying affinities to acyl-CoA esters and show different subcellular localizations, suggesting that they do not have redundant roles in plant lipid metabolism. While ACBP1 and ACBP2 are membrane-associated proteins, ACBP3 is targeted extracellularly. Here we report that ACBP4 and ACBP5 are localized in the cytosol. Each of ACBP4 and ACBP5 was translationally-fused to an autofluorescent protein for particle gun bombardment of onion epidermal cells and tobacco BY-2 cells, and for agroinfiltration of tobacco leaves. Observations by confocal laser scanning microscopy revealed that ACBP4::DsRed, ACBP5::DsRed and ACBP5::GFP fusions were present predominantly in the cytosol. To further confirm the localizations of ACBP4 and ACBP5, stable transgenic plants expressing eGFP::ACBP4, ACBP5::eGFP and ACBP5::Red were generated. Confocal microscopy and western blot analyses using subcellular protein fractions from transgenic Arabidopsis revealed that the ACBP4 and ACBP5 fusions occur in the cytosol. Hence, other than the well-studied 10-kDa cytosolic ACBP that is prevalent in eukaryotes, we have identified two more cytosolic ACBPs in Arabidopsis. ACBP4 and ACBP5, which binds oleoyl-CoA ester, could participate in its transfer from the plastid (the site of de novo fatty acid biosynthesis) to the endoplasmic reticulum for the eukaryotic pathway in lipid biosynthesis.

P-528

Genetic Characterization and Functional Analysis of the *Arabidopsis* Mutants Disrupted in Endoplasmic Reticulum to Chloroplast Lipid Trafficking. Changcheng Xu¹, Koichiro Awai¹, Jilian Fan¹, Binbin Lu¹, Adams Cornish¹, Christoph Benning¹. ¹Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA

The photosynthetic (thylakoid) membrane of plants is one of the most extensive biological cell membrane systems found in nature. It harbors the photosynthetic apparatus which is essential to life on earth as carbon dioxide is fixed and atmospheric oxygen released by photosynthesis. Lipid biosynthetic enzymes of different subcellular compartments participate in the biogenesis of the thylakoid membrane system. This process requires the extensive exchange of lipid precursors between the chloroplast and the endoplasmic reticulum (ER). The underlying lipid trafficking phenomena are not yet understood at the mechanistic level, but genetic mutants of the model plant *Arabidopsis thaliana* with disruptions in lipid trafficking between the ER and the chloroplast have recently become available. The TGD1-TGD3 proteins are similar to components of multipartite ABC transporters as found in bacteria. They are localized in the inner chloroplast envelope membrane. The *tgd1-1* mutant shows an increase in the level of phosphatidic acid. The rate of phosphatidic acid incorporation into galactolipids by isolated *tgd1-1* chloroplast is decreased. The TGD2 protein specifically binds phosphatidic acid. The TGD3 protein has ATPase activity in vitro. Based on this evidence, we hypothesize that the respective complex transfers phosphatidic acid from the outer envelope membrane to the phosphatidic acid phosphatase at the inside of the inner envelope membrane, thereby providing the ER-derived DAG precursor of galactoglycerolipid biosynthesis. A fourth protein, TGD4, is ER-associated. We hypothesize that it mediates the direct interaction of ER and the outer chloroplast envelope membrane and/or facilitates the transfer of lipids between the membrane systems.

P-529

Identification and Characterization of Pyruvate Decarboxylase (pdc) Gene Family Members in *Arabidopsis*. Songqing Ye¹, Jerry Cohen¹. ¹University of Minnesota, Saint Paul, US

The indole-3-pyruvic acid (IPA) pathway has long been proposed to be one of the major tryptophan-dependent pathways in auxin biosynthesis. However, the details of this pathway have not been clearly defined and no gene involved in this pathway has been fully described in plants. We have found that there are six genes in *Arabidopsis* having high sequence similarity to the *ipdc* (EC 4.1.1.74) or *pdc* (pyruvate decarboxylase, EC 4.1.1.1) from microbes. I have identified the *Atpdc2* is a functional *pdc* gene yielding a protein product with the predicted biochemical function. PDC converts, by decarboxylation, pyruvate into acetaldehyde and then alcohol dehydrogenase (ADH, EC 1.1.1.1) reduces acetaldehyde into ethanol. This set of reactions shunts the main glycolytic pathway into ethanol fermentation, instead of entry into the tricarboxylic acid cycle, so it is very important for plants as they respond to anaerobic stress such as flooding. Most interesting, this active enzyme does not have the classical bi-functional activity (*pdc/ipdc*) found with all known bacterial enzymes and it shows around 40% of its activity with only a single cofactor TPP (Thiamine Pyrophosphate) or Mg²⁺ or without any cofactor supplied as compared to the rate obtained with a combination of both cofactors.

We have designed a new highly specific GC-MS method to analyze plant indole pyruvate decarboxylase (IPDC) activity. We found that this method works well for the IPDC from bacteria, but as yet has not revealed plant IPDC activity from any of the genes cloned to date. In addition, antibodies against the whole PDC family and to specific protein sequences have been generated independently such that the native proteins can be immunopurified from extracts prepared from plant tissues. Hopefully, their roles in auxin biology as well as the kinetics of their turnover can be determined.

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Responses to the Environment

P-530

COP9 Signalosome Interacts with RNA Processing Factors in *Arabidopsis*. Shiori AKI¹, Atsuhiro OKA¹, Tomohiko TSUGE¹. ¹ICR Kyoto University, Kyoto, JAPAN

The COP9 signalosome (CSN) is a nuclear protein complex essential for plant and animal development, regulating proteasome-mediated proteolysis. To characterize novel mechanisms of CSN, CSN subunit 1 (CSN1) binding proteins were isolated and analyzed in *Arabidopsis*.

SAP130, one of the identified CSN1 binding proteins, is a component of the SF3b complex which is involved in mRNA splicing. In *Arabidopsis*, AtSAP130 was coded by two genes (*AtSAP130a* and *AtSAP130b*) which both map on chromosome 3. The mRNAs for both genes were detected in all tested organs. The expression level of *AtSAP130b* was found to be significantly higher than that of *AtSAP130a* in all tissues. Pull down experiments *in vitro* revealed that AtSAP130 directly binds AtCSN1 at the N terminal portion of AtCSN1. Here we discuss the possible roles of CSN in post-transcriptional regulation in *Arabidopsis*.

P-531

Identification of putative oxidative stress-associated genes in *Arabidopsis* by reverse genetics. Herländar Azevedo¹, Vitor Amorim-Silva¹, Sara Laranjeira¹, Rui Tavares¹. ¹Biology Dep., Minho University, Braga, Portugal

With the onset of aerobic life, cells have had to cope with the by-products of oxygen metabolism: reactive oxygen species (ROS). These species result from the transfer of energy or electrons to ground state oxygen, being extremely toxic to the cell by easily reacting with proteins, lipids and nucleic acids. Under standard conditions, the production of ROS in cells is maintained in homeostatic levels, with many abiotic stresses disrupting this condition and enhancing the production of ROS. The major progress in the identification of determinants involved in the homeostasis of ROS is due primarily to the use of *Arabidopsis* as a molecular genetic model system. Functional screening in the *Arabidopsis* biological model makes use of phenotype-centred forward and reverse genetic approaches, usually using loss-of-function mutant analysis. Based on a reverse genetics approach, the expression analysis of oxidative stressed *Arabidopsis* plants, available through the Affymetrix microarray database (NASC), led to the identification of putative oxidative stress-related genes. Through loss-of-function mutants we are currently screening these genes for phenotype.

P-532

MPK3/6 Protein Accumulation Primes *Arabidopsis* for Enhanced Stress Resistance. Gerold Beckers¹, Yidong Liu², Shuqun Zhang², Uwe Conrath¹. ¹Plant Biochemistry & Molecular Biology Group, Department of Plant Physiology, RWTH Aachen University, Aachen, 52056, Germany. ²Department of Biochemistry, 371G Life Sciences Center, University of Missouri-Columbia, Columbia, MO 65211, USA.

In plants, induced resistance to biotic and abiotic stress is associated with an enhanced capacity for more rapid and intense activation of defense responses, a phenomenon called "priming". The mechanism of priming and the cellular components that mediate priming remained obscure. We show that in *Arabidopsis* priming is associated with the accumulation of transcripts and protein of mitogen-activated protein kinase 3 and 6 (MPK3/6). MPK3/6 accumulation is associated neither with dual phosphorylation of the MPK3/6 TEY activation motif nor with MPK3/6 enzyme activity. However, upon exposure to stress, activation site phosphorylation and MPK3 activity are induced to high levels in primed plants, and this leads to boosted defense gene expression and disease resistance. Furthermore, mpk3 and mpk6 mutants cannot be primed for induced resistance. Thus, we provide the first evidence that provision of a latent signaling component is a mechanism for priming plants to better fend off stress.

P-533

Responses of sexual reproduction pattern and leaf traits of *Arabidopsis thaliana* to elevated temperature from global warming. Jin Biao¹, JIANG Ke-zhen¹, WANG Jing¹, NI Cheng-yang¹, QI Yi-ming¹, TENG Nian-jun². ¹College of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China, ²College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

It is predicted that the mean global temperature will increase 2-5°C during the next 50-100 years according to the report of IPCC Fourth Assessment Report (2007). The issue of global warming has been appealed to many people and governments in the world. How future plants will respond to this global change?

In the present study, *Arabidopsis thaliana*, will be continuously grown under the temperature of 23°C /18°C (day/night temperature) (ambient temperature) and 28°C /23°C (elevated day/night temperature by 5°C) (elevated temperature) for multi-generations. The changes in various reproductive characteristics of an individual generation will be investigated and their overall changes in multi-generation will be further investigated. In addition, the changes in physiological, biochemical, and cytological traits of leaves of *Arabidopsis* grown under two different temperature conditions will be compared. The main results of the first generation are listed as follows: compared with ambient temperature, elevated temperature significantly advanced the flowering time and shortened the life cycle of *Arabidopsis*, and resulted in less flowers, less fruits, less seeds, less reproductive organ biomass, less vegetative organ biomass, and less total biomass as well. In addition, seed weight, seed number per siliques, siliques length, and the mass allocated to reproduction were significantly decreased, whereas seed germination rate was significantly increased. Furthermore, elevated temperature also had a negative effect on leaf growth and development. The content of chlorophyll in leaf cell and the numbers of grana thylakoid, grana thylakoid lamelle and stroma thylakoid in the chloroplast were decreased under elevated temperature. All these results suggest that global warming in the near future will result in possible consequences of plant growth and development.

P-534

PHOSPHATE STARVATION SIGNALLING IN ARABIDOPSIS. Re-gia Bustos¹, Jose Manuel Franco-Zorrilla¹, Pablo Catarecha¹, Gabriel Castrillo¹, Isabel Mateos¹, Mabel Puga¹, Vicente Rubio¹, Antonio Leyva¹, Javier Paz-Ares¹. ¹Centro Nacional de Biotecnología-CSIC, Madrid, Spain

Plants have evolved adaptive responses to cope with growth under phosphate (Pi) limiting conditions. This rescue system is under the control of a highly elaborated regulatory mechanism, which, besides Pi, is modulated by sugars, cytokinins and other unknown signal(s) mediating long distance systemic repression. We have found that transcription of Pi starvation-induced genes is repressed by arsenate, potentially reflecting the existence of a savage system to protect plants from arsenate, particularly in Pi poor soils. One component of the Pi starvation regulatory system is transcription factor PHR1, which recognises an imperfect palindromic motif (GNATATNC, P1BS) that is over-represented in the promoter region of Pi starvation induced genes. We have shown that PHR1/P1BS acts as key integrator in Pi starvation signalling. Thus, a minimal promoter containing a multimerised P1BS motif is specifically induced by Pi starvation and is responsive to the stimulatory effect of sugars and the inhibitory effects of cytokinins, long distance repression signals and arsenate. Control of Pi starvation responses also involves the participation of Pi starvation responsive miRNAs (miR399) and other noncoding RNAs, namely the IPS1 family members. Functional characterisation of IPS1 has disclosed a novel mechanism of inhibition of miRNA activity, in which a non-coding RNA sequesters a miRNA and reduces its effective levels. We coin the term target mimicry to define this riboregulatory mechanism of miRNA activity.

P-535

Functional analysis of Arabidopsis transcriptional factor AtHsfA6a in heat stress tolerance. Xu Chao^{1,2}, Dong-Ping Wang¹, Xia Zhang¹, Zhen-Hui Gong², Yun-liu Fan¹, Jun Zhao¹. ¹Biotechnology Research Institute , National Key Facility for Crop Gene Resources and Genetic Improvement ,Chinese Academy of Agricultural Sciences ,Beijing 100081, China. ²College of Horticulture, Northwest Agricultural and Forestry University, Yangling 712100, China;

To dissect the molecular mechanism underling plant tolerance to heat, a genomic fragment containing the ORF of putative Arabidopsis heat shock transcriptional factor AtHsfA6a was cloned by PCR amplification, and homozygous transgenic Arabidopsis lines containing either over-expression (OE) or antisense (AS) constructs of AtHsfA6a were generated through Agrobacterium-mediated genetic transformation. The performance of OE, AS and wild type (WT) plants under heat stress was analyzed. The results showed that the survival rate of OE plants (86%) was much higher than that of WT (59%) after treatment under 43°C for 2 hours, in contrast to that of AS plants of which only 48% survived. Measurement of electric conductivity revealed that the level of electrolyte leakage after treated under 43°C for half an hour was significantly reduced in OE plants comparing to that in WT, whereas that in AS plants was largely increased. Gene expression analysis indicated that AtHsfA6a was induced by heat stress and Hsp70 was the downstream target up-regulated by AtHsfA6a. These data suggests that AtHsfA6a functions to enhance heat stress tolerance through activating the expression of Hsp70.

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Functional characterization of AtATM1, AtATM2 and AtATM3, a subfamily of Arabidopsis half-molecule ABC transporters implicated in iron homeostasis. Sixue Chen¹, Rocío Sánchez-Fernández², Elise Lyver², Andrew Dancis², Philip A. Rea². ¹University of Florida, Gainesville, FL32610, USA, ²University of Pennsylvania, Philadelphia, PA19104, USA

Arabidopsis AtATM1, AtATM2 and AtATM3 are half-molecule ATP-binding cassette (ABC) proteins that are homologous to the yeast ATM1 (ScATM1) protein. The ScATM1 localizes to the inner mitochondrial membrane and is implicated in the export of mitochondrially synthesized iron-sulfur (Fe/S) clusters to the cytosol for the assembly of redox-active Fe/S proteins. Yeast ATM1-deficient (atm1) mutants grow very slowly, are respiration-deficient, accumulate toxic levels of iron in the mitochondria and show enhanced high-affinity iron uptake. Of the three AtATMs, AtATM3 bears the closest functional resemblance to ScATM1. AtATM3 is not only able to complement the growth phenotype of yeast atm1 mutants but is also able to suppress the high-affinity iron uptake associated with the mutation of ScATM1, abrogate intramitochondrial iron hyperaccumulation, and restore mitochondrial respiratory function and cytochrome c levels. By comparison the functional resemblance of AtATM1 and AtATM2 to ScATM1 is weaker. AtATM1 only partially suppresses the atm1 phenotype, exerts little effect on high-affinity iron uptake, and only partially alleviates mitochondrial iron hyperaccumulation and cytochrome c levels. AtATM2 exerts little suppressive action on the yeast atm1 phenotype but instead is toxic when expressed in this system. These differences between AtATM3 and AtATM1 are maintained after exchanging their target peptides and these proteins as well as AtATM2 colocalize with the mitochondrial fluor MitoTracker Red when expressed in yeast as GFP fusions. Of the three AtATM::GFP fusions, those derived from AtATM3 exhibit the most pronounced mitochondrial localization versus AtATM1::GFP and AtATM2::GFP fusions when ectopically expressed in Arabidopsis. While its toxicity when heterologously expressed in yeast except when fused with GFP precluded the functional analysis of native AtATM2, a common function, mitochondrial export of Fe/S clusters or their precursors for the assembly of cytosolic Fe/S proteins, is inferred for AtATM3 and AtATM1.

P-538

Functional characterization of soybean SLTI114 gene encoding matrix metalloproteinase protein induced by abiotic stresses. Chang-Woo Cho¹, Eunsook Chung¹, Kyoungmi Kim¹, Hyun-A So¹, Boh-Hyun Yun¹, Jee-Sook Kang¹, Yao Ran¹, Jai-Heon Lee¹. ¹Dong-A University, Busan, Republic of Korea

Matrix metalloproteinase proteins (MMPs) are involved in remodeling of plant extracellular matrix in association with plant growth, development, and possibly defense processes. A novel soybean (*Glycine max*) metalloprotein gene, SLTI114 was identified. The complete cDNA sequence of SLTI114 comprised of 1,443 bp with an open reading frame of 1,179 bp which encodes 43.2 kDa polypeptide consisting of 393 amino acid residues. The nascent SLTI114 polypeptide contained N-terminal signal peptide with a central hydrophobic core between amino acids Asp29 and Ser30, and predicted cleavage site between amino acids Asp153 and Val154. The deduced SLTI114 polypeptide is a pre-pro-enzyme that has all of the hallmark motif characteristic of matrix metalloproteinases. To confirm the expression of the SLTI114 gene at the transcriptional level, northern blot analysis was also carried out using the mRNA prepared from developing soybean cotyledons and the soybean leaves exposed to various stresses and hormone. The expression of SLTI114 was induced by LT (5°C), NaCl, wounding stresses and ABA. SLTI114 protein expressed in *E. coli* cells showed protease activity in zymography assay. Further *in vivo* function of SLTI114 was investigated in *SLTI114* overexpressing Arabidopsis plants.

P-539

An Arabidopsis mutant uvt34 exhibits enhanced tolerance to UV-B stress. SeungWon Choi¹, YeRim Kwon¹, Suk-Whan Hong², Hojoong Lee¹. ¹Division of Biotechnology, College of Life Sciences and Biotechnology, Korea university, Seoul, Republic of Korea,²Division of Applied Plant Science, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Republic of Korea

One of the severe abiotic stresses encountered by plants is solar radiation which includes UV light. However, plants should cope with this type of stress because of the photosynthetic reaction. It is reported that UV radiation causes harmful effects on cellular metabolisms via damaging DNAs, proteins and lipids, leading to cell death. One of the prime candidates for cellular damage by UV stress includes reactive oxygen species (ROS) which are known to be increased in the cell exposed to UV radiation. Studies with UV sensitive mutants have aided in our understanding on the mechanisms by which plants protect themselves against UV-induced cellular damage. Some mutants have defects in the biosynthetic pathway of phenolics, flavonoids or saponines, which are known to help plants by serving as sunscreen. Up to date, four Arabidopsis mutants have been identified, uvt1, uvi1, rcd1-2 and uvi4 that are tolerant to UV-B. To enhance our understanding in the UV tolerance mechanisms, we have isolated a new UV-B tolerant mutant, uvt34 (UV-B tolerant 34), of Arabidopsis (EMS mutant). The uvt34 is a recessive mutant and exhibit tolerance to high UV-B radiation which is lethal level to wild-type plants. Possible reasons for this enhanced tolerance in the uvt34 were explored.

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P-541

Functional characterization of At398 (unknown protein) which is homology to Om398, that Induced by Mechanical Wounding Based on cDNA Microarray Data in Wild Rice. Mei Hua Cui¹, Yun Young Kim¹, Kwang Wook Jung¹, Kyoung Shin Yoo¹, Jeong Sheop Shin¹. ¹Graduate School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

In this study, *O. minuta* (BBCC) of wild rice species, which has been known to have useful resistance-related genes against brown planthopper (BPH), bacterial blight and green leafhopper (GLH), etc, was used to screen and identify the differentially expressed genes induced by insect feeding and wounding. And we found Om398 was strongly induced by wounding and insect feeding compared to controls. Om398 protein showed sequence homology to At398 in Arabidopsis. And these proteins have no reported homology with any known animal protein. To identify Om398 functions, first we focus on the At398 in Arabidopsis. Through screening now we have three At398-overexpression lines and one knock out line in Arabidopsis. On germination assays under various stress conditions, At398-overexpressed Arabidopsis plants showed salt, ABA, mannitol and glucose stress tolerance. With the ProAt398::GUS lines, we identified that At398 specially expressed in the senescence leaves and open flowers. Based on these data, we have determined the biological functions in defense system, including regulatory mechanism as well as the effects of gene itself. Moreover, we will define the components of regulatory networks that mediate defense signaling using the yeast hybrid system.

P-542

STH2, a B-box protein in Arabidopsis, activates transcription and positively regulates light mediated development. Sourav Datta¹, Chamari Hettiarachchi², Henrik Johansson¹, Verena Lorenz¹, Magnus Holm¹. ¹CMB-Molecular Biology, Gothenburg University, Sweden,²Department of Chemistry, Colombo University, Sri Lanka

COP1 and HY5 are two major regulators of light signaling in plants. Proteins interacting with either could therefore be important regulators of light dependent development. Using COP1 or HY5 as bait in yeast two-hybrid screens several putative regulators of light signaling, all containing B-boxes, were identified. One of them STH2 was found to interact with HY5. Mapping studies confirmed that the bZIP domain of HY5 and the B-boxes in STH2 are important for this interaction. STH2 and HY5 when co-expressed in onion epidermal cells show FRET. Furthermore, although STH2 is uniformly nuclear by itself, it localizes to speckles when co-expressed with unfused COP1. We identified two independent T-DNA insertion lines in STH2. Northern blots confirmed that both sth2-1 and sth2-2 are null alleles. Both alleles are hyposensitive to red and blue light.

Double mutants between sth2 and hy5 show an additive effect on hypocotyl elongation in light whereas sth2 partially suppresses the hypocotyl phenotype of dark grown cop1 alleles. The sth2 mutant, like hy5, shows enhanced number of lateral roots and accumulates less anthocyanin. Furthermore, it can suppress the reduced number of lateral roots and high anthocyanin levels of the cop1 alleles. Interestingly we found that STH2 can activate transcription. Transient transfection assays in protoplasts using LUC reporter driven by the Chalcone Isomerase promoter show that the B-boxes in STH2 and a functional G-Box element in the promoter are required for this activity. In conclusion we have identified STH2, a B-box protein in Arabidopsis, as a positive regulator of photomorphogenesis and for the first time report that the B-box domain plays a direct role in activating transcription in plants.

P-540

Dual targeting of mungbean (*Vigna radiata* L.) MLT107 encoding 2-Cys Prx to mitochondria and chloroplasts. Eunsook Chung¹, Jee-Eun Heo¹, Chang-Woo Cho¹, Hyun-A So¹, Bo-Hyun Yun¹, Jee-Sook Gang¹, Yao Ran¹, Kyoungmi Kim¹, Jai-Heon Lee¹. ¹Dong-A University, Busan, Republic of Korea

We isolated low temperature inducible genes using suppression subtractive hybridization (SSH) method and were able to obtain to clone MLT107 gene encoding peroxiredoxin and aminotransferase. The full-length cDNA of MLT107 is 1,049 bp with an open reading frame (ORF) consisting of 261 amino acid (aa). Genomic southern blot confirmed that mungbean genome has two copies of MLT107 gene. Northern blot analysis was also carried out for the gene expression during ABA, NaCl, drought, wounding and H2O2 stresses. The expression of MLT107 gene significantly decreased by ABA, NaCl and drought stress, but wounding and H2O2 stress significantly induced MLT107 gene expression. Especially, H2O2 strongly induced the MLT107 gene expression. The expression of MLT107 gene during low temperature stress started to increase in 3 h after treatment, and than slightly decreased and again increased at 24 h. Using GFP fusion vector, smGFP-MLT107 was targeted both to mitochondria and chloroplast. However, it was mostly targeted to mitochondria and partially targeted to chloroplast. For the functional analysis of MLT107, MLT107 recombinant protein was heterologously expressed in *E. coli*. The MLT107 recombinant cells showed enhanced antioxidant activity compared to that of vector control cells.

P-543

Genetic dissection of the plant circadian clock. Seth Davis¹.¹

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Proper plant growth and development requires a robust detection of the diurnal environment. This occurs through a coupling mechanism of light detection and the circadian clock. We recently took a genetic approach to confirm the core of the circadian oscillator, which is comprised of the morning-acting genes CCA1 and LHY that work with the evening-acting gene TOC1 (Ding, *Genetics* 2007a). From this core, we have further characterized elements that work at the dawn and dusk boundaries. For dawn, we genetically isolated the TIC locus required for proper detection of the dark to light transition. The cloning of this gene provides an understanding that TIC regulates dawn sensation through LHY regulation (Ding, *Plant Cell* 2007b). We have further found that TIC responds through post-transcriptional processes that include phosphorylation and ubiquitin, suggesting that dawn detection is molecularly signaled through regulated proteolysis. To further our understanding of dusk perception, we are working on the ELF3 and ELF4 loci required for detection of the light to dark transition. Various molecular-physiological analyses have been used to place the ELF3 and ELF4 proteins within a model framework of the clock (McWatters, *Plant Phys.* 2007). We have also uncovered an allelic series of elf3 and elf4 mutations. Characterization of these mutations at physiological and biochemical levels is assisting our understanding of the protein function of these pioneer proteins. The current model from these collective efforts is that a multiple-protein transcriptional-regulator complex forms to regulate initiation of CCA1 and LHY. The mode of this mechanism of action will be discussed. Our collective use of genetics and protein analyses is enhancing our understanding of the biochemical mechanisms plants use to detect the boundaries ever present every day.

P-545

Characterisation of dracula (no avoidance of shade) and icasrus (extreme avoidance of shade) mutants in Arabidopsis

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Plants are capable of detecting red-depleted light reflected from neighbouring vegetation which induces a classical, shade avoidance response, including an increase in elongation growth, a decrease in the degree of branching and a shortening of the time to flowering. The phytochrome photoreceptors involved in the perception of vegetative shade have been well characterised but we know little about the signal transduction events downstream of these.

We used a transcriptomic approach to identify a number of genes differentially expressed under simulated shade in *Arabidopsis thaliana* and have used this knowledge to generate a shade responsive marker for a high throughput, non-invasive, real-time bioluminescent assay.

We have carried out a mutant screen using this marker and have identified several mutants in which the response to shade is defective. These are divided into two classes: dracula (no avoidance of shade) and icasrus (extreme avoidance of shade). In many cases, these mutants show additional, novel defects in light signalling. We have mapped the approximate location of a number of these mutations. Curiously, one of these mutations is the result of a novel mutation in one of the phytochrome photoreceptors that appears to result in a constitutive non-shade-avoiding phenotype with respect to our marker.

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The molecular basis of vernalization. Caroline Dean¹.¹The John

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At a certain stage in their life-cycle plants flower and undergo the transition from vegetative to reproductive development. The correct timing of flowering is crucial for reproductive success so plants integrate multiple environmental and endogenous signals. The Dean laboratory is studying the importance of prolonged cold or winter for flowering, a process known as vernalization. The need for vernalization ensures plants over winter in a vegetative form and flower in the favourable conditions of spring. Vernalization requirement has been bred into many crop species to extend their geographical range and variation in vernalization is a key parameter in adaptation of plants to different climates.

We have used a molecular genetic analysis in *Arabidopsis thaliana* to identify genes involved in determining both the need for vernalization and the ability to vernalize. The pathways we study share a common downstream target, FLC, a gene encoding a MADS box repressor of flowering. The talk will describe our current understanding of these pathways, how FLC chromatin regulation has been identified as a central mechanism and how the functioning of the pathways has changed as *Arabidopsis* has adapted to different climates.

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The role of cell identity in plant salt stress response. Jose

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The survival of an organism is dependent on its ability to respond appropriately to environmental changes. In organs, where different cell- and tissue-types perform unique biological functions, cell-identity is likely to determine aspects of differential stimulus perception and response. Most studies however, have analyzed the transcriptional impact of environmental stimuli on single-cells, whole organs, or organ systems. Here we show that, in *Arabidopsis* roots, the majority of the response to a well characterized environmental stress (high salinity) is cell-type specific resulting in the differential regulation of particular biological functions in subsets of tissue layers. Our analysis is based on three microarray data sets we have generated that explore transcriptional changes spatially among 6 tissue layers and 4 longitudinal regions or temporally along 5 time points after salt treatment. Analysis of cis-element enrichment in the promoters of salt responsive genes shows that known stress regulatory elements such as the ABRE and DRE likely regulate semi-ubiquitous responses to salt occurring in multiple cell types whereas the W-box may control stele specific responses. We functionally test the role of cell-identity in regulating salt responses in the epidermis and reveal cell-autonomous and non-autonomous effects when cell identity is altered. Together, these data reveal previously unknown spatial and temporal aspects of a stress response and demonstrate the important role cell-identity plays in guiding changes in transcriptional states.

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HARDY, an Arabidopsis gene conferring drought and salt tolerance in Arabidopsis. Shital Dixit¹, Raffaella Greco^{2,1}, Asaph Aharoni³, Jelle Hiemstra¹, Nayelli Marsch-Martinez¹, Andy Pereira^{4,1}. Plant Research International, Wageningen University, The Netherlands,²Universit degli Studi di Milano, Italy,³Weizmann Institute of Science, Israel,⁴VBI, Virginia Tech, Blacksburg, USA

Fresh water scarcity is one of the principal global problems of this century, and plants account for around 65 % of global fresh water use. Water scarcity often gives rise to drought and salinity which can cause severe and irreversible damage to crop yields. Identification and characterization of genes and mechanisms for tolerance to multiple stress factors is therefore desirable. In a phenotypic screen of an *Arabidopsis En/1(Spm)* transposon activation tagged mutant collection, we identified a semi-dominant mutant *hardy* (*hrd-D*) with the remarkable feature of having roots with enhanced strength, branching and extra starch-storing cortical cells. Overexpression of *HRD* produced dark green leaves that were smaller and thicker with more chloroplast bearing mesophyll cells. A quantitative root-pull assay showed that the *hrd-D* mutant has 20-50 % more root-strength compared to wild-type.

When tested for drought tolerance, the *hrd-D* mutant showed drought tolerance by surviving a prolonged period of progressive drought compared to the wild type (9 vs. 12 days). To eliminate the effect of reduced growth and size of the mutant in the drought tolerance assay, we developed an inducible *HRD* overexpression genotype (*HRD-GR*, glucocorticoid receptor fusion) and showed that steroid DEX induced *HRD-GR* plants, which did not show a small-plant phenotype, were also drought tolerant. In a salt tolerance assay *hrd-D* gave tolerance to as high as 300mM of NaCl and could reach full maturity in contrast to the wild-type. The salinity tolerance of the *HRD* overexpressors is accompanied by lower accumulation of Na⁺ ions. *HRD* overexpression lines also displayed a significant tolerance to a root-infecting pathogen (*Verticillium*) suggesting a general stress tolerance mechanism is active due to *HRD* overexpression. Expression of *HRD* is predominantly found in developing seed and mature pollen, suggesting that *HRD* might play a role in priming seeds for desiccation tolerance.

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The Universal Stress Protein (USP)-A possible link between, stress, early flowering and accelerated senescence. Natalia Edison¹, Shimon Gepstein¹. ¹Technion-Israel Institute of Technology, Haifa, ISRAEL

Universal Stress Proteins have been described widely in prokaryotes, where their production is stimulated by starvation or large variety of stresses. In eukaryotes very little is known about these proteins and their function has not been revealed. Some genes with homology to USP family have been briefly described in plants and in *Arabidopsis* USP gene family has been identified.

We have identified USP gene that was preferentially expressed during senescence. The USP transcript levels were found to be the highest during the initiation of flowering, a stage known to be related to the induction of senescence. Stress-induced senescence displayed dramatic up-regulation of the USP gene with synergistic effect of dark and leaf detachment. Periodic rhythm, with peak in the afternoon, of USP transcript levels has been demonstrated. Addition of ACC, the precursor of ethylene, caused an up-regulation of the USP transcript, whereas addition of cytokinins and ozone caused down-regulation of the expression of the USP gene. Over expression of the USP gene exhibited significant acceleration of life cycle. Sub-cellular immunolocalization analysis suggest chloroplast localization of the USP. Taken together, the results raise the possibility that the USP gene (At3g62550) serves as a link between stress, early flowering and premature senescence.

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Chloroplast Signal Modifies the Light Signal Transduction. Aurora Piñas Fernández¹, Åsa Strand¹. ¹Umeå University, Umeå, Sweden

Phytochrome A (PHYA) mediates the far-red block-of-greening response and mutants in the phyA gene can survive a far-red block-of-greening treatment. We have shown that the *gun5* mutant can also survive this treatment indicating that chloroplast retrograde signal mediated by intermediates of chlorophyll synthesis is involved in the far-red block-of-greening response. Thus, we used two mutants impaired in the chlorophyll biosynthesis pathway, *gun5* and *crd*, in order to identify intermediates implicated in this response. The *gun5* mutant has a mutation in the H-subunit of Mg-chelatase and it has a reduced amount of Mg-protoporphyrin IX (Mg-ProtoIX). The *crd* mutant has reduced cyclase activity and as a result it accumulates large amounts of Mg-ProtoIX-monomethyl ester (Mg-ProtoIXMe) under stress conditions. In contrast to the *gun5* mutant, the *crd* mutant demonstrated the same phenotype as wild type seedlings. Biochemical analysis of *Arabidopsis* and barley demonstrated that the chlorophyll biosynthesis intermediate, Mg-ProtoIXMe, accumulates in response to a far-red block-of-greening treatment. The *gun5* and *phyA* mutants both accumulated lower amounts of Mg-ProtoIXMe compared to the *crd* mutant or wild type seedlings. Our observation is in contrast to the hypothesis that the far-red block-of-greening response is an effect of photo-oxidative damage in cells caused by an excess of protochlorophyllide accumulation driven by PHYA. At the expression level, we found that LHC2 and RBCS transcript levels were negatively correlated with the kinetics of Mg-ProtoIXMe accumulation in wild-type seedlings. Transcript levels of LHC2 and RBCS were maintained in the *gun5* and *phyA* mutants. Our results suggest that PHYA regulates chlorophyll biosyntheses by controlling cyclase activity and that whereas PHYA initiates the far-red block-of-greening response, the component necessary to fulfil this response is Mg-ProtoIXMe. In summary, we describe an interaction between light signalling components and plastid signals that are necessary to fine-tune the response of seedlings to environmental cues.

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ENGINEERING STOMATAL ACTIVITY FOR DROUGHT TOLERANCE IN PLANTS. Massimo Gabiati¹, Eleonora Cominelli¹, Laura Simoni¹, Priscilla Francia¹, Chiara Tonelli¹. ¹University of Milan, Milan, Italy

Land plants lose over 95 % of their water via transpiration through stomatal pores, distributed on the surface of leaves and stems. The opening and closing of the pore is mediated by turgor-driven volume changes of two surrounding guard cells. Engineering of stomatal responses in mutant or transgenic plants represents a valuable tool to design new crops with a more sustainable water use and opens new possibilities to improve plant survival and productivity during drought. We employed large scale genomic screens to identify guard cell-specific mutations and promoters in *Arabidopsis*. Reverse genetic screen of T-DNA mutagenized lines, allowed the identification of a null allele of the guard cell-specific transcription factor AtMYB60. Analysis of stomatal movements in wild type and mutant plants revealed that the *atmyb60-1* mutation results in the constitutive reduction of stomatal aperture, and thus in reduced transpirational water loss during drought. Microarray analysis of gene expression indicated that a limited number of stress-related gene is altered in the *atmyb60-1* mutant. We also analyzed the GUS expression pattern of approximately 20,000 *Arabidopsis* gene trap lines, and identified 5 lines in which the reporter gene was specifically expressed in guard cells. We established the genomic insertion sites of the gene trap element and investigated the expression profile of the tagged genes. Our findings indicate that the gene trap lines isolated in the screen provide valuable marker lines to study pathways that are unique to guard cells, a powerful tool for reverse genetics analyses of guard cell-specific genes, and a precious source of guard cell-specific regulatory regions.

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Cloning and Characterization of SLTI98 encoding Ribosomal Protein Genes S6 from Soybean (*Glycine max*). Jee-Sook Gang¹, Eunsook Chung¹, Kyoungmi Kim¹, Chang-Woo Cho¹, Jee-Eun Heo¹, Bo Hyun Yun¹, Hyun-A So¹, Yao Ran¹, Jai-Heon Lee¹.¹ Dong-A University, Busan, Republic of Korea

In an attempt to better understand translational control during abiotic stresses, we isolated and characterized a stress inducible gene designated as SLTI98 encoding ribosomal protein S6 in soybean. The derived amino acid sequence of SLTI98 showed the highest identity of 93% with ribosomal protein S6 from *Medicago truncatula* (ABD32373). The size of the full-length genomic clone of SLTI98 is 2,701 bp containing 6 exons and 5 introns, of which structure is similar to that of *Arabidopsis* ribosomal protein S6. Genomic southern blot analysis confirmed that soybean genome has two copies of SLTI98 gene. RNA expression of SLTI98 was mildly induced by salt stress, ABA and wounding stress, but not by dehydration stress. According to localization study using GFP fusion expression system, we were able to confirm that SLTI98-smGFP was restricted mostly to nucleus and partly to cytoplasm. The present study implies that the nuclear SLTI98, ribosomal protein S6 play an important role in translational control during abiotic stresses.

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Toward physiological function of AtFtsH4-mitochondrial ATP dependent metalloprotease from *Arabidopsis*: mutation in the AtFtsH4 gene leads to altered morphology, elevated level of ROS and accumulation of oxidized proteins. Marta Gibala¹, Wataru Sakamoto², Alicja Antonczak¹, Hanna Janska¹.¹ Department of Biotechnology, University of Wroclaw, Przybyszewskiego 63/77, Wroclaw, Poland,²Research Institute for Bioresources, Okayama University, Kurashiki, Japan

AtFtsH4 is one out of four *Arabidopsis* mitochondrial ATP-dependent metalloproteases, known to possess proteolytic and chaperone-like activities. AtFtsH4 is anchored to the inner mitochondrial membrane with the catalytic site exposed to the intermembrane space. Here we demonstrate morphological and molecular consequences of AtFtsH4 loss by studying two independent homozygous lines with T-DNA insertion in the AtFtsH4 gene.

Comparative studies of the wild type and ftsH4 mutant growth were performed under short (SD) and long day (LD) conditions at 22 C or 30 C. We examined phenotypic changes at different stages: germination, early seedling growth on agar plates and soil-based growth over the entire life of plants. A lack of serious defects was observed under LD conditions at 22 C. However, phenotypic differences were observed under SD photoperiod at normal temperature as well as during continual moderate heat stress (30 C) under LD conditions. Interesting, most of the striking phenotypic changes (asymmetric leaves, late bolting, shorter inflorescence) were similar between both treatments and were detected at the same time of development: at the end of vegetative phase and during flowering. We found a correlation between morphological abnormalities of rosette and cauline leaves and the elevated level of reactive oxygen species. Furthermore, we noticed a substantially higher amount of two mitochondrial membrane proteins (prohibitin and porin) in the mutant compared with the wild type. These proteins are known to be targets of reactive oxygen species in plant mitochondria. We are now testing hypothesis that AtFtsH4 protease is involved in degradation of oxidatively damaged proteins prior to bolting. Taken together, our data suggest that under LD conditions AtFtsH4 is required for a normal growth and development at temperature only slightly above the optimal growth for *Arabidopsis*.

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Molecular analysis of FT orthologs defines distinct mechanisms for controlling photoperiodic flowering in *Arabidopsis* and *Pharbitis*. Ryosuke Hayama¹, Bhavna Agashe¹, Rod King², George Coupland¹.¹ Max Planck Institute for Plant Breeding Research,²Commonwealth Scientific and Industrial Research Organization

The photoperiodic response of flowering is mediated by the interaction between environmental light signals and the circadian clock. Recent molecular studies revealed that in the long-day plant *Arabidopsis* the length of the day is measured by a mechanism that involves the post-transcriptional activation of a clock-controlled gene *CONSTANS* (*CO*) by light. Under LDs *CO* transcripts begin to rise in the middle of the day, and expression of *CO* while the plants are exposed to light induces the protein resulting in activation of transcription of the *FLOWERING LOCUS T* (*FT*) gene, and flowering occurs. In contrast under SDs *CO* activation does not occur because the transcripts of *CO* only rises in the night and therefore *FT* is not induced.

Here we illustrate at the molecular level a distinct mechanism for controlling the photoperiodic response of flowering in the short-day plant *Pharbitis*, which measures the length of the night. We isolated the ortholog of *FT* (*PhFT*) from *Pharbitis*. The short-day induction of the *PhFT* mRNA expression, as well as the early-flowering phenotype of transgenic *Arabidopsis* and *Pharbitis* plants that overexpresses the gene demonstrated the participation of *PhFT* in the photoperiodic response of flowering in *Pharbitis*. *PhFT* transcript abundance rises during the night and shows a circadian rhythm in constant dark, and this rhythm is set by light off at dusk. Exposure to continuous light damped this rhythmicity and suppressed *PhFT* expression. We propose that in *Pharbitis* a light-sensitive circadian clock set by dusk is dedicated to measure the length of the night. This clock generates a rhythm and if this rhythm oscillates sufficiently long in the dark *PhFT* is activated and flowering occurs.

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Defects in Chloroplast Purine Biosynthesis alter Metal Homeostasis in *Arabidopsis thaliana*. Yan He¹, Alberto Maurer¹, Elizabeth Rogers¹.¹ Department of Biochemistry and Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, United States

Iron is essential for various proper biological functions of most living organisms. It is also important for hormone perception, enzyme activities, signaling, membrane transport and osmoregulation. However, excessive iron is toxic since iron can catalyze the formation of reactive oxygen species. The chloroplast is the major site for the use and storage of iron and other metals in plants. In a screen for altered iron homeostasis in a mutagenized population of *Arabidopsis thaliana*, we identified two new allelic lines, called SP46 and CS557, with mutations in AtATase2, a gene encoding a chloroplast-localized enzyme that catalyzes the first step in purine biosynthesis. Mutations in ATase2 confer variegated chlorosis and stunted growth, especially under high light growth conditions. Chlorophyll concentration and ATP concentration in leaves of those mutants are lower than wild type. Additionally, SP46 and CS557 display a delayed induction of ferric-chelate reductase activity when compared with wild type; ferric chelate reductase is a key activity in iron acquisition. The SP46 mutant also over-accumulates some metals, including manganese, cobalt, and cadmium, while underaccumulating others (zinc and copper); overall iron levels in SP46 are unchanged. We are currently testing the hypothesis that because photosynthesis in these mutants is impaired, they need less iron than wild type plants. Therefore, it takes longer under iron deficient conditions for the SP46 and CS557 mutants to become iron deficient and upregulate their ferric chelate reductase activity. These mutants provide the first evidence linking disruption of chloroplast function with general metal homeostasis in *Arabidopsis*.

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Transcriptome changes in response to magnesium deficiency and restoration in *Arabidopsis thaliana*. Christian Hermans¹, Adrian Craciun¹, Nathalie Verbruggen¹. ¹Université Libre de Bruxelles, Brussels, Belgium

Magnesium plays a key role in plant metabolism. In addition to being a central atom of the chlorophyll molecule, Mg is also essential for the functioning of many enzymes, including RNA polymerases, ATPases, protein kinases and phosphatases. Despite the wide recognition of Mg deficiency in several soil types, relatively little is known about Mg deficiency in plants. The first physiological analysis of Mg deficient *Arabidopsis* was recently reported [1], however proper knowledge of the molecular mechanisms underpinning Mg homeostasis is currently scarce. One dramatic effect during the early response to Mg deficiency is sucrose and starch accumulation in young source leaves, before any noticeable effect on photosynthetic activity. A later effect of Mg deficiency is a reduction in plant growth, with the shoot biomass being more affected than root.

We were primarily interested in the identification of genes involved in rapid responses to external Mg deprivation. The roots and the most recently expanded leaves of the rosette constituted the material for our transcriptomic analysis. A cDNA-AFLP approach (covering on estimation 5% of the transcriptome) was chosen for a pilot experiment, in order to decide about the sampling time for further microarray studies. Several time points following the removal of Mg from the nutrient solution, were analyzed in order to increase the temporal resolution and thus to discriminate between early and late Mg response gene. In this analysis, microarray hybridizations were done using Affymetrix Ath1 chips on three independent biological replicates. We also analyzed the transcriptomic response to Mg resupply (re-addition of Mg in the nutrient solution of 7 days deficient plants). The analysis of microarrays data generated will provide information for a long-term goal: uncovering the relationship of specific downstream events to the signaling pathways that control Mg deficiency responses in *Arabidopsis*.

[1] Hermans and Verbruggen (2005) J. Exp. Bot. 56: 1983-1989.

P-556

HY5 interacting B-box proteins are members of a novel class of transcription factors and regulate plant growth and development. Magnus Holm¹, Henrik Johansson¹, Chamari Hettiarachchi¹, Sourav Datta¹. ¹Gothenburg University, CMB-Molecular Biology

Light is an important factor for plant growth and development and a dramatic example of light signaling can be seen during seedling de-etiolation. This process is induced by wavelength specific photoreceptors and entails a dramatic transcriptional reprogramming. We study a group of *Arabidopsis* proteins that were identified through their interactions with two key regulators of light signaling, COP1 and HY5. COP1, an E3 ubiquitin ligase, represses light dependent development by targeting positive factors such as the transcription factor HY5 for degradation in the dark. Our genetic and molecular analysis of the HY5 and/or COP1 interacting proteins indicate that they are positive downstream regulators of light signaling and one of our goals is to characterize how light regulates these proteins and how they in turn regulate light dependent development. Furthermore, five of the proteins have tandem repeated Zn²⁺ binding B-boxes. The B-box is generally regarded as a protein-protein interaction domain and is often found in the so-called RBCC proteins, which also contain RING finger and Coiled-Coil domains. RBCC proteins such as the tumor suppressor PML in humans and LIN-41, which is involved in developmental timing in *C. elegans*, are important regulators in animals. On the other hand, no RBCC proteins exist in *Arabidopsis*. Our findings that B-box containing proteins interact with the RING finger and Coiled-Coil containing COP1 protein is therefore interesting. However, our recent results, which strongly suggest that the B-box proteins constitute a novel class of DNA binding transcription factors, are remarkable and have important implications for the interpretation of RBCC protein function. Taken together, we have genetic, functional and cell biological data suggesting that these B-box proteins are transcription factors that positively regulate HY5 activity in light and are currently investigating if the B-box domain, previously considered a protein-protein interaction domain, is a novel DNA binding domain. The characterization of this novel B-box function is an important goal for our research.

P-557

Correlation analysis and QTL identification for some photosynthetic factors in rice under different environment -watered conditions. Songping Hu^{1,2}, Lijun Luo². ¹ College of Resource and Environmental Science, Jishou university, Hunan province, China,² Shanghai Agrobiological Gene Center, Shanghai , China.

Methods:

A total of 195 RIL population developed between the paddy rice Zhenshan97B and upland rice IRAT109 and their parents. All materials were planted randomly with three replications in the drought screen facility in Shanghai , China

The facility was set up in a rainproof greenhouse including two islands separated by 1.8 m depth canal. When starting stress, the sprinkler irrigation was stopped while the drip irrigation was still working and underground water was drained out through the canal. A water gradient from island edge to center was formed and drought treatment would also be built naturally. The plants in the center were in well -watered condition while the plants near edge were in the stress.

Phenotyping and genotyping

A total of 213 microsatellite markers were used to genotype the population. A linkage map was constructed by using MapMaker/Exp V3.0 (Lincoln et al. , 1992) to span 1825.0 cM of genome size with an average distance of 8.6 cM between adjacent markers.

Results :**The relationship between CC and PR:**

There was significant positive correlation ($r = 0.1857 * *$) between CC and PR of rice RILs' leaves in well-watered condition, while not significant in drought($r = 0.0766$), indicating that the enhancing function of high CC to PR could be exerted only in well-watered condition.

The main QTLs: A total of 13 QTLs related to CC were detected and located on chromosomes 1, 2, 3, 4, 5, 6 and 10; The phenotypic variations explained by single QTL ranged from 3.54 % to 14.53 %. Seven QTLs were found in well-watered condition accounting totally 56.19% phenotypic variation. Six QTLs were detected in drought explaining totally 47.39% phenotypic variation. Six QTLs were detected in both two conditions.

Four QTLs, of which three in stress and one in well-watered related to PR were located on chromosome 2, 10 and 11 respectively. qPR10 was detected in both conditions. The phenotypic variance explained by 3 QTLs of PR in stress was 11.03 % together, while in well-watered it was 2.91%.

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Cooperation and Functional Diversification of Two Phospholipase A1 Genes for Jasmonate Biosynthesis. Youbong Hyun¹, Sungwook Choi¹, Hyun-Ju Hwang¹, Jihyeon Yu¹, Sang-Jip Nam², Jaeyoung Ko², Ju-Young Park³, Hyoung Yool Lee⁴, Stephen Beungtae Ryu⁴, Yong-Hwan Lee³, Yoo-Sun Noh⁵, Sunghwa Choi¹, Heonjoong Kang², Ilha Lee^{1,5}. ¹National Research Laboratory of Plant Developmental Genetics, Department of Biological Sciences, Seoul National University, Seoul 151-742, Korea, ²Center for Marine Natural Products and Drug Discovery, School of Earth and Environmental Sciences, Seoul National University, NS80, Seoul 151-747, Korea, ³Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea, ⁴Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Ochang, Chungbuk 363-883, Korea, ⁵Global Research Laboratory for Flowering at SNU and UW, Seoul 151-742, Korea

Jasmonic acid (JA) plays pivotal roles in diverse plant biological processes including wound response. The release of a-linolenic acid is the committed step for JA biosynthesis, but the mechanism of this process remains elusive. We report here that two genes encoding chloroplast-targeted phospholipase A1, DONGLE (DGL) and DEFECTIVE IN ANTER DEHISCENCE1 (DAD1), are both necessary and sufficient for a-linolenic acid production. These two genes, despite being functionally redundant, show different induction kinetics following wounding, indicating temporally separated roles in the wound response DGL for the early phase and DAD1 for the late phase. Moreover, DGL has a specific role in maintaining basal JA content under normal conditions, which regulates vegetative tissue growth and is required for a rapid JA burst after wounding. Our results indicate that PLD produces a wound-inducible signal for the transcriptional activation of DGL and DAD1, but that it does not provide the precursor of a-linolenic acid.

P-559

Genetic architecture of a Genotype * Environment interaction using *Arabidopsis thaliana* natural variation. Anna Ihnatowicz¹, Sigi Effgen¹, Maarten Koornneef¹, Matthieu Reymond¹, ¹Max Planck Institute for Plant Breeding Research, Cologne, Germany

Arabidopsis thaliana natural accessions have been collected in a wide range of habitats. It is assumed that selection for adaptation to the local environments has occurred, providing genetic variation in responses to environmental factors. Consequently, analysis of natural variation is a powerful tool to identify which allelic variants are adaptive under specific environmental conditions. A set of *Arabidopsis* accessions originating from different locations, grown together at several light and temperature conditions, has shown contrasting responses of traits such as growth, flowering time, pigmentation and photosynthetic performance. Phenotypic variation for some of these traits was characterized using spectroscopic analysis and by measuring chlorophyll content.

Interestingly, under particular environmental conditions (low temperatures) one high-altitude accession Hodja-Obi-Garm (Hog-collected around 1800m) showed a chlorotic phenotype linked with a large reduction in photosynthetic performance. When Hog plants were grown at 4 °C, the fraction of QA, the primary electron acceptor of PSII, present in the reduced state was significantly increased, whereas Fv/Fm and photosynthetic yield were reduced. These results indicate photoinhibitory effects.

An advanced-backcrossed population has been developed using the Hog accession and Ler as a non-chlorotic recurrent parental line. The aim of this study is to introduce only the gene(s) from Hog responsible for the chlorotic phenotype into the genetic background of Ler, creating near-isogenic lines. The obtained near-isogenic lines (BC2F2) are being further genotyped by using tilling array and illumina techniques. To further explore the functional relationship between genotype and phenotype, differences in gene expression between parental and near-isogenic lines grown at different temperatures are being examined. The mapping strategy combined with gene chip experiments and further physiological characterization of the observed phenotype will enable us to select candidate genes. The molecular basis of the underlying G * E interaction will be further studied.

P-560

Molecular evolution of clock genes in *Arabidopsis*. Aziz Jamaï¹, Katherine R Amato¹, Kathryn E. Hacker¹, Mark Borsuk¹, Mark A. McPeek¹, C. Robertson McClung^{1,1} Dartmouth College, Hanover, (NH), USA

The circadian clock is an endogenous timing mechanism with a period of ~24 hr. It serves to synchronize an organism with the externally imposed cycle associated with the daily rotation of the earth on its axis. One of the central premises of the study of circadian rhythms is that a functional circadian clock enhances organismal fitness and recent studies (e.g., Dodd et al. 2005 *Science* 309:630) have provided experimental proof that this is so. It is abundantly clear that there is considerable natural variation in clock function among *Arabidopsis* accessions (e.g., Michael et al. 2003 *Science* 302:1049). Presumably some of this natural variation arises from mutational differences in clock genes among accessions and, given that clocks enhance fitness, one might hypothesize that these sequence variations might be subject to selection. We have tested this hypothesis through sequence analysis of *PSEUDO-RESPONSE REGULATOR 7* (*PRR7*; At5g02810) from >100 accessions. There is a significant excess of replacement mutations relative to synonymous mutations (20 and 5, respectively; p<0.005), consistent with diversifying selection at the *PRR7* locus. This cannot be attributed to selection occurring at adjacent loci, as one flanking gene (*DNA TOPOISOMERASE*; At5g02820) shows excess synonymous mutations (6 replacement and 31 synonymous; p<0.001) and the other flanking gene (*PROTEIN KINASE*; At5g02800) shows roughly equal numbers of replacement and synonymous mutations (10 and 14, respectively; p>0.5), consistent with purifying and neutral selection, respectively. This suggests selection for multiple allelic variants at the *PRR7* locus, which is consistent with distinct *PRR7 alleles contributing differentially to organismal fitness in different environments*. Indeed, two *PRR7* clades show distinct spatial distributions within Europe. We are currently testing whether these different alleles are functionally equivalent through complementation of mutants defective in *PRR7* activity. This work is supported by a grant from the National Science Foundation (MCB-0343887) to CRM.

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Elicitor and UV light related transcription factor (OgElr) derived from Wild Rice (*Oryza grandiglumis*) gave resistance against UV-B radiation. Eun-Hee Jeon¹, Jung-Hun Pak¹, Mi-Jin Kim¹, Hye-Jeong Kim¹, Ki-Jung Lee¹, Young-Soo Chung¹, Kyung-Ho Kang². ¹Dept. of Genetic Engineering, Dong-A University, Busan, Korea, ²National Crop Experiment Station, Suwon, Korea

One of the wild rice species *Oryza grandiglumis* is tetraploid (2n=48) and has CCDD genome. It has been known to own fungal resistance against sheath blight, rice blast, bacterial leaf blight and insect resistance against brown plant hopper (*Nilaparvata lugens*).

The multiple techniques were used to identify the genes differentially expressed at wounding and fungal elicitor treated wild rice (*Oryza grandiglumis*). This technique is a combination of suppression subtractive hybridization (SSH) and GenomeWalker PCR. Here we report the gene cloning and expression characterization of OgElr gene. Whole sequences of OgElr gene from *Oryza grandiglumis* (CCDD, 2n = 48) were obtained by GenomeWalker method. OgElr contains 447 nucleotides and 148 amino acids. The cDNA has 96% sequence homology to *Oryza sativa* elicitor and UV light related transcription factor (Elr). The OgElr gene induced by wounding, fungal elicitor, jasmonic acid (JA) and salicylic acid (SA), protein phosphatase inhibitors cantharidin (CN) and endotall (EN) as well as UV-B. To identify in vivo function of OgElr gene, the gene was transformed into *Arabidopsis thaliana*. The OgElr transgenic plants were exposed to UV-B for 4 hours and UV-C for 2 hours and a root-bending assay was carried out. The survival rate of OgElr transgenic plants under UV exposure greatly increased. And also root growth was less inhibited by UV-B exposure in OgElr transgenic plants compared to the wild type. Our results suggest that OgElr gene may involve in tolerant response of plant against UV irradiation.

P-562

Transcriptomic and proteomic profiling reveals new insights into salt stress responses in *Arabidopsis* root. YQ Jiang¹, MK Deyholos¹. ¹University of Alberta, Edmonton, Canada

Salt stress is a major abiotic stress limiting the productivity and the geographical distribution of many plants. Roots are an attractive system for genomic and post-genomic studies of NaCl responses, due to their primary importance to agriculture and relative structural and biochemical simplicity. We used Qiagen Operon 70-mer oligonucleotide microarrays representing 23,686 *Arabidopsis* genes to identify the transcripts in 18d-old *Arabidopsis* roots that changed in relative abundance following 6 h, 24 h, or 48 h of hydroponic exposure to 150 mM NaCl. We verified 15 selected genes by qRT-PCR. Enrichment analysis identified groups of structurally or functionally related genes whose members were statistically over-represented among up- or down-regulated transcripts. Our results are consistent with generally observed stress response themes, and highlight potentially important roles for underappreciated gene families, including several groups of transporters, signalling molecules and transcription factors. As the mRNA level is not correlated well with protein level as a result of alternative splicing and post-translational modifications, we also carried out a comparative proteomic analysis of *Arabidopsis* roots treated with 150 mM NaCl for 6 and 48 h using two-dimensional electrophoresis and LC-MS/MS. We detected ~1,000 protein spots reproducibly on each gel. In contrast to the transcriptomic analyses, the greatest differences between control and treated samples were detected 48 h after NaCl treatment. Mass spectrometry analysis allowed the identification of 86 differentially expressed proteins, including well-known and novel salt-responsive proteins. The identified proteins are involved in several processes, i.e. ROS scavenging, signal transduction, protein translation, processing and degradation, primary and hormone metabolisms, and metabolism of amino acids. We chose a few novel salt-responsive genes for further characterization. In conclusion, our study provides new insights into salt stress responses in *Arabidopsis* root and will facilitate mapping of regulatory networks and extend our ability to improve salt tolerance in plants.

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The Functional Characterization of Novel *Arabidopsis* CBS-DOMAIN CONTAINING PROTEIN1 Gene That Acts on Ethylene & Jasmonate Biosynthetic Pathways during Anther Dehiscence, also Is Required for Biotic and Abiotic Stress Responses. Kwang Wook Jung¹, Kyoung Shin Yoo¹, Mei Hua Cui¹, Yun Young Kim¹, Jeong Sheop Shin¹. ¹School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

The programmed cell death (PCD) process is an integral part of development and the defense response, but the underlying mechanisms of its executors and regulators remain largely unknown in planta. We report the identification a novel gene, CBS-DOMAIN CONTAINING PROTEIN1 (AtCDCP1), which is believed to be involved in determining the timing of JA and ET biosynthesis. The loss-of-function mutant of AtCDCP1 had well-developed anther cells and showed an increased accumulation of JA and ET in its flower bud clusters. Conversely, overexpression of AtCDCP1 caused a delay in the development of the floral organs, arrested anther cells on secondary cell-wall biosynthesis in endothecium, and a reduction in the accumulation of JA and ET in the flower, resulting in male sterility through anther indehiscence. Moreover, those overexpression lines are much more tolerant to salt and mannitol stresses. Based on these data, we are trying to characterize the biological roles of novel genes in defense system or developmental process, including regulatory mechanism as well as the effects of gene itself. Subsequently, these data will be applicable to the molecular breeding of cultivated rice.

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The barley ERF-type transcription factor HvRAF confers enhanced pathogen resistance and salt tolerance in *Arabidopsis*. Jinwook Jung¹, So Youn Won², Seok Cheol Suh³, Youn Soo Cheong¹, Sang Ryong Park¹, Hyeran Kim⁴, Rod Wing⁴, Yeonhwa Jeong¹, Ingyu Hwang¹, Minkyun Kim¹. ¹School of Agricultral Biotechnology, Seoul National University, Seoul, Republic of Korea, ²GMO Safety Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon, Republic of Korea, ³Cell and Genetics Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon, Republic of Korea, ⁴Arizon Genomics Institute, University of Arizona, Tucson, USA

We isolated HvRAF (*Hordeum vulgare* Root Abundant Factor), a cDNA encoding a novel ERF-type transcription factor from young seedlings of barley. In addition to the most highly conserved AP2/ERF DNA-binding domain, the encoded protein contained an N-terminal MCGAIL signature sequence, a putative nuclear localization sequence, and a C-terminal acidic transcription activation domain containing a novel mammalian hemopexin domain signature-like sequence. RNA blot analyses revealed that HvRAF transcripts were more abundant in roots than in leaves. HvRAF expression was induced in barley seedlings by various treatment regimes such as SA, ethephon, MeJA, cellulase, and methyl viologen. In a subcellular localization assay, the HvRAF-GFP fusion protein was targeted to the nucleus. The fusion protein of HvRAF with the GAL4 DNA-binding domain strongly activated transcription in yeast. Various deletion mutants of HvRAF indicated that the transactivating activity was localized to the acidic domain of the C-terminal region, and that the hemopexin domain signature-like sequence was important for the activity. Overexpression of the C-terminus region of the HvRAF conferred enhanced cadmium tolerance to yeast. Furthermore, overexpression of the HvRAF gene in *Arabidopsis* plants induced the activation of various stress-responsive genes.

Furthermore, the transgenic *Arabidopsis* plants showed enhanced resistance to *Ralstonia solanacearum* strain GMI1000, as well as seed germination and root growth tolerance to high salinity. These results collectively indicate that HvRAF is a transcription factor that plays dual regulatory roles in response to biotic and abiotic stresses in plants.

P-565

Boron transcriptome analysis-profiles of boron nutrition-regulated genes and role of WRKY6 in regulation. Ichiro Kasajima^{1,2,3}, Yoko Ide^{1,2,3}, Masami Yokota Hirai⁴, Toru Fujiwara^{1,2,5,1} Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo,² Biotechnology Research Center, The University of Tokyo,³ JSPS Research Fellow,⁴ RIKEN Plant Science Center,⁵ Solution Oriented Research for Science and Technology, Japan Science and Technology Agency

Boron (B) is an essential nutrient for plants but is toxic in excess. We performed transcriptome analysis under conditions of both low B (0.3 μ M borate) and high B (3,000 μ M borate) in *Arabidopsis thaliana* using Affymetrix 22K array. Induction by high B was also confirmed for nine genes in an independent experiment. Transcripts of At1g03770 (transcription factor) and At5g57340 were found to be elevated in roots, whereas At2g04040, At2g04050, At2g04070 (all encoding multidrug and toxic compound extrusion transporters), At1g32870 (transcription factor), At5g51440 (heat shock protein-like), At2g41730 and At2g21640 are induced in the shoots. The accumulation of both At2g04050 and At5g51440 transcripts was further found to be elevated by more than 100-fold after two days of high B treatment. Although the roles of these genes in high B conditions remain unknown, our findings represent the first identification of high B-induced genes. We also identified WRKY6 as a low-B induced gene. Its transcript is upregulated in root tips under low B condition. This induction was confirmed with the WRKY6 promoter-GUS plants, suggesting that regulation is at the transcriptional level. To elucidate target genes of WRKY6, we performed transcriptome analysis with 8-mm root tip samples of wrky6-3, a mutant carrying T-DNA in WRKY6. We identified several genes whose mRNA accumulations are affected in the root tips of wrky6-3. Preliminary experiments suggested that root elongation of wrky6-3 mutant plants are reduced only under low-B conditions. The downstream genes may be involved in maintenance of root elongation under low-B conditions.

P-566

ZEITLUPE is a circadian photoreceptor stabilized by a blue-light-enhanced protein-protein interaction. Woe-Yeon Kim¹, Sumire Fujiwara¹, Sung-Suk Suh¹, Jeongsik Kim², Yumi Kim², Lin-Qu Han¹, Hong Gil Nam², David E. Somers¹. ¹ Department of Plant Cellular and Molecular Biology/Plant Biotechnology Center, Ohio State University, Columbus, OH 43210, USA, ² Department of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, South Korea

Appropriate regulation of rhythmic cycling of key elements of the clock is essential to maintain robust oscillations. Cyclic oscillation of ZEITLUPE (ZTL) is required to sustain normal circadian cycling via the proteasome-dependent degradation of TOC1, a key element of the central oscillator in plants. ZTL is an F-box protein and its cyclic oscillation is post-transcriptionally regulated by the proteasome. We have identified a factor which regulates ZTL protein abundance. In its absence, the normal four-fold diurnal cycling in ZTL is post-transcriptionally eliminated, resulting in constitutively low ZTL accumulation. Their interaction occurs through the N-terminal ZTL LOV domain, which is necessary and sufficient for the interaction. Further, this interaction is strongly and specifically enhanced by blue light, via the N-terminal flavin-binding LIGHT, OXYGEN OR VOLTAGE (LOV) domain of ZTL. Mutations within the LOV domain that greatly diminish the interactions also lead to strongly reduced ZTL levels. A C82A transition in the LOV domain, implicated in the flavin-dependent photochemistry, eliminates blue-light enhanced binding. These data establish ZTL as a blue-light photoreceptor, by which light absorption facilitates its own stability via a blue-light enhanced protein-protein interaction. Circadian control of message levels of the ZTL-interacting protein, and subsequent cycling of the protein, confers a post-translational rhythm on ZTL protein abundance, defining a novel mechanism to establish and sustain circadian oscillations. Additional progress on this mechanism will be presented.

P-567

Functional characterization of DEAD-box RNA helicases in *Arabidopsis thaliana* under various abiotic stress conditions. Kyung Ae Kim¹, Chul Min Park¹, Hunseung Kang¹. ¹ Chonnam National University, Gwangju, Korea

Although DEAD-box RNA helicases have been implicated to play roles during stress adaptation processes, the functions of RNA helicases in the stress responses of plants are poorly understood. To understand the functions of DEAD-box RNA helicases in *Arabidopsis thaliana* in responses to environmental stimuli, the stress-related expression patterns, nucleic acid-binding properties, and functional roles of the three RNA helicases, AtRH9, AtRH14, and AtRH25, were investigated. The transcript levels of the three RNA helicases were up regulated in response to cold stress, whereas their transcript levels were down regulated by salt or drought stress. The RNA helicases have different nucleic acid-binding specificity, and contain an RNA helicase activity in vitro.

Phenotypic analysis of the transgenic plants revealed that constitutive overexpression of AtRH9, AtRH14, or AtRH25 resulted in retarded germination compared with the wild-type plants under salt or dehydration stress. AtRH25 contributed to enhance freezing tolerance in *Arabidopsis* plants. In situ poly(A) hybridization analysis indicated that the export of mRNAs was impaired in the loss-of-function mutants of RNA helicases at low temperatures. AtRH14 and AtRH25 complemented the cold sensitivity of BX04 mutant *E. coli* cells, had a transcriptional anti-termination activity in RL211 *E. coli* cells, and contained nucleic acid-melting activity in vitro, demonstrating that AtRH14 and AtRH25 exhibit an RNA chaperone activity during the cold adaptation process. The present results demonstrate that AtRH9, AtRH14, and AtRH25 impact on germination and growth of *Arabidopsis* plants under various stress conditions, and strongly imply that AtRH14 and AtRH25 exhibit an RNA chaperone activity during the cold adaptation process. [Supported by KOSEF]

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Cold shock domain proteins and zinc finger-containing RNA-binding proteins play roles as an RNA chaperon during the cold adaptation process. Won Yong Kim¹, Tae Rin Oh¹, Su Jung Park¹, Yeon Ok Kim¹, Hunseung Kang¹. ¹ Chonnam National University, Gwangju, Korea

Despite the fact that cold shock domain proteins (CSDPs) and zinc finger-containing RNA-binding proteins (GRPs) have been implicated to play a role during the cold adaptation process, their importance and function in plants are largely unknown. To get better insight into the functional roles of CSDPs and GRPs in the cold response, four CSDPs (CSDP1 to CSDP4) and three GRPs (AtRZ-1a to 1c) from *Arabidopsis thaliana* were investigated. AtRZ-1a confers cold and freezing tolerance in *Arabidopsis* plants. Heterologous expression of CSDP1, CSDP3, AtRZ-1a, or AtRZ-1b complemented the cold sensitivity of BX04 mutant *Escherichia coli*. By contrast, CSDP2, CSDP4, and AtRZ-1c had very weak ability. The CSDPs and GRPs that showed complementation ability at low temperatures had DNA melting activity, RNA melting activity, and transcription anti-termination activity. By contrast, CSDP2, CSDP4, and AtRZ-1c did not have these activities. To understand the importance of the N-terminal RRM and CSD, and the C-terminal glycine-rich region containing CCHC-type zinc fingers, a domain swapping experiment between the N-terminal and C-terminal domains was performed, and the RNA chaperone activity of the chimeric proteins was analyzed. Together, these results strongly imply that CSDPs and GRPs exhibit an RNA chaperone activity during the cold adaptation process. [Supported by KOSEF and KRF]

P-569

Characterization of GIGANTEA expression patterns in the nucleus. Yumi Kim¹, Jeongsik Kim¹, Miji Yeom¹, Hong Gil Nam^{1,2}. ¹Division of Molecular Life Sciences and National Core Research Center for Systems Bio-Dynamics, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea, ²The I-BIO graduate program, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Circadian regulation in plant is essential for adaptation to environment. GIGANTEA (GI) was isolated through forward genetics as a circadian regulator. GI, as a nuclear protein, is post-translationally regulated by light/dark via 26S proteasome, and required for maintaining circadian amplitude and proper period length. Several circadian regulators including GI express in the nucleus and make distinctive nuclear bodies. However, little is known about the roles of nuclear bodies in circadian regulation. Here we describe nuclear expression patterns of GI using GI-GFP fusion protein and their regulation. GI has three typical nucleus expression patterns; dispersed in the nucleus, small and numerous nuclear bodies and large and hollow nuclear bodies. Three nucleus expression patterns have distinct portions during a day. Our results suggest that proper nuclear expression of GI might be another mechanism to explain its function in circadian and photoperiodic flowering regulation.

P-571

Functional Characterization of Stress-Related Putative Caleosin (SRC) that Plays on Biotic and Abiotic Stress Response. Yun Young Kim¹, Mei Hua Cui¹, Kwang Wook Jung¹, Kyoung Shin Yoo¹, Jeong Sheop Shin¹. ¹School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

With cDNA microarray experiments, we have previously identified a group of genes differentially expressed in wild rice by fungal infection, insect infestation and wound treatment. Based on expression profiling, Northern and Southern analysis, OmSRC (*Oryza minuta* Stress-Related Putative Caleosin) was selected for the further study. Caleosins, which contain a single Ca^{2+} -binding EF-hand, have been identified in a number of plant species. In *Arabidopsis*, until now, it was only reported that caleosins play on seed embryo development.

However, our studies demonstrated that SRC protein could act on stress response. To intensive study, we have also characterized *ARABIDOPSIS STRESS-RELATED PUTATIVE CALEOSIN1* (AtSRC1) as a *Arabidopsis* ortholog of OmSRC. Interestingly, strong expression of ProAtSRC1:GUS was detected in all tissues, including leaves, flower and stem, along the veins. On germination assays under various osmotic stresses condition, AtSRC1-overexpressed *Arabidopsis* plants show multiple abiotic-stress tolerance. Moreover, we confirmed that AtSRC1 gene is required to restrict to spread of necrotrophic fungal pathogen *Botrytis cinerea*. Based on these data, we have determined the biological functions of novel AtSRC1 in defense system, including regulatory mechanism as well as the effects of gene itself.

P-570

An essential role of FIONA1 for circadian clock function in *Arabidopsis*. Jeongsik Kim¹, Yumi Kim¹, Miji Yeom¹, Hong Gil Nam^{1,2}. ¹Division of Molecular Life Sciences and National Core Research Center for Systems Bio-Dynamics, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea. ²The I-BIO graduate program, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Summary

The circadian clock is a time-keeping mechanism to support and give organisms to prepare the metabolic and physiological processes with daily cycles of light and dark. These daily information processes also determine proper flowering time by recognizing the day-length in higher plants. In order to facilitate to isolate putative clock components, we isolated mutants of the circadian clock by screening early flowering phenotypes. One of these mutants, fiona1 exhibited extremely early flowering both in long day and short day. The fio1 mutation affects the essential photoperiodic genes, CO and FT in *Arabidopsis*, and also lengthens the period of multiple output rhythms of the circadian clock in constant light free-running condition including leaf movement and expression of various clock controlled genes. The 'FIO1' gene acts independently of light- and temperature-dependent pathway. Furthermore, transient expression of FIO1 gene in LL free-running condition lengthened the period length of wild type plants. Collectively, these results suggest that FIO1 plays an essential role for sustaining the master oscillator activity. Map-based cloning of FIO1 identified a novel nuclear protein which is well conserved in other prokaryotes and eukaryotes, implicating a common and dispensable role for this protein in diverse organisms.

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***rcd3*-a novel guard cell membrane transport mutant.** Triin Kolist¹, Hannes Kollist^{1,2}, Heino Moldau², Yong-Fei Wang³, Radhika Desikan⁴, Julian Schroeder³, Jaakko Kangasjärvi¹. ¹University of Helsinki, Helsinki, Finland, ²University of Tartu, Tartu, Estonia, ³University of California, San Diego, (CA), USA, ⁴University of the West of England, Bristol, UK

Ozone (O₃) is a predominant air pollutant, which has also been used as a tool to induce the formation of reactive oxygen species (ROS) in the apoplast, and this way identify components and processes regulated by apoplastic ROS. We have previously isolated a series of O₃-sensitive *rcd* (for radical induced cell death) mutants. Here we describe an O₃-sensitive mutant *rcd3*, the phenotype of which is caused by a change in stomatal function. *rcd3* has constitutively higher stomatal conductance than the Col-0 wild type and O₃-induced stomatal closure is largely suppressed in *rcd3*. The number of stomata in the abaxial side of the leaf is not altered. Detailed analysis of stomatal responses immediately after the onset of O₃-treatment revealed that O₃ induces a rapid stomatal closure and subsequent reopening within 30 minutes in several mutants with altered ozone sensitivity or stomatal function, and in several ecotypes including Col-0 and Ler. This fast stomatal response, which is induced by even as short as 60-180 sec O₃-pulse, is most likely an indication of the activity of ROS in the guard cell apoplast. During the reopening the guard cells loose responsiveness to the ROS-pulse and gain it again after 90 minutes. In the abscisic acid (ABA) insensitive mutants *abi1*, *abi2*, and *ost1*, and in *rcd3*, the transient closure was absent. The phenotype of *rcd3* is not caused by altered ABA content since a six hour treatment with O₃ caused similar ABA induction in both Col-0 and *rcd3*. However, guard cells of *rcd3* are insensitive to ABA, nitric oxide, and hydrogen peroxide, and their responses to light, humidity and CO₂ are altered. Map-based cloning of *rcd3* revealed a plasma membrane transport protein which is a novel component in membrane transport processes involved in stomatal closure.

P-573

Physiological activities of the N-terminal photosensory and the C-terminal kinase domains of phototropin 2 in transgenic *Arabidopsis*. Sam-Geun Kong^{1,2}, Toshinori Kinoshita³, Ken-ichiro Shimazaki³, Nobuyoshi Mochizuki¹, Tomomi Suzuki¹, Akira Nagatani¹. ¹Kyoto University, Kyoto, Japan, ²National Institute for Basic Biology, Okazaki, Japan, ³Kyushu University, Fukuoka, Japan

Phototropins (phot1 and phot2) are membrane-associated blue light photoreceptors that mediate various physiological responses such as phototropism, chloroplast relocation, and stomatal opening in plants. We have recently reported that phot2 associates with the Golgi apparatus in a light-dependent manner and its C-terminal kinase domain is essential for the localization (Kong et al., 2006). In this study, we further analyzed biological activities of the N-terminal photosensory and C-terminal kinase domains of phot2 in transgenic *Arabidopsis*. For this purpose, these domains were fused to green fluorescent protein (GFP) and ectopically expressed in the wild type and a *phot1/phot2* double mutant of *Arabidopsis*. Consequently, the kinase domain fused to GFP (P2CG) was localized to the plasma membrane and the Golgi apparatus, whereas the photosensory domain fused to GFP (P2NG) was uniformly localized in the cytosol. Hence, the kinase domain but not the photosensory domain was responsible for the membrane association. Interestingly, the P2CG plants exhibited constitutive blue light responses even in dark conditions. Namely, stomata were open and chloroplasts were in the avoidance position. By contrast, P2CG, with a mutation that abolishes the kinase activity (P2C[D720/N]G), failed to exhibit these activities. However, it inhibited the blue light-induced stomatal opening in the wild type background. Hence, P2CG appears to be correctly localized to the functional sites in the cell and trigger the light signal transduction through its kinase activity. By contrast to P2CG, P2NG failed to affect the phot2 responses except that P2NG partly inhibited the phototropic response caused by the endogenous phototropins.

P-574

HIGH LEVEL OF ARABIDOPSIS EXPANSIN EXPRESSION IN TRANSGENIC PLANTS CAUSES ENHANCED HORMONE OR SALT STRESS SENSITIVITY. YeRim Kwon¹, Hee-Jin Lee², Eun-Ji Koh¹, Jee Eun Oh¹, Kyoung-Heon Kim², Suk-Whan Hong³, Hojoung Lee¹. ¹Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea., ²Division of Food Science, College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea., ³Division of Applied Plant Science, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Korea.

Cellulose is the most abundant and renewable natural resource which can be obtained from less costly producible plant lignocellulosic materials. A number of wall-modifying proteins were discovered to have crucial roles in the molecular basis of cell wall expansion. Expansin, one of cell wall loosening proteins, appears to permit the microfibril matrix network to slide in growing plant cell walls, thereby enabling the wall to expand. Hence, as one strategy of improving cellulase performance leading to an increase in sugar yields, we intended to express expansins in various protein expression systems such as *E. coli* or yeast. Although we obtained a fare amount of expansins in these systems, there was no activity detected in terms of cell wall extension. This led us to establish plant expression systems for the production of higher amount of expansins. However, expansins are involved in the cell expansion process, it may cause harmful defects in plant growth and development when tried to over-express these proteins in *planta*. Hence, to scrutinize possible impacts of expansin over-expression in plant growth and development, we generated two different types of transgenic plants constitutively expressing *Arabidopsis EXP3* or *EXP-β1* under the control of 35S-CaMV promoter. Here, we report that transgenic plants showing high level of *EXP-β1* expression exhibit enhanced sensitivity to salt stress. Possible reasons for this phenomenon were explored.

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P-575

A protein-protein interaction network model for salt stress in *Arabidopsis thaliana*. Xingguo Lan¹, Yu-zhe Nie¹, Guang Li¹, Tie-liu Shi², Qijiang Xu¹, Yuhua Li¹. ¹College of Life Science, Northeast Forestry University, Harbin, China, ²Bioinformation Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Protein-protein interactions play key roles in protein function and the structural organization of a cell. We constructed a protein-protein interaction network using a naive Bayes classifier for integrating disparate data types in predicting *Arabidopsis thaliana* protein-protein interactions. These methods included gene expression method, shared biological function method, protein domain method, phylogenetic profile method, gene fusion method, gene neighbor method and GO annotation method. Here, we collected 2645 non-redundant salt related proteins through consulting drastic database (<http://www.drastic.org.uk/>), GO database (<http://www.geneontology.org>) and texts mining. And a protein-protein interaction network for salt stress in *Arabidopsis thaliana* was constructed with 2645 proteins. In the network, we found that 898 proteins formed 1778 interaction pairs. We performed analysis that showed a dynamical explanation for the SOS pathway and MAPK pathway. Furthermore, relationships of salt induced proteins were discussed. This protein-protein interaction network model provides a framework for exploring molecular mechanism in plant salt stress response.

P-576

Reverse genetics approach on the identification of putative salt and osmotic stress determinants in *Arabidopsis*. Sara Laranjeira¹, Herlinder Azevedo¹, Vitor Amorim-Silva¹, Rui Tavares¹. ¹Biology Dep., Minho University, Braga, Portugal

In the face of a global scarcity of water resources and the increased salinization of soil and water, abiotic stress is already a major limiting factor in plant growth and will soon become even more severe as desertification covers more and more of the world's terrestrial area. Drought and salinity are already widespread in many regions, and are expected to cause serious salinization of more than 50 % of all arable lands by the year 2050. The major progress in the identification of salt/osmo-tolerance determinants is due primarily to the use of *Arabidopsis* as a molecular genetic model system. Functional screening in the *Arabidopsis* biological model makes use of phenotype-centred forward and reverse genetic approaches, usually using loss-of-function mutant analysis. Based on a reverse genetics approach, the expression analysis of salt and osmotic stressed *Arabidopsis* plants made available through the Affymetrix microarray database (NASC), led to the identification of genes of unknown function. Through loss-of-function mutant strategy we are searching for salt/osmotic stress related phenotypes.

P-577

The roles of microRNAs in *Arabidopsis thaliana* under various abiotic stress conditions. Hwa Jung Lee¹, Yeon Ok Kim¹, Won Yong Kim¹, Hunseung Kang^{1,1} Chonnam National University, Gwangju, Korea

Micro RNAs (miRNAs) are 20 to 22-nucleotide-long RNAs that regulate the expression of a large number of genes by mRNA cleavage or translational repression of target mRNAs. Although the importance of miRNAs in plants is becoming more evident, our understanding on the importance and function of miRNAs in the growth, development, and stress responses of plants are severely limited. To better understand the functions of miRNAs in the responses of plants to various stresses, we examined the transgenic *Arabidopsis* plants that overexpress miR393, miR397, or miR406 under the control of cauliflower mosaic virus 35S promoter. Under normal growth conditions, no significant changes in growth pattern were observed between the genotypes. However, the 35S: :miR393 and 35S: :miR397 transgenic plants displayed promoted germination compared with the wild-type plants under glucose or abscisic acid treatment. The 35S: :miR406 plants survived better than the wild-type plants under salt stress conditions. The expression of putative target genes was analyzed in the wild-type and transgenic plants. The biogenesis and functions of natural miRNAs and artificial miRNAs were compared. These results suggest that these miRNAs play roles in the responses of plants to various environmental stimuli. [Supported by KRF]

P-579

Natural variation in genes determining high-latitude adaptation in *Arabidopsis thaliana*. AM Lewandowska-Sabat¹, S Fjellheim¹, R Nestestøl¹, JE Olsen¹, OA Rognli¹. ¹Norwegian University of Life Sciences P. O. Box 5003, 1432 Aas, Norway

Vernalization (exposure to prolonged cold) accelerates flowering by suppressing expression of the floral repressor FLC. Analyses of vernalization responses help to reveal how these responses are influencing flowering and local adaptation in *Arabidopsis*. The objective of this study is to understand the effect of vernalization on flowering in natural populations of *A. thaliana* at high latitudes. *A. thaliana* populations have been collected from range of high-latitude and high-altitude locations in Norway. These locations have unique combinations of photoperiod, light quality and temperature found nowhere else where *A. thaliana* naturally occurs. Flowering time after 5 different vernalization treatments (0, 3, 6, 9, 12 wks of vernalization) was scored in 27 (0, 3, 6, 9 wks) or 36 (12 wks) populations of *A. thaliana* using 5-12 lines per population. Sequence analysis of flowering pathway genes such as PHYC, FLC, CRY1 and CRY2 has been performed in 15-25 populations. Variation for flowering time among populations and among lines within populations was high. Statistical analysis revealed significant clinal variation in flowering time regressed against temperature, precipitation, altitude and latitude. No genetic variation in CRY1/2 was found, and SNPs in PHYC did not show any geographic/phenotypic pattern. However, analysis of the FLC gene in 25 populations revealed two main genetic clusters among Norwegian populations. Northern populations cluster together and flower significantly later than Southern ones. However the bootstrapping values of the phylogenetic tree are low and in addition not all late or rapid flowering populations are clustered together. This can be due to population structure, outcrossing or recent introduction of these accessions in Norway. Further genetic and phenotypic screening confirming the local adaptation and elucidating the origin of Norwegian populations are necessary. Consequently, phenotypic screening for responses to 5 different photoperiods of 10 selected populations is currently being conducted. Extreme individuals will be crossed with standard Col-0 and used to establish QTL mapping populations.

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Analysis of transcriptome for *Deschampsia antarctica* Desv. from King George island in the Antarctic. Hyoungseok Lee¹, Hyun Hee Cho¹, Yoo Kyung Lee¹, Il-Chan Kim¹, Joung Han Yim¹, Hong Kum Lee^{1,1} Polar BioCenter, Korea polar Research Institute (KOPRI), Song Do Techno Park, Incheon, 406-840 Korea

Deschampsia antarctica Desv. is the only monocot that thrives in the rough conditions of the Antarctic region and represents an invaluable resource for identifying genes associated with tolerance against various environmental pressures. In order to identify genes differentially regulated between greenhouse-grown and Antarctic field-grown plants, we have initiated a detailed analysis of its gene expression. Two different cDNA libraries were constructed with these plants. A total of 2,112 cDNA clones were sequenced and grouped into 1,199 non-redundant (nr) ESTs consisting of 242 consensus and 957 singleton sequences. Using similarity searches against several public databases we constructed a functional classification of the ESTs various categories such as stress-responsive genes, genes related to carbon and nitrogen metabolisms. Real-time PCR analysis of DaWRAB1, DaCOR413, DaCOR2, and DaCORU, showed a distinct regulation pattern in different environments indicating that they are involved in responses for specific environmental factors. The significance of these results during the cold-acclimation process will be discussed.

P-580

Dissecting P-type H⁺-mediated proton extrusion in Arabidopsis. Wenfeng Li¹, Simonetta Santi², Chris Tan¹, Wolfgang Schmidt¹. ¹IPMB, Academia Sinica, Taipei, Taiwan, ²University of Udine, Italy

As part of a concerted response to iron (Fe) deficiency, comprising developmental, physiological and metabolic reactions, the acquisition of sparingly soluble iron compounds is aided by P-type ATPase-mediated extrusion of protons across the plasma membrane of apical rhizodermic cells. This response is critical for Fe uptake but under-studied in *Arabidopsis* because the response is not very pronounced in the lab strains commonly used. Screening of 96 natural accessions for enhanced proton extrusion revealed several lines that showed substantially higher acidification capacity when compared to Col-0. Four ATPase genes, *AHA2*, *AHA3*, *AHA4*, and *AHA7* appeared to be particularly responsive to Fe deficiency. Accessions with higher proton extrusion activity showed a generally higher induction of *AHA2* and *AHA7* in response to Fe deficiency. In addition, other key genes of the Fe deficiency response such as *IRT1* and *FRO2* showed higher message levels in strong acidifying lines. Time-course experiments revealed that full induction levels were reached earlier in active lines. Light affected the expression of all Fe-responsive genes. The effects of light and Fe deficiency on *IRT1* and AHA were additive and revealed strict co-regulation of *AHA7*, but not of *AHA2*, with *IRT1*. Transcripts of all Fe-regulated genes were lower at high external pH. Regulation of iron-responsive genes was impaired in *aha7* mutants, indicating a regulatory function of *AHA7* in iron homeostasis.

In order to generate RILs with high proton extrusion activity in response to Fe deficiency, we have developed a high-throughput system to screen for highly active individuals. Crosses from parents that have been selected from the initial screening showed a more pronounced response when compared to Col-0. Backcrosses with high proton extrusion capacity exhibited an increased frequency of root hairs, indicating a close linkage of root hair formation and acidification capacity. The role of ATPase-mediated proton extrusion in the regulation of physiological and developmental responses to Fe deficiency is discussed.

P-581

Interaction between *Arabidopsis* HSF-A1a and HSF-A1b in vivo. Ming Li¹, Fritz Schoeffl¹. ¹Zentrum für Molekulare Biologie der Pflanzen / Allgemeine Genetik, Universität Tübingen, auf der Morgenstelle 28, 72076 Tübingen, Germany

The class A Heat Shock Factors HSF-A1a and HSF-A1b have been identified as early response regulators activating the transcription of several target genes upon heat stress in *Arabidopsis thaliana* (Busch et al., 2005, Plant J., 41, 1-14). The analysis of gene knock out mutations suggests that both HSF may interact in vivo and that they can functionally replace each other (Lohmann et al., 2004 Mol Gen Genomics, 271, 11-21). Using bimolecular fluorescence complementation we tried to investigate whether these HSF can interact in vivo.

Arabidopsis thaliana (Columbia) cell culture protoplasts were transformed with suitable gene fusion constructs between full length HSF and C- or respectively N-terminal parts of the yellow fluorescence protein. Confocal microscopic imaging and fluorescence activated cell sorting (FACS) analysis indicate that HSF-A1a can interact in a homomeric and with HSF-A1b in a heteromeric combination. Interaction can also be observed between truncated versions of HSF, representing only the oligomerization domains (OD: hydrophobic repeat region). These interactions are localized in the cytoplasm, with no preference for the nucleus. However, no homo- and heteromeric combinations were observed when the oligomerization domain HSF-A1a was deleted. Our data indicate that these two structurally and functionally related HSF can interact in vivo, possibly through hetero-oligomerization. This interaction may be instrumental in stress sensing and signalling in the very early phase of the heat shock response.

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Isolation and Characterization of an *Arabidopsis* Temperature-dependent Mutant *lls*. Xiaofang Li¹, Xinghua Shao¹, Bin Yao¹, Yue Sun¹. ¹The School of Life Science, East China Normal University

It is well known that temperature controls a number of aspects of plant development. However, the mechanism of which is still unknown. We isolated a long life span (*lls*) mutant from *Arabidopsis thaliana* L. through a T-DNA tagging approach. The phenotype of *lls* plant includes loss of apical dominance, reduced plant size and dwarfism, dark-green and curved leaves, severe late flowering, growing slowly and long life span at 22-26 °C. However, these phenotypes lost at 28 °C or higher than 28 °C, suggesting that the *lls* mutant is temperature dependent. Many mutant phenotypes at room temperature indicate that *lls* may be involved in certain phytohormones metabolism, transport or signalling. Using a reporter gene driven by an auxin-responsive promoter, we found that the expression pattern of auxin response element was not altered in *lls*. The auxin sensitivity and transport did not change either. While the sensitivity of *lls* plants to cytokinin was reduced. Physiological assay indicated that sugar content in *lls* was higher than that in wild-type by at least 3 times. These results suggest that the *lls* may connect with temperature and sugar and phytohormone metabolism, transport or signaling to control plant development. Genetic analysis demonstrates that *lls* is a recessive mutant and segregates with a T-DNA insertion, which indicates that *LLS* gene could not be cloned by iPCR approach. We have finely mapped *LLS* between two markers on chromosome 4 by mapped-clone method and the cloning of *LLS* will provide us more valuable information on how this gene controls plant development related with temperature.

P-583

Microtubule dynamics in *Zea mays* roots in response to drought stress. Jiansheng Liang¹, Bing Lü¹, Feng Chen¹. ¹College of Bioscience and Biotechnology, Key Laboratory of Crop Genetics and Physiology of Jiangsu Province, Yangzhou University, Yangzhou, People's Republic of China, 225009

Microtubules play important roles in plant physiological responses to environmental stress, including drought stress, etc. When plants are exposed to drought stress, dynamic changes of microtubules occur. Plenty of evidence has showed that plants have developed complicated mechanisms to adapt to these environmental stress conditions. Abscisic acid (ABA) accumulates significantly in plants in response to drought conditions, which has been considered a major mechanism for plants to enhance drought tolerance. However, little has been known whether the dynamic changes of microtubule are correlated to ABA accumulation under a drought condition. In the present study, we have focused on the role of microtubules in the induction of abscisic acid biosynthesis as a response of *Zea mays* to drought stress. The functions of microtubules in response to drought stress were investigated by immuno-fluorescence localization, enzyme-linked immuno-sorbent assay and reverse transcription-polymerase chain reaction. The microtubule-destabilizing (oryzalin) and stabilizing reagents (taxol) were used in combination with osmotic stress treatment. Results indicated that microtubule dynamics correlated with the ABA accumulation under osmotic stress. Microtubule-destabilizing and stabilizing reagent treatments also led to significant changes of ABA accumulation. Our results suggest that the dynamic arrangement of microtubules is involved in the accumulation of ABA under osmotic stress. A possible mechanism of plant response to drought stress through microtubules is proposed that provides an explanation for the increased abscisic acid biosynthesis under drought stress.

P-584

Identification and Characterization of Transcription Factors involved in Salt and Osmotic Stress Response. Felix Lippold¹, Magdalena Musialak¹, Diego Sanchez¹, Franziska Schwabe¹, Tomasz Kobylko¹, Rosa Morcuende², Dirk Hincha¹, Wolf R diger Scheible¹, Michael Udvardi³. ¹ Max Planck Institute for Molecular Plant Physiology, Wissenschaftspark Golm, Am M hlenberg 1, Potsdam-Golm, 14476, Germany, ² Instituto de Recursos Naturales y Agrobiolog a de Salamanca, Consejo Superior de Investigaciones Cient ficas, 37008 Salamanca, Spain, ³Samuel Roberts Noble Foundation, 2510 Sam Noble Pky., Ardmore, OK 73401, USA

Transcription factors (TFs) are master-control proteins in all living cells. They often exhibit sequence-specific DNA binding and are capable of activating or repressing transcription of multiple target genes. In this way, they control or influence many biological processes, including cell cycle progression, metabolism, growth and development, and responses to the environment.

To identify TFs that have an essential regulatory impact in response to salt stress, we used a qRT-PCR based platform to screen almost all *Arabidopsis thaliana* TFs (Czechowski et al. , 2004). *Arabidopsis thaliana* seedlings have been grown for nine days in a liquid culture system and subsequently stressed with 100 mM NaCl respectively 200mM Mannitol for three hours. Material from three biological replicates has been used to screen approximately 2000 TFs. Among them, twenty candidate genes displaying an altered gene expression with respect to salt stress or osmotic treatment were selected. To confirm the results additional experiments have been carried out including a hydroponic growth system and time course experiments. Six out of this 20 TFs showed an significant expression change combined with an interesting expression pattern. They have been selected for further analysis using a reverse genetic approach. Overexpressors were analyzed on a physiological and molecular level. The results from these experiments revealed potential regulators of early salt stress response whose impact on gene expression is now examined on a genome wide level.

Czechowski, T. et al. (2004) "Real-time RT-PCR profiling of over 1400 *Arabidopsis* transcription factors: unprecedented sensitivity reveals novel root- and shoot-specific genes", *The Plant Journal* 38, 366-379.

P-585

ER stress activates the processing and relocation to the nucleus of ER membrane-bound transcription factors. Jianxiang Liu¹, Renu Srivastava¹, Ping Che¹, Stephen Howell¹. ¹ Plant Sciences Institute, Iowa State University, Ames IA 50011, USA. ER stress in eukaryotic cells results from the disruption of processes leading to the folding or modification of secreted proteins. This form of stress is perceived and acted upon by sensor/transducers located in the ER. We have identified three ER-resident bZIP transcription factors in *Arabidopsis* that are candidates for ER stress sensors/transducers. Those candidates are type II proteins with a cytosol-facing b-ZIP 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, is activated by salt stress while another, AtbZIP28, is activated in the typical manner of an unfolded protein response (UPR) triggered by stress agents, such as tunicamycin or DTT. Following different ER stresses, the transcription factors are processed, releasing their N-terminal b-ZIP domains, which are then translocated to the nucleus. Both responses require the function of a subtilisin-like serine protease (subtilase), AtS1P. AtS1P is a Golgi-localized protease, and it is thought that following stress, the bZIP sensor/transducers are translocated from the ER to the Golgi where they are processed by AtS1P and other proteases. We have demonstrated processing of epitope-tagged forms of AtbZIP17 and AtbZIP28 in vitro using recombinant AtS1P. In *Arabidopsis*, AtbZIP17 activation directly or indirectly up-regulated the expression of many salt stress response genes, including the homeodomain transcription factor ATHB-7. Expression in transgenic seedlings of a truncated form of AtbZIP28, containing only the N-terminal cytosol-facing domain, up-regulated the expression of several genes associated with UPR, such as BIP3. Thus, two different types of ER stress are sensed by two different sensor/transducers, which activate different sets of genes that act to mitigate the corresponding stress.

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P-586

Proteomic analysis of the response of *Arabidopsis* proteins to polycyclic aromatic hydrocarbons stress. Hong Liu¹, Yuejun Yang², Bo Cui¹, Yuanbei Ye¹, Xuanming Liu², Chentao Lin², Yanhe Huang¹, Zonghua Wang *¹. ¹Fujian Agriculture and Forestry University, Fuzhou, P. R. China, ²Hunan University, Changsha, P. R. China

Polycyclic aromatic hydrocarbons (PAHs) are of serious environmental issues because they cause many health problems in humans including cancer and mutation . Plants are important in removing PAHs from the atmosphere; yet, information on biochemistry and molecular biology of PAH stress responses in plants is lacking. In order to study the difference at the protein expression level, a proteome approach based on two-dimensional gel electrophoresis (2-DE) was used to compare the protein patterns of the phenanthrene-treated plants and control. Thirty of these 53 of protein spots which were differentially expressed in phenanthrene-treated plants were identified by Matrix Assisted Laser Desorption/Ionization-time of Flight Mass Spectrometry-Mass Spectrometry (MALDI-TOF-TOF-MS) and database analysis. Among the differentially expressed 30 proteins, 25 were up-regulated and 5 were down-regulated respectively when treated with 1 mM phenanthrene. According to the function, these proteins can be classified into seven categories: metabolism /cellular component (26.6%), energy(16.6%), cellular communication / signal transduction(3.3%), protein fate(3.3%), defense stress and detoxification(23.3%), miscellaneous function(3.3%), and unclassified protein(23.3%). It indicated that photosynthetic processes are strongly inhibited and oxidative stress signaling is induced.

P-587

DIG5, a novel plastidic protein, regulates auxin polar transport and lateral root development in *Aidopsis thaliana*. Wei Liu^{1,2}, Ruigang Wang¹, Liming Xiong^{1,1} Donald Danforth Plant Science Center, St. Louis, Missouri 63132, USA,² High-Tech Research Center, Shandong Academy of Agricultural Sciences, Jinan 250100, China

Environmental conditions such as nutrients, water, and soil mechanical properties have dramatic impacts on postembryonic root development of higher plants. However, the molecular mechanisms underlying root development are largely unknown. To understand the mechanism involved in root adaptation to water deficit, we initiated a genetic analysis of root response to osmotic stress and abscisic acid (ABA) in *Arabidopsis* (*Plant Physiol* 142: 1065). One of the loci defined in the study, DIG5 (for Drought Inhibition of lateral root Growth 5) was studied in detail. The dig5 mutants have significantly fewer lateral roots than the wild type either with or without ABA treatments. Their leaves are pale green and have irregular venation patterning. Analyses of hormonal responses in the dig5 mutant found the seedlings are able to respond to auxin in promoting lateral root growth but are less sensitive to ethylene in inducing apical hook formation. Assays of auxin transport in the mutant found that both acropetal and basipetal auxin transport are impaired. Map-based cloning and molecular complementation revealed that DIG5 encodes a novel protein that shows no overall sequence homology to other proteins but contains a catalytic domain found in nucleotide deaminases that may participate in RNA editing. Sequence profiling of all major RNA editing sites in the chloroplast and mitochondrial transcriptomes, however, failed to find any changes in RNA editing in the mutant. We also were unable to detect the deaminase activity of the recombinant full-length or truncated DIG5 proteins. Nonetheless, HPLC analysis of the mutant root extracts revealed that dig5 accumulates several-fold flavonoids including kaempferol, naringenin, and dihydroflavone. We propose that the DIG5 enzyme catalyzes a key step in a novel metabolic pathway whose blockage causes its substrates to flow to the flavonoid pathway. As a result, the accumulation of flavonoids impairs auxin polar transport and root development. Supported by USDA-ARS (grant # 2004-02111 to LX).

P-588

Identification and Phenotyping of T-DNA Insertion lines of AtGLRs in *Arabidopsis*. Lai-Hua Liu¹, Pia Walch-Liu², Xiao-Qing Qu¹, Feng-Qiu Cao¹, Wei-Hong Wang¹, Brian Forde², Julia Davies³, Mark Tester⁴. ¹College of Resources and Environmental Sciences, China Agricultural University, 100094 Beijing, China, ²Biological Sciences, Lancaster University, Bailrigg, Lancaster LA1 4YQ, United Kingdom, ³Department of Plant Sciences, University of Cambridge, Downing Street, CB2 3EA, United Kingdom, ⁴Australian Centre for Plant Functional Genomics and The University of Adelaide, Glen Osmond, Adelaide SA 5064, Australia

Eight years ago, 20 genes encoding AtGLRs were identified and were predicted to be glutamate receptor-like ion channels based on sequence similarity to animal iGluRs. However, a clear understanding of the biological or physiological function of any member of the AtGLR family is still lacking.

Using a reverse genetic approach, by PCR-based detection and gene expression analysis, we identified and isolated homozygous T-DNA insertion lines of the 20 AtGLR genes from "Salk" and "GABI" seed sources. To understand potential roles in the plant physiology of these genes, we performed phenotype-screening assays under a variety of growth conditions, including treatments with different nitrogen sources, sugar, amino acids, plant hormones, micro-nutrients, low temperature. Several treatments revealed different growth phenotypes in the mutant lines compared with wildtype (wt) plants. Among all of the 20 Atglr mutants, only Atglr3.2 mutants displayed a reduced growth rate when treated with a combination of ammonium and no sugar in the medium. Aluminium, zinc and cold inhibited root growth to a greater extent in several Atglr lines than in controls. Atglr1.3, Atglr3.2 and Atglr3.4 mutants were more sensitive to NaCl compared to the other lines and wt. No Atglr line showed a distinct growth phenotype with IAA or ABA application compared with the wt. More detailed experimental data will be discussed in the conference. To further confirm and characterize observed plant phenotypes caused by T-DNA insertions in the Atglr mutants, approaches such as histochemical analysis, pharmacological tests, heterologous expression for a survey of the channel function, studies of protein localization and promoter activity as well as functional complementation in plants are being taken.

P-589

Iron Deficiency in Arabidopsis Roots: High-resolution Transcriptional Profiling to Decipher Regulatory Networks. Terri Long¹, Jean Wang¹, Joel Burill¹, Philip Benfey¹. ¹Duke University

Plant roots undergo a host of developmental alterations in response to environmental perturbations. When *Arabidopsis thaliana* is exposed to iron deficient conditions (-Fe), we find that roots and root hairs respond by growing in a wavy growth pattern. Our objectives are to determine the spatio-temporal global transcriptional responses to -Fe, to characterize how these responses may lead to developmental alterations, and explore how root transcriptional regulatory networks control these responses.

As alterations in root development can be seen within the first 24 hours of exposure to -Fe, we performed time-course microarray analysis at 0, 3, 6, 12, and 24 hours exposure to -Fe to determine how the transcriptome responds to -Fe over time. We found that over 600 genes are differentially expressed during this time and that these genes are most responsive at 24 hours -Fe. To determine the spatial responses to -Fe, we have performed microarray analysis of 4 major longitudinal growth stages of the root after 24 hours -Fe, and found that two oldest stages of growth are the most responsive. We have also performed microarray analysis of 6 of the major radial zones of the root at 24 hours -Fe, and found distinct patterns of gene expression within specific cell-types.

Results will be presented on the transcriptomes spatio-temporal responses to -Fe, with emphasis on patterns of gene expression, enriched cis elements and, particularly, transcription factor (TF) responses. Based on our microarray analyses we have selected TFs that respond to -Fe, which may play roles in iron sensing, for further characterization. We will present results on how several TF insertion mutants respond to -Fe, and whether we have uncovered new key regulators of the -Fe response. Future studies include determining how possible regulators of the -Fe response control expression of downstream targets.

P-590

Intracellular Localization of Integrin-like Protein and Its Roles in Osmotic Stress-induced ABA Biosynthesis in Zea Mays

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Plants have evolved many mechanisms to cope with adverse environmental stresses. Abscisic acid (ABA) accumulates significantly in plant cells in response to drought conditions, which has been considered as a major mechanism for plants to enhance drought tolerance. In this study, we explore the possible mechanisms how plant cells perceive the osmotic stress, and as a consequence, induce cells to biosynthesize ABA. Western Blotting and immuno-fluorescence localization experiment, using an anti-human Integrin β 1 polyantibody, showed that there existed a protein in roots of *Zea mays*, which was similar to integrin protein of animals and it mainly localized in insoluble fraction of plant cells. Treatment with GRGDS, a synthetic pentapeptide containing RGD domain, which interacted specifically with integrin protein and thus blocked the cell wall-plasma membrane interaction, significantly inhibited osmotic stress induced ABA biosynthesis in cells, but not in protoplasts, and the RGD analog, which did not contain the RGD domain, had no effects on them. Our results showed that a strong interaction existed between cell wall and plasma membrane and this interaction was largely mediated by integrin-like proteins. A great difference in responses to osmotic stress in induction of ABA biosynthesis was observed between protoplasts and cells, implying that cell wall and/or cell wall-plasma membrane interaction play important roles in perceiving osmotic stress. Accordingly, we concluded that cell wall and/or cell wall-plasma membrane interaction mediated by integrin-like protein played important roles in osmotic stress- induced ABA biosynthesis in *Zea mays*.

P-591

Proteome analysis of the cold stress response in *Arabidopsis thaliana*. Andrea Matros¹, Hans-Peter Mock¹. ¹Institute of Plant Genetics and Crop Plant Research

Environmental influences such as high light, drought, high or low temperature and salinity affect growth and yield of crop plants by leading to altered gene and protein expression, metabolic changes, and growth retardation [1]. How such environmental stimuli are perceived and trigger the complex defensive and adaptive signalling networks, and how these events result in resistance/tolerance is of major practical interest. A complementary proteome study was started to analyse the response of *Arabidopsis* plants exposed to cold stress (6 °C). We first carried out a comparison of protein patterns by using DIGE technology [2]. Proteins displaying significant changes in abundance included RNA-binding protein CP29, a glycine-rich protein, carbohydrate metabolism-associated proteins, dehydrins and low-temperature-induced protein 78. Taking advantage of recent developments in proteome analysis samples from these studies were also analysed by a label free quantification method using LC-based separation combined with ESI-Q-TOF MS. This novel approach confirmed earlier results obtained by using DIGE technology but also lead to the identification of a number of additional proteins with changed expression pattern after cold stress exposure. In addition we have started to investigate fractions of phosphorylated proteins from these experiments in order to evaluate regulatory circuits of cold stress responses in *Arabidopsis*. Many of the proteins identified have been described previously in the context of cold stress responses [3,4], indicating the validity of our complementary approach for further in depth studies.

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P-592

Characterization of three *Arabidopsis* AP2/EREBP family transcription factors that were involved in ABA sensitivity, freeze and salt tolerance. Wenqian Mei¹, Pei Han¹, Xiang Jin¹, Huimin Tan¹, Yuxian Zhu¹. ¹National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Science, Peking University, Beijing, China

AP2/EREBP transcription factors (TFs) play very important roles in plant development, hormonal regulation and stress response. Upon genome-wide cDNA cloning, phylogenetic and expression pattern analyses of this plant specific TF family, we found that three of the members including At1g71450, At1g50680 and At5g13910 were likely involved in ABA, cold and salt responses. Complementary DNAs containing putative full length ORFs of these three TFs were obtained and fused individually to the GAL4 DNA-binding domains. All three genes functioned effectively as trans-activators using yeast one-hybrid assay. RT-PCR experiments showed that At1g71450 gene was induced by ABA and low temperature, At1g50680 gene was responsive to quite a few stress conditions especially to freezing temperature and At5g13910 gene was induced by high salt treatment, drought and ethylene. By searching the ABRC T-DNA insertion mutant stocks, we obtained knockout lines for these TFs. Homozygous ko1 (At1g71450) plants showed hypersensitive response to ABA during seed germination and also in stomata movement. Homozygous ko2 (At1g50680) plants showed a significant reduction in plant freezing tolerance compared to the wild type after chilling treatment. Homozygous ko3 (At5g13910) were less tolerant to high salinity than wild type plants. Our data suggest At1g71450 is a negative regulator in ABA signaling while At1g50680 and At5g13910 are positive regulators in cold and salt stress responses

P-593

A Dunaliella viridis trehalose-6-phosphate synthase increases abiotic stress tolerance in Arabidopsis. Xiangzong Meng¹, Sai-fan Luo², Nan Zhang², Zhengkai Xu^{1,2}, Rentao Song². ¹Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Science, Shanghai, China. ²School of Life Sciences, Shanghai University, Shanghai, China.

In many organisms, trehalose protects against harsh environmental stresses, such as freezing, drought, heat, and salt, probably by stabilizing protein structures and lipid membranes. The biosynthesis of trehalose consists of two enzymatic steps catalyzed by trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP). In this study, a cDNA (designated as DvTPS1), encoding a TPS homologue, was isolated from the unicellular green alga *Dunaliella viridis*, which is highly tolerant to salt and cold. Protein-sequence comparison revealed that DvTPS1 belongs to class II type of TPS, which has a N-terminal TPS domain and an extended C-terminal TPP domain. RT-PCR showed that DvTPS1 mRNA was constitutively expressed in *D. viridis*. The heterologous expression of DvTPS1 in the yeast mutant *ena1* suppressed Na⁺ hypersensitivity and demonstrated the salt-resistant function of DvTPS1. Transgenic *Arabidopsis* overexpressing DvTPS1 under the control of the CaMV 35 S promoter showed increased tolerance to salt, cold and drought stresses, without growth inhibition or visible phenotypic alterations. This study documents a useful strategy for improving abiotic stress tolerance of plants.

P-594

Unwinding the biological clock with systems biology.

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The 24-hour circadian clock is a regulatory module with complex inputs from the environment. It controls many 24-hour rhythms in plants and other eukaryotes, including organ growth, photosynthetic capacity and the photoperiodic response. The clock mechanisms in all taxa include complex gene circuits with interlocking negative feedback loops. Genome-wide, at least 15% of RNAs are targets for circadian regulation (Edwards et al., *Plant Cell* 2006). Mis-timing of circadian rhythms under light-dark cycles can cause 50% deficit in the growth of *Arabidopsis* in the lab (Dodd et al., *Science* 2005), so correct temporal regulation can significantly enhance plant growth rate.

We use systems biology approaches to understand the clock mechanism. To develop systems-level models, we combine timeseries of molecular data and luciferase (LUC) reporter gene imaging, with analysis of plant clock mutants, statistical parameter estimation and global parameter searches. The resulting differential equation models of the clock and the photoperiod sensor allowed us to predict the properties of unidentified clock components. One gene corresponding to a predicted clock component was identified by experiment, validating its location in the model (Locke et al., *Mol. Syst. Biol.* 2005 and 2006), though additional predictions remain to be validated. The models are clearly incomplete but they facilitate reasoning about the clock network and aid experimental design. The challenges are now mathematical simplification with more biological realism. For realism, we need to incorporate additional molecular components and processes and also to expand the range of rhythmic phenomena that the models can match.

The analysis of clock models is helping to address why all circadian clocks have multi-loop circuits. In this area, we have focussed on 1. flexibility in the evolution of deterministic regulation (Rand et al., *Interface* 2004; *J. Theor. Biol.* 2006), 2. flexibility in phase and waveform in the face of daily or seasonal changes in photoperiod, 3. temperature compensation - the remarkable constancy of clock speed across a range of temperatures. I will discuss new experimental and mathematical results on these aspects of clock function.

P-595

Oxidative damage to respiratory function in plants: understanding the mechanisms behind selective protein damage in mitochondria during environmental oxidative stress. A. Harvey Millar¹, Alison M. Winger¹, Yew-Foon Tan¹, Nicolas L. Taylor¹, Adam Carroll¹, Joshua L. Heazlewood¹. ¹The University of Western Australia, Perth, WA, Australia

Respiration is a fundamental process in plant cell function and is also a site of damage during environmental stress leading to loss of energy generation and restriction of both carbon and nitrogen metabolism. Analysis of the changing role of the plant mitochondrial compartment under oxidative stress is providing new insights into the nature and mechanisms of oxidative stress and damage to plant cellular function. We have been analysing the mitochondrial proteome (1) and have now defined links between lipid peroxidation, protein damage and protease activation using proteomic approaches (2-4). We have coupled this to evidence of metabolic changes (using GC-MS profiling) to identify the particular mitochondrial processes that are most susceptible to damage and degradation during stress induced by oxidative conditions in *Arabidopsis*. We show that a series of cumulative factors: differential susceptibility of proteins to lipid peroxidation modification due to surface reactive amino acid moieties; affinity of specific proteins for transition metals that then catalyse localised ROS formation and lipid peroxidation reactions; and the presence of proteases that selectively cleave oxidatively-modified proteins, play integrated roles in the nature and extent of damage. We are using this to build evidence for elements in retrograde signaling to the nucleus of mitochondrial oxidative stress status. This provides a model system for understanding the mechanism of selective protein damage within a plant organelle and provides tools to consider engineering stress tolerance in respiratory energy generation in plants.

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2. Taylor et al (2005) *Molecular and Cellular Proteomics* 4:1122-1133.
3. Winger et al (2005) *Biochemical Journal* 387:865-870.
4. Millar et al (2005) *Trends in Plant Science* 10:36-43.

P-596

Alterations in the plasma membrane microdomains during cold acclimation in *Arabidopsis thaliana*. Anzu Minami¹, Akari Furuto², Matsuo Uemura^{1,2}. ¹The 21st Century Center of Excellence (COE) Program, Iwate University, Morioka, Japan, ²Cryobiosystem Research Center, Iwate University, Morioka, Japan

Arabidopsis thaliana increases freezing tolerance upon exposure to low, non-freezing temperatures, which is known as cold acclimation. Cold acclimation results in substantial changes in compositions, functions and structure of the plasma membrane. Among these changes, a decrease in the proportion of sphingolipids (i.e., glucocerebrosides) and dynamic changes in protein composition have been widely reported occurring in many cold-acclimated plant species including both herbaceous and woody plants. Recently, it has emerged that the plasma membrane contains microdomains that are enriched in sphingolipids and sterols and have specific protein compositions. The microdomains can be practically isolated as low-density detergent-resistant membrane fraction (DRM) from the plasma membrane. Although DRM is thought to play important roles in plasma-membrane mediated biological processes, DRM functions in environmental stress adaptation are largely unknown. To investigate whether DRM-associated proteins involve in cold-acclimation-induced enhancement of freezing tolerance of plants, we isolated DRM and compared DRM-associated protein profiles before and after cold acclimation in *Arabidopsis thaliana* seedlings. When separated with SDS-PAGE and 2D-SDS-PAGE, DRM-associated proteins showed quantitative changes during cold acclimation, suggesting a possible involvement in cold acclimation process in *Arabidopsis thaliana*. In addition, DRM lipid composition seems to respond to cold treatment, which is different from the responses of the plasma membrane as a whole. We will present a progress report of identification of cold-responsive DRM-associated proteins using mass spectrometry and discuss the molecular aspect of the DRM-associated proteins in plant cold acclimation. (Supported by grants (to M. U. and A. M.) and the 21st Century COE program (to Iwate University) from MEXT, Japan)

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Great promoting effect of high irradiance from germination on flowering in *Arabidopsis thaliana* a process of photo-acclimation
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Arabidopsis thaliana (L.), ch1-1 (chlorophyll b-less mutant), gi-1 (GI deficient mutant), cry2-1 (blue-light-photoreceptor CRY2 deficient mutant) and Columbia (Col) (wild ecotype) were grown under broad range of irradiance from the beginning of germination and the effect of light intensity on the survival, development and flowering was studied. Under low and moderate light intensities (< 300 mol m⁻²s⁻¹), flowering time and plant size at flowering showed great variations among ch1-1, gi-1, cry2-1 and Col, whereas under higher light intensities (> 500 mol m⁻²s⁻¹), these were almost the same. These results suggest that under high light intensities, development and flowering of ch1-1, gi-1, cry2-1 and Col converge to almost the same state. Flowering time was negatively correlated with light intensity and under higher light intensities, acclimation in *A. thaliana* is associated with a decrease in chlorophyll (Chl) content and increases in xanthophyll cycle pool and membrane-bound APX activity (EC 1.11.1.11), suggest that an increase in oxidative stress induces earlier flowering. The plants of gi-1 and cry2-1 survived but Col and ch1-1 died under 1000 mol m⁻²s⁻¹, suggest that mutants deficient in GI or CRY2 are more photo-stress-tolerant than Col and Chl b-less mutant. In this study, we demonstrate that high light intensity exerts on plants of *Arabidopsis* raised from germination till flowering, a promoting effect on development and flowering time involving modulation of the photosynthetic apparatus, and this promoting effect seems to be independent of the functions of flower-inducing GI or CRY2 gene. This can be regarded as photo-acclimation of *A. thaliana* for survival and reproduction under high light conditions.

P-598

Transgenerational memory of stress in plants. Jean Molinier¹, Gerhard Ries², Cyril Zipfel³, Barbara Hohn⁴.¹ Institut de Biologie Moléculaire des Plantes, Strasbourg, France,² BioMedinvestor AG, Basel, Switzerland,³ The Sainsbury Laboratory, John Innes Centre, Norwich, UK,⁴Friedrich Miescher Institute, Basel, Switzerland

Plants are constantly exposed to stresses from the environment. Both biotic and abiotic stresses can result in tolerance to conditions such as unbalanced water supply, light stress, extreme temperatures and pathogens. Influences such as these have also been documented to result in elevated rates of genomic changes such as transposition or homologous recombination. However, it has not been analysed whether the impact of these influences could also be inherited. Experiments using *Arabidopsis thaliana* plants transgenic for markers for genomic changes, however, showed that exposure of these plants to UV-B or to the bacterial elicitor flagellin exhibited changes of homologous recombination far exceeding those of unexposed plants. Quite unexpectedly, however, the progeny of these plants still exhibited elevated recombination rates, although they themselves were not influenced by the agents their parents were exposed to. This change in the recombination frequency must be due to an epigenetic modification of an unknown locus or of unknown loci; it was the whole population that reacted to the challenge. If a mutation would have caused the described effect only a very small number of plants would have shown the changed behavior. The change was not located at the genomic position of the transgene used to monitor recombination, since plants lacking the transgene transmitted their experience of being treated to an untreated crossing partner containing the recombination monitoring transgene. This resulted in increased recombination frequencies in the progeny of these plants. These experiments may point to the importance of epigenetic changes of plant chromatin in permitting evolutionarily important flexibilities useful for adaptation.

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endodermal-amloplast less 1 is a novel allele of SHORT-ROOT.
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endodermal amyloplastless 1 (eal1) is a unique mutant that completely lacks gravitropism in inflorescence stems and exhibits reduced gravitropism in hypocotyls, whereas its roots showed normal gravitropism.

Previously, it was suggested that differentiation or development of amyloplasts in shoot statocytes (endodermal cells) is affected by the eal1 mutation. Here, we have identified EAL1 as a SHORT-ROOT (SHR) allele based on map position. Three nucleotides in the SHR coding region were deleted in the eal1 mutant, resulting in the deletion of just one amino acid. The protein encoded by the novel allele of SHR appears to have retained its function as a transcription factor since the endodermal cell layer was formed both in roots and in shoots of eal1. SCARECROW (SCR) promoter activity monitored by reporter protein expression was significantly decreased in eal1, suggesting that the activity of SHR lacking one amino acid is reduced. In addition, transcription level of SHOOT GRAVITROPISM 5 (SGR5), which is mainly expressed in the endodermis of inflorescence stems, was markedly decreased. Together with the presence of abnormal endodermal amyloplasts in eal1, these results strongly suggest that the endodermis observed in eal1 is not sufficiently differentiated to execute shoot gravitropism.

P-600

The *Arabidopsis DAY NEUTRAL FLOWERING* gene and its role in the control of flowering time. Karl Morris¹, Sarah Thomber¹, Lesley Codrai¹, Stephen Jackson¹.¹ Warwick University, Warwick, UK

Mutation of the *Arabidopsis DAY NEUTRAL FLOWERING* (DNF) gene causes the mutant plants to flower as early in 8h short days (SD) as they would if grown in 16h long days (LD), the dnf mutant is therefore essentially apotropical. The reason for this is because expression of the FT gene is induced in SD as it is in LD. Analysis of co, dnf double mutants indicates that an active CONSTANS (CO) gene is required for early flowering of the dnf mutant, furthermore CO expression has been shown to be altered in the mutant. This places DNF above CO in the photoperiodic pathway. The DNF gene is expressed transiently at a specific time of the day when its role is to repress CO transcription, in the mutant this does not occur resulting in increased CO expression earlier in the day. The effect of the dnf mutation on CO expression is correlated to the observed shift in critical nightlength for flowering in the mutant.

P-601

Phytochromes and cryptochromes regulate the differential growth of *Arabidopsis* hypocotyls in both PGP19-dependent and independent manners. Akitomo Nagashima¹, Genki Suzuki¹, Kensuke Saji², Toshiko Furukawa³, Tomokazu Koshiba³, Masayo Sekimoto⁴, Shozo Fujioka⁴, Takeshi Kuroha¹, Mikiko Kojima¹, Hitoshi Sakakibara¹, Noriko Fujisawa¹, Yukiko Uehara¹, Kiyotaka Okada^{1,2}, Tatsuya Sakai¹.¹RIKEN Plant Science Center,² Department of Botany, Graduate School of Science, Kyoto University,³Department of Biological Sciences, Tokyo Metropolitan University,⁴RIKEN Discovery Research Institute

Photoreceptors, phytochromes and cryptochromes regulate hypocotyl growth under specific conditions by suppressing negative-gravitropism, modulating phototropism and inhibiting elongation. Although those effects seem to be partially caused via the regulation of the phytohormone auxin, the molecular mechanisms underlying this process are still poorly understood. In our present study, we demonstrate that the flabby mutation enhances the phytochrome-inducible hypocotyl bending in *Arabidopsis*. The FLABBY gene encodes the ABC-type auxin transporter, PGP19, and its expression is suppressed by the activation of phytochromes and cryptochromes. These results therefore reveal that phytochromes and cryptochromes have at least two effects upon the tropic responses of the hypocotyls in *Arabidopsis*; one is the enhancement of hypocotyl bending through the suppression of PGP19, and another is a hypocotyl bending-inducing mechanism, which is independent of PGP19. The auxin polar transport assay and the DR5::GUS expression analysis suggest that phytochromes inhibit the basipetal auxin transport and induce the asymmetric distribution of auxin in hypocotyls. Further, activations of phytochromes and cryptochromes decrease auxin content in the aerial portion of the seedlings. These results thus suggest that the control of auxin transport and accumulation by phytochromes and cryptochromes is a critical component of the regulation of hypocotyl growth in response to light.

P-602

Identification of ecotype diversity between different accessions of *Arabidopsis* using CAPS and SSLP molecular markers. RUDRA NAIK¹.¹UAS, DHARWAD, KARNATAKA, INDIA

Arabidopsis is a small flowering plant adapted to various ecological niches of the world. It is a member of the mustard (Brassicaceae) family, which includes cultivated spp like cabbage and radish. This genus is of great interest since it contains Thale Cress (*Arabidopsis thaliana*), one of the model organisms used for studying plant biology and the first plant to have its entire genome sequenced. *Arabidopsis* is not of major economic significance, but it offers important advantages for basic research in genetics and molecular biology since the changes in the plant are easily observed, making it a very useful model. Molecular markers are available that are polymorphic for *Arabidopsis*.

DNA was isolated from four accessions of *Arabidopsis* collected from the Netherlands (Wageningen, Landsberg, Rhenen, and Oosterberg) and used for CAPS and SSLP mapping. Wageningen accessions were found to be different by the SSLP marker while the others were identical. CAPS revealed the presence of one Hind III restriction site in Landsberg, but absent in all the other accessions studied. In this experiment, the resolving power of genetic markers is determined by the level of polymorphism detected, which is primarily affected by the mutation rate at the genomic sites involved. The 30bp difference detected between Wageningen accessions could be due to DNA polymerase slippage during DNA replication creating some mismatches by using the primers.

There is a genetic variation in the selected *Arabidopsis* accessions as revealed by CAPS and SSLP markers. The Wageningen accessions though collected from the same ecological niche are different accessions, which may be due to ecotype selection over some alleles as revealed by the SSLP. All the accessions have no Hind III except Landsberg SSLP and CAPS are essential marker tools in studying the genetic diversity in *Arabidopsis*.

P-603

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P-605

The expression of senescence-enhanced genes in response to oxidative stress. SAEID NAVABPOUR¹, VICKY BUCHANAN-WOLLASTON². ¹Dpt. of Agronomy & Plant Breeding, University of Agricultural Sciences and Natural Resources, Gorgan, IRAN, ²Horticultural Research International, Wellesbourne, UK

Senescence is a genetically regulated oxidative process that involves a degradation of cellular structures and the mobilization of the products of degradation to other parts of the plant. Environmental stress is a major limiting factor in plant productivity. Much of the injury to the plant caused by stress exposure is associated with oxidative damage at the cellular level. The role of Reactive Oxygen Species (ROS) during abiotic stress has become a subject of considerable interest given that ROS have been implicated in processes leading to plant stress acclimation. High concentrations of ROS lead to phytotoxicity, whereas relatively low levels can be used for acclimatory signalling. Therefore, ROS are not simply toxic by-products of metabolism but also can function as signalling molecules. Thus, the controlled modulation of ROS levels in plants is extremely important. The metallothionein gene, "LSC54", is expressed at high levels during leaf senescence in "*Brassica napus*" and "*Arabidopsis thaliana*". The promoter region of this gene has been cloned, fused to GUS gene and transformed into "*Arabidopsis*". The aim of this project was to analyse the regulation of gene expression in stress induced and developmental senescence. For this purpose transgenic "*Arabidopsis*" plants containing the "LSC54" promoter: GUS fusion were treated with numerous chemicals known to generate ROS. Silver nitrate was the most consistent treatment, resulting in strongly elevated levels of "LSC54" expression. Treatment with 3-Amino-Triazole (catalase inhibitor) also gave measurable and consistent increases in GUS expression. The role of silver nitrate (AgNO_3) in "LSC54" expression was investigated by treatment with several Reactive Oxygen Species (ROS) scavengers combined with silver nitrate treatment. It was found that "LSC54" expression is induced by a combination of ROS. Northern hybridisation experiments showed that many senescence-enhanced genes are induced by AgNO_3 and 3-AT treatment, in many cases the levels of expression are comparable to those seen during senescence.

P-606

Constitutive over-expression of the MYB-related transcription factor PHR1 results in accumulation of high levels of phosphate in shoots of Arabidopsis. Tom Hamborg Nielsen¹, Lena Nilsson¹, Renate M Iler². ¹Dpt. Plant Biology, University of Copenhagen, Copenhagen, Denmark, ²Dpt. Agricultural Sciences, University of Copenhagen, Copenhagen, Denmark

Plants have evolved a number of adaptive strategies to cope with fluctuations in phosphate supply. The current knowledge of the transcriptional regulation of the phosphate starvation response in plants is limited. However, the MYB-related transcription factor PHR1 (At4g28610) is known to be involved in the P-starvation response (Rubio et al., 2001, Gene Dev 15: 2122-2133). In this study we characterise a T-tagged knock-out phr1-mutant, and a series of transgenic plant lines which over-express PHR1 in wild type and phr1 mutant background. The knock-out mutant has an altered phosphate allocation between root and shoot, accumulates less sugar and starch than P-starved wildtype and has a lower AGPase activity. The mutant is impaired in induction of a subset of phosphate-starvation induced genes including a transporter gene (PHT1-7), a ribonuclease gene (RNS1) and several genes involved in anthocyanin biosynthesis. Over-expression of PHR1 in the phr1 mutant rescues the responsiveness to P-starvation and leads to wild type levels of sugars and starch during phosphate starvation. This confirms the involvement of PHR1 in adjusting carbon metabolism. Furthermore, over-expression of PHR1 in both wild type and phr1 mutant leads to strongly increased phosphate content irrespective of P-regime.

This study emphasises the importance of PHR1 in regulation of phosphate starvation responses, and shows that targeting a key regulatory element in the phosphate starvation regulatory network may represent a useful approach for molecular breeding of plants toward more efficient phosphate uptake and assimilation.

P-607

Screening of rice genes for salinity or high-temperature tolerance using *Arabidopsis* expression library of rice full-length cDNAs
Kenji Oda¹, Naoki Yokotani¹, Naka Fujimoto¹, Tsutomu Saito¹, Takanari Ichikawa², Youichi Kondo², Minami Matsui², Hirohiko Hirochika³, Masaki Iwabuchi¹. ¹Research Institute for Biological Sciences, Okayama, Japan, ²RIKEN Plant Science Center, Yokohama, Japan, ³National Institute of Agrobiological Sciences, Tsukuba, Japan

To identify useful genes of rice, we started large-scale functional analysis of rice genes using a gene screening procedure named full-length cDNA over-expressor gene (FOX) hunting system. About 13,000 full-length cDNAs of rice were introduced into *Arabidopsis* by Agrobacterium-mediated floral dip transformation method. More than 20,000 of transgenic *Arabidopsis* were generated and seeds of each line were harvested separately. Using this expression library, we tried to identify rice genes related to salinity or high-temperature tolerance. To isolate salinity tolerant lines, T2 seeds were sowed on agar medium containing 1/2 x MS salt and 150 mM of sodium chloride. Germination and growth were assessed visually for two weeks. About 200 tolerant lines were selected and 52 lines showed better germination and growth even on agar medium with 180 mM of sodium chloride. For isolation of thermotolerant lines, 4 day-old T2 plants were treated transiently at 42 °C. Following 10 days after heat treatment, survivability was checked. Three lines were selected as thermotolerant lines. Rice cDNAs introduced in about 80 % of selected lines were amplified by genomic PCR. To confirm direct relation between these cDNAs and the phenotype, re-introduction of these cDNAs into *Arabidopsis* is in progress. This work is supported by Special Coordination Funds for Promoting Science and Technology in Japan.

P-608

Characterization of Physiological Function of *Arabidopsis* Methionine Sulfoxide Reductase 4 and Identification of its Signal Transduction Pathway. Jee Eun Oh¹, Eun-Ji Koh¹, YeRim Kwon¹, Ki Deok Kim¹, Suk-Whan Hong², Hojoong Lee¹. ¹Korea University, Seoul, Republic of Korea, ²Chonnam National University, Gwangju, Republic of Korea

Owing to its cellular respiration and metabolic processes in aerobic environments, living organism is susceptible to abundant oxidative stresses, which result in accumulation of damaged proteins, cellular senescence and fundamentally even more catastrophic event, death. To protect themselves against both biotic and abiotic stresses, organisms have developed extensive repair mechanisms for improperly modified proteins. Methionine sulfoxide reductase (MSR) is an enzyme to restore oxidized free and/or bound methionine residues. In *Arabidopsis*, two types of methionine sulfoxide reductase, Msra and Msrb, and their function in control of oxidized methionine residues are now characterized. In our former study, we depicted molecular regulation of MSR4 gene induction in abiotic stress conditions. Thus, we generated overexpressors and mutants including point mutation and antisense to understand the physiological role of MSR4. Interestingly we observed formation of large siliques in the overexpressor lines under stress conditions. Moreover, among the transgenic plants, we were able to distinguish lines that are specifically responsive to high salt condition. These were found to have amino acid alterations on the conserved region of MSRs from various organisms. Thus, we suppose this observation may be the result of dominant negative mutation. We also scrutinized the relationship between the control of MSR4 and the proteasome-mediated degradation because, in order to maintain "protein homeostasis", removal of oxidized proteins and rapid turnover of repairing enzymes must be carefully regulated at all times. Fine adjustment of gene expression will be a better, feasible and reliable way to improve stress tolerance of plants and thus to approach to the application of GM crops and phyto-products.

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P-609

Continuous mechanical impedance enhances ethylene response rather than ethylene production in *Arabidopsis* roots. Takashi Okamoto¹, Abidur Rahman², Yutaka Ono³, Seiji Tsurumi¹. ¹Center for Supports to Research and Education Activities Isotope Division, Kobe University, ²Cryobiosystem Research Center, Faculty of Agriculture, Iwate University, ³Department of ion-beam-applied biology, Japan Atomic Energy Research Institute

We investigated the role of hormones 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 show a characteristic ethylene phenotype; 0.5-fold reduction in root growth, 1.2-fold increase in root diameter, 0.5-fold 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 chemical inhibitor studies, gene expression analyses and the ethylene production assay, altogether suggested that enhanced ethylene response plays a primary role in changing the root morphology and development during mechanical impedance. We also provided evidence that ethylene signaling rather than ethylene synthesis plays a pivotal role in enhancing the ethylene response. Taken together, our results provide a mechanistic explanation of the role of ethylene in changing the root morphology during mechanical impedance.

P-611

Mapping of QTL for *Pieris* resistance and for defense-related traits in *Arabidopsis* Da(1)-12 × Ei-2. Marina Pfalz¹, Heiko Vogel¹, Juergen Kroymann¹. ¹Max Planck Institute for Chemical Ecology

Natural genetic variation among *Arabidopsis* accessions has been successfully exploited to map and clone QTL (quantitative trait loci) for many quantitative traits, including plant resistance against generalist insect herbivores such as *Spodoptera exigua* or *Trichoplusia ni*. Typically, plant damage caused by generalist insect herbivores is negatively correlated with increasing glucosinolate concentration or with increasing myrosinase activity, and resistance QTL co-localize with glucosinolate or myrosinase QTL. These results demonstrate that the glucosinolate-myrosinase system plays a major role in the defense of cruciferous plants against generalist insect herbivores.

Less clear, however, is whether and how cruciferous plants defend themselves against insects that have specialized on crucifers, such as *Plutella xylostella* and *Pieris brassicae*. These insects possess counteradaptations that help avoiding deleterious effects arising from toxic glucosinolate hydrolysis products. Nonetheless, highly replicated insect feeding trials on 16 *Arabidopsis* accessions revealed substantial genetic variation within *Arabidopsis* for resistance against specialist herbivores. We are now using a recombinant inbred line (RIL) population developed from a cross between the *Arabidopsis* accessions Da(1)-12 and Ei-2 to map QTL controlling resistance against crucifer specialists, and to investigate a potential impact of the glucosinolate-myrosinase system on *Pieris brassicae* herbivory.

P-612

A functional genomics approach reveals TCP21 as a new component of the *Arabidopsis* clock transcriptional network. Jose Pruneda-Paz¹, Ghislain Breton¹, Steve Kay¹. ¹The Scripps Research Institute, La Jolla, (CA), USA

Two MYB transcription factors (CCA1/LHY) are considered key components in the current model of *Arabidopsis* circadian network. Although it has been well documented that CCA1/LHY regulate the expression of several core clock genes, the molecular mechanisms governing CCA1/LHY transcription remains poorly understood. In an attempt to identify new members of the *Arabidopsis* clock transcriptional network, we have generated a library of circadian-regulated transcription factors. The library was screened against different core clock gene promoters in a yeast one-hybrid system. Here we present the identification of TCP21, a class I TCP transcription factor, as a new regulator of CCA1 expression. TCP21 binds to a TCP class I binding site present in CCA1 promoter and represses the expression of a CCA1 promoter::LUC + reporter in transient assays. TCP21 mRNA expression peaks four hours before the trough in CCA1 expression, which is consistent with TCP21 functioning as a repressor of CCA1 promoter activity. In addition, low amplitude rhythmic CCA1 mRNA is observed in TCP21 over-expressing transgenic lines. Although CCA1 and LHY are co-expressed, we were not able to identify any TCP21 binding activity to the LHY promoter. This finding suggests that CCA1 and LHY might not be regulated by the same mechanism. A similarity of clock architecture among different organisms is that their main components are part of feedback loops. Interestingly, a CCA1 binding site (CBS) is present in TCP21 promoter region. Specific binding of CCA1 to this element was observed in gel shift assays and elevated TCP21 mRNA levels were found "in planta" in a CCA1 over-expression background. In conclusion, using a novel alternative approach we have identified TCP21 as a transcription factor directly regulating CCA1 promoter activity. We propose a transcriptional feedback loop, where CCA1 is inducing the expression of TCP21 that constitutes the negative arm of the loop.

P-610

Cytochrome P450s as reporters for circadian-regulated pathways Yinghong Pan¹, Matthew E. Hudson¹, Todd Michael², Joanne Chory², Mary A. Schuler¹. ¹University of Illinois, Urbana, Illinois, USA, ²The Salk Institute, La Jolla, California, USA

Cytochrome P450s play important roles in the synthesis of diverse secondary compounds in '*Arabidopsis thaliana*'. Comparing four datasets that included seedlings harvested over a 2-day period of constant conditions after growth with varying photo and thermocycles, a total of 98 P450 loci were circadian-regulated for at least one of the four different conditions. Here, we further describe the different circadian-regulated pathways using, as reporters, individual P450 loci that are likely to be rate-limiting in secondary metabolic pathways. Using RT-PCR analysis, we have confirmed circadian regulation for P450s involved in phenylpropanoid, carotenoid, oxy-lipin, glucosinolate and brassinosteroid biosynthesis. Using bioinformatics approaches, we have identified conserved motifs in the promoters of different circadian-regulated pathways by defining their over-representation compared to all promoters in the '*Arabidopsis*' genome. Analysis of these promoters will provide us with the opportunity to functionally characterize novel promoter motifs that might be important in circadian regulation of cytochrome P450s.

P-613

GIGANTEA and regulation of flowering by day length in *Arabidopsis* and ryegrass. Jo Putterill¹, Karine David¹, Moyra Black¹, Vic Aracus¹, Milan Gagic², Igor Kardailsky². ¹University of Auckland, Auckland, New Zealand, ²AgResearch, Palmerston North, New Zealand

Major seasonal cues such as changing day length and winter cold help to synchronise the reproduction of many plants to favourable seasons of the year and enable outcrossing. Summer annual ecotypes of *Arabidopsis* germinate and flower rapidly in spring, while winter annuals grow vegetatively over the winter and need an extended period of cold (vernalisation) in order to be able to respond to the long day signal in spring. Molecular-genetic analysis has revealed the photoperiod flowering time pathway which regulates flowering in response to day length in *Arabidopsis*. Components of this pathway that promote flowering such as GI and CO act to upregulate the floral integrator gene FT. These genes are conserved in other evolutionarily-diverse plants including rice although their regulation can be different. Relatively little is known about the molecular and biochemical role of the flowering-time protein GI, which has pleiotropic effects and is a very large (127kD), nuclear, plant-specific protein with no domains of known biochemical function. Here we report on our work on tracking *Arabidopsis* GI in plants by immunoblotting which shows that GI protein oscillates through the day/night cycle, accumulating during the day and that it is subjected to degradation by the 26S proteasome at night. We present recent work on analysis of the effect of photoreceptor mutations on GI accumulation. We also report on progress on biophysical characterization of the GI protein including initial Cryo EM pictures and present results on the identification and characterization, of flowering time genes from the important forage crop ryegrass which is induced to flower by vernalisation followed by longer daylengths in spring. Interestingly, over expression of a rye grass FT gene in *Arabidopsis* rescues an ft mutation and leads to an increase in the abundance of the endogenous gene supporting the idea of positive feedback regulation.

P-614

YUCCA genes are required for shade-induced increases in auxin signalling. Melissa Pytlak¹, Kazunari Nozue¹, Youfa Cheng², Michael Covington¹, Stacey Harmer¹, Yunde Zhao², Julian Maloof¹. ¹Section of Plant Biology, University of California, Davis, CA, USA, ²Division of Biological Sciences, University of California, San Diego, CA, USA

Because plants depend on light for energy they have developed the ability to detect adjacent plants and alter their growth to compete for light. Neighbor detection can lead to the shade-avoidance syndrome, a suite of traits that includes increased stem and petiole elongation, early flowering, and changes in resource allocation. Work by others has established that auxin is involved in shade-induced increases in cell elongation, but how shade alters auxin signaling is unclear. One model suggests that shade increases lateral transport of auxin; other data suggests that shade increases auxin biosynthesis. To examine shade/auxin interactions in real-time, we have used the synthetic auxin responsive promoter DR5 to drive expression of firefly luciferase (LUC). We observed a strong increase in DR5:LUC bioluminescence after treating plants with end-of-day far red (EOD-FR) to induce the shade-avoidance syndrome. Auxin transport inhibitors did not prevent the shade-induced increase in DR5 expression, consistent with shade increasing auxin biosynthesis rather than changing transport. To test this idea further we examined extant microarray data and found three putative auxin biosynthesis genes, YUCCA 5, 8, and 9, that are all induced by shade treatment. Analysis of a quintuple mutant that removes function of YUCCA5, 8, and 9, along with two other YUCCA genes in the same clade, shows that these genes are required for shade-induced increases in auxin signaling. In addition, we find that although inhibition of auxin transport does not prevent increased DR5:LUC bioluminescence, it does prevent increases in cell elongation. Together our data suggests that shade causes an increase in auxin biosynthesis by increasing YUCCA expression, and that auxin transport is required to bring the newly synthesized auxin to target tissues.

P-615

SCABP8/CBL10, a Putative Calcium Sensor, Interacts with the Protein Kinase SOS2 to Protect *Arabidopsis* Shoots from Salt Stress. Ruidang Quan¹, Huixin Lin¹, Imelda Mendoza², Yuguo Zhang^{1,3}, WanHong Cao¹, Yongqing Yang¹, Mei Shang¹, Shouyi Chen³, Jos M. Pardo², Yan Guo¹. ¹National Institute of Biological Sciences, Beijing, 7 Science Park Road, Zhongguancun Life Science Park, Beijing 102206, P. R. China, ²Instituto de Recursos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, Sevilla 41012, Spain, ³Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Chaoyang District, Beijing 100101, China

The SOS (Salt Overly Sensitive) pathway plays an essential role in conferring salt tolerance in *Arabidopsis*. Under salt stress, the calcium sensor SOS3 activates SOS2, a protein kinase, which in turn positively regulates the activity of a plasma membrane sodium/proton antiporter, SOS1. Here we show that SOS3 acts primarily in roots under salt stress. By contrast, the SOS3 homologue, SCABP8/CBL10 (SOS3 like Calcium Binding Protein 8, and Calcineurin B-Like 10), functions mainly in the shoot response to salt toxicity. While root growth is reduced in the sos3 mutants in the presence of NaCl, the salt sensitivity of scabp8 is more prominent in the shoot tissues. SCABP8 is further shown to bind calcium, interact with SOS2 both *in vitro* and *in vivo*, recruit SOS2 to the plasma membrane, enhance SOS2 activity in a calcium dependent manner, and activate SOS1 in yeast. In addition, the sos3 scabp8 and sos2 scabp8 double mutants display a phenotype similar to sos2, which is more sensitive to salt than either the sos3 or scabp8 single mutant. Over-expression of SCABP8 in the sos3 mutant partially rescues the sos3 salt sensitive phenotype. However, over-expression of SOS3 in the scabp8 mutant fails to complement scabp8. These results suggest that SCABP8 and SOS3 are only partially redundant in their function and each play additional and unique roles in the plant salt stress response.

P-616

STO (Salt Tolerance protein) participates in the light signalling pathway in plants. Marta Rodriguez-Franco¹, Martin Indor¹, Katrin Marquardt¹, Felipe Samiento¹, Hui Li Yan¹, Eija Schulze¹, Gunther Neuhaus¹. ¹Institute of Cell Biology, University of Freiburg, Schaenzlestrasse 1, 79104 Freiburg, Germany

The Salt Tolerance protein (STO), and its homologue STH, of *Arabidopsis* were identified as proteins conferring salt tolerance to yeast cells. In order to uncover their function, we isolated STO and STH T-DNA insertion lines and generated RNAi and overexpressor *Arabidopsis* plants. Here we present data of these lines indicating that STO acts as a negative regulator in phytochrome and blue light signalling. Transcription analysis of STO uncovered a light and circadian dependent regulation of gene expression and analysis of light regulated genes revealed that STO is involved in the regulation of CHS expression during de-etiolation. In addition, we could show that CONSTITUTIVE PHOTOMORPHOREGULATION 1 (COP1) represses the transcription of STO and contributes to the destabilization of the protein in etiolated seedlings. Microscopic analysis revealed that the STO:eGFP and STH:eGFP fusion protein are located in the nucleus, and STO:eGFP accumulates in a light-dependent manner. Colocalisation experiments of these two proteins with COP1 in transient transformation assays will be presented and the role in the light signalling pathway will be discussed.

P-617

RCI1A negatively regulates constitutive freezing tolerance and cold acclimation in *Arabidopsis*. Julio Salinas^{1,2}, Rosa L pez-Cobollo¹. ¹Departamento de Biotecnología, INIA, Madrid, Spain, ²Departamento de Biología de Plantas, CIB-CSIC, Madrid, Spain

Low temperatures constitute one of the most influential environmental factors on plant development and survival. In the case of cultivated species, they also limit their growing season and yield, originating important economical losses. Plants exhibit a large diversity of responses to low temperatures, and in some cases have evolved adaptive mechanisms allowing them to survive to low (0-10 °C) and freezing (< 0 °C) temperatures. The central element in the adaptation to freezing temperatures is the process of cold acclimation, an adaptive response by means of which many plants from temperate regions are able to increase their freezing tolerance after being exposed some days to low-nonfreezing temperatures. Understanding the molecular mechanisms that control cold acclimation is important from both basic and practical points of view. RCI1A is an *Arabidopsis* gene whose expression is induced in response to low temperature and encodes a 14-3-3 protein. We have identified a mutant, rci1a-1, which does not show cold induction of RCI1A. The characterization of rci1a-1 revealed that it has increased capacity to tolerate freezing before and after cold acclimation, indicating that RCI1A negatively regulates constitutive freezing tolerance and cold acclimation response. In order to understand these phenotypes at molecular level we are analyzing the transcriptome of the rci1a-1 mutant under both control and cold conditions. On the basis of these results, the role of RCI1A in freezing tolerance and cold acclimation will be discussed.

P-618

MLO function(s) in plants: Towards the genetic control of the "root curling" phenotype associated with Atmlo4 and Atmlo11 mutants. Noir Sandra¹, Alan M. Jones², Zhongying Chen², Ralph Panstruga¹. ¹Max Planck Institute for Plant Breeding Research -Dept. Plant Microbe Interactions- Carl-von-Linn Weg 10, D-50829 Cologne - Germany, ²The University of North Carolina -Dept. of Biology- CB# 3280 Coker Hall, Chapel Hill, North Carolina 27599-3280 - USA

MLO proteins constitute a plant-specific family of seven-transmembrane domain proteins comprising approximately 10-15 members per plant species. Barley Mildew-resistant Locus O (MLO) protein is the "founder" of this protein family, and based on amino acid similarity, 15 MLO homologs have been identified in *Arabidopsis thaliana*. To date, barley MLO and its functional *Arabidopsis* 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 indicate putative functional diversification within the protein family. To elucidate function(s) of MLO proteins in plants, we characterized Atmlo4 and Atmlo11 mutant root growth behaviour since roots are the predominant organ in which these genes are expressed. We observed that Atmlo4 and Atmlo11 mutants exhibit a dramatic circling root growth pattern under *in vitro* culture conditions. 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. As a first step, we identified *Arabidopsis* mutants that suppress the "root curling" phenotype using a chemical re-mutagenesis approach. We identified and characterized nine suppressor mutants. A map-based cloning of selected suppressing genes is on-going and an update on our progress will be provided. In addition, we took a directed chemical-genetic approach. By screening chemical libraries, we found that compounds described as auxin transport, vesicle trafficking-inhibitors, and flavonoids reverse the "root curling" phenotype of Atmlo mutants.

P-619

REGULATORY GENE NETWORK IN DROUGHT AND ABA RESPONSES. Kazuo Shinozaki¹, Kazuko Yamaguchi-Shinozaki², Motoaki Seki¹. ¹RIKEN Plant Science Center, Yokohama, Japan, ²The University of Tokyo, Tokyo & Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

Drought stress induces a variety of genes of which products function in drought stress tolerance and response in plants. Many stress-inducible genes have been used to improve stress tolerance by gene transfer. In this meeting, we present our recent studies on regulatory networks in drought and ABA responses. We have identified complex regulatory systems in stress-responsive gene expression: ABA-dependent and ABA-independent systems. In one of the ABA-independent pathways, a cis-acting element (DRE/CRT) and its binding proteins, DREB2s, are important cis- and trans-acting elements in drought-responsive gene expression, respectively. DREB2 is also involved in heat stress response. In the ABA-dependent pathways, bZIP transcription factors (AREB/ABF) are involved in the major process. Protein phosphorylation is important for the activation of AREB proteins. The MYB/MYC and NAC transcription factors are involved in ABA-responsive gene expression and jasmonic acid responses.

In the ABA-dependent pathway, stress-inducible NCED3 is mainly involved in the ABA biosynthesis during drought stress. We analyzed metabolic profiles regulated by ABA using T-DNA tagged mutant and with GC-MS and LC-MS. We discuss the function of CYP707A3 in the regulation of ABA metabolism during stress responses. We also report the functions of SnRK2 protein kinases in drought and ABA responses using mutants and transgenic overexpressors. In addition, recent progress in transcriptome analysis for novel transcripts including noncoding RNAs, miRNAs, and sense-antisense RNAs will be presented based on tiling array analysis and 454 sequencing of small RNAs.

Umezawa et al. *Curr Opin Biotech* 17: 113-122 (2006)

Yamaguchi-Shinozaki and Shinozaki. *Ann Rev Plant Bio* 57: 781-803 (2006)

P-620

N-terminal 21 amino acid region of soybean (*Glycine max*) SLTI25 encoding Ribosomal Protein Genes S6 is necessary but not sufficient for chloroplast targeting. Hyun-A So¹, Jee-Sook Gang¹, Eunsook Chung¹, Kyoungmi Kim¹, Chang-Woo Cho¹, Jee-Eun Heo¹, Bo Hyun Yun¹, Yao Ran¹, Jai-Heon Lee¹. ¹Dong-A University, Busan, Republic of Korea

The suppression subtractive hybridization (SSH) was used to isolate low temperature stress inducible genes from soybean. Functional Analysis of stress inducible genes SLTI25 showed high homology with genes encoding Ribosomal protein S13. The full-length with intron of SLTI25 is 1,858 bp containing 5 exon and 6 intron. The full-length cDNA of SLTI25 is 742bp contained an open reading frame (ORF) consisted 151 amino acid (aa). The derived amino acid sequence showed the highest identity of 95% with ribosomal protein S13 from *P. ginseng* (BAA96366). SLTI25 gene showed one copy number in the soybean genome using Southern blot analysis. The expression of SLTI25 gene during low temperature stress, salt, wounding, ABA, and drought showed various induction. Wounding is early induction and LT, salt, ABA are late induction. Cellular localization of SLTI25 (full amino acids) is chloroplast, 21 amino acids at N-terminal is cytosol, and the reminder 130 amino acids is cytosol.

P-621

Functional analysis of dominant GAF-domain tyrosine mutants of *Arabidopsis* phytochromes in transgenic plants. Yi-shin Su¹, J. Clark Lagarias^{1,2}. ¹ University of California, Davis, (CA), USA

Light sensing by phytochromes, a family of biliprotein photoreceptors that are widely distributed nature, exploits the reversible photoisomerization of their covalently bound linear tetrapyrrole (bilin) prosthetic groups. Initially undertaken to examine the biological activity of a recently identified class of highly fluorescent, poorly photoactive phytochrome mutants in transgenic plants, the present investigation led to the unexpected discovery of constitutively active phytochrome alleles that possess mutations in a conserved GAF domain tyrosine (YGAF) residue. Most pronounced gain-of-function activities were observed for the Y276H allele of *Arabidopsis* phyB (YHB) whose expression conferred dominant constitutively photomorphogenic (COP) phenotypes as well as constitutive, light-insensitive phyB signaling activities to both dark- and light-grown transgenic plants. YHB-mediated COP development paralleled constitutive nuclear localization of the YHB protein and expression of light-regulated genes in darkness - both of which are consistent with its light-independent activation. Moreover, the COP phenotype was suppressed in bilin-deficient genetic backgrounds, indicating that the YHB allele encodes a bilin-dependent regulator of photomorphogenesis. Phenotypic analysis of transgenic plants expressing YQB, YIB and YRB alleles further revealed that the signaling activity of phyB critically depends on the amino acid at the YGAF position. By comparison with YHB, the COP phenotype conferred by the Y242H allele of phyA (YHA) was less pronounced, and a dominant-negative response to YHA expression was observed in wild-type genetic backgrounds. Taken together, these results implicate participation of this conserved YGAF residue in the transduction of light-driven bilin chromophore isomerization to phytochrome-mediated regulation of plant growth and development. Dominant, constitutively active phytochrome alleles are of potential agronomic significance since their introduction into any transformable crop plant species represents a practical approach to suppress deleterious responses to light quality in field environments.

P-623

Root tip contact with low-phosphate media reprogrammes plant root architecture. Sergio Svistoonoff^{1,2}, Audrey Creff¹, Matthieu Reymond^{1,3}, Cécile Sigoillot-Claude¹, Lilian Ricaud¹, Aline Blanchet¹, Laurent Nussaume¹, Thierry Desnos¹. ¹ CEA, ²IRD, ³Max Planck Institute for Plant Breeding Research

In plants, the roots are able to sense soil nutrients availability. In order to acquire heterogeneously distributed water and minerals, they optimize their root architecture. One poorly understood plant response to soil phosphate (Pi)-deficiency is a reduction in primary root growth with an increase in the number and length of lateral roots. Here we show that physical contact of the *Arabidopsis thaliana* primary root tip with low-Pi medium is necessary and sufficient to arrest root growth. We further show that loss-of-function mutations in LPR1 and its close parologue LPR2 strongly reduce this inhibition. LPR1 was previously mapped as a major quantitative trait locus (QTL); the molecular origin of this QTL is explained by the differential allelic expression of LPR1 in the root cap. These results provide strong evidences for the involvement of the root cap in sensing and/or responding to nutrient deficiency. LPR1 and LPR2 encode multicopper oxidases (MCO), highlighting the essential role of MCO for plant development.

P-622

Role of an auxilin-like J-domain protein, JAC1, on the regulation of actin filaments during chloroplast avoidance response. Noriyuki Suetsugu¹, Noboru Yamada², Akeo Kadota², Masamitsu Wada¹. ¹National Institute for Basic Biology, Okazaki, Japan, ²Tokyo Metropolitan University, Hachioji, Japan

Chloroplasts relocate in plant cells according to the ambient light conditions. Chloroplasts accumulate in weak light to capture light efficiently (accumulation response) and they escape from strong light to avoid photodamage (avoidance response). In *Arabidopsis thaliana*, the accumulation response is mediated by two phototropins (phot1 and phot2) that act redundantly and the avoidance response is mediated by phot2 alone. Dynamic organization of short actin filaments on chloroplasts (cp-actins) by blue light mediates chloroplast photorelocation movement. Using a new screening method, we recently isolated a mutant, jac1 (J-domain protein required for chloroplast accumulation response 1), which lacks the accumulation response but retains the avoidance response (Suetsugu et al., 2005, *Plant Physiol.*). JAC1 gene encodes a C-terminal J-domain protein similar to clathrin uncoating factor auxilin at its C-terminus. In this study, we analyzed in detail chloroplast avoidance movement and cp-actin dynamics during the avoidance in petiole cells of wild type and jac1 mutant.

P-624

Regulation of boron transport in *Arabidopsis thaliana*. Junpei Takano¹, Kyoko Miwa¹, Mayuki Tanaka¹, Toru Fujiwara². ¹University of Tokyo, ²University of Tokyo, SORST, JST

Boron is an essential element in plants. We have been working on identification and characterization of boron transporters/channels. A member of MIP family, NIP5;1, is involved in boron uptake in roots and BOR1, an efflux transporter of boron, is required for efficient boron transport from roots to shoots. Genes homologous to these transporters are differentially expressed and their roles in B transport have been elucidated. Moreover, accumulation of these transporters are regulated in response to B conditions and some members show polar localization patterns. Our studies suggest that these transporters function coordinately to transport boron from soil solutions to sites in plants/cells where boron is necessary. We have also been successful in generating transgenic *Arabidopsis* that are tolerant to boron stress. We will discuss an updated model of overall mechanisms of boron transport and their regulation in *Arabidopsis*.

Takano et al., *Nature* 2002

Takano et al., *PNAS* 2005

Takano et al., *Plant Cell* 2006

Miwa and Takano et al., *Plant Journal* 2006

P-625

Plant tolerance to drought and salinity: modulation of transcription factors. Chiara Tonelli¹, Eleonora Cominelli¹, Domenico Allegra¹, Massimo Galbiati¹. ¹Dip Scienze Biomolecolari e Biotecnologie, University of Milan Italy

Plant growth and productivity are greatly affected by abiotic stresses such as drought, high salinity, and low temperature. In the signal transduction network from perception of stress signals to stress-responsive gene expression, a crucial role is played by transcription factors. Here we report the characterization of two R2R3MYB genes of *Arabidopsis thaliana*. AtMYB60 is specifically expressed in stomata guard cells and its expression is negatively modulated during drought. A null mutation in AtMYB60 results in the constitutive reduction of stomatal opening and in decreased wilting under water stress conditions. Transcript levels of a limited number of genes are altered in the mutant and some of them are known to be involved in plant response to stress. AtMYB60 promoter sequence have been dissected and the minimal region for guard cell expression identified.

AtMYB90 is up-regulated in response to drought, salt and ABA treatments. Transgenic plants overexpressing AtMYB90 showed enhanced salt tolerance compared to wild-type while plants, in which AtMYB90 was silenced through antisense approach, resulted more sensitive to salt treatment. Our data indicate that modulation of At-MYB60 and AtMYB90 opens new possibilities to engineer plant responses to improve survival during drought and salt stress.

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P-627

Comprehensive analysis of ABA-regulated metabolome and transcriptome in drought response of *Arabidopsis*. Kaoru Ura-no¹, Kyonoshin Maruyama², Migiwa Takeda³, Nozomu Sakurai³, Hideyuki Suzuki³, Kazuki Saito^{3,4,5}, Daisuke Shibata³, Kazuko Yamaguchi-Shinozaki^{2,6}. ¹RIKEN PSC, Tsukuba, Japan, ²JIRCAS, Tsukuba, Japan, ³Kazusa DNA Res. Inst., Kisarazu, Japan, ⁴Chiba Univ., Chiba, Japan, ⁵RIKEN PSC, Yokohama, Japan, ⁶The Univ. of Tokyo, Tokyo, Japan

ABA is an important phytohormone involved in drought stress response in higher plants. 9-CIS-EPOXYCAROTENOID DIOXYGENASE (NCED) is a key enzyme for ABA biosynthesis. Drought-inducible *Arabidopsis* NCED3 is a key gene for ABA accumulation under drought stress. To understand ABA-regulated metabolic network in response to drought stress, we analyzed metabolome and transcriptome using *Arabidopsis* wild-type plants and NCED3 knockout mutant (nc3-2) under drought stress. Of 200 metabolites were detected by GC-TOF-MS (gas chromatography time-of-flight mass spectrometry) and CE-MS (capillary electrophoresis mass spectrometry), 64 metabolites were found to increase in *Arabidopsis* wild-type plants in response to drought stress. Of these 64 metabolites, 37 metabolites less increased in nc3-2. Accumulation of amino acids and organic acids depended on ABA accumulation under drought stress. The transcript levels of many drought-inducible genes were also altered in nc3-2 under stress conditions. These genes have the ABA responsive elements (ABREs) in their promoter regions. ABRE dependent gene expression is a major regulation system under drought stress. Integrated analysis of metabolome and transcriptome revealed the ABA dependent transcriptional regulation of branch chain amino acids, saccharopine, proline and polyamine biosynthesis. Our study provides the endogenous ABA functions to control the metabolic network in response to drought stress.

P-628

TOR kinase pathway in plants: Linking stress to growth signals and adaptation of the plant. D.P.S. Verma¹. ¹Ohio State University, Columbus, OH, USA

Plants grow under a variety of environmental conditions facing both abiotic and biotic stresses as well as nutrition limitations. Multiplexing of these diverse signals is essential for a plant to optimize its growth. TOR (Target Of Rapamycin) kinase is recognized as a central regulator for nutrition and stress signals in yeast and animal cells, however our understanding of the role of this kinase in plants is very limited. We have recently cloned all major players of this pathway from *Arabidopsis*, including pTOR, pRapTOR, pGbetaL, pS6K1, pS6K2 and demonstrated that this pathway is operative in plants (Mahfouz et al., 2006). In addition, we have demonstrated a direct link of ribosomal protein S6 (S6p) phosphorylation by pS6K and the possible control of ribosome biogenesis and protein synthesis. This occurs via binding of a histone deacetylase with S6p. The fact that G(L) homolog protein that interacts with TOR is induced by auxin, suggests a possible link of TOR with growth signaling. We observed that the ineffectiveness of rapamycin to inhibit pTOR in plants is apparently due to the inability of plant FKBP12 to interact with rapamycin and thus fail to form a stable complex with pTOR. We have shown that if pFKBP12 is replaced by human FKBP12, a stable complex can be formed with pTOR (Mahfouz et al., 2006). We are developing a stable line of *Arabidopsis* expressing human FKBP12 and would like to use this line to carry out chemical genomics studies followed by the isolation of suppressors of the TOR kinase pathway in plants. This novel approach will allow us to dissect the entire TOR kinase pathway and may facilitate the linking of stress signals to the growth signal pathways, a fundamental problem in plant development and adaptation to a given environment. This study is likely to have a major impact on our understanding of plant growth and development, particularly under stress. This may lead to our understanding of the adaptation and speciation through epigenetic changes that are accelerated under stress conditions.

P-626

Function of de-phosphorylated NPH3 in phototropic response. Tomoko Tsuchida-Mayama¹, Michiharu Nakano¹, Yukiko Uehara¹, Noriko Fujisawa¹, Kiyotaka Okada², Tatsuya Sakai¹. ¹RIKEN Plant Science Center, Yokohama, Japan, ²Kyoto University, Kyoto, Japan

The phototropic response of *Arabidopsis thaliana* seedlings is initiated by the blue-light photoreceptors, phototropin (phot) 1 and phot2, and by their signal transducers, NONPHOTOTROPIC HYPOCOTYL (NPH) 3 and ROOT PHOTOTROPISM (RPT) 2. Although previous studies have indicated that phot1 contains blue-light-activated auto-phosphorylation activity and binds to NPH3 and RPT2, its biochemical function during the induction of the phototropic response remains unclear. In this report, we show that the NPH3 protein is phosphorylated under dark conditions and is de-phosphorylated under blue light, and that phot1 is necessary for blue-light-dependent de-phosphorylation of NPH3 but not phot2 under any fluence rates of blue light that we tested. Since seedlings of phot1 mutant showed that partially phototropism under high fluence rate of blue light, de-phosphorylation of NPH3 is not essential to phototropism at least in phot2 signaling pathway. The phosphorylation sites of NPH3 were predicted using prediction server, NetPhos 2.0 (<http://www.cbs.dtu.dk/services/Net-Phos/>). The functional significance of each of the predicted phosphorylation sites in NPH3 was assessed by site-directed mutagenesis of each specific Ser or Thr to Ala followed by analysis of NPH3 phosphorylation state. Four candidates of NPH3 phosphorylation sites were identified. We are also testing abilities of the construct, altered or deleted phosphorylated region, to rescue the null nph3 (SALK_110039) mutant. We will discuss function of de-phosphorylated NPH3 in phototropic response here.

P-629

Genetic engineering of farnesylation for plant drought tolerance and yield protection. Jiangxin Wan¹, Yang Wang¹, Jifeng Ying¹, Monika Kuzma¹, Michelle Beath¹, Maryse Chalifoux¹, Angela Sample¹, Charlene McArthur¹, Tina Uchacz¹, Carlene Sarvas¹, David Dennis¹, Peter McCourt², Yafan Huang^{1,1} Performance Plants, Inc., ²University of Toronto

Protecting crop yield under drought stress is a major challenge for modern agriculture. One biotechnological target for improving plant drought tolerance is the genetic manipulation of the stress response to the hormone abscisic acid (ABA). Previous genetic studies have implicated the involvement of the b-subunit of the Arabidopsis farnesyltransferase (ERA1) in the regulation of ABA sensing and drought tolerance. Here we show that molecular manipulation of protein farnesylation in Arabidopsis, through down-regulation of either the a- or b-subunit of farnesyltransferase, enhances the plant's response to ABA and drought tolerance. To test the effectiveness of tailoring farnesylation for drought tolerance and yield protection in a crop plant, transgenic Brassica napus carrying down-regulation constructs of either the a- or b-subunit of farnesyltransferase were examined. In comparison with the non-transgenic control, the transgenic canola showed enhanced ABA sensitivity, as well as significant reduction of stomatal conductance and water transpiration under drought stress conditions. The transgenic plants were more resistant to water deficit-induced seed abortion during flowering. Results from multiple years and multiple locations of field trial studies suggest that with adequate water, the transgenic canola plants produced the same amount of seed as the parental control. However, under moderate drought stress conditions at flowering, the seed yields of the transgenic canola were significantly higher than the control. These results represent a successful demonstration of engineered drought tolerance and yield protection in a crop plant under laboratory and field conditions. Using protein farnesyltransferase as an effective target, we have also made significant progresses on engineering drought tolerance in other important crop species.

P-630

Cloning and characterisation of dracula I2-164, an Arabidopsis mutant showing no avoidance of shade. Xuewen Wang¹, Irma Roig-Villanova², Jaime Martinez-Garcia², Paul Devlin¹. ¹Royal Holloway University of London, ²Institut de Biologia Molecular de Barcelona

The shade avoidance response is a response to competition from neighbouring plants. Plants are able to detect light that has been reflected from a neighbouring plant by perceiving the change in light quality. Leaves absorb strongly in the red and blue wavelengths due to absorption by chlorophyll but they reflect far-red wavelengths. Plants are able to monitor the red:far red (R:FR) ratio of incident light and can interpret a reduction in red:far red ratio as evidence of a neighbour growing closely alongside that may overtop it in future. Low R:FR ratio triggers a pronounced elongation growth response; reduction in branching; and reduction in leaf area to prevent future shading. The photoreceptors detecting this change in R:FR ratio are the phytochrome family of R and FR-responsive photoreceptors. We have carried out a high throughput mutant screen using a shade responsive bioluminescent marker and have identified several shade avoidance mutants. One such mutant, dracula I2-164, constitutively shows no avoidance of shade in this screen. Dracula I2-164 is a novel phytochrome A mutant. In addition to showing a long hypocotyl in far red light, this mutant also shows a long hypocotyl in red light. Physiological analysis suggests that this red light phenotype is the result of interference with phyB signalling. The nature of the mutation points to a possible disruption of normal signal transduction leading us to predict that dracula I2-164 could yield important clues in understanding phytochrome signalling.

P-631

The drought tolerant *Arabidopsis "alx8"* mutant has altered leaf morphology as well as changes in stress response signalling pathways under normal growth conditions and during abiotic stress. Pip Wilson¹, Gonzalo Estavillo¹, Katie Fields², Peter Crisp¹, Daniel Harris-Pascal¹, Barry Pogson¹. ¹The ARC Centre for Plant Energy Biology, The Australian National University, Canberra, Australia, ²Department of Plant and Animal Sciences, University of Sheffield, Sheffield, UK

The development of tolerance to abiotic stress requires a combination of physiological, morphological and molecular responses. These responses are regulated by complex signalling pathways, which often coalesce or antagonise each other. In this study, we use the Arabidopsis "alx8" mutant to examine the relationship between the development of stress tolerance and the pathways that regulate it.

The "alx8" mutant has increased drought tolerance, elevated ABA, and constitutive expression of the antioxidant enzyme, APX2 (Rossel "et al.", 2006). The drought tolerance of alx8 seems to be due to a combination of physiological, morphological and molecular changes both under normal growth conditions and stress treatment. For example, alx8 has changes in stomatal function under both normal and high light stress conditions and altered leaf and stomatal morphology. There are also changes in stress response signalling compounds, such as ABA, and microarray studies have revealed significantly altered gene expression. The relationship between these changes and the drought tolerance is currently being investigated.

The "alx8" gene has been identified by positional cloning; the details of which will be discussed at the conference. Interestingly, despite the ALX8 protein being a key player in regulation of multiple stress response pathways, not all of the expected down-stream targets have altered expression. Furthermore, the altered leaf morphology and gene expression indicate a role for this protein under normal growth conditions. Hence, the "alx8" mutant may be very useful in elucidating the regulation of the various components of stress responses and the development of stress tolerance.

Reference: Rossel "et al.", 2006, Plant, Cell and Environment 29 (2): 269-281

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P-632

SDIR1 Is A Novel RING Finger E3 Ligase That Positively Regulates Stress-responsive ABA Signaling in Arabidopsis. Qi Xie¹, Yiyue Zhang¹, Chengwei Yang², Yin Li², Nuoyan Zheng², Hao Chen¹, Qingzhen Zhao¹, Ting Gao¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Science, Beijing 100101, China, ²Zhongshan University, Guangzhou 501275, China

Ubiquitination plays an important role in plant hormone signal transduction. Here we show that the novel RING finger E3 ligase, *Arabidopsis SDIR1* (Salt and Drought Induced RING finger 1), is involved in abscisic acid (ABA)-related stress signal transduction. The expression of SDIR1 was detected in all tissues of *Arabidopsis* by RT-PCR. SDIR1 expression is up-regulated by drought and salt stress, but not by ABA. Transgenic *Arabidopsis* plants carrying the SDIR1 promoter fused to the GUS gene confirmed the results, in particular, strong induction of GUS expression was found in stomatal guard cells and leaf mesophyll cells under drought stress. The GFP-SDIR1 fusion protein is co-localized with intracellular membranes. We confirmed that SDIR1 is an E3 ubiquitin ligase and that the RING finger conservation region is required for its activity. Overexpression of SDIR1 leads to ABA hypersensitivity and ABA-associated phenotypes, such as salt hypersensitivity in germination, enhanced ABA-induced stomata closing, and enhanced drought tolerance. The expression levels of a number of key ABA and stress marker genes are altered both in SDIR1-overexpression and *sdir1-1* mutant plants. Cross complementation experiments showed that the *ABI5*, *ABF3* and *ABF4* genes can rescue the ABA insensitive phenotype of the *sdir1-1* mutant, whereas SDIR1 could not rescue the *abi5-1* mutant. This suggests that SDIR1 acts upstream of those bZIP family genes. Our results indicate that SDIR1 is a positive regulator of ABA signaling.

P-633

Changes in DNA base sequences in the mutant of arabidopsis thaliana induced by low-energy Ar + implantation. Min Xu¹, Bian Po¹, Yuejin Wu¹, Zengliang Yu¹. ¹Key Laboratory of Ion Beam Bio-engineering of Institute of Plasma Physics, Chinese Academy of Sciences, Hefei 230031, China

As ion beams are a type of high linear energy transfer ionizing radiation, they can deposit high energy on a target as opposed to low LET radiations such as electron beams, γ -rays and x-rays. It has been suggested that high LET radiation causes clustered damage on DNA produced several novel mutants, in rice (semi dwarf, early maturity and high yield mutants) with agron ion. They also indicated that substantial DNA alterations might be involved in profucing these mutants. To reveal the mutation effect of low-energy ion implantation on *Arabidopsis thaliana*. Tc243, a stable male-sterility mutant, derived from the seeds irradiated by 30KeV Ar+ with the dose of 1.5 1017 ions/cm², was used for genetic mapping and DNA sequence analysis. The results indicated that repetitive insertion of "TTATA" mutation in + 624bp was happened in the AUXIN RESISTANT 1 gene, encoding one of a subunit of a heterodimeric RUB-activating enzyme of the jasmonic signaling pathway. The possibility reason resulted in the changes in DNA sequences may be double stand break. The mechanisms of low-energy bio-effect on DNA sequences were discussed.

P-635

HSP90 regulates heat shock response that is responsible for heat adaptation. Kenji Yamada¹, Yoichiro Fukao², Makoto Hayashi¹, Mitsue Fukazawa¹, Iku Suzuki¹, Mikio Nishimura¹. ¹National Institute for Basic Biology, Aichi, Japan, ²Nara Institute of Science and Technology, Nara, Japan

Plant survival requires the ability to adapt to heat. When plants are subjected to heat shock (a slightly higher temperature than the germinating temperature), the expression of various genes is induced, and the plants become tolerant to much higher temperatures. The molecular mechanisms responsible for the induction of such heat tolerance remain poorly understood. Here, we found that HSP90 negatively regulates heat-inducible genes and that transiently inhibiting HSP90 in *Arabidopsis* seedlings induces heat-inducible genes and heat adaptation. Moreover, in transgenic *Arabidopsis*, heat shock reduced the activity of exogenously expressed glucocorticoid receptor (GR). As GR activity depends on HSP90, this suggests that heat shock reduces HSP90 activity *in vivo*. Microarray analysis revealed that many of the genes that are upregulated by both heat shock and HSP90 inhibitors are involved in protein folding and degradation, suggesting that the activation of a protein maintenance system is a crucial part of this response. A motif searching revealed that most of these genes have heat shock response element (HSE)-like motifs in their promoters, which suggests that heat shock transcription factor (HSF) is involved in the response to HSP90 inhibition. Several HSF genes are expressed constitutively in *Arabidopsis* in the absence of heat shock, including AtHsfA1d and AtHsfA4c, whose constitutive expression is unchanged by heat shock or HSP90 inhibitor treatment. Recombinant AtHsfA1d protein recognizes the HSE motif, indicating that AtHsfA1d is involved in the heat shock response. Thus, it appears that in the absence of heat shock, HSP90 actively suppresses HSF function. Upon heat shock, HSP90 is transiently inactivated, which leads to HSF activation.

P-634

AKT1 Regulation and Potassium Uptake In Arabidopsis. Jiang Xu¹, Hao-Dong Li¹, Li-Qing Chen¹, Yi Wang¹, Li-Li Liu¹, Liu He¹, Wei-Hua Wu¹. ¹China Agricultural University, Beijing 100094, China

Potassium is an essential mineral element for plant growth and development. Although it is known that plants absorb and transport K⁺ through the membrane transporters, it remains unclear how these transporters are regulated. Here we show that a protein kinase CIPK23, encoded by the LKS1 gene cloned from the low-K⁺ sensitive *Arabidopsis* mutant lks1, significantly regulates K⁺-uptake particularly under low-K⁺ conditions. Lesion of LKS1 significantly reduced K⁺-uptake and caused leaf chlorosis and growth inhibition, whereas overexpression of LKS1 significantly enhanced K⁺-uptake and low-K⁺ tolerance. It was further demonstrated that CIPK23 positively regulated K⁺ transporter AKT1 and two calcineurin B-like proteins CBL1 and CBL9 were upstream positive regulators of CIPK23. The activation of AKT1 by CIPK23 and CBL1 or CBL9 was further confirmed by electrophysiological recordings using *Xenopus* oocyte expression system and also in root cell protoplasts. The AKT1-mediated and CIPK23- and CBL1/CBL9-regulated K⁺-uptake pathway in *Arabidopsis* under low-K⁺ stress will be discussed.

P-636

Molecular architecture of circadian clock system in *Arabidopsis*. Miji Yeom¹, Jeongsik Kim¹, Yumi Kim¹, Hong Gil Nam^{1,2}. ¹Division of Molecular Life Sciences and National Core Research Center for Systems Bio-Dynamics, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea, ²The I-BIO graduate program, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Circadian clocks give organisms the ability to anticipate environmental changes that arise due to the rotation of the Earth on its axis. In *Arabidopsis*, many aspects of physiology, metabolism and development are controlled by circadian clock. Also, a large part of the transcriptome shows circadian regulation. The core components of circadian clock such as TOC1, CCA1, and LHY were suggested to make a transcriptional feedback loop. Recently, it is proposed that molecular architecture of circadian clock is composed of well-coordinated multiple loops. However, previous reports using the mutant plants are insufficient to explain the well-coordinated multiple circuits. Therefore, we would introduce the inducible gene expression system which elevates the specific clock gene mRNA at specific circadian time (CT). We would analyze the response kinetics among the other clock components mRNA expression in time series and find out the closely or weakly regulations in circadian clock system. And then, we would make mathematical model using these data.

Through this approach, we could describe accurate the molecular architecture of circadian clock.

P-637

Cyclic Electron Transport Protects against Moderate Heat Stress in *Arabidopsis thaliana*. Ru Zhang¹, Thomas Sharkey^{1,1}.
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Photosynthesis is among the most heat-sensitive functions in plants. However, the mechanism by which moderate heat stress reduces photosynthetic capacity is unclear. Photosynthesis uses both linear and cyclic electron flows to convert light energy into the transthalakoid proton motive force, composed of the proton gradient and the electric field. Heat stress frequently causes increased cyclic electron transport. Here we used two *Arabidopsis thaliana* mutants, each impaired in one of the two pathways of cyclic electron flow — *crr2* (chlororespiratory reduction, deficient in the chloroplast NAD (P) H dehydrogenase dependant cyclic flow from NADPH to plastoquinone) and *pgr5* (proton gradient regulation, deficient in the antimycin A sensitive cyclic flow from ferredoxin to plastoquinone). Heat stress experiments were done in light with intact leaves by switching leaf temperature from 23°C to 40°C in 2 minutes and electrochromic shift was measured to monitor transthalakoid proton fluxes during photosynthesis. The electrochromic shift results from an electric field effect on carotenoid absorbance bands at 518 nm. Our result indicated that these two cyclic-electron-transport mutants were more sensitive to heat stress and had less ability to recover from heat damage than wildtype, especially *pgr5*. What's more, the electrochromic shift data showed that the transthalakoid electrical gradient was significantly reduced in *pgr5* mutants. We propose that cyclic electron transport protects against moderate heat stress in *Arabidopsis thaliana*.

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Effect of DDB1A Overexpression on DET1 Genetics and Biochemistry. Yu Zhang¹.¹University of Manitoba, Winnipeg, Manitoba, Canada

Yu Zhang and Dana F. SchroederDepartment of Botany, University of Manitoba, Winnipeg, MBR3T 2N2 CanadaAbstractLight is an important regulator of *Arabidopsis* growth. Dark-grown wildtype *Arabidopsis* seedlings exhibit elongated hypocotyls and small closed cotyledons, whereas light-grown seedlings develop photomorphogenically, exhibiting short hypocotyls and expanded green cotyledons. De-Etiolated 1 (DET1) is an important negative regulator of *Arabidopsis* photomorphogenesis. In loss-of-function DET1 mutants, dark-grown seedlings look photomorphogenic: they have short hypocotyls and open cotyledons. Transformation of det1 mutants with epitope-tagged DET1 can partially rescue the det1 mutant phenotype. UV-Damaged DNA Binding protein 1 (DDB1) is a component of the 350 kD DET1 complex in plant cells. DDB1A, one of two DDB1 genes in the *Arabidopsis* genome, has been found to genetically interact with DET1.

To further study the biochemical and genetic interaction between DET1 and DDB1A, we generated *Arabidopsis* lines with overexpressed DDB1A-3HA in wildtype, det1, and Myc-DET1 and GFP-DET1 rescued genetic backgrounds. Overexpression of DDB1A-3HA in wildtype and det1 backgrounds did not result in significant phenotypic changes. However combination of DDB1A-3HA and Myc-DET1 overexpression resulted in decreased rescue of dark-grown hypocotyl and adult height and rosette width phenotypes. This result is consistent with the decreased levels of Myc-DET1 detected in the double overexpressor in both dark-grown seedlings and adults. Interestingly, the GFP-DET1 DDB1A-3HA double overexpressor exhibits increased rescue of dark hypocotyl and light chlorophyll phenotypes relative to GFP-DET1 alone, despite the fact that GFP-DET1 also decreases in this background. Variation in DDB1A-3HA levels and complex formation may account for this difference. Overall, DDB1A overexpression reduces levels of epitope-tagged DET1 and modulates rescue of det1 mutants by the DET1-DDB1 complex.

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Characterization of Lipid-related Novel Stress/Defense Genes - from Model Higher Plant towards Crops. Shilan Zhao¹, Zheng Qing Fu², Da Luo¹, Wei Ma¹, James R. Alfano², Donald F. Becker³, Qi Cheng³.¹School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200030, China,²Plant Science Initiative and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68588, USA,³Department of Biochemistry, Redox Biology Center, University of Nebraska, Lincoln, Nebraska 68588, USA

Plant productivity is significantly impacted by metabolic activities and environmental stress. Our research is to characterize 4 long chain fatty omega-alcohol oxidases homologues (AtFAOs) found in model higher plant *Arabidopsis* in concert with 8 *Arabidopsis* long chain omega-hydroxylases. In some industrial yeast, omega-oxidation pathways in lipid metabolism have been characterized but in higher plants the omega-oxidation pathway has been overlooked. We identified long chain fatty alcohol oxidases in higher plant and have initiated the characterization of all 4 AtFAOs in *Arabidopsis*. Some pioneering studies have already shown that the omega-hydroxylase could play important roles in response to pathogenic stress but the detail mechanism is yet to be elucidated. Recent microarray and experimental data increasingly suggest that AtFAO genes may be regulated by several abiotic or biotic stresses, including cold stress and pathogenic attack. We hypothesize that the AtFAOs may participate in such defense network in concert with other defense candidate proteins, resembling omega-oxidation pathways in yeast. Such pathway(s) play extensive roles in higher plants in response to the environmental stress. One of our long term goal is to study the biochemical and biophysical aspects of these novel proteins and relevant metabolic/signaling pathway(s) in *Arabidopsis* and/or oil crops. This will help contribute to better understanding of higher plant lipid metabolism, defense mechanisms and explore more ways of crop engineering.

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The *Arabidopsis abi2-1* Mutant Demonstrates an Enhanced Beta-Aminobutyric Acid-Mediated Acquired Thermotolerance and Root Growth Inhibition. Laurent Zimmerli¹, Bi-Huei Hou², Chia-Hong Tsai¹, Gabor Jakab³, Brigitte Mauch-Mani⁴, Shauna Somerville².¹Department of Life Science, Institute of Plant Biology, National Taiwan University, Taipei, Taiwan.,²Department of Plant Biology, Carnegie Institute, Stanford, CA 94305, U.S.A.,³Department of Plant Physiology, Institute of Biology, University of Pecs, H-7601 Pecs, P. O. Box 266, Hungary.,⁴Department of Science, Laboratory of Molecular and Cellular Biology, University of Neuch tel, 2009 Neuch tel, Switzerland.

The non-protein amino acid beta-aminobutyric acid (BABA) primes *Arabidopsis* plants to respond more quickly and strongly to pathogen and osmotic stress. Here, we report that BABA also significantly enhanced acquired, but not basal, thermotolerance in *Arabidopsis*. An *Arabidopsis* mutant deficient in heat shock protein 101, a protein essential for thermotolerance, did not demonstrate a BABA-enhanced thermotolerance. Microarrays were used to analyze the global gene expression patterns of water- and BABA-treated *Arabidopsis*. BABA-treated plants demonstrated elevated transcript levels for several transcription factors and DNA binding proteins regulating abscisic acid (ABA) and ethylene signalings. To test the role of ABA signaling in BABA mode of action, we evaluated the BABA response of ABA insensitive mutants *abi1-1* and *abi2-1*. BABA-enhanced thermotolerance was partially compromised in *abi1-1*, but was augmented in *abi2-1*. Like ABA, BABA inhibited root growth in wild type and the level of inhibition was roughly additive in roots treated with both compounds. Root growth of both *abi1-1* and *abi2-1* was also inhibited by BABA, with *abi2-1* roots being highly sensitive. Unexpectedly, *abi1-1* and *abi2-1* root growth was inhibited more strongly by combined ABA and BABA treatments than by BABA alone. Our results together with previously published data suggest that BABA is a general enhancer of plant stress resistance. In addition, our data illustrate cross-talk between BABA and ABA signaling cascades. Specifically, the BABA-mediated accumulation of ABA transcription factors without concomitant activation of a downstream ABA response could represent one component of the BABA-primed state in *Arabidopsis*.

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Effects of Phosphorus Signaling on Auxin Transport as Related to Root Development. Zhou Guoquan¹, Wang Jinxiang¹, Yan Xiaolong¹, Liao Hong¹. ¹Root Biology Center, South China Agricultural University, Guangzhou 510642, China

Phosphorus (P) is an essential nutrient element for plant growth, and it is well established that P availability regulates root architecture. Low P availability inhibits primary root growth and promotes lateral root growth of *Arabidopsis*, and induces a more branching root system. The interactions between P availability and auxin biosynthesis as well as auxin signaling were documented. However it remains unclear that the effects of spatial availability of phosphorus on auxin transport as related to root development. In the present study, we elucidated the interactions between the spatial P availability and auxin transport in *Arabidopsis* through stratified P treatment approach. Four treatments, namely LP/LP, LP/HP, HP/LP and HP/HP are employed. We found that primary root growth could be rescued in two stratified P treatments, LP/HP and HP/LP; and the lateral root proliferation in the high P patch was induced in these two stratified P treatments. More auxin accumulation and cell division activities of the lateral root primordia in the high P patch were found as indicated by the expressions of the cell cycle marker gene CycB1::GUS and auxin reporter gene DR5::GUS, but the effects were decreased after the primordia emerged, implying that the effects of spatial P availability on cell division and auxin transport is dependent on root development stage. We conclude that P availability has a close relationship with auxin distribution, and P availability affects root development through altering auxin transport or/and synthesis. We thank Drs Tom J. Guilfoyle (University of Missouri) and Tom Beeckman (University of Gent) for providing seeds of transgenic lines of *Arabidopsis* with DR5::GUS and CycB1::GUS. This work is supported by the National Natural Science Foundation of China (30571111 and 30600380).

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Involvement of *Arabidopsis* HOS15 in histone deacetylation and cold tolerance. Jianhua Zhu¹, Jaecheol Jeong², Yanmei Zhu³, Irina Sokolchik³, Saori Miyazaki⁴, Jian-Kang Zhu⁵, Paul M. Hasegawa³, Hans J. Bohnert⁴, Huazhong Shi⁶, Dae-Jin Yun², Ray A. Bressan³. ¹Purdue University, West Lafayette, IN; University of California, Riverside, CA, USA, ²Gyeongsang National University, Jinju 660-701, Korea, ³Purdue University, West Lafayette, IN, USA, ⁴University of Illinois, Urbana, IL, USA, ⁵University of California, Riverside, CA, USA, ⁶Texas Tech University, Lubbock, TX, USA

Histone modification in chromatin is one of the key control points in gene regulation in eukaryotic cells. Protein complexes composed of histone acetyltransferase or deacetylase, WD40 repeat protein and many other components have been implicated in this process. The WD40 repeat protein in these complexes functions as a bridge through protein-protein interactions to link histones in chromatin with other proteins in the complex including histone modifying enzymes. Here, we report the identification and functional characterization of HOS15, a WD40 repeat protein crucial for repression of abiotic stress-responsive genes through histone deacetylation in *Arabidopsis*. The HOS15 gene was identified by forward genetic screening for mutations that alter the expression of abiotic stress-responsive genes. The mutation in the HOS15 gene renders the mutant hypersensitive to freezing temperatures and causes late flowering. HOS15 shares high sequence similarity with human transducin-beta like proteins (TBL), a component of a repressor protein complex involved in histone deacetylation. HOS15 is localized in the nucleus and specifically interacts with histone H4. The level of acetylated histone H4 is higher in the hos15 mutant than in wild type plants. Moreover, the RD29A promoter in the hos15 mutant is associated with a substantially higher level of acetylated histone H4 than in wild type under cold stress conditions. This is consistent with enhanced induction of the RD29A gene in the hos15 mutant. Our results suggest a critical role for gene activation/repression by histone acetylation/deacetylation in plant acclimation to cold stress.

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Constitutive Expression of ThIPK2, an Inositol Polyphosphate kinase, from *Thellungiella halophila*, Enhances Multiple Abiotic Stress Tolerance, and Alters Seed Oil Fatty Acid Composition in Transgenic *Brassica napus* L. Jingqi Zhu¹, Jiantao Zhang¹, Qiuqing Wang¹, Lei Yang¹, Renjie Tang¹, Hongxia Zhang¹. ¹Shanghai Institute of Plant Physiology and Ecology, SIBS, CAS, Shanghai, 200032, P. R. China

It has been reported that the inositol polyphosphate 6-/3-kinase (AtIPK2β) in *Arabidopsis* plays a possible role as a transcriptional control mediator in higher plants. And some evidence suggested that AtIPK2β plays an essential role in abiotic stresses, such as salt, drought and ABA.

Thellungiella halophila (Salt Cress), a halophyte and cryophyte *Arabidopsis* relative model plant, shows 90-95% identity with *Arabidopsis* genomic DNA and higher salt tolerant ability than *Arabidopsis*. To further study the homogenous gene's functions and characterizations, we first isolated an inositol polyphosphate kinase gene (ThIPK2) was from *Thellungiella halophila*, and investigated its functions in yeast and *Brassica napus* L. cv. Huyou No. 1 (canola). The results shows that ThIPK2 shares 85% amino acid identity (91% similarity) with *Arabidopsis* AtIPK2β (GenBank accession no. AY147936). Transient expression of a ThIPK2-green fluorescent protein (GFP) fusion protein in onion (*Allium cepa*) cells indicated that the ThIPK2-GFP fusion protein was localized to both nucleus and plasma membrane. Expression of ThIPK2 in *Saccharomyces cerevisiae* arg82Δ/lpk2Δ, a mutant lacking lpk2 activity, rescued the mutant's normal growth at 37°C and growth under high salt or osmotic stress condition. Constitutive expression of ThIPK2 in canola enhanced tolerance to salt, dehydration and oxidative stress of transgenic plants. Furthermore, expression of ThIPK2 increased the transcripts of stress responsive genes and altered fatty acid constituents of transgenic canola, resulting in an increased level of oleic acid (C18:1) and a decreased level of linoleic acid (C18:2) in transgenic seeds. Taken together, the results in this study implicated a mutual correlation between ThIPK2 and abiotic-related defense responses, and a possible regulatory role of ThIPK2 in fatty acid metabolic syntheses in canola.

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Glyco-activation of a pro-drug in vivo. Yang Zhao¹, Tszfung Chow¹, Rachel Puckrin¹, Simon Alfred¹, Sean Cutler². ¹Department of Cell and Systems Biology, University of Toronto, ²Center for Plant Cell Biology, University of California, Riverside

Sequence variation in human drug metabolism and target genes can cause pharmacogenetic variation. Given the extensive metabolic variation documented between *Arabidopsis* accessions, we reasoned that *Arabidopsis* would be a powerful system for identifying natural alleles that modulate drug sensitivity, which in turn could be used to unravel the mechanism of action of compounds discovered in chemical genetic screens. Toward this goal, we subjected several *Arabidopsis* accessions to chemical genetic screens for inhibitors of cell expansion and discovered 12 molecules that induce strong strain-selective effects. The natural variation approach revealed that hypostatin, a new inhibitor of etiolated hypocotyl cell expansion, requires *in vivo* glycosylation for bioactivity. Many *Arabidopsis* strains are resistant to hypostatin due to mutations in HYR1, which encodes a UGT that glycosylates hypostatin, thus activating it. We additionally show that a prevalent SNP is sufficient for hypostatin resistance and, like other pharmacogenetic factors, HYR1 modulates sensitivity to multiple xenobiotics. Our results show that pharmacogenetic variation can be uncovered efficiently with small molecule screens and that natural alleles can yield mechanistic insights for new bioactive compounds. Because HYR1 is a member of the UGT-superfamily of enzymes that are pharmacogenetic factors in humans, our results demonstrate that intra-specific variation in UGT-function modulates xenobiotic sensitivity across biological kingdoms.

Responses to Microbials

P-645

A phage-display approach for high throughput screening of cDNA libraries expressed during plant-pathogen interactions
 Ines Arrieta-Aguirre¹, Florencia Lucca¹, Keith A. Charlton²,
 Susana Garcia-Sanchez¹. ¹NEIKER,²Haptogen Ltd

Arabidopsis response to microorganisms involves a large number of plant proteins. Although genome sequence is fully available, many of the microbial-response proteins remain unknown because there is not experimental evidence for their function. The physical affinities of these proteins for pathogen molecules can be the key to evidence their role in the plant-pathogen interactions. The phage-display technology allows the phage-mediated expression of a large number of proteins and their selection on the bases of the physical affinity to a ligand molecule. In this work we have applied the phage-display technology to selection of a large number of proteins related to plant-pathogen interactions. The strategy has been proven to be a efficient tool to select proteins from "Allium sativum" that bind microbial living cells and has lead to the identification of a putative defensin-like protein (AS2-3P) able to recognize "Pseudomonas aeruginosa" cells with high affinity. We constructed cDNA libraries from "Arabidopsis thaliana" after infection with "P. syringae" pv. "tomato" DC3000 and "P. aeruginosa" PA14, an opportunistic pathogen of humans which is also able to infect Arabidopsis. The cDNA-encoded genes were expressed as functional proteins fused to the capsid of a viral bacteriophage. These phage-displayed proteins and their corresponding genes were rescued by the ability of phagemic particles to bind microbial cells as a ligand, in a so-called "biopanning" selection. The construction of a library of 10 * 6 clones in combination with microarray hibridization allowed to test the capacity of an equivalent number of proteins for pathogen interaction. The value of the phage-display technology to genome-wide exploration of plant defence mechanisms is discussed.

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Investigating roles of RAR1 and SGT1 in TIR-NB-LRR immune receptor function. Shigeyuki Betsuyaku¹, Lennart Wirthmueller¹, Jane Parker¹. ¹ Max-Planck Institute for Plant Breeding Research, Koeln, Germany

Animal and plant immune receptors (R protein) of the NOD/NB-LRR type are assembled inside the cell as structurally constrained proteins that recognize specific pathogen effectors. *RAR1* (*Required for Mla12 conditioned resistance*) and *SGT1* (*Suppressor of G2 allele of skp1*) are positive regulators of plant immune responses mediated by R proteins. *RAR1* and *SGT1* have molecular features of cochaperones and are required for accumulation of Coiled-coil (CC)-NB-LRR type receptors most likely through their cooperation with HSP90 during assembly. Another major class of plant NB-LRR receptors has N-terminal homology (the TIR domain) to mammalian Toll-like receptors. The roles of *RAR1* and *SGT1* in function of plant TIR-NB-LRR immune receptor signalling remain unclear. In this study, we are investigating the functions of *RAR1* and *SGT1* in the assembly, activities and sub-cellular localizations of *Arabidopsis* TIR-NB-LRR proteins using genetic and biochemical approaches. We find that transcript levels of *EDS1* (*Enhanced Disease Susceptibility1*), a key positive regulator of TIR-NB-LRR mediated and basal defence, are reduced in both *rar1* and *sgt1b* mutants, resulting in depleted *EDS1* protein accumulation in the absence of pathogen. This correlates with defects in basal resistance of the *rar1* and *sgt1b* mutants. We are now analyzing how accumulation of TIR-NB-LRR proteins is affected in these mutants to establish more precisely where *RAR1* and *SGT1* operate in the TIR-NB-LRR triggered plant immune response.

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Hormonal control of cell death and defense responses in the *Arabidopsis* lesion mimic mutant *vad1*. Olivier Bouchez¹, Carine Huard¹, Séverine Lorrain², Dominique Roby¹, Claudine Balagué¹. ¹LIPM, Cactanet-Tolosan, France,² University of Lausanne, Switzerland

The lesion mimic mutant *vad1* (*Vascular Associated Death*) exhibits propagative HR-like lesions along the vascular system, presents expression of defense-related genes and increased resistance to different strains of *Pseudomonas syringae* pv *tomato*. *VAD1* encodes a novel putative membrane-associated protein containing a GRAM domain (a lipid- or protein-binding signaling domain). *VAD1* might constitute a new potential component of the signaling pathways leading to HR/ resistance (Lorrain et al., 2004).

To elucidate the function of *VAD1*, we performed overexpression tests in the heterologous system *Nicotiana benthamiana*. Results indicate that *VAD1* overexpression leads to a delay in HR symptoms appearance. The placement of this gene within defense signaling pathways was also addressed. We previously demonstrated that crosses of *vad1* with mutants affected in the SA pathway (*NahG*, *sid1*) drastically alter the *vad1*-associated phenotypes (Lorrain et al., 2004). These results, confirmed by the analysis of the double mutant *vad1/sid2*, indicate that SA is a key component in the *vad1*-associated phenotypes. We demonstrate here that ethylene (ET), an other defense signal, is also an essential component in the *vad1*-associated phenotypes. Analysis of the progeny from crosses between *vad1* plants and ET biosynthesis and signaling mutants revealed that the *vad1* cell death and defense phenotypes are dependent on ET biosynthesis and signaling pathways. These results suggest that *VAD1* could act as an integrative node in hormonal signalling, with ET acting in concert with SA, as a positive regulator of cell death propagation. In order to identify new actors of cell death and defense pathways, search for suppressors of *vad1*-associated phenotypes was initiated. Preliminary results will be presented on the characterization of identified mutants. (Lorrain et al., 2004, *Plant Cell*, 16, 2217-2232).

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Aspartic protease modulation of stress signalling in *Arabidopsis*. Matthieu Chabannes¹, Yiji Xia², Xinwei Chen¹, Chris Lamb¹. ¹John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK,²Donald Danforth Plant Science Center 975 North Warson Road St. Louis, Missouri 63132, USA.

We have used activation tagging with T-DNA carrying cauliflower mosaic virus 35S enhancers to investigate the complex signalling networks underlying disease resistance in *Arabidopsis*. From the screen of ~ 5000 lines we have identified *CDR1* (constitutive disease resistance) that encodes an apoplastic aspartate protease involved in local peptide signalling in basal disease resistance. Here we describe the characterisation of a second activation tagged allele *CDS1-D* which shows enhanced disease susceptibility. Remarkably, *CDS1* (constitutive disease susceptibility) encodes a second apoplastic aspartic protease closely related to *CDR1*. Over-expression of *CDS1* causes some drastic effects on the plant phenotype, which are totally abolished when point mutations are introduced into the protease active site indicating that the phenotypes require *CDS1* protease activity. The *CDS1-D* activation tagged line exhibits enhanced disease susceptibility to virulent *P. syringae* and is also compromised for localised resistance to isogenic avirulent strains. Recent experiments show that over-expression of *CDS1* compromises also the resistance to the biotrophic fungus *Peronospora parasitica* and to the necrotrophic fungus *Fusarium graminearum*. On the contrary *CDS1-D* shows an increase of resistance to chewing insects and interestingly increased tolerance to different abiotic stresses. Finally, we have shown that *CDS1* over expression leads to a potentiation of abscisic (ABA) and jasmonic (JA) acid accumulation in response to biotic and abiotic stresses. Taken together these emerging data suggest a model involving proteinase-mediated regulation of mobile peptide signal molecules in the modulation of biotic and abiotic stress responses.

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Galactinol as a novel priming component on induced systemic resistance elicited by *Pseudomonas chlororaphis* O₆. Song Mi Cho¹, Mi Seong Kim¹, Young Cheol Kim¹, Kwang Yeol Yang¹, Baik Ho Cho¹. ¹Agricultural Plant Stress Research Center, Biotechnology Research Institute, Department of Plant Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Republic of Korea

Root colonization by *Pseudomonas chlororaphis* O₆ elicited induced systemic resistance (ISR) in cucumber against *Corynespora cassiicola*. To understand O₆-mediated ISR, a suppressive subtractive hybridization method was undertaken and led to isolation of several distinct genes including a cucumber galactinol synthase (CsGolS1) gene. The transcriptional level of the CsGolS1 and the resulting galactinol content showed an increase 12 h earlier under O₆ treatment than in water control plants only after challenge with *C. cassiicola*, while no difference detected on the plants without pathogen challenge. To examine the role of galactinol on ISR, we employed *Arabidopsis* mutants at the AtGolS1 gene, an ortholog of the CsGolS1 gene, and AtGolS1-overexpressing transgenic *Arabidopsis*. The transcriptional level of the AtGolS1 was also primed by showing an increase 12 h earlier under O₆ treatment than in water control plants after challenge with *Botrytis cinerea* on *Arabidopsis*. The AtGolS1 mutant plant compromised ISR against *B. cinerea*, while the AtGolS1 overexpressor showed constitutive ISR against *B. cinerea* and *Erwinia carotovora* coupling to increased accumulation of galactinol content. Pharmaceutical application of 5 mM galactinol was sufficient to elicit ISR in *Arabidopsis* Col-0, npr1-1, etr1-1, and ctr1-1 plants against *B. cinerea*, but did not in the jar1-1 mutant that was validated to detect transcriptional expression of two JA indicator genes, AtVSP and AtPDF5.2. Taken together, our results indicate that primed accumulation of galactinol in the cucumber and *Arabidopsis* when *P. chlororaphis* O₆ colonized on the root can play a critical role in the JA-associated ISR against fungal and bacterial pathogens.

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Induced systemic drought tolerance elicited by *Pseudomonas chlororaphis* O₆ in *Arabidopsis thaliana*. Song Mi Cho¹, Kwang Hyun Min¹, Song Hee Han¹, Beom Ryong Kang¹, Kwang Yeol Yang¹, Baik Ho Cho¹, Young Cheol Kim¹. ¹Department of Plant Biotechnology and Environmental-friendly Agriculture Research Center, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea

Root-associated bacteria have been reported as possible inoculants for eliciting enhancement of plant growth and induced systemic resistance in several plant species. In this study, here we report that root colonization of *Pseudomonas chlororaphis* O₆ elicit tolerance referred to as induced systemic tolerance (IST) to abiotic stresses such as drought and salt stress in *Arabidopsis thaliana*. To identify the genes that are involved in IST we performed microarray analysis using the 22,800-gene Affymetrix GeneChip probe array with RNA extracted from leaf tissue. Different clusters of genes were expressed or repressed by growth of the plant with and without root colonization by *P. chlororaphis* O₆ and with and without water holding. Root colonization in watered plants increased genes associated with defense, response to activated oxygen species and auxin-responses, and jasmonic acid signaling but decreased transcription factors associated with ethylene and ABA signaling. Withholding water from colonized plants caused changes in expression of more genes. Analysis of two gene clusters that showed different regulation analysis showed down-regulation of genes involved in pectin catabolism and up-regulation of genes for sugar metabolism, ACC synthase and a transcription factor for ABA, ATHB-7. Clusters of genes that were up-regulated in the *P. chlororaphis* O₆-water-stressed plants were observed as being down-regulated in comparison with the drought-stressed non-colonized plants and vice versa. Other authenticated stress and defense responsive genes also were regulated by *P. chlororaphis* O₆ root colonization with some enhanced response under drought stress. These findings confirm that root colonization alters gene regulation in a systemic manner in IST. We speculate that jasmonic acid as well as ethylene and salicylic acid regulated genes were important in the systemic induction of the both abiotic and biotic stress by *P. chlororaphis* O₆ root colonization.

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Virulence function of *Pseudomonas syringae* effector AvrB on *Arabidopsis*. Haitao Cui¹, Yujing Wang¹, Xiaoyan Tang², Jian-Min Zhou¹. ¹National Institute of Biological Sciences, Beijing, China,² Department of Plant Pathology, Kansas State University, Manhattan, KS

Pathogenic bacterial effectors suppress pathogen-associated molecular pattern (PAMP)-triggered host immunity (PTI) to promote parasitism. Previously we show that the *Pseudomonas syringae* effector AvrB enhances virulence on *Arabidopsis* in a RAR1- and jasmonate pathway-dependent manner. RAR1 is known to be a HSP90 cochaperone that stabilizes many resistance proteins in effector-triggered immunity (ETI).

However, rar1 mutants exhibit an enhanced cell wall defense response to flg22, indicating that RAR1 is a negative regulator of basal defense. Co-immunoprecipitation experiments indicated that AvrB and RAR1 are in the same protein complex. Experiments are underway to test the hypothesis that RAR1 also stabilizes some negative regulators in plant immunity. These negative regulators may be exploited by AvrB to enhance plant susceptibility. Progress will be presented.

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LRA5 an EPIGENETIC component of ARABIDOPSIS disease resistance. Albor Dobon¹, Brande B. H. Wulff¹, Jefferey L. Dangl². ¹IBMC (UPV-CSIC), Valencia, Spain, ²University of North Carolina, Chapel Hill, (North Carolina), USA

The Arabidopsis mutant LRA5 was isolated in a screen for loss of resistance to the model bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000. Intriguingly, further characterisation of the mutant has revealed it to be impaired in several resistances (including basal; some, but not all gene-for-gene; and SAR). The most prominent feature however, is that LRA5 lacks non-host resistance against several isolates of *Pseudomonas*.

Another important characteristic of this mutant was discovered when it was shown by genetics to be a dominant gain-of-function mutation. Analysis of the transcriptome of non-challenged LRA5 plants has revealed that the basal expression of defence genes was greatly reduced in LRA5 plants, suggesting that LRA5 operates at a nodal point in defence signalling.

LRA5 has been mapped to a ~11.5 kb interval on chromosome II containing three known genes: two transcription factors (TF5 and TF2) and a gene without homology to any gene with a known function (UNKNOWN). None of these three genes have previously been implicated in disease resistance. Sequence analysis of the LRA5 mapping interval did not reveal any nucleotide change, indicating that LRA5 is an epigenetic mutant. Furthermore, LRA5 is genetically meta-stable (1 revertant per 3,000 mutant chromosomes), which is another hallmark of epigenetic mutants.

In a second round of mutagenesis of LRA5 we isolated six suppressors, three of which were defined by genetics to be intragenic. One of these carries a mutation in the UNKNOWN, suggesting this gene may be LRA5. However, qRT-PCR analysis has so far not revealed a significant change in the transcript level of this gene in mutant plants, whereas, surprisingly, transcript levels of TF5 are significantly down in LRA5 compared to wild-type.

To identify LRA5 we are currently complementing the above analyses with the phenotyping of transgenic plants (RNAi, T-DNA and overexpression) as well as comparative methylation analysis of the mapping interval.

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S-Nitrosoglutathione Reductase Affords Protection against Pathogens in Arabidopsis, Both Locally and Systemically. M. Carme Espunya¹, Christine Rustucci², Maykelis D'az¹, Matthieu Chabannes³, M. Carmen Martínez¹. ¹Universitat Autònoma de Barcelona, ²Université Jules Verne-Picardie Sciences, ³John Innes Center

Nitric oxide and S-nitrosothiols (SNOs) are widespread signaling molecules that regulate immunity in animals and plants. Levels of SNOs "in vivo" are controlled by nitric oxide synthesis (which in plants is achieved by different routes) and by S-nitrosoglutathione turnover, which is mainly performed by the S-nitrosoglutathione reductase (GSNOR). GSNOR is encoded by a single-copy gene in *Arabidopsis*. We report here that transgenic plants with decreased amounts of GSNOR (using antisense strategy) show enhanced basal resistance against "*Peronospora parasitica* Noco2" (oomycete), which correlates with higher levels of intracellular SNOs and constitutive activation of the pathogenesis related gene, PR-1. Moreover, systemic acquired resistance is impaired in plants overexpressing GSNOR and enhanced in the antisense plants, and this correlates with changes in the SNO content both in local and systemic leaves. We also show that GSNOR is localized in the phloem and, thus, could regulate systemic acquired resistance signal transport through the vascular system. Our data corroborate the data from other authors that GSNOR controls SNO "in vivo" levels, and shows that SNO content positively influences plant basal resistance and resistance-gene-mediated resistance as well. These data highlight GSNOR as an important and widely utilized component of resistance protein signalling networks conserved in animals and plants (1).

(1) Rustucci "et al". (2007) Plant Physiol. 143:1282–1292

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Abscisic acid plays an important role in plant disease susceptibility. Jun Fan¹, Chris Lamb¹. ¹Department of Disease and Stress Biology, John Innes Centre, Norwich, U.K.

We have used activation tagging to investigate the complex signaling networks underlying disease resistance in *Arabidopsis*. From a screen of ~ 8,000 lines, we identified a constitutive disease susceptibility (*cds2*) mutant showing enhanced susceptibility to virulent *Pseudomonas syringae* pv. *maculicola* ES4326 (*Psm*) and the equivalent strain carrying avirulence gene *avrRpm1* (*PsmavrRpm1*). Systemic acquired resistance and systemic induction of PR protein transcripts were also compromised in *cds2* plants. The T-DNA insertion adjacent to *AtNCED5*, one of the six genes encoding the abscisic acid biosynthetic enzyme 9-cis-epoxycarotenoid dioxygenase, led to a marked increase in transcript level and ~ 2 fold enhanced abscisic acid levels. Over expression of *AtNCED* on either constitutive or inducible promoters recreated the enhanced disease susceptibility phenotype. These findings prompted further investigation of the role of abscisic acid in disease susceptibility. Several *AtNCED* genes are induced by pathogen and abscisic acid accumulates strongly in a compatible interaction. Furthermore, exogenous abscisic acid increases bacterial growth and the abscisic acid biosynthetic mutant *aba3* shows reduced susceptibility to virulent *P. syringae* pv. *tomato* DC3000. Finally, abscisic acid synergises with jasmonic acid and exhibits a complex antagonistic relationship with salicylic acid. Our findings provide genetic evidence that abscisic acid has an important role in disease susceptibility.

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Study of ABA-seed priming effects on germination and ionic distribution in Arabidopsis plant under salt stress. rozbéh Farhoudi¹, Adel Modhei¹. ¹Agronomy Dep., Faculty of Agriculture, Islamic Azad University, Shoushtar Branch, Iran

An experiment was carried out to study *Arabidopsis thaliana* ABA-seed priming effects on seed germination and ionic distribution in seedlings under salt stress. Twenty five (25) seeds were planted in each Petri dish (7cm in diameter). Salt stress was applied at four levels including: 4, 8, 12 and 16ds/m. Sodium chloride (NaCl)/calcium chloride (CaCl₂) mixture at 3 : 1 ratio was used to apply salt stress. Distilled water was used for control Petri dishes. In order to prime, seeds were soaked in 10μmol ABA solution for 12hrs. The experiment was carried out in terms of factorial-completely random design with 8 treatments at four replications. 8-16ds/m salt stress significantly decreased germination rate, vigor index and coleoptiles length, but increased mean germination duration. However, coleorhizae length was not affected by salt stress conditions except at 16ds/m level. ABA-priming of stressed seeds increased germination rate and seed vigor index, decreased mean germination duration, whereas did not significantly affect coleoptiles and coleorhizae length. Salt stress condition significantly decreased K⁺ and Ca²⁺ and increased Na⁺ content of seedling tissues. ABA-priming increased K⁺ and Ca²⁺ resulting from salt stress, whereas had no effect on Na⁺ content of seedlings. ABA application decreased Na⁺ level and increased K⁺ and Ca²⁺ content of seedling tissues.

Keywords: *Arabidopsis*, salt stress, ABA, seed priming

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Ferredoxin plays an essential role in plant innate immunity and fruit ripening. Teng-Yung FENG¹, Hsiang-En HUANG¹. ¹Institute of plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

There are many isoforms of ferredoxin proteins in plant cells. In this communication, we would like to present that ferredoxin(Fd), is not only involving in electron flow of photosystem I, but also highly linked to plant biotic/abiotic stress monitoring. We found that that Fd1-mediated disease resistance is established through the regulation of evolution of active oxygen species (AOS). The disease immunity of various transgenic plants was enhanced by the over-expression of Fd1 protein(note 1). The "fd1" gene was transferred to wild-type and resistance-defective Arabidopsis to reveal the mechanism of Fd1-mediated resistance. The wild-type plant with elevated amount of Fd1 behaves more resistant to bacterial pathogen infection. However, "fd"-transgenic plant becomes less tolerant under abiotic stress (light and heat). Under the experimental conditions the "fd1"-transgenic Arabidopsis plants are more sensitive to the treatment of heat, salicylic acid (SA), harpin or "*Pseudomonas syringae*". As the non-transgenic Arabidopsis plants were shifted into dark condition, both the transcripts of Fd1 and Fd2 are quickly diminished in a similar fashion, although Fd2 is in a slower way. Under this condition, heat treatment would slow down this decline phenomenon. In tomato plants elevating Fd3 content artificially would speed up fruit ripening process in terms of pigment formation. In contrast, the increment of Fd1 would slow down this ripening process. These findings would help us to understand how the contents of different Fd proteins in plant could monitor some important stress physiology, even including fruit ripening.

Note 1: reference cited

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Role of the *Arabidopsis* MYB transcription factor AtMYB30 in the control of disease resistance and hypersensitive cell death. Solène Froidure¹, Carine Huard¹, Amandine Leger¹, Dominique Roby¹, Susana Rivas¹. ¹ Laboratoire des Interactions Plantes-Microorganismes, UMR CNRS/INRA 2594/441, BP 52627, 31326 Castanet Tolosan, FRANCE.

The Hypersensitive Response (HR), characterized by a rapid and localized cell death at the inoculation site, is one of the most efficient resistance reactions to pathogen attack in plants. We previously found AtMYB30 as specifically, rapidly and transiently expressed during incompatible interactions between *Arabidopsis* and bacterial pathogens. AtMYB30 presents homology to plant MYB transcription factors which play a central role in the regulation of a variety of developmental and metabolic responses. We also demonstrated that AtMYB30 is a positive regulator of the hypersensitive cell death. Results from a transcriptome analysis, together with recent molecular, genetic and biochemical studies, show that putative AtMYB30 target genes are involved in the lipid biosynthesis pathway leading to the production of very long chain fatty acids (VLCFAs), suggesting a role of this pathway in the control of the HR and plant defence responses. New strategies aimed at (i) studying the subcellular localization of AtMYB30, (ii) characterizing posttranslational modifications within the protein, and (iii) identifying proteins that may interact and work together with AtMYB30 in the initiation of the HR will be presented.

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The *Pseudomonas syringae* type III effector HopU1 ADP-ribosylates RNA-binding proteins and suppresses plant immunity

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Pseudomonas syringae is a bacterial plant pathogen and its pathogenicity depends on the type III protein secretion system (T3SS) and the type III effectors it injects into host cells. The predicted product of the *hopU1* effector gene carried a potential mono-ADP-ribosyltransferases (ARTs) active site. These enzymes have been well characterized in animal pathogens and are also found in mammals but have not been demonstrated to be present in plants or plant pathogens. RT-PCR indicated that *hopU1* was expressed in *P. syringae* and translocation assays indicated that HopU1 was injected into plant cells by the T3SS. HopU1 inhibited several outputs of plant innate immunity. Partially-purified HopU1-His ADP-ribosylated poly-L-arginine, which indicates it is an arginine specific ART. We found that HopU1 ADP-ribosylated several proteins in plant extracts and this activity required its putative ART active site. Using two dimensional gel electrophoresis and mass spectrometry, we identified the *in vitro* substrates of HopU1-His to be three chloroplast RNA-binding proteins and two glycine-rich RNA-binding proteins (GR-RBPs) in *Arabidopsis* extracts. *Arabidopsis* T-DNA knockout plants of one of the GR-RBPs, AtGRP7, were more susceptible to *P. syringae* than wild type plants, indicating that this substrate plays a role in innate immunity. When co-expressed in plants, HopU1 was capable of ADP-ribosylating AtGRP7 indicating HopU1 could modify AtGRP7 in planta. Two arginine residues within AtGRP7's RNA-binding domain were required for it to be ADP-ribosylated by HopU1-His, suggesting that ADP-ribosylation interferes with AtGRP7's ability to bind RNA. Our results suggest a novel strategy employed by a bacterial pathogen where ADP-ribosylation of plant RNA-binding proteins results in posttranscriptional inhibition of host innate immunity.

P-659

Suppression of Dicer-like 4 by the P6 protein of Cauliflower mosaic virus: relevance of sub-cellular localization and link to symptom induction. Gabrielle Haas¹, Guillaume Moissiard¹, Christophe Hieber¹, Mario Keller¹, Olivier Voinchet¹. ¹Institut de Biologie Moléculaire des Plantes, ULP/CNRS UPR2357, 12 rue du Général Zimmer, 67084 Strasbourg cedex, France

RNA silencing is a mechanism of gene regulation with antiviral roles in plants and insects. A common counter-defensive strategy of viruses against RNA silencing is the production of viral factors referred to as silencing suppressor. Although RNA silencing and its suppression have been well documented for plant RNA viruses, less data are available for DNA viruses.

Cauliflower mosaic virus (CaMV) has a DNA genome (8kbp) which is replicated by reverse transcription of a pregenomic RNA (35S RNA), a feature of the pararetrovirus supergroup. This 35S RNA is polycistronic and encode for all the viral proteins. Recently, it has been shown that the Arabidopsis silencing machinery targets an extensively base-paired region of the 35S RNA (referred to as leader), which is hierarchically processed by the four Arabidopsis Dicer-like proteins (DCLs) to produce populations of viral siRNAs (vsRNAs). Although it remains unclear if these molecules are effectively used to guide viral transcript degradation, it has been shown that Dicing is one limiting factor of 35S RNA accumulation in infected cells. It was thus expected that CaMV would deploy a suppressor activity to limit siRNA biogenesis in order to preserve intact 35S RNA for viral replication.

The P6 protein of CaMV is a pathogenicity factor, which is a common feature of viral silencing suppressors. To test P6 ability to suppress silencing, we introduced its ORF in several silencing reporter systems available in *Arabidopsis*. Molecular analyses of the resulting transformants and of infected plants suggest that P6 has a strong and specific inhibitory effect on the DCL4 activity, the primary antiviral dicer of plants. Transgenic plants expressing various P6 alleles, defective for translational activation, dsRNA binding, nucleo-cytoplasmic trafficking, or viroplasm formation, have been generated and the analyses of those plants now allow us to define which features of P6 are involved (or not) in suppression of RNA silencing in *Arabidopsis*.

P-660

Genetic analysis of suppressors of rar1. Yijian He¹, David Hubert¹, Pablo Tornero², Jeff Dangl¹, ¹Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, ²IBMCV, Universidad Politécnica de Valencia, Valencia, Spain

Disease resistance in plants is controlled by a gene-for-gene mechanism. Products of plant disease resistance (R) genes specifically recognize, directly or indirectly, avirulence (Avr) proteins encoded by pathogens. Most R genes encode NBS-LRR proteins that have some domain similarity to animal NOD/CARD proteins. NBS-LRR-mediated recognition triggers plant defense responses. Mutational analysis in *Arabidopsis* has been used to identify many components required for R gene function. RAR1 is required for hypersensitive cell death (HR) and disease resistance mediated by many NBS-LRR proteins, even those that do not genetically require RAR1. To find additional genetic elements involved in RAR1 function in NBS-LRR signaling, we isolated disease resistance following infection with bacteria *Pseudomonas syringae* pathovar tomato strain DC3000 (*avrPphB*). This strain triggers the RPS5 NBS-LRR protein. While disease resistance mediated by RPS5, RPM1 and RPS2 is diminished in rar1 plants, it is recovered in all five mutants. Three of these suppressors also recover RPM1-mediated HR, while the other two mutants do not. However, none of these five suppressor mutants can recover RPS5-mediated HR in a rar1 background. These findings support previous findings that show the roles of RAR1 in R gene-mediated disease resistance and HR are genetically separable. Genetic analysis demonstrates that these five suppressors belong to three loci which are localized on Chromosome I, II and V. In addition, the non-allelic noncomplementation between Chromosome II and V loci suggests that the corresponding protein products interact with each other.

P-661

A Dual Regulation Mechanism for Disease Resistance and Susceptibility through Modulation of Salicylic Acid and Auxin Signaling by A GH3 in *Arabidopsis*. Zuhua He¹, Zhong Zhang¹, Qun Li¹, Paul E. Staswick², Muyang Wang¹, Ying Zhu¹, Zhimiao Li¹, ¹National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China, ²Department of Agronomy and Horticulture, University of Nebraska, Lincoln, Nebraska, USA

Salicylic acid (SA) plays a central role in plant disease resistance, and emerging evidence indicates that auxin, an essential growth hormone, is involved in plant disease susceptibility. GH3.5, a member of the GH3 family of early auxin-responsive genes in *Arabidopsis*, encodes a protein possessing an in vitro adenylation activity on both IAA and SA and playing a role in modulating IAA homeostasis. Here we show that GH3.5 acts as a bifunctional modulator in both the SA-mediated disease resistance and auxin-elicited disease susceptibility. Overexpression of the GH3.5 gene in an activation-tagged mutant *gh3.5-1D* leads to elevated accumulation of SA and increased expression of PR-1 in local and systemic tissues in response to avirulent pathogens. By contrast, two T-DNA insertional mutations of GH3.5 partially compromised the systemic acquired resistance (SAR) associated with the diminished PR-1 expression in systemic tissues. The *gh3.5-1D* mutant accumulated high levels of free IAA after pathogen infection and impaired multiple R gene-mediated resistance, which was also observed in the GH3.6 activation-tagged mutant *df1-1D* that impacted the auxin pathway, indicating an important role of GH3.5/GH3.6 in disease susceptibility. Microarray and northern blot analysis showed that the SA and auxin pathways were simultaneously augmented in *gh3.5-1D* upon infection with an avirulent pathogen. The SA pathway was amplified by GH3.5 through inducing SA-responsive genes, basal defense components, and multiple R gene homologs; while the auxin pathway was de-repressed through up-regulating IAA biosynthesis and transport genes and down-regulating auxin repressor genes, resulting in altered metabolism and transport of various nutrients that might favor pathogen infection. Taken together, our data revealed novel regulatory functions of GH3.5 in the plant pathogen interaction.

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Arabidopsis-Botrytis interactions: using a model plant system to reveal diversity of a plant pathogen. Heather Rowe¹, Dan Kliebenstein¹. ¹University of California, Davis, (CA), USA

Arabidopsis genetic resources can be used to gain understanding of plant pathogen diversity and infection mechanisms. The molecular bases of host plant interactions with necrotrophic fungal pathogens such as *Botrytis cinerea* are unknown relative to the well-described avr/R interactions that mediate Arabidopsis relations with biotrophic pathogens. We use a combined approach to investigate naturally variable molecular mechanisms controlling *Arabidopsis-Botrytis interactions*: *Arabidopsis* mutants with identified genetic lesions in defense-related pathways allow direct tests of the effects of candidate genes, while natural variation among *Arabidopsis* accessions can be exploited to identify novel components of the plant-pathogen interaction via QTL mapping. *B. cinerea* is genetically diverse and infects numerous plant hosts. We measure intra-specific diversity of *B. cinerea* pathogenesis mechanisms by testing multiple *Botrytis* isolates on both *Arabidopsis* mutants and near-isogenic lines differing at QTL influencing *Arabidopsis susceptibility to Botrytis* infection. *B. cinerea* isolates differed in their inhibition by polygalacturonase-inhibiting proteins (PGIPs) and their ability to induce the phytoalexin camalexin in planta. Additionally, isolates varied for increases in virulence on *Arabidopsis* mutants deficient in JA-mediated signaling, suggesting that some isolates are detected by a JA-independent signal transduction network. Tests of 2 QTL using near-isogenic lines showed that one QTL influenced lesion growth on *Arabidopsis* leaves for 90% of isolates tested, while a second QTL influenced camalexin production for only 60% of isolates tested. These data indicate that studies of plant defenses using single pathogen isolates may not produce results broadly informative of the mechanisms of plant defense. Measuring diversity among *B. cinerea* isolates may allow us to understand how this pathogen can have such a broad host range, while identifying conserved elements of the host-pathogen interaction to assist control efforts.

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Tzs, a host range factor, is required for Agrobacterium tumefaciens growth under infection conditions. Hau-Hsuan Hwang^{1,2}, Yu-Chen Liao¹, Yi-Chun Chen¹, Erh-Min Lai¹. ¹Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan,² Department of Life Sciences, National Chung-Hsing University, Taichung, Taiwan

Agrobacterium tumefaciens is an organism capable of trans-kingdom DNA transfer, transforming mainly plants but also other eukaryotic species, from fungi to human cells. Genetic transformation by *A. tumefaciens*, which in plants causes neoplastic growths called "crown gall", results from the transfer and integration of a specific DNA fragment (transferred DNA or T-DNA) from the bacterium into the plant genome. Here, we characterized a Ti-plasmid encoded gene, *tzs* (trans-zeatin synthesizing), that is responsible for the synthesis of a plant hormone cytokinin in *A. tumefaciens* when bacteria were induced by a phenolic compound acetosyringone (AS). To determine the role(s) of *tzs* in *A. tumefaciens* virulence, *tzs* deletion mutants ($\Delta tzs - 277$ and $\Delta tzs - 278$) were generated and characterized. Quantitative tumor assays demonstrated that *tzs* mutants decreased their ability to cause tumors on *Arabidopsis* roots and potato tubers. Additionally, *tzs* mutants reduce transient transformation efficiency in *Arabidopsis* roots, suggesting that *Tzs* is likely involved in step(s) prior to T-DNA integrations. Interestingly, the *tzs* mutants are impaired in cell viability and/or growth in both AS-induced and infection conditions. *Tzs* protein is constitutively expressed and is up regulated by AS either in rich or minimal media. Tumor assays on various plant species were also tested to determine if *Tzs* is a host range factor. The *tzs* mutants were able to enhance transformation efficiency on green pepper and cowpea stems, reduce transformation efficiency on white radish, but not on other plant species tested. In summary, *tzs* may be required for *A. tumefaciens* growth under infection conditions and may play different roles when *A. tumefaciens* infects different kinds of plants.

P-664

Characterization of hypersensitive mutant (*stop1*) to proton-rhizotoxicity. Satoshi Iuchi¹, Hiroyuki Koyama², Atsuko Iuchi¹, Yasufumi Kobayashi², Sadako Kitabayashi¹, Yuriko Kobayashi², Takashi Ikka², Takashi Hirayama³, Kazuo Shinozaki⁴, Masatomo Kobayashi¹. ¹RIKEN BRC, ²Gifu University, ³Yokohama City University, ⁴RIKEN PSC

Acid soil represents a limiting factor of crop production. Plant growth on acid soil is limited mostly because of the rhizotoxicities of ions such as aluminum (Al^{3+}). Although proton (H^+) could be the major rhizotoxicant in some soil types, molecular mechanisms of plant tolerance against H^+ rhizotoxicity have not been identified yet. We have isolated a mutant that is hypersensitive to H^+ rhizotoxicity from EMS mutagenized seed stock of *Arabidopsis thaliana* by root bending assay system. The isolated mutant, *stop1* (Sensitive TO Proton rhizotoxicity), was defect in root growth under low pH conditions. This phenotype of *stop1* was attributed to a missense mutation at the zinc-finger domain in a predicted Cys2/His2 type zinc-finger protein, designated as *STOP1*. The *STOP1* belongs to a functionally unidentified subfamily of zinc-finger proteins in *Arabidopsis*. The treatments of cadmium, copper, lanthanum, manganese and sodium chloride gave no significant difference in root growth between *stop1* mutant and wild type, however, *stop1* mutant show hypersensitivity to Al^{3+} treatment. The *stop1* mutant lacked the induction of the *AtALMT1* gene that encodes a malate transporter, and was unable to induce malate exudation by Al^{3+} treatment. The *AtALMT1* knockout mutant shows hypersensitivity to Al^{3+} , whereas the *stop1* mutant is hypersensitive to both Al^{3+} and low pH treatment. These results suggest that *STOP1* play a key role in both Al^{3+} tolerance and low pH tolerance.

P-665

Functional analysis of the wound-inducible *Arabidopsis* blue copper-binding protein gene promoter in transgenic tobacco plants. Jin An Jeong¹, Kwang Hyun Min¹, Eun Kyung Jang¹, Jin A Jeong¹, Young Cheol Kim¹, Baik Ho Cho¹, Kwang Yeol Yang¹. ¹Department of Plant Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea

The plant wound response has to be a rapid and coordinated process in the local region of injury, since wound sites are known to be the main entry points for many kinds of plant pathogens such as fungi, bacteria and viruses. The promoters of the genes that are induced by wounding could be valuable in regulating the expression of heterologous beneficial genes. The transcriptional regulatory region (from -1294 to +247) of a wound-inducible *Arabidopsis* gene encoding a blue copper-binding protein was fused to the β -glucuronidase gene in plant transformation vector. Stable integration of the single *Arabidopsis BCB/GUS* gene into tobacco genome resulted in strongly induced GUS activity upon wounding in the homozygous plants. The BCB promoter was activated at the site of infection with bacterial, fungal and viral pathogens. It was also activated after treatment with salicylic acid and ultraviolet. The cis-acting elements of the BCB promoter region were analyzed by online programs. The promoter analysis revealed several potential regulatory elements that are related to biotic and abiotic stress regulation. These data suggest that, although the BCB promoter activity is reduced in the heterologous host, its pathogen-responsiveness could be useful in driving the expression of transgenes to promote disease resistance in plant.

P-666

Turnip crinkle virus resistance in *Arabidopsis* requires CRT1, a new member of the GHKL ATPase family. Hong-Gu Kang¹, Joseph Kuhl¹, Pradeep Kachroo², Daniel F. Klessig¹. ¹Boyce Thompson Institute for Plant Research, Ithaca, New York, USA, ²University of Kentucky, Lexington, Kentucky, USA

HRT is a CC-NBS-LRR-type resistance (R) protein that is required for resistance to turnip crinkle virus (TCV) in *Arabidopsis*. To gain insights into HRT-mediated signaling, a genetic screen was performed to identify mutants compromised for recognition of the TCV avirulence factor, which is encoded by an endogenously expressed TCV coat protein (CP) transgene. One of the mutants identified, crt1 (compromised recognition of TCV), carries a prematurely terminated novel ATPase protein, in which a recently recognized GHKL ATPase motif termed a Bergerat fold has been deleted. Following TCV infection, crt1 plants developed a spreading hypersensitive response (HR) and failed to prevent viral replication and spread to the systemic portions of the plants. Systemic viral movement and disease symptoms were even more pronounced when two closely related CRT1 homologs were partially silenced. In addition, silencing CRT1 and its homologs in the Col-0 background led to delayed HR against avirulent *Pseudomonas syringae* carryingavrRpt2 and the crt1 mutation suppressed spontaneous cell death in the ssi4 mutant, which contains a gain-of-function mutation in a TIR-NBS-LRR type R gene. In summary, this novel CRT1 family appears to play an important function in activating defenses against pathogen infection.

P-668

Functional Analysis of *Arabidopsis* WRKY38 and WRKY62 Transcription Factors in Plant Defense Responses. Kang-Chang Kim¹, Baofang Fan¹, Zhixiang Chen¹. ¹Purdue University, West Lafayette, IN 47907 USA

Arabidopsis WRKY38 and WRKY62 encode two structurally similar Type III WRKY transcription factors. WRKY38 and WRKY62 are induced by salicylic acid (SA) in an NPR1-dependent manner. The two WRKY genes also exhibit SA- and NPR1-dependent induction by the bacterial pathogen *Pseudomonas syringae*. WRKY38 and WRKY62 function as transcriptional activators in plant cells. Both loss-of-function mutants and overexpression lines for WRKY38 and WRKY62 have been generated for functional analysis of their roles in plant defense responses. WRKY38 and WRKY62 interact with a histone deacetylase in both yeast and plant cells. Expression of the gene encoding the histone deacetylase is also induced by *P. syringae* and the stability of its transcripts in pathogen-infected plants is dependent on SA and NPR1. T-DNA insertion mutant and overexpression lines for the histone deacetylase gene have also been generated. Characterization of the mutants and overexpression lines for the genes encoding WRKY38, WRKY62 and their interacting histone deacetylase partner will be described.

P-667

A microarray assisted screen to identify mutants that compromise nonhost resistance. Li Kang¹, Keri Wang¹, Kirankumar Mysore¹. ¹The Samuel Roberts Noble Foundation, Ardmore, OK USA

Transgenic *Arabidopsis* lines overexpressing a fatty acid amide hydrolase (FAAH) were more susceptible to bacterial pathogens and lost resistance against nonhost pathogens. Uninoculated and mock inoculated AtFAAH overexpressors had lower amounts of both free and conjugated salicylic acid (SA) when compared with the wild-type. Surprisingly, AtFAAH overexpressors accumulated significantly higher amounts of free SA, 12 hours after inoculation with bacteria, while the total SA (free + conjugated) was still low. Gene expression studies revealed that transcripts of a number of disease resistance (R) genes as well as genes involved in SA biosynthesis and signaling were considerably lower in AtFAAH overexpressors compared to those in wild-type plants. To further dissect the signal pathway mediated by AtFAAH in *Arabidopsis*, 130 genes that were expressed most differently between Col-0 and AtFAAH overexpressor were further characterized. We obtained T-DNA knockout lines for these 130 genes and screened them for compromised resistance against nonhost pathogen *Pseudomonas syringae* pv. tabaci. Five mutants that exhibited enhanced susceptibility to a nonhost pathogen were

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The plant innate immune system, role of phospholipids and oxylipins.. Olga Kourtchenko¹, Mats X Andersson¹, Cornelia Göbel², Mats Hamberg³, Jefferey L Dangl⁴, David Mackey⁵, Ivo Feussner², Mats Ellerström¹. ¹Göteborg University, Gothenburg, Sweden, ²Universität Göttingen, Göttingen, Germany, ³Karolinska Institutet, Stockholm, Sweden, ⁴University of North Carolina, Chapel Hill, USA, ⁵The Ohio State University, Columbus, USA

Bacterial pathogens deliver type III effector proteins into the plant cell during the course of infection. On susceptible hosts, type III effectors contribute to virulence, but in the case of resistant host plants, they betray the pathogen to the plants immune system. These proteins are functionally termed avirulence (Avr) proteins. Recognition induces a complex suite of cellular and molecular events comprising the plant's inducible defense response. As recognition of the bacterial Avr proteins occurs inside host cells, the response can be elicited by "in planta" expression of bacterial type III effectors. Genes encoding two such effector molecules, AvrRpm1 and AvrRpt2 of "*Pseudomonas syringae*" were introduced as chemically inducible constructs in wild type *Arabidopsis* and in the corresponding resistance-gene mutants, "rpm1" and "rps2". We demonstrate that recognition of either of the two Avr proteins induces biphasic accumulation of phosphatidic acid (PA) via the sequential activation of phosphoinositide specific phospholipase C and D. Inhibition of phospholipases blocks the response, and feeding PA and phospholipase D directly to leaf tissue causes cell death and defense-gene activation. These results indicate that PA is an integral part of the disease resistance signaling. Avr-protein-elicited defense response also includes the induction of 9- and 13-lipoxygenase (LOX)-dependent oxylipin synthesis. The 13-LOX products 12-oxo-phytodienoic acid (OPDA) and dinor-oxo-phytodienoic acid (dinor-OPDA) are the most prominent oxylipins accumulated. Interestingly, the majority of OPDA and dinor-OPDA (>90 %) are esterified to glycerolipids and, in particular, to a novel galactolipid. The structure of this compound was determined and given the trivial name Arabidopside E. We will discuss possible roles of Arabidopside E in the context of plant immune responses.

P-670

A negative effect of RPM1 on the AvrRpt2/RPS2-mediated gene-for-gene resistance. Tack-Min Kwon¹, Soon-Jae Jeong¹, Young-Byung Yi¹, Jaesung Nam¹. ¹Faculty of Molecular Biotechnology, Dong-A University, Busan 604-714, Korea

The TTSS effector, AvrRpt2, targets and eliminates RIN4 that is not only a positive regulator for basal defense but also a negative regulator for RPS2-mediated resistance. Therefore, if *P. syringae* expressing avrRpt2 infects host plant lacking resistance gene RPS2, AvrRpt2-mediated elimination of RIN4 suppresses a basal defense and results in a hospital environment for propagation of pathogen. However, when RPS2 is present in the host plants, the elimination of RIN4 triggers RPS2-mediated effective defenses including hypersensitive response (HR) and lead to resistance against pathogens. Kinetics of RPS2-mediated HR reveals that AvrRpt2-mediated elimination of RIN4 occurs in 3 – 5 hr post infiltration of *P. syringae* (avrRpt2), which sequentially destabilize RPM1 and eliminate RPM1 in 12 – 20 hrs independent on RPS2. When RPS2 is present, the elimination of RPM1 is tightly linked with RPS2-mediated HR time point. Interestingly enough, RPS2-mediated HR is accelerated in rpm1 mutant, suggesting that RPM1 may function as a negative regulator for AvrRpt2/RPS2-mediated gene-for-gene resistance. We will discuss that what kinds of plant factors involve in AvrRpt2-mediated RPM1 elimination and what their functions are in the plant defense mechanism.

P-672

Repression of defense and cell death by the BON1/CPN1 family likely involves multiple R genes in *Arabidopsis*. Yongqing Li¹, Huijun Yang¹, Jian Hua¹. ¹Cornell University, Ithaca, NY 14853, USA

The *Arabidopsis* *BON1/CPN1* gene is a negative regulator of defense responses. It belongs to an evolutionarily conserved copine gene family encoding two calcium-dependent phospholipid binding C2 domains and a VWA domain. The loss of *BON1* function leads to constitutive defense responses apparently through activating an accession-specific TIR-NBS-LRR type of disease resistance (*R*) gene *SNC1*. To investigate the intriguing repression of defense responses by *BON1*, we set out to identify the cellular processes *BON1* regulates.

We found that *BON1* functions together with *BAP1* and its homolog *BAP2* that both encode small proteins with one C2 domain. *BAP1* and *BAP2* appear to be a general inhibitors of programmed cell death across kingdoms, as their overexpression suppresses *R*- and mammalian apoptotic *Bax*-induced programmed cell death (PCD) in plants as well as hydrogen peroxide-induced PCD in yeasts. Co-expression of *BON1* is required in some cases for the inhibition of PCD by *BAP1* and *BAP2*, suggesting the involvement of both *BON1* and *BAP1/BAP2* in PCD modulation.

In addition, we identified potential target genes of *BON1* other than *SNC1*. *BON1* has an overlapping function with its homolog *BON3* in *Arabidopsis* and the *bon1bon3* double mutant is seedling lethal in the *Col* accession but wild-type looking in the *Ws* accession. Using recombinant inbred lines, we mapped three major QTLs responsible for this natural variation. Interestingly, they all coincided with clusters of NBS-LRR type of *R* genes. It is likely that some of these *R* genes become activated in *bon1bon3*, leading to lethality. We propose that *BON1* is involved in modulating PCD and the *BON1* gene family is guarded by multiple *R* genes.

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Cadmium stress alters gene expression of *Arabidopsis* seedlings. Wan Liu¹, YS Yang². ¹Institute of Applied Ecology, Chinese Academy of Sciences, ²Cardiff University

Cadmium (Cd) is a non essential element, and is a widespread environmental pollutant. Exposure to Cd can result in a variety of adverse health effects in plant and humans. In this study, *Arabidopsis* seedlings were used as bioindicator of Cd pollution in the range of 0-6.0 mg L⁻¹ for 18 days and gene expression patterns were used to link increased Cd exposure with progressive biological effects. Seven genes known to be involved in cell division and DNA mismatch repair (MMR) were investigated by RT-PCR and normalized according to 18S rRNA gene expression. Expression of proliferating cell nuclear antigen 2 (PCNA2) or MutS3 homolog (MSH3) genes in shoots was induced by exposure to Cd of 0.75 ppm or 1.5 ppm Cd significantly, but was repressed by other Cd concentration, respectively; whereas exposure to 0.75-6 ppm of Cd obviously decreased expression of PCNA1, MutL1 homolog (MLH1) and MSH6 independent of any observable health effects, including survival, gross morphology and height of shoots. This work demonstrated that specific gene expression changes could serve as useful molecular biomarkers of exposure and related health effects.

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Involvement of lipase in R gene-induced increase in salicylic acid. Hyoung-Yool Lee¹, Hong Gil Nam², Inhwan Hwang², Stephen B. Ryu¹. ¹Korea Research Institute of Bioscience and Biotechnology, Korea, ²POSTECH, Korea

The establishment of local host defense response in plants is R gene-dependent and associated with the elevated levels of salicylic acid (SA). SA is a necessary and sufficient plant hormone for local host defense response. In spite of functional importance of SA, cellular mechanism of R gene-induced SA increase remains to be much unknown. Here we report that lipase A and its lipid products are involved in upstream signaling between gene-for-gene interaction and SA increase in plant host defense response. The lipase A mutant showed the defect in R gene-induced expression of ICS1, a key enzyme in SA biosynthesis, and PR genes.

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Arabidopsis TGA1 and TGA4 are required for NPR1-independent Disease Resistance. Jinman Liu¹, Yu Ti Cheng², Dongling Bi¹, Xin Li², Yuelin Zhang¹. ¹National Institute of Biological Science, Beijing, China,² Michael Smith Labs, University of British Columbia, Canada

Systemic acquired resistance (SAR) is a secondary response following the R protein-mediated HR and it provides general systemic resistance against a broad-spectrum of pathogens. Salicylic acid (SA) is a necessary and sufficient signal to induce the SAR response, which includes the up-regulation of PR (pathogenesis-related) genes. NPR1 is required for SA-induced PR gene expression and pathogen resistance. NPR1 interacts with a subgroup of TGA transcription factors, TGA2, TGA5 and TGA6. We previously showed that induction of PR gene expression and pathogen resistance by the SA analog INA is blocked in the tga2-1 tga5-1 tga6-1 triple knockout mutant, indicating that TGA2, TGA5 and TGA6 are essential for SAR like NPR1. The Arabidopsis genome contains seven additional TGA transcription factors in addition to TGA2, TGA5 and TGA6. Sequence analysis indicates that TGA1 is closely related to TGA4. We found that the tga1 tga4 double mutant plants were more susceptible to the virulent bacterial pathogen *Pseudomonas syringae* pv *maculicola* ES4326 (P. s. m. ES4326), although the SA-induced PR gene expression is not affected in the double mutant. To determine whether TGA1 and TGA4 function in the NPR1-independent resistance pathway, we crossed the tga1 tga4 double mutant with the snc1 npr1 double mutant in which NPR1-independent resistance against P. s. m. ES4326 is constitutively activated. snc1 contains a gain-of-function mutation in a TIR-NBS-LRR-type of R-protein. In the snc1 npr1 tga1 tga4 quadruple mutant, the constitutive resistance to P. s. m. ES4326 in snc1 npr1 is blocked by the tga1 tga4 double mutation, suggesting that TGA1 and TGA4 are required for the NPR1-independent resistance. We have also created the tga1 tga4 npr1 triple mutant. The triple mutant is more susceptible to P. s. m. ES4326 than the tga1 tga4 and npr1 alone, and displayed enhanced susceptibility to the oomycete pathogen *Paranospora parasitica* EMMA1 compared to the tga1 tga4 and npr1 mutants, further suggesting that TGA1 and TGA4 is important for the regulation of NPR1-independent resistance.

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Screening for resistance to bacterial and fungal pathogens in Rice-Arabidopsis FOX lines overexpressing full-length rice cDNA. Masaki Mori¹, Joseph Gogo Dubouzet¹, Satoru Maeda¹, Miki Otake¹, Takanari Ichikawa², Youichi Kondo², Minami Matsui², Kenji Oda³, Hiroshi Takatsuki¹, Hirohiko Hirochika¹. ¹National Institute of Agrobiological Sciences, Tsukuba, Japan, ²RIKEN PSC, Yokohama, Japan, ³RIBS, Okayama, Japan

About 13,000 full-length cDNAs of rice were mixed and ligated to an expression vector cassette. They were introduced into Arabidopsis by Agrobacterium-mediated floral dip transformation to generate Rice-Arabidopsis FOX (full-length cDNA over-expressor) lines. Three-week-old T2 seedlings of approximately 20,000 FOX lines were screened for resistance to a bacterial pathogen, *Pseudomonas syringae* pv *tomato* DC3000 (Pst3000), by dip inoculation. About 100 resistant lines were selected after 2-3 rounds of screening and the rice cDNAs inserted in their genomes were identified. Some of these genes that passed further evaluation have been re-introduced into Arabidopsis and rice for overexpression to confirm the disease resistance phenotypes. At present, overexpression of three transgenic Arabidopsis (Columbia) lines expressing rice cDNAs exhibited repeatable resistance to Pst3000. Two of the rice cDNAs have, upon overexpression in rice (Nipponbare), provided resistance to *Xanthomonas oryzae* cryzze, the pathogen that causes Rice Blight, in T0 generation. Overexpression of one cDNA that enabled resistance to *Xanthomonas* in rice had marked pleiotropic effects that included weak root regeneration, low fertility, and large grain. Seed progeny of these resistant lines will be re-screened for resistance to *Xanthomonas* and other rice pathogens. In addition, approximately 12,000 FOX lines were screened for resistance to the fungal pathogen *Colletotrichum higginsianum*, whose infection mechanisms in Arabidopsis are similar to those of Magnaporthe grisea in rice. Both of these fungi form appressoria and penetration pegs. About 30 resistant lines were selected after 2-3 rounds of screening and the rice cDNAs inserted in their genomes were identified. The selected lines are being subjected to other requisite analytical processes similar to that applied to the Pst3000-resistant lines.

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Arabidopsis EDR1, EDR2 and EDR3 regulate plant defense responses to powdery mildew. Haozhen Nie¹, Huirong Pan¹, Yiping Wang¹, Yingying Wu¹, Chunpeng Yao¹, Ting Zhao¹, Roger Innes², Dingzhong Tang¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing China 100101, ²Department of Biology, Indiana University, Bloomington, IN, USA 47405

The edr1, edr2, and edr3 mutants of Arabidopsis display enhanced disease resistance to the powdery mildew pathogen *Erysiphe cichoracearum*. Leaves from edr mutant plants form dramatic lesions upon infection with *E. cichoracearum*. Powdery mildew resistance mediated by edr1, edr2 and edr3 is SA dependent, but JA and ethylene independent. Among them, EDR1 encodes a CTR1-like protein, consisting of an N-terminal putative regulatory domain and a C-terminal kinase domain. EDR1 displays kinase activity in vitro. EDR2 encodes a novel protein, consisting of a putative pleckstrin homology (PH) domain and a StAR (Steroidogenic Acute Regulatory protein)-related lipid-transfer (START) domain. The PH and START domains are implicated in lipid binding, suggesting that EDR2 may regulate defense responses through lipid signaling. EDR3 encodes a dynamin like protein (DRP1E), consisting of a N-terminal GTPase and a C-terminal GTPase effector domain. Analysis of EDR3-GFP fusion proteins revealed that EDR3 is at least partially localized to mitochondria, suggesting that EDR3 may regulate programmed cell death via modification of mitochondrial function. The edr3 phenotype suggests a mechanistic link between salicylic acid signaling, mitochondria and programmed cell death in plants. Currently, we are performing molecular and biochemical analyses on the EDR1, EDR2, EDR3 and hope to identify other components in these pathways using genetic and molecular approaches.

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A BRASSINOLIDE-INDEPENDENT ROLE FOR THE BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) IN PLANT CELL DEATH CONTROL AND IMMUNITY TO NECROTROPHIC FUNGAL INFECTION. Thorsten Nürnberger¹, Birgit Kemmerling¹, Anne Schwedt¹, Sara Mazotta¹, Patricia Rodriguez¹, Sandra Postel¹. ¹ZMBP Plant Biochemistry, University of Tuebingen, Germany

Programmed cell death (PCD) is a common host response to microbial infection. In plants, PCD has been associated with immunity to biotrophic pathogens, but has also been shown to promote disease upon infection by necrotrophic pathogens. Therefore, execution and extent of plant cell suicidal programs must be strictly controlled. We have demonstrated that the Arabidopsis thaliana Brassinosteroid Insensitive 1 (BRI1)-Associated receptor Kinase 1 (BAK1), which operates as a co-receptor of BRI1 in brassinolide-dependent plant growth control, also regulates the containment of microbial infection-induced cell death. BAK1-deficient plants develop spreading necrosis upon microbial infection, which is accompanied by production of reactive oxygen intermediates and which results in enhanced susceptibility to necrotrophic fungal pathogens. Exogenous application of BL rescues growth defects of bak1 mutants, but fails to restore immunity to fungal infection. Moreover, BL-insensitive and deficient mutants do not exhibit enhanced susceptibility to fungal infections. Together, these findings suggest that plant steroid hormone signalling is not required for the containment of infection-induced PCD. We propose a novel, BL-independent function of BAK1 in plant cell death control that is distinct from its BL-dependent role in plant development.

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Differential expression of cell wall metabolism-, cell cycle- and hormone response-related genes in the interactions between "Arabidopsis" and geminivirus. Jungan Park¹, Yuchul Jung¹, Seungmi Kim¹, Jongbum Park², Kenn Buckley³, Keith R. Davis⁴, Sukchan Lee¹. ¹Dept. of Genetic Engineering, Sungkyunkwan University, Suwon, South Korea, ²Dept. of Life Science, Silla University, Busan, South Korea, ³Dept. of Molecular Genetics, Plant Biotechnology Center, and Program in Molecular, Cellular and Developmental Biology, The Ohio State University, Columbus, Ohio, USA, ⁴Mitchell Memorial Cancer Center, KY, USA

Beet severe curly top virus (BSCTV) has the monopartite genome of single-stranded DNA as a member of the geminivirus. BSCTV is transmitted by leafhopper, and can infect broad host plants of dicot. BSCTV causes systemic symptoms such as stunted growth, callus formation, curling and the accumulation of anthocyanin pigments in "Arabidopsis thaliana". Particularly, BSCTV induces abnormal cell divisions on tips of inflorescent stems and roots in "Arabidopsis". The internal structures of the BSCTV infected or BSCTV C4 ORF expressing transgenic "Arabidopsis" showed that cell divisions were induced on phloem cells and its surrounding cortex cells. To elucidate basis of the symptom development caused by BSCTV, real-time quantitative RT-PCR and DNA microarray were carried out to analyze the transcription profile involved in symptom development. Altered gene expressions such as hormone metabolism-, cell wall biosynthesis- and cell cycle-related genes shown altered cell structures caused by BSCTV infection or C4 expression may play a critical role on typical symptom development by the interaction between "Arabidopsis" and BSCTV.

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"Arabidopsis thaliana" homeobox gene, "ATHB12" is induced by Beet Severe Curly Top Geminivirus infection. Jungan Park¹, Kun-sup Lee¹, Hyunsik Hwang², Yong Rhee¹, Choong-Il Cheon³, Chungkyoon Auh⁴, Sukchan Lee¹. ¹Dept. of Genetic Engineering, Sungkyunkwan University, Suwon, South Korea, ²Dept. of Molecular Genetics, Plant Biotechnology Center, and Program in Molecular, Cellular and Developmental Biology, The Ohio State University, Columbus, Ohio, USA, ³Dept. of Biological Science, Sookmyung Womens University, Seoul, South Korea, ⁴Dept. of Life Science, Mokpo National University, Mokpo, Korea

"ATHB12" is an "Arabidopsis thaliana" homeobox gene, and a member of the homeodomain-leucin zipper (HD-Zip) family. It has been generally reported that "ATHB12" was induced by abscisic acid (ABA) and water stress. Moreover, recent findings strongly suggest that "ATHB12" expression is also induced by the infection of beet severe curly top geminivirus (BSCTV) to "Arabidopsis". BSCTV develops an array of morphological abnormalities such as leaf curling, stunting, and callus-like structure on the infected "Arabidopsis". However, on molecular level, the direct correlation was not yet established between patho-morphogenesis and HD-Zip gene in BSCTV infected "Arabidopsis". At this time, regulation/expression of "ATHB12" gene under the environmental (biotic & abiotic) stress was studied with an array of transgenic "Arabidopsis" with over/under-expressed "ATHB12" gene. The real-time quantitative RT-PCR results indicated that expression of HD-Zip protein members as well as cell cycle- and hormone-related genes were regulated by BSCTV infection.

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Over-expression of wild rice (*Oryza grandiglumis*) OgPAE1 conferred fungal resistance against *Botrytis cinerea* infection. Jung-Hun Pak¹, Eun-Hee Jeon¹, Mi-Jin Kim¹, Hye-Jeong Kim¹, Ki-Jung Lee¹, Young-Soo Chung¹, Kyung-Ho Kang². ¹Dept. of Genetic Engineering, Dong-A University, Busan, Korea, ²National Crop Experiment Station, Suwon, Korea

Oryza grandiglumis (CCDD, 2n=48), one of the wild rice species, has been known to possess fungal resistance against sheath blight, rice blast, bacterial leaf blight and insect resistance against brown plant hopper(*Nilaparvata lugens*). Here we report the cDNA cloning, expression characterization and resistance against *Botrytis cinerea* of OgPAE1 gene. OgPAE1 contains 714bp of nucleotides and 237 amino acids. The highest homology was observed with OsPAE1 mRNA for alpha 5 subunit of 20S proteasome from *Oryza sativa* (japonica cultivar-group). The 20S proteasome is the proteolytic complex that is involved in removing abnormal proteins and other various biological functions. In this study, we observed resistance of OgPAE1 gene against *Botrytis cinerea*. The OgPAE1 gene was induced by wounding, yeast extract, jasmonic acid (JA) and salicylic acid (SA), protein phosphatase inhibitors cantharidin (CN) as well as entodol (EN). To identify in vivo function of gene OgPAE1, the gene was transformed into *Arabidopsis thaliana* and high concentration of *Botrytis cinerea* (5 × 10⁵/mL) was inoculated on the OgPAE1 transgenic plants. The growth of *Botrytis cinerea* was suppressed in OgPAE1 transgenic plants but most of control plants destroyed completely. Average necrosis sizes of transgenic plants were 3.8 through 6.4 mm², whereas that of control was 11.2 mm². Our results suggest that OgPAE1 may contribute to partial resistance against fungal diseases, even though clear in vivo function of the gene has not been reported yet from any plant species.

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Toward isolation and characterization of the protein complex containing the *Arabidopsis* disease resistance protein RPS2. Yiping Qi¹, Fumiaki Katagiri¹. ¹University of Minnesota-Twin city, USA

The *Arabidopsis* resistance (R) gene RPS2 encodes a NB-LRR protein which recognizes the avirulence protein AvrRpt2 from the bacterial pathogen *Pseudomonas syringae*. Accumulating evidence suggests that NB-LRR proteins function in a large protein complex which may change dynamically after activation. Identification of unknown members of the RPS2 protein complex will provide insights into the mechanisms controlling early events in RPS2-mediated gene-for-gene resistance.

To facilitate purification of R protein complexes, we devised a tripartite sequence termed the HPB tag. This tag, based on the concept in Zhong et al. (2003), consists of one copy of the HA epitope tag, a Prescission protease recognition site, and the biotinylation site of the *Arabidopsis* 3-methylcrotonyl-CoA carboxylase. The biotinylation sequence facilitates high affinity purification using a streptavidin matrix, while the HA tag allows protein visualization using a Western Blot. We have demonstrated that the C-terminal RPS2-HPB fusion protein is functional, is biotinylated in planta, and can be purified from plant extracts using a streptavidin matrix. Protein fractions purified from RPS2-HPB-expressing plants were compared to those from untransformed plants using SDS-PAGE and silver staining. Several unique protein bands were identified from RPS2-HPB-expressing plants. We are currently identifying the RPS2-HPB-specific proteins using mass-spectrometry-based methods.

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The Arabidopsis MAP kinase 4 substrate, MKS1, Contribute to Basal Resistance and Exist in Complex with WRKY33 in planta. Jin-Long Qiu¹, Klaus Petersen¹, Berthe Katrine Friis¹, Juri Lutje¹, John Mundy¹, Morten Petersen¹.¹ Department of Molecular Biology, Copenhagen University, Copenhagen Biocenter, Ole Maaløes Vej 5, 2200 Copenhagen N, Denmark

Recently, we identified MKS1 as a MPK4 substrate and analyses of transgenic plants and genome-wide transcript profiling indicated that MKS1 is required for full SA-dependent resistance in mpk4 mutants, and that overexpression of MKS1 in wild-type plants is sufficient to activate SA-dependent resistance (Andreasson et al., 2005). In addition, the transcription factors WRKY25 and WRKY33 were found to interact with MKS1 in yeast suggesting that these two WRKY factors could function downstream of MPK4 in controlling the expression of various defense genes (Andreasson et al., 2005). Here we demonstrate that MKS1 also play a pivotal role in basal resistance. Loss of MKS1 leads to enhanced growth of virulent strains of *P. syringae* and PR1 accumulation is severely repressed in the initial phases of infection on mks1 mutants. In addition, we find that MKS1 interacts with WRKY33 in vivo suggesting that MPK4 may control gene expression through WRKY33 via MKS1. PR1 accumulates to wild-type levels in wrky33 mutants infected with both virulent and avirulent strains of *P. syringae*. However, comparative microarray analysis of wrky33 and wild type shows that other defense genes fails to accumulate in wrky33 after SA treatment but also after virulent and avirulent infections. Thus WRKY33 is required for induction of only a small subset of SA-regulated defense genes.

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Insights about the role of CDR1 in the complex reiterative signal networks underlying disease resistance. Maria C. Romero-Puertas¹, Chris J. Lamb¹.¹ Disease and Stress Biology Department, John Innes Center, Norwich NR4 7UH, UK

From a screen of ~ 5,000 lines with a T-DNA carrying cauliflower mosaic virus 35S enhancer, we identified Constitutive Disease Resistance 1 (CDR1) encoding an apoplastic aspartic protease, over expression of which causes dwarfing and resistance to virulent *Pseudomonas syringae*. These phenotypes reflect salicylic acid-dependent activation of micro oxidative bursts and various defence-related genes. Antisense CDR1 plants were compromised for resistance to a-virulent *P. syringae* and more susceptible to virulent strains than wild type. The CDR1 protein accumulates in the apoplast in response to pathogen attack. Induction of CDR1 generates a small mobile signal, and CDR1 action is blocked by the protease inhibitor pepstatin and by mutations in the protease active sites. Thus CDR1 mediates a peptide signal system involved in the activation of inducible resistance mechanisms. We have explored how CDR1 is integrated within the disease resistance signal network to position CDR1 action stage relative to other key players such as small molecule signals like ROS, salicylic acid and signal transduction proteins. Thus, transgenic plants containing CDR1 under the control of the dexamethasone inducible TA promoter (TApr::CDR1) have been crossed with the following key mutant/transgenic phenotypes: *npr1-1*, *eds1-1*, *pad4-5*, *dir1-1*, *rboh D*, *rboh F*, *rboh D/F* and *sid2-2*. As final goal, ROS production, cell death and gene induction have been analyzed to determine if RBOH D/F, NPR1, EDS, DIR, PAD4 and SA are required for both, local and systemic effects of CDR1.

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Signaling crosstalk between PAMP-triggered immunity and UVB/sucrose stress responses. Yusuke Saito¹, Nico Tintor¹, Francesca Ceron¹, Hye Sup Yun¹, Silke Robatzek¹, Paul Schulze-Lefert¹.¹ Max-Planck Institute for Plant Breeding Research, Cologne, Germany

Plants recognize encounters with microbes by detection of molecular patterns that are conserved in many microbial species, designated pathogen-associated molecular patterns (PAMPs). PAMP perception triggers immune responses that restrict microbe invasion and growth. Bacterial flagellin and elongation factor Tu (EF-Tu) act as effective elicitors of PAMP-triggered immune responses in *Arabidopsis*. However, constitutive stimulation of PAMP-signaling leads to growth retardation, suggesting that defense activation is achieved at the cost of growth-related processes. We show that UVB- and sucrose-induced flavonoid accumulation, a characteristic response to these abiotic stresses, is abolished in young seedlings in the presence of PAMPs.

PAMP-signaling activation diminishes UVB- and sucrose-induced elevation in the mRNA levels of "chalcone synthase" ("chs"), encoding for a key enzyme in the flavonoid biosynthetic pathway. Accordingly, CHS steady-state levels do not increase upon exposure to PAMPs and UVB/sucrose. The two PAMPs Flagellin and EF-Tu confer similar repression of abiotic stress-induced flavonoid accumulation that is dependent on the presence of cognate PAMP receptors FLS2 and EFR, respectively. Thus, it seems likely that PAMP-mediated suppression of flavonoid accumulation occurs downstream of a presumed convergence point for multiple PAMP receptor-initiated signaling pathways. Furthermore, the ease and robustness of flavonoid visualization and quantification in *Arabidopsis* seedlings provide an ideal system for mutant screens. We present the preliminary characterization of mutant plants defective in PAMP-signaling and its crosstalk with UVB/sucrose-mediated control of flavonoid metabolism.

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TRILATERAL TIES: PLANT GROWTH PROMOTION BY INTERACTION OF SOIL BACTERIA AND AMOEBA. Tony R. Schaeffner¹, Kristin Krome², Katja Rosenberg², Birgit Geist¹, Stefan Scheu², Michael Bonkowski².¹ GSF Research Center for Environment and Health - Institute of Biochemical Plant Pathology, 85764 Neuherberg/München, Germany,² Darmstadt University of Technology-Institute of Zoology-Rhizosphere Ecology Group, 64287 Darmstadt, Germany

Increased plant growth due to soil protozoa-soil bacteria interactions in the rhizosphere is well documented and generally has been assigned to nutrient release from consumed bacterial biomass. However, protozoa are highly selective grazers on bacteria and more recent investigations indicate that protozoa-induced changes in bacterial community composition, rather than nutrient effects may be additionally responsible for plant growth promotion in presence of protozoa.

In order to gain a better understanding of the underlying mechanisms in protozoa-bacteria-plant interactions, we established a sand-litter system with *Arabidopsis thaliana* grown either completely sterile, in presence of soil bacteria or bacteria plus the amoeba *Acanthamoeba castellanii*, an abundant protozoan predator of bacteria. Compared to sterile treatments, plant rosette diameter, shoot biomass and seed production increased significantly in presence of bacteria, but was further enhanced in presence of amoebae. Measurements of plant-available nitrogen in soil indicated that the plant growth promoting effect of amoebae cannot be exclusively explained by an enhanced nutrient availability. However, analyses of the bacterial communities demonstrated shifts in bacterial species composition in presence of amoebae.

Analyses of the plant transcriptome revealed that a number of genes involved in secondary metabolism, which were up-regulated by soil bacteria, were down-regulated in presence of amoebae. In addition, several growth associated genes were up-regulated under the latter conditions. We hypothesize that strong grazing-induced shifts in bacterial community composition most likely ameliorated the plant defense-response to bacteria by down-regulation of plant defense genes and genes involved in the production of plant secondary metabolites, resulting in a plant growth promoting effect of protozoa.

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Nuclear activity of MLA immune receptors links isolate-specific and basal resistance responses. Qian-Hua Shen¹, Yusuke Saito¹, Stefan Mauch¹, Christoph Biskup², Stephan Bieri³, Beat Keller³, Hikaru Seki¹, Bekir Ülker¹, Imre Somssich¹, Paul Schulze-Lefert¹. ¹ Max Planck Institute for Plant Breeding Research, Köln, Germany, ²Friedrich-Schiller-University of Jena, Jena, Germany, ³University of Zürich, Zürich, Switzerland

Plant immune responses are triggered by pattern recognition receptors that detect conserved pathogen-associated molecular patterns (PAMPs) or by resistance (R) proteins recognizing isolate-specific pathogen effectors. The polymorphic barley MLA R locus encodes allelic intracellular receptors containing an N-terminal coiled-coil (CC) structure, a central nucleotide binding (NB) site, and a leucine-rich repeat (LRR) region. MLA receptors share >90% sequence identity but recognize isolate-specific *B. graminis f sp hordei* effector. We show that in barley MLA R proteins function in the nucleus to confer resistance against the powdery mildew fungus. The recognition of the fungal AVRA10 effector by MLA10 induces nuclear associations between receptor and the barley transcription factors. The identified WRKY proteins act as repressors of PAMP-triggered basal defence in barley. Two *Arabidopsis* WRKY TFs, AtWRKY18/40 that are the closest homologs of *HvWRKY1/2*, also function as repressors of basal defence in *Arabidopsis*. Genome-wide gene expression profiling data obtained upon inoculated with virulent *P. syringae* DC3000 in *Arabidopsis* reveals that 21 out of 23 up-regulated genes are PAMP-responsive genes, including the 6-fold up-regulated *SID2*. MLA appears to interfere with the WRKY repressor function, thereby de-repressing PAMP-triggered basal defence. Our findings imply a mechanism by which the MLA NB-LRR immune receptors integrate distinct pathogen signals, and suggest that the MLA immune receptors affect the transcription machinery to activate an efficient immune response.

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Arabidopsis SNI1 Complex Regulates both Gene Transcription and DNA Recombination During the Defence Response. Jungi Song¹, Wendy Durrant¹, Ek Han Tan¹, Xinnian Dong¹. ¹ Duke University, Durham, NC 27708, USA

Systemic acquired resistance (SAR) is a general defence mechanism that confers long-lasting and broad-spectrum plant disease resistance that occurs after an initial infection. Induction of SAR involves salicylic acid (SA)-mediated activation of pathogenesis-related (PR) genes. SNI1 (suppressor of NPR1, inducible) functions as a negative regulator of SAR required to dampen the basal PR gene expression. A suppressor of SNI1, RAD51D has been recently cloned that is involved in SA-dependent NPR1-independent PR gene expression. Here we report the identification and characterization of another SNI1 suppressor, SSN2. Map-based cloning of SSN2 revealed that it encodes a novel protein containing a SWIM domain which is predicted to have DNA-binding and protein-protein interaction functions in different contexts. Physical interaction between SSN2, SNI1 and RAD51D indicate that these proteins can form a complex that plays a dual role in regulating both PR gene transcription and DNA recombination during the defense response.

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Characterization of a Chinese cabbage cyclophilin with anti-fungal activity. Mi Rim Shin¹, Jin Ho Park¹, Ho Byoung Chae¹, Deok Ho LEE¹, Kyun Oh Lee¹, Woe-Yeon Kim¹, Sang Yeol Lee¹. ¹ Environmental Biotechnology National Core Research Center, PMB-BRC & Division of Applied Life Science(BK21), Gyeongsang National University, Jinju 660-701 Korea

An antifungal protein that inhibits the growth of filamentous fungal pathogens was isolated from Chinese cabbage (*Brassica campestris* L. ssp. pekinensis) by affinity chromatography on Affi-gel blue gel and ion exchange chromatography on CM-Sepharose. The N-terminal amino acid sequence of the protein was highly homologous to that of plant cyclophilins and consequently the protein was denoted as C-CyP. To understand the antifungal activity of C-CyP, we isolated a cDNA encoding its gene from a Chinese cabbage leaf cDNA library. The Chinese cabbage genome bears more than one C-CyP gene copy and C-CyP mRNA is highly expressed in all tissues except the seeds. Recombinant C-CyP catalyzed the cis-trans inter-conversion of the Ala-Pro bond of the substrate, which indicates this protein has peptidyl-prolyl cis-trans isomerase activity. It also inhibited the growth of several fungal pathogens.

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'Ascophyllum nodosum' extract induces resistance in 'Arabidopsis thaliana' against 'Pseudomonas syringae' pv 'tomato' DC3000. Sowmyalakshmi Subramanian¹, Bruce A. Gray¹, Rudra P. Singh², Simon D. Hankins³, Alan T. Critchley³, D. Mark Hodges⁴, Balakrishnan Prithiviraj¹. ¹ Nova Scotia Agricultural College, Truro, NS, Canada B2N 5E3, ²Agriculture and Agri-Food Canada, 850 Lincoln Rd., Fredericton, NB Canada, E3B 4Z7, ³Acadian Seaplants Limited, 30 Brown Avenue, Dartmouth, NS, Canada B3B 1X8, ⁴Agriculture and Agri-Food Canada, 32 Main St. Kentville, NS, Canada B4N 1J5

Experiments were conducted to study the effect of extracts of a brown seaweed 'Ascophyllum nodosum' on the induction of resistance in 'Arabidopsis thaliana' against a bacterial pathogen 'Pseudomonas syringae' pv 'tomato' DC3000 (Pst). 'A. thaliana' was treated with 'A. nodosum' extracts by root irrigation followed by inoculation of the leaves of the treated plants with Pst. 'A. nodosum' extracts significantly reduced the area of leaf infected and subsequent disease intensity (DI). This reduction in the DI correlated with a decline in the bacterial titer in the leaf apoplast. Surprisingly, addition of seaweed extracts to the bacterial culture medium enhanced the growth of Pst, suggesting that 'A. nodosum' induced systemic disease resistance in 'A. thaliana'. PR1 (Pathogen related protein 1) gene, a marker gene associated with induced resistance was upregulated by seaweed extract treatments. Taken together the results suggest that the extracts of 'A. nodosum' elicit systemic acquired resistance in 'A. thaliana'.

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"MtERF5", a regulator of rhizobial symbiosis in "Medicago truncatula". Tatiana Vernié¹, Sandra Moreau¹, Françoise De Billy¹, Fabienne Vailleau², Marie-Françoise Jardinaud², Giles Oldroyd³, Christian Rogers³, Pascal Gamas¹, Andreas Niebel¹. ¹Laboratoire des Interactions Plantes Micro-organismes, INRA-CNRS, Castanet Tolosan, France, ²Symbiosis and Plant Pathologies, INP-EN-SAT, Castanet-Tolosan, France, ³John Innes Center, Norwich, UK

"Arabidopsis" has been successfully used as a model plant to study plant-pathogen interactions during the last years leading to the functional characterisation of key regulators during this type of interaction. Unfortunately species of the Brassicaceae are unable to undergo mutualistic interactions such as mycorrhizal or nitrogen-fixing symbioses. Leguminous plants for which various genetic and genomic tools have been recently developed open the unique opportunity to study molecular dialogues between symbiotic bacteria of the Rhizobiaceae family and the plant host. Using "Medicago truncatula" as a model plant, we performed transcriptomic approaches to get better insights into the way legumes control symbiotic rhizobial infection and nodule development. Among a set of genes up-regulated during nodulation, we identified "MtERF5", a transcription factor (TF) belonging to the ERF (Ethylene Response Factor) subfamily of the AP2/EREBP family, known to play a role in biotic stresses. Expression studies by RT-QPCR showed an induction in roots 3 days post "Sinorhizobium meliloti" inoculation and a maximal expression in young nodules. We localized "MtERF5" expression in dividing cells of young nodule primordia and in the infection zone of mature nodules by "in situ" hybridisation and promoter-Gus fusions. We also analysed "MtERF5" expression in the context of various symbiotic bacterial and plant mutants. "MtERF5" appears as a regulator of symbiosis as suggested by the phenotype of transgenic roots expressing RNAi or over-expression constructs, and more recently of a deletion mutant. "MtERF5" may also be a regulator of pathogenic infections, since it is up-regulated by "Ralstonia solanacearum", a bacterial root pathogen. We are now looking for MtERF5 targets by transcriptomic approaches and preliminary results suggest that "MtERF5" could play a role in the cytokinin signalling pathway.

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Perception and Signaling of Chitooligosaccharides in Arabidopsis. Jinrong Wan¹, Xuecheng Zhang¹, Steve Clough², Katrina Ramonell³, Sung-Yong Kim¹, Minviluz G. Stacey¹, Shuqun Zhang¹, Geon Hui Son⁴, Jong Chan Hong⁴, Gary Stacey¹. ¹University of Missouri, Columbia, MO, USA, ²USDA-ARS, University of Illinois, Urbana, IL, USA, ³University of Alabama, Tuscaloosa, AL, USA, ⁴Gyeongsang National University, Jinju, Korea

Chitin is a polymer of N-acetyl-D-glucosamine. It is found in the cell walls of plant pathogenic fungi and is recognized by the plant as a pathogen-associated-molecular pattern (PAMP), which triggers a general defense response. To study chitin signaling and its role in plant defense, we took advantage of the model plant Arabidopsis thaliana. In Arabidopsis, there are five LysM RLKs which have an extracellular LysM motif-containing domain, a transmembrane domain and an intracellular serine/threonine kinase domain. Mutation in one of them blocked the induction of almost all chitin-responsive genes by chitin, suggesting that this LysM RLK may play a critical role in perceiving chitin signal. The mutation also leads to more susceptibility to fungal pathogens, suggesting a connection between chitin signaling and plant defense. Further studies demonstrate that a MAPK cascade and several WRKY transcription factors appear to be involved in the chitin signaling pathway to mediate gene regulation and defense.

P-692

Salicylic Acid Antagonizes the Auxin Signaling Pathway.
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The phytohormone auxin regulates almost every aspect of plant development. A growing body of evidence indicates that during pathogen infection, auxin homeostasis is perturbed. Many plant pathogens either produce auxin themselves or manipulate host auxin biosynthesis to interfere with the host normal developmental processes. Disruption of bacterial auxin synthesis genes compromised virulence, suggesting that manipulation of auxin signaling be taken by pathogenic microbes as a virulence strategy. In response, plants likely evolved mechanisms to regain control over auxin signaling. Auxin molecules are recognized by the auxin receptor TIR1 and related proteins; these auxin-bound F-box proteins then target the Aux/IAA family of transcriptional repressors of auxin response for degradation. Bacterial flagellin has been shown to trigger rapid degradation of auxin receptor transcripts via a microRNA, resulting in down regulation of the auxin signaling pathway and an increase in basal resistance to the pathogen. Recognition of flagellin and other pathogenesis-associated molecular patterns often leads to long-lasting and broad-spectrum resistance mediated by the defense signaling molecule salicylic acid (SA). Here, we demonstrate that activation of the SA pathway strongly desensitize plants responses to both exogenous and endogenous auxin. Furthermore, we provide evidence that SA exerts its inhibitory effect on auxin by stabilizing the Aux/IAA repressor proteins. This antagonism between a major defense signal and a key growth hormone may enable the plant to adjust global homeostasis in response to a changing environment.

P-693

Chip-based cloning of "Suppressor of SNI1 3" ("SSN3").
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The Arabidopsis "Nonexpresser of PR genes 1" ("NPR1") is a key positive regulator of salicylic acid (SA). The "npr1" mutant abolishes the SA-induced expression of "Pathogenesis-related" ("PR") genes. In the "npr1-1" background, the "sni1" ("suppressor of npr1-1, inducible 1") mutant restored the inducibility of the "PR" genes. This indicates that an SA-dependent and "NPR1"-independent pathway is involved in the expression of PR genes. To identify those genes belong to this pathway, a genetic screen was conducted for "Suppressors of SNI1" ("SSN") genes. The "SSN1" ("RAD51D") and "SSN2" have successively been cloned. Here, we report the cloning of "SSN3" gene. The "ssn" mutants were generated by fast neutron bombardment and the "ssn3" locus has 6 alleles: 6C-1, 9F-1, 13F-1, 13G-1, 17A-1 and 21B-1. Using Arabidopsis Tiling Array (Affymetrix), a deletion (more than 10 kb) was found in 9F-1 allele. In addition, mutations were identified in other alleles (6C-1, 13F-1, 13G-1 and 21B-1) by sequencing their genomic DNA. So far, the "ssn3" mutants have been complemented by the "SSN3" cDNA driven by the "3S5" promoter. According to our data, SSN1, SSN2 and SSN3 may form a chromatin remodeling complex to sense the SA signal and to promote the expression of "PR" genes.

P-694

Complex regulatory pathways control the basal defense response. Yiji Xia¹, Huifen Zhu¹, Yongdun Xie^{1,2}, Shengbing Wang¹, Xiaochun Ge^{1,3}, Guojing Li¹. ¹Danforth Plant Science Center, ²China Agricultural University, ³Fudan University

The basal defense response/innate immune response can be triggered when host cells recognize microbe-associated molecular patterns (MAMPs) and is delicately regulated through interplay of both positive and negative pathways. The regulatory components that control basal resistance remain largely unknown. Using functional genomics and reverse genetic approaches, we have identified several *Arabidopsis* genes that play positive or negative roles in basal immunity. AtNUDT7, a Nudix protein that hydrolyzes nucleotide derivatives including NADH and ADPR, was found to be a negative regulator of basal immunity. NUDT7 modulates two distinct branches of the defense response pathway and functions to prevent excessive cellular stimulation. The *nudt7* mutation leads to perturbation of cellular redox homeostasis which potentiates an amplified defense response when provoked by biotic and other stresses. We have also found that heterotrimeric G protein play a positive role in activation of the basal defense response. In addition, a nuclear gene encoding a transcriptional regulator that acts in chloroplast has been found to be involved in the defense response. The transcription factor likely enhances expression of some plastid genes encoding ribosomal proteins. The study may shed light on how the photosynthetic organelle plays a role in disease resistance. Our findings further reveal the complexity of the mechanisms that regulate basal disease resistance.

P-696

A *Pseudomonas syringae* Effector Inactivates MAPKs to Suppress PAMP-Induced Immunity. Jie Zhang^{1,2}, Feng Shao², Yan Li², Haitao Cui², Linjie Chen², Hongtao Li², Yan Zou², Chengzu Long², Lefu Lan³, Jijie Chai², She Chen², Xiaoyan Tang³, Jianmin Zhou². ¹Institute of plant physiology and ecology, Shanghai, China, ²National institute of biological sciences, Beijing, China, ³Department of Plant Pathology, Kansas State University, USA

Pseudomonas syringae effector proteins suppress plant immunity to promote parasitism. However, their biochemical function and virulence targets in the plant remain largely unknown. Here we show that HopA11, an effector widely conserved in both plant and animal bacterial pathogens, inhibits the *Arabidopsis* mitogenactivated protein kinases (MAPKs) stimulated by bacterial flagellar peptide flg22, consequently suppressing two independent downstream defenses - one leads to cell wall reinforcement through the AtrbohD-mediated oxidative burst, the other activates the transcription of a large number of genes. The MAPKs MPK3 and MPK6 interact with HopA11 in vitro and in vivo, indicating that they are direct targets of HopA11. HopA11 inactivates the MAPKs by removing the phosphate group from phosphothreonine through a unique phosphothreonine lyase activity, uncovering a novel mechanism by which the bacterium overcomes host innate immunity.

P-695

Negative Regulation of Pathogen Resistance by a Replication Factor Subunit-like Protein in *Arabidopsis thaliana*. Shitou Xia¹, JinGui Chen², Xin Li³, Yuelin Zhang¹. ¹National Institute of Biological Science, Beijing, China, ²Department of Botany, University of British Columbia, Canada, ³Michael Smith Labs, University of British Columbia, Canada

Systemic acquired resistance (SAR) is a plant immune response induced after a local infection by necrotizing pathogens. Expression of SAR in plants involves up-regulation of many Pathogenesis-Related (PR) genes, which work in concert to confer resistance to a broad spectrum of pathogens. Salicylic acid (SA) is an essential signal for the onset of SAR. Because constitutive activation of SAR is detrimental to plants, expression of PR genes must be tightly regulated. In an EMS mutagenesis screen to look for negative regulators of SAR, we found a mutant hypersensitive to INA, an analogue of SA. This mutant has enhanced induction of PR genes and display enhanced resistance against *Peronospora parasitica* (P. p.) Noco2, an oomycete pathogen. The gene was identified by map-based cloning and found to encode a replication factor subunit-like protein (RFL) and the mutant was named rfl-1. rfl-1 mutant plants are smaller than wild type plants with narrower leaves and petals. In the epidermis of cotyledons and true leaves, there are less cells. In the root elongation zone, the number of cortex cells is also lower, indicating that the mutated RFL slows down the cell proliferation and RFL plays important roles in DNA replication. Transforming wild type RFL gene into rfl-1 revert the mutant phenotypes of rfl-1 to wild type, suggesting that the rfl-1 mutation is responsible for the mutant phenotypes observed. As RFL genes may be involved in the replication-coupled chromatin assembly, we propose that chromatin assembly and remodeling are required for the negative regulation of PR genes.

P-697

Gene expression in *Brassica napus* in response to infection by *Sclerotinia sclerotiorum* and identification of WRKY transcription factors. Jianwei Zhao¹, Roger Rimmer¹, Lone Buchwaldt¹, Dwayne Hgedus¹. ¹Agriculture and Agri-Food Canada, Saskatoon, Canada

Sclerotinia sclerotiorum is a pathogenic fungus that can infect a wide range of plant species, including *Arabidopsis thaliana* and several important crop species. The temporal response of a partially resistant genotype of *Brassica napus*, Zhong You 821, to this pathogen was examined using a *Brassica* gene oligo-array representing 15,000 unique genes. RNA was extracted from inoculated stem tissues at 6, 12, 24, 48 and 72 h post-infection and used for micro-array analysis. A total of 7,744 genes were found to be significantly differentially expressed across times. The largest proportion of these genes was differentially expressed at 48 h post-infection, while a smaller group was induced at the earlier times. Hierarchical clustering analysis revealed a temporal expression pattern across times. Exploiting the genome synteny between *B. napus* and *A. thaliana* together with *A. thaliana* gene annotation, the function of proteins encoded by these genes were categorized into pathogenesis-related proteins, protein kinase pathways, secondary metabolism and maintenance of cell wall integrity, abiotic stress, transcription factors as well as some proteins with unknown function. In particular, we found several genes encoding WRKY transcription proteins that were highly induced at the early stages of *sclerotinia* infection. Three homologous WRKY genes were isolated from *B. napus* and over-expressed in *A. thaliana*. These transgenic lines are being screened for phenotypic changes in response to pathogen infection.

Signal Transduction

P-698

The Arabidopsis Somatic Embryogenesis Receptor-like Kinase 1 interacts with the hexameric ATPase CDC48A in a larger complex. Jose Aker¹, Renske Hesselink¹, Rumyana Karlova¹, Jan Willem Borst¹, Ruchira Engel¹, Antonie, J. W. G. Visser¹. ¹Laboratory of Biochemistry, Wageningen University, The Netherlands

The *Arabidopsis thaliana* Cell Division Cycle protein CDC48A was previously shown to interact with the Somatic Embryogenesis Receptor-like Kinase 1 (SERK1) and to co-immunoprecipitate with SERK1 in *Arabidopsis* cultured cells and seedlings. In living cells the CDC48A protein co-localizes with SERK1 at peripheral ER based membranes and the plasma membrane (PM). Förster Resonance Energy Transfer-Fluorescence Lifetime Imaging Microscopy (FRET-FLIM) showed that CDC48A interacts with SERK1 at the PM.

CDC48A is a member of the family of AAA ATPases (ATPases associated with various cellular activities), shown to have various functions in cell division, membrane fusions and in proteasome and ER associated degradation (ERAD) of proteins. The role of AAA ATPases is to generate mechanical force to disrupt or fuse molecular structures by means of ATP binding and hydrolysis. The AtCDC48A protein was shown by others to play a role in ERAD and in membrane fusions. AAA proteins are present as stacked hexameric rings that are stabilized by the binding of ATP. They are only reported to be active in hexameric form. Employing Fluorescence Correlation Spectroscopy, it was shown that in protoplasts the CDC48A hexamers are part of a much larger complex. SERK1 was shown to associate with the active form of the fluorescently tagged CDC48A protein in *Arabidopsis* protoplasts, therefore implicating a functional relationship between both proteins.

P-699

Physiological functions of two structurally distinct tyrosine-sulfated peptides in *Arabidopsis* growth and development. Yukari Amano¹, Mari Ogawa¹, Hidefumi Shinohara¹, Hiroko Tsubouchi¹, Yoshikatsu Matsubayashi¹. ¹Graduate School of Bio-agricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan

Post-translational modification is a major mechanism by which peptides undergo specific structural changes at certain residues that confer special functions to the molecule. In particular, tyrosine sulfation is one of the common post-translational modifications of proteins secreted through the trans-Golgi network. Phytosulfokine (PSK) is a 5-amino-acid tyrosine-sulfated peptide that has been identified in the medium of plant cell cultures, based on the results of assays of growth-promoting activity of cultured cells. Overexpression of PSK or direct treatment of seedlings with PSK peptide stimulates plant growth by promoting cell division and expansion. Genes encoding PSK precursors are expressed at considerable level in a variety of tissues throughout plant life cycle. The PSK receptor PSKR1 was purified from microsomal fractions of carrot cells by ligand-based affinity chromatography and identified as a member of leucine-rich repeat receptor kinase (LRR-RK).

Disruption and overexpression of *Arabidopsis* ortholog of PSKR1 (At-PSKR1) affects cellular potential for growth during plant development. We have also developed a novel procedure for specifically enriching sulfated peptides based on ion-selective interaction of sulfate ions with anion exchangers. By using this procedure, we searched for sulfated peptides contained in culture medium of *Arabidopsis* cells and identified a novel tyrosine-sulfated peptide named PSY1. PSY1 is a 18-amino-acid secreted peptide with no sequence similarity with PSK. Overexpression of PSY1 or direct treatment of seedlings with PSY1 peptide promotes cell expansion. A gene encoding PSY1 precursor is expressed at considerable level in a variety of tissues. We discuss physiological functions of PSY1 and PSK peptide in *Arabidopsis* growth.

P-700

The role of WRKY transcription factors in plant innate immunity. Rainer Birkenbihl¹, M. Shahid Mukhtar¹, Imre Somssich^{1,2}. ¹Max-Planck-Institute for Plant Breeding, Cologne, Germany

The plant innate immune system consists of two interconnected branches termed PTI (PAMP-Triggered Immunity) and ETI (Effector-Triggered Immunity) that initiate massive transcriptional reprogramming. Recently, firm genetic evidence has been obtained demonstrating that WRKY transcription factors play a pivotal role in regulating the plant defense transcriptome. In *Arabidopsis* over 70 % of the entire WRKY gene family of 74 members respond to pathogens or pathogen-mimicking stimuli. Loss-of-AtWRKY33 function renders plants susceptible to infection by two necrotrophs [1], whereas Atwrky18/Atwrky40 and Atwrky11/Atwrky17 double mutants show enhanced resistance towards *P. syringae* DC3000 [2,3]. Moreover, we recently demonstrated that Atwrky18/Atwrky40 double mutants are resistant towards the virulent fungus *G. orontii* and that very likely these WRKY factors form an interface that links terminal PTI and ETI signaling to the transcriptional machinery [4].

Our current studies are focused on: a) using reverse genetics to identify WRKY genes associated with defense and leaf senescence, b) to identify regulators of the immediate-early class of WRKY genes, whose activation does not require de novo protein biosynthesis, and c) use of ChIP to identify *in vivo* targets of candidate WRKY genes. Data will be presented showing that loss-of-AtWRKY27-function results in enhanced tolerance of Col-0 plants towards two vascular bacterial pathogens. For another WRKY transcription factor, At-WRKY33, our results reveal that rapid pathogen-dependent activation of AtWRKY33 itself is almost exclusively controlled by a subset of WRKY factor binding sites (W box elements) within its promoter region. AtWRKY33 functions as a regulator of pathogen defense responses and interacts with MKS1, a putative nuclear coupling factor acting downstream of MAP kinase 4 (MPK4; [5]).

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P-701**RING-type ubiquitin E3 ligase family in *Arabidopsis thaliana*.**

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The ubiquitin pathway catalyzes covalent attachment of the 76-amino acid ubiquitin, typically to epsilon amino groups of substrate proteins, and includes proteins that recognize and catabolize ubiquitylated proteins. Ubiquitylation can affect the activity, localization and/or longevity of the substrate protein. The ubiquitin E3 ligases play an important role in determining specificity of ubiquitylation by interacting with the E2 carrying activated ubiquitin and the substrate. One type of E3 contains a conserved domain called a RING (for Really Interesting New Gene) domain that serves, in part, to interact with the E2. Bioinformatics searches of the predicted *Arabidopsis thaliana* proteome identified over 470 proteins with RING or RING-like domains. Our major goal is to identify the *in vivo* functions for selected RING and RING-like type E3 ligases. cDNAs were isolated and *in vitro* activity assays of recombinant proteins were used to determine whether RING proteins could function as E3 ligases. Publicly available T-DNA insertion lines in over 100 RING domain genes were propagated to isolate homozygous individuals that were subsequently subjected to phenotypic analyses. We have focused detailed studies on three different RING genes that are seedling lethal when the insertion is homozygous. Interacting partners for one group of RING proteins were identified by Y2H analyses and results verified by *in vitro* interaction assays. Altogether, these studies will identify new E3 ligases, their function and aid in our understanding of how ubiquitylation by E2 and RING E3 ligases is regulated. Supported by NSF 2010 program.

P-702**Over-expression of a truncated *Arabidopsis thaliana* heterotrimeric G protein γ subunit results in a phenotype similar to α and β subunit knockouts.**

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Heterotrimeric G proteins (G-proteins) are a diverse class of signal transducing proteins which have been implicated in a variety of important roles in plants. When G-proteins are activated, they dissociate into two functional subunits (α and the $\beta\gamma$ dimer) that effectively relay the signal to a multitude of effectors. In animal systems, the $\beta\gamma$ dimer is anchored to the plasma membrane by a prenyl group present in the γ subunit and membrane localization has proven vital for heterotrimer function. A semi-dominant negative strategy was designed aiming to disrupt heterotrimer function in *Arabidopsis thaliana* (ecotype Columbia) plants by over-expressing a truncated γ subunit lacking the isoprenylation motif (γ^*). Northern analysis shows that the levels of expression of the mutant γ subunit in several transgenic lines (35S- γ^*) are orders of magnitude higher than that of the native subunits. In-depth characterization of the 35S- γ^* lines has been carried out, specifically focusing on a number of developmental characteristics and responses to several stimuli previously shown to be affected in α - and β -deficient mutants. In all cases, the transgenic lines expressing the mutant gamma subunit behave in the same way as the α - and/or the β -deficient mutants, albeit with reduced severity of the phenotype. Our data indicates that signaling from both functional subunits, α and the $\beta\gamma$ dimer, is disrupted in the transgenic plants. Even though physical association of the subunits has been previously reported, our research provides evidence of the functional association of α and β with the γ subunits in *Arabidopsis*, while also suggesting that plasma membrane localization may be critical for function of plant heterotrimeric G proteins.

P-703**Plastidial oleic acid levels modulate defense signaling by regulating expression of resistance genes.**

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Oleic acid (18:1) is one of the major monounsaturated fatty acids (FA) of membrane glycerolipids and its biosynthesis is catalyzed by the soluble stearoyl-acyl-carrier-protein-desaturase (S-ACP-DES). We have previously shown that changes in the levels of 18:1 results in the alteration of salicylic acid (SA)- and jasmonic acid-mediated defense responses (1, 2, 3, 4, 5, 6). This is evident in the *Arabidopsis ssi2* mutant, which encodes a defective S-ACP-DES and consequently accumulates high levels of stearic acid (18:0) and low levels of 18:1. Consequently replenishing 18:1 levels results in restoration of wild-type-like signaling in the *ssi2* mutant (2, 4, 5, 6). Plants carrying low levels of 18:1 exhibit enhanced resistance to virulent pathogens, as well as R-gene specific resistance to viral (Turnip crinkle virus-TCV) and bacterial pathogens (7, 8). We have recently shown that the 18:1-mediated pathway regulates defense signaling by upregulating expression of multiple R genes (9). Normalizing 18:1 levels by second-site mutations restores R gene expression.

Intriguingly, TCV inoculation does not activate the 18:1-regulated pathway in resistant plants, instead it results in the induction of several genes that encode 18:1-synthesizing isozymes. Consequently 18:1 levels in the plant remain constant during a resistance response to TCV. These data suggest that the 18:1-regulated pathway may be specifically targeted during pathogen infection and that alterations of 18:1 levels may serve as a novel strategy for promoting disease resistance.

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P-704**A Novel Regulator of Ethylene Receptor Signaling.**

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The gaseous hormone ethylene plays an important role in plant growth and development and responses to environmental stresses. We have uncovered a negative regulator of ethylene responses that acts by positively regulating ETR1 ethylene receptor function in *Arabidopsis*. *Arabidopsis* has five ethylene receptors, which exhibit functional redundancy and negatively regulate ethylene responses. In a genetic screen for suppressors of the dominant ethylene-insensitive receptor mutant *etr1-2*, we identified *REVERSION-TO-ETHYLENE SENSITIVITY1* (*RTE1*), which encodes a novel integral membrane protein found in plants, animals and protists. The *rte1* null mutant displays ethylene hypersensitivity, which appears to be caused by reduced *ETR1* function, whereas over-expression of *RTE1* confers ethylene insensitivity that is largely dependent on *ETR1*.

Interestingly, loss of *rte1* function can suppress only certain *etr1* dominant alleles; 7 alleles were suppressed out of 13 *etr1* alleles tested, suggesting that *RTE1* and *ETR1* interact at the protein level. The other four ethylene receptors do not appear to be affected by *rte1*. A functional version of RFP-tagged *RTE1*, expressed under the native *ETR1* promoter, localizes to the ER and Golgi, and shows partial co-localization with CFP-tagged *ETR1*. Preliminary biochemical data suggests that *RTE1* can interact with *ETR1* in living plant cells.

P-705

Glutathione S-Transferase Interacting with FIN219 Is Involved in Phytochrome A-Mediated Signaling in Arabidopsis. Ing-Chien Chen¹, I-Ching Huang¹, Ming-Jung Liu¹, Hsu-Liang Hsieh¹. ¹Institute of Plant Biology, College of Life Science, National Taiwan University, Taipei 106, Taiwan

Far-red insensitive 219 (FIN219) was previously shown to be involved in phytochrome A-mediated far-red light signaling. To further understand its function and regulatory relation with other light signaling components, a yeast two-hybrid approach was used to isolate FIN219-interacting partners. Here, we demonstrate that a FIN219-interacting protein 1 (FIP1) interacts with FIN219 in vitro and in vivo and is composed of 217 amino acids that belong to the tau class of the large glutathione S-transferase (GST) gene family. FIP1 was further shown to have GST activities. The gain-of-function and partial loss-of-function of FIP1 resulted in a hyposensitive hypocotyl phenotype under continuous far-red light (cFR) and a delayed flowering phenotype under long-day conditions, which suggests that FIP1 may exist in a complex to function in the regulation of *Arabidopsis* development. As well, FIP1 mRNA was down-regulated in the spa1 mutant and differentially expressed in cop1-4 and cop1-5 mutants under cFR. Intriguingly, FIP1 expression was up-regulated in the fin219 mutant under all light conditions except cFR. Furthermore, promoter activity assays revealed that FIP1 expression was light dependent, mainly associated with vascular tissues, and developmentally regulated.

Subcellular localization studies revealed that the GUS-FIP1 fusion protein was localized in the nucleus and cytoplasm. Taken together, these data indicate that FIP1 may interact with FIN219 to regulate cell elongation and flowering in response to light.

P-706

***Arabidopsis* Cullin 4 Forms an E3 Ubiquitin Ligase with RBX1 and the CDD Complex in Mediating Light Control of Development.** Haodong Chen^{1,2,3}, Yunping Shen^{1,3}, Xiaobo Tang⁴, Lu Yu², Jia Wang², Lan Guo^{2,1}, Yu Zhang^{1,2}, Huiyong Zhang², Suhua Feng^{2,3}, Elizabeth Strickland³, Ning Zheng⁴, Xing Wang Deng^{1,2,3,1} Peking-Yale Joint Center of Plant Molecular Genetics and Agrobiotechnology, College of Life Sciences, Peking University, Beijing 100871, China., ²National Institute of Biological Sciences, Zhongguancun Life Science Park, Beijing 102206, China., ³Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8104, USA., ⁴Department of Pharmacology, Box 357280, University of Washington, Seattle, WA 98195, USA.

Repression of photomorphogenesis in *Arabidopsis* requires activity of the COP9 signalosome (CSN), CDD, and COP1 complexes, but how these three complexes work in concert to accomplish this important developmental switch has remained unknown. Here we demonstrate that *Arabidopsis* cullin 4 (CUL4) associates with the CDD complex and a common catalytic subunit RBX1 to form an active E3 ubiquitin ligase both in vivo and in vitro. The partial loss-of-function of CUL4 resulted in a constitutive photomorphogenic phenotype in respect to morphogenesis and light regulated gene expression. Further, CUL4 exhibits a synergistic genetic interaction with COP10 and DET1. Therefore this CUL4-based E3 ligase is essential for the repression of photomorphogenesis. This CUL4-based E3 ligase appears to physically associate with COP1 E3 ligase and positively regulates COP1-dependent degradation of photomorphogenesis-promoting transcription factors, while the CSN controls biochemical modification of CUL4 essential for E3 activity. This study thus suggests a biochemical activity connection between CSN and CDD complexes in their cooperation with COP1 in orchestrating the repression of photomorphogenesis.

P-707

ProMEX-a protein and phosphoprotein mass spectral reference library. Yanmei Chen¹, Jan Hummel¹, Stefanie Wienkoop¹, Michaela Niemann¹, Waltraud Schulze¹, Dirk Walther¹, Joachim Selbig², Wolfram Weckwerth¹. ¹Max-Planck-Institute of Molecular Plant Physiology, ²University of Potsdam

Here we present a plant protein reference library ProMEX (<http://promex.mpimp-golm.mpg.de/cgi-bin/peplib.pl> [1]) containing about 3000 plant proteins including several hundreds of *Arabidopsis thaliana* phosphoproteins detected in a novel phosphoprotein enrichment method termed MOAC (Metal Oxide Affinity Chromatography). When compared to commercial phosphoprotein enrichment kits, the method is more cost effective and easily applicable to method optimization [2]. We coupled the enrichment procedure to two-dimensional gel electrophoresis [3]. *Arabidopsis thaliana* cell cultures are investigated in response to different treatments and differential in vivo phosphorylation of proteins. The data are assembled in ProMEX. ProMEX is a reference mass spectral library consisting of tryptic peptide product ion spectra generated by mass spectrometry and was developed using protein samples of *Arabidopsis thaliana*, *Medicago truncatula*, *Chlamydomonas reinhardtii*, tomato and potato. An algorithm is implemented allowing for protein identification in uncharacterized samples. Protein identification based on the reference peptide mass spectra showed improved true positive rates as well as improved phosphoprotein detection compared to commercial algorithms. ProMEX integrates proteomics data with other levels of molecular organization including metabolite, pathway, and transcript information and may thus become a useful resource for plant systems biology studies.

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P-708

At5PTase13 involves in blue light responses and acts in phototropin 1 signalling through altering cytosolic Ca²⁺. Xu Chen¹, Wen-Hui Lin¹, Yuan Wang¹, Hong-Wei Xue¹. ¹Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Inositol polyphosphate 5-phosphatase (5PTase), a key enzyme in phosphatidylinositol signaling pathway, plays critical roles in hormone signaling, cotyledon vein pattern formation, and secondary wall synthesis and actin organization in fiber cells. Our previous studies indicated the roles of At5PTase13, a WD40-containing type II 5PTase, in hormone-mediated cotyledon vein development, and here we further showed its involvement of blue light responses. Compared to the expression under dark, that of At5PTase13 is suppressed by blue light irradiation and deficiency of which results in shortened hypocotyls and expanded cotyledons. Further, At5PTase13 is significantly enhanced under deficiency of phototropins (PHOTs), and suppressed At5PTase13 expression rescued the elongated hypocotyls of phot1 or phot1/phot2 under blue light. Measurement of cytosolic Ca²⁺ concentrations ([Ca²⁺]_{cyt}) revealed the reduced or increased [Ca²⁺]_{cyt} under blue light irradiation in phot1 and at5pt13 seedlings respectively, demonstrating the role of At5PTase13 in mediating PHOT1 effects and [Ca²⁺]_{cyt}-regulated hypocotyl elongation under blue light.

P-709

JASMONATE-INSENSITIVE 3 Is A Nuclear Target Of SCFCO11 Negatively Regulating JA Signalling. Andrea Chini¹, Sandra Fonseca¹, Gemma Fernández¹, Bruce Adie¹, Jos M. Chico¹, Oscar Lorenzo², Gloria García-Casado¹, Irene L. Pérez-Vidriero¹, Francisca M. Lozano³, María R. Ponce³, Jos L. Micó³, Roberto Solano¹. ¹ Departamento de Genética Molecular de Plantas. Centro Nacional de Biotecnología - CSIC, Madrid, Spain, ² Departamento de Fisiología Vegetal. Centro Hispano-Luso de Investigaciones Agrarias, Salamanca, Spain, ³ División de Genética and Instituto de Bioingeniería, Elche, Spain

Jasmonates (JAs) are essential phytohormones structurally similar to metazoan prostaglandins and potent anti-cancer agents in humans. In spite of their importance for plant development and survival the molecular details of their signalling pathway remain largely unknown. The identification of COI1 as an F-box protein almost a decade ago suggested the existence of a repressor of JA responses targeted by SCFCO11 for degradation by the proteasome in response to JA. Here we report the identification of JASMONATE-INSENSITIVE 3 (JAI3) and show that it belongs to a novel family of JA-regulated nuclear targets of SCFCO11, named JAIL (JAI3-Like), which share conserved domains with CONSTANS (CO) and CO-related proteins. JAI3 and other JAIL proteins physically interact with COI1, and JA treatment induces their SCFCO11-dependent, proteasome degradation. The jai3-1 allele encodes a mutant protein resistant to degradation that also inhibits degradation of the wild-type JAI3 and other JAILs, explaining its dominant JA-insensitive phenotype. In addition, we show that JAI3 and other JAILs physically interact with the key transcriptional activator of JA-regulated gene expression, AtMYC2, which suggests a model of JAI3/JAILs action as co-repressors of AtMYC2. Our results demonstrate that JAILs are direct targets of the E3 ligase SCFCO11, linking ubiquitin-mediated protein degradation to transcriptional activation of JA-responses. Moreover, our results show the existence of a negative regulatory feed-back loop involving AtMYC2 and JAILs that provides a mechanistic explanation for the pulsed response to the hormone and the subsequent desensitization of the cell.

(A.C., S.F. and G.F. contributed equally to this work)

P-710

Role of CAX genes in plant hormonal signaling. Daeshik Cho¹, Sangmee Lee¹, Jenny Seo¹, Kendal Hirsch², June Kwak¹. ¹ Dept. of CBMG, University of Maryland, ²Baylor College of Medicine, Texas

Guard cells consist of stomatal pores that are responsible for gas exchange and water transpiration in plants. Thus, guard cell play an essential role for plant growth and development. Plants have developed complicated signaling mechanisms controlling stomatal opening and closing in response to changing environments. Abscisic acid (ABA) and Ca²⁺ are well-studied signaling molecules which mediate stomatal closure. It was shown that Ca²⁺ signatures encode necessary information for stomatal closure. The information in Ca²⁺ oscillations is encoded in amplitudes and frequencies that are regulated in part by cellular Ca²⁺ transporters. Arabidopsis Ca²⁺/H⁺ transporters (CAX) are important antiporters that control intracellular Ca²⁺ levels. A single-cell type functional genomics approach identified that two CAX genes are highly expressed in guard cells. To investigate the role of these CAX genes in guard cell signaling, stomatal responses to various hormones and environmental cues were examined in the cax single and double mutants. Furthermore, consistent with the stomatal response, seedling of the cax single and double mutants show that these mutants are defective in the hormone signaling. Our results suggest that CAX proteins play a central role in plant hormone signaling by contributing to the cellular hormone and Ca²⁺ homeostasis. Further biochemical and physiological results will be discussed.

P-711

Bio-Dynamics of Cytokinin Response Regulators in Arabidopsis. Seung Hee Choi¹, Hyo Jung Kim¹, In Chul Lee¹, Hong Gil Nam¹. ¹ POSTECH, Pohang, South Korea

Cytokinins have many critical functions in plants, such as cell proliferation, shoot formation, nutrient relocation, shoot branching, and longevity control as a "master regulator" of plant growth and development. In *Arabidopsis*, cytokinin signal is known to be initiated by the three cytokinin receptors, AHK2, AHK3, and AHK4/CRE1/WOL, with the structural features of hybrid histidine kinases, and transduced to downstream response regulators via a His-to-Asp phosphorelay cascade.

For better understanding of cytokinin signaling, we have been trying to see cytokinin signaling at a systems level and with a dynamic view. We are constructing dynamic regulatory networks among cytokinin response regulators. The *Arabidopsis* genome has 22 genes for response regulators, which are grouped into two classes, type-A *Arabidopsis* Response Regulator (ARR) and type-B ARR. While the type-A ARR genes have been examined for cytokinin inducibility, type-B ARRs are not induced by cytokinins. It has been suggested that the type-B ARRs might function as transcription factors. By examining the expression of type-A ARR genes in ARR1, ARR2, and ARR10 overexpression lines, we found that these type-A ARR genes are differentially regulated by type-B ARR proteins at the transcriptional level. We also have been generating constitutive and inducible overexpression lines for these response regulators and observing the noticeable phenotypes. Based on the phenotypes, we will construct transcriptional regulatory network in the specific organ. Through this systemic approach, we will get the significant advances in understanding cytokinin signaling in *Arabidopsis*.

P-712

A gain-of-function mutation of elongated-D suppress the dwarf phenotypes of both brassinosteroids biosynthetic and signaling mutants. Yuhee Chung¹, Mi Kwon¹, Ok Sun Lee¹, Woo Suk Cho¹, Sungwha Choe¹. ¹ School of biological sciences, College of natural sciences, Seoul National University, Seoul 151-747, Korea

Brassinosteroids (BRs) stimulate plant growth through a receptor kinase-mediated signaling pathway. Contrast to the conventional BR dwarf phenotypes, tall plant stature can be induced due to a gain-of-function mutation in a gene for positive regulator or a loss-of-function mutation in a negative regulator in BR signaling pathways.

We obtained a list of long hypocotyl mutants from *Arabidopsis* Biological Resources Center (ABRC), and made double mutants with a BR biosynthetic mutant *dwf7-1*. Among the mutants tested, elongated-D (*elg-D*) caused a significant suppression of a dwarf phenotype seen in *dwf7-1*. Thus, we hypothesized that elongated-D has a dominant mutation in one of the BR signaling components. Using a map-based cloning method, we identified that *ELG* encodes for BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1 (BAK1). The *elg-D* mutation caused a substitution of Asp at the 123th amino acid residue for Ile, which is located in an extracellular Leucine-rich repeat domain of BAK1. To further understand the effect of this mutation, we introduced the *elg-D* mutation into BR signaling mutants including *bri1-5*, *bin2-1D/dwf12-1D*, *bzr1-D*, and *bes1-D* through genetic crossing. *elg-D* mutation suppressed the dwarfism of *bri1-5* more than three folds in the whole plant height. However, the level of suppression of the *bin2-1D/dwf12-1D* mutant was about 30% that of a single mutant, confirming that the *bin2-1D/dwf12-1D* mutation is epistatic to the *elg-D*. Currently, we hypothesize that the *elg-D* mutation constitutively transmits signal even in an absence of brassinolide possibly due to structural change in the LRR domain into active form, which is normally shaped by brassinolide binding.

P-713

Genetic approaches to understand the initiation of jasmonic acid biosynthesis in response to wounding. Lucie Dubugnon¹, Edward E. Farmer¹. ¹Departement of Plant Molecular Biology, University of Lausanne, CH-1015 Lausanne, Switzerland

Oxylipins such as jasmonic acid (JA) play central roles in the wound response and during pathogenesis and several studies have confirmed the important role of the canonical jasmonate pathway in plant defense.

However, little is known about the initial steps that regulate JA production early in attack. Lipoxygenases (LOXs) are non-heme dioxygenases that catalyse the hydroperoxydation of triunsaturated fatty acids to initiate JA synthesis. We recently isolated the fatty acid oxygenation upregulated ("fou2") mutant in which leaf LOX activity is elevated. When wounded "fou2" overproduces JA. Levels of AtLOX2, 3, 4 and 6 transcripts are elevated in "fou2" leaves. The "fou2" mutant implicates cation flux in the regulation of JA production (Bonaventure "et al.", 2007).

AtLOX2 is a good candidate for a potential regulation target of the JA pathway, because it is the main LOX occurring in expanded *Arabidopsis* leaves. Also several plant LOXs have been shown to possess putative calcium-binding domains.

A single recessive mutant of AtLOX2 was characterised. These plants are fully fertile and do not show altered susceptibility to "Botrytis cinerea" infection. Quantitative oxylipin analysis showed that "lox2" can still accumulate JA after wounding, which suggests that LOX2 is not the only LOX involved in JA synthesis. Moreover, LOX2 activity was shown to be activated "in vitro" by addition of divalent cations. In further "in vitro" and "in vivo" experiments we will investigate how cations may affect LOX2 activity and how this related to the initiation of JA synthesis.

P-715

Interplay of negative regulators of photomorphogenesis, MYC2, COP1 and SPA1, in Light, Abscisic Acid and Jasmonic Acid signaling pathways in *Arabidopsis thaliana*. Seeramaiah N. Ganagappa¹, Sudip Chattopadhyay^{1,2}. ¹National Centre for Plant Genome Research, Arun Asaf Ali Marg, New Delhi 110067, India.

Arabidopsis MYC2/ZBF1/JIN1 is a negative regulator of cryptochrome-mediated blue light signaling, and is a point of cross talk in light, abscisic acid (ABA), jasmonic acid (JA) and jasmonate-ethylene signaling pathways. Although many regulatory components of light signaling have been functionally characterized, only a few of them have been reported to cross talk with other signaling cascades. Here we report the functional relationships of three important negative regulators: MYC2, COP1 and SPA1 in light signaling pathways and their interplay in light, ABA and JA signaling. The genetic and molecular studies using *atmyc2 cop1* and *atmyc2 spa1* double mutants suggest that *atmyc2* act synergistically with *cop1* or *spa1* suppressing the photomorphogenic growth in the dark. The negative regulatory role of MYC2 in blue light is although dependent on COP1, it enhances SPA1 function in far-red light probably acting in parallel pathway. This study further reveals that COP1 and SPA1 act in ABA and JA signaling pathways. Although *atmyc2* can partially suppress some of the altered physiological responses of *cop1* and *spa1* in light signaling, mutation in *atmyc2* exerts opposite effects on *cop1* and *spa1* in ABA and JA responsiveness. The genetic, molecular and physiological studies in this study collectively suggest that MYC2 plays a balancing role in maintaining the ABA and JA responsiveness controlled by COP1 or SPA1 in a light specific manner.

P-716

The heterotrimeric G-protein α subunit GPA1 acts independently of brassinosteroid receptor BRI1 to regulate cell division in *Arabidopsis*. Ya-Jun Gao^{1,2}, Shucui Wang¹, Tadao Asami³, Jin-Gui Chen¹. ¹Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada, ²College of Resources and Environment, Northwest A & F University, Yangling, Shaanxi 712100, China, ³Plant Science Center and Plant Functions Laboratory, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan

The heterotrimeric G-proteins modulate cell division in diverse eukaryotes. Compared to vertebrates, the simpler repertoire of G-protein complex and accessory components in *Arabidopsis* offers a unique advantage over all other multicellular, genetic-model systems for dissecting the mechanism of the action of G-proteins in cell proliferation. Loss-of-function mutants of *Arabidopsis* heterotrimeric G-protein α subunit (GPA1) display defects in cell division throughout development. Hormone sensitivity assays suggest that heterotrimeric G-proteins may have a role in brassinosteroid (BR)-mediated pathways. Here we use *Arabidopsis* hypocotyl as a model system to dissect the role of GPA1 and BR receptor BRI1 in cell division. We provide genetic evidence that GPA1 acts independently of BRI1 in the regulation of cell division in hypocotyl epidermal cells. Loss-of-function mutants of GPA1 (*gpa1-2* and *gpa1-4*), weak mutant allele of BRI1 (*bri1-5*), and BR biosynthesis mutant *det2-1* all have short hypocotyls. However, we found that the short hypocotyl of *gpa1* mutants is largely due to a reduction in cell division, whereas the short hypocotyls of *bri1-5* and *det2-1* mutants are due to reductions in both cell elongation and cell division. An additive effect on the reduction of cell division was found in *gpa1-2 bri1-5* and *gpa1-4 det2-1* double mutants when compared with *bri1-5* and *det2-1* single mutants. These results provide direct genetic evidence that GPA1 and BRI1 act in parallel pathways to regulate cell division.

P-714

Genetic and biochemical studies of the auxin receptor reveal a novel mechanism of hormone perception. Mark Estelle¹. ¹Indiana University, Bloomington, IN, USA

The auxin class of plant hormones, including the endogenous auxin IAA, are a relatively heterogeneous collection of molecules. These compounds regulate diverse aspects of plant growth and development by promoting the degradation of transcriptional regulators called Aux/IAA proteins through the action of the ubiquitin protein ligase SCFTIR1. In recent work the F-box protein subunit of SCFTIR1, a protein called TIR1, was shown to function as an auxin receptor. Auxin binds directly to TIR1 to promote binding of the Aux/IAA proteins. Structural studies of the ASK1-TIR1 complex indicate that auxin binding does not produce a conformational change in TIR1. Rather, auxin appears to function as a molecular glue" to stabilize a weak interaction between TIR1 and the Aux/IAA substrates. These studies also suggest how structurally diverse compounds function as auxins. In addition, genetic analysis of TIR1 and other members of the TIR1/AFB auxin receptor family, provide new insight into the complexity of auxin signaling.

P-717

A Signaling Complex Constituted by the Heterotrimeric G-proteins and the Scaffold Protein RACK1. Jianjun Guo¹, Jin-Gui Chen¹. ¹ University of British Columbia, Vancouver, British Columbia, Canada

Signaling through the heterotrimeric G-proteins (G-proteins) is conserved in diverse eukaryotes. Receptor for Activated C Kinase 1 (RACK1) is a tryptophan-aspartic acid-domain repeat (WD40 repeat) protein that serves as a versatile scaffold protein which binds numerous signaling molecules from diverse signal transduction pathways in mammals. Mutational analysis indicated that both G-proteins and RACK1 mediate hormone responses and play regulatory roles in multiple developmental processes in plants. Here we demonstrate how G-proteins and RACK1 may constitute a signaling complex to regulate the responsivenesses to auxin and abscisic acid (ABA) in *Arabidopsis*. Loss-of-function mutations in the *Arabidopsis* G-protein alpha (GPA1) subunit, beta subunits (AGB1), and RACK1 all confer alterations in auxin-induced lateral root formation and ABA-mediated seed germination inhibition. Therefore, we use lateral root formation and seed germination as two model systems to dissect the roles of G-proteins and RACK1 in auxin and ABA responses respectively. We provide biochemical, molecular and cellular, and genetic evidence that G-proteins and RACK1 could work dependently or independently in a tissue/cell-specific manner. These findings offer a possible fine-tuning molecular mechanism through which the G-proteins modulate hormonal signaling and regulate phenotypic and developmental plasticity.

P-718

EIN3-like1 is a critical signaling component in EBF1/2-mediated ethylene response and plant development in *Arabidopsis*. Hongwei Guo¹. ¹ Peking University

The *Arabidopsis* EIN3 protein is a key transcription factor mediating ethylene-regulated gene expression and morphological responses. We have previously shown that the level of EIN3 protein rapidly increases upon ethylene treatment. In the absence of ethylene, EIN3 is quickly degraded through a ubiquitin/proteasome pathway mediated by two F-box proteins, EBF1 and EBF2. In this study, we demonstrated that loss of EIN3 function can only partially suppress various morphological defects (including the triple response, plant height, leaf epinasty, seed fertility, floral development) observed in the *ebf1 ebf2* double mutants. We further showed that ethylene positively regulates the level of EIL1 protein, the closest homolog of EIN3 in *Arabidopsis*, through a ubiquitin/proteasome-dependent pathway. Consistently, we found that although loss of EIL1 function can only slightly suppress *ebf1 ebf2* double mutant, loss of both EIN3 and EIL1 functions are able to completely rescue myriad defects caused by *ebf1 ebf2* double mutations. Genome-wide gene profiling analysis revealed that EIN3 and EIL1 are two critical transcription factors mediating most, if not all, of ethylene-regulated gene expression in either wild-type or *ebf1 ebf2* mutant backgrounds. Furthermore, our data revealed that ethylene is not required to activate EIN3 and EIL1 when EILN3/EIL1 protein is constitutively present in the nucleus, suggesting that control of protein level is the primary mechanism of ethylene regulation on EIN3/EIL1 activity. Taken together, these results indicate that, as EIN3, EIL1 is a primary target for the EBF1/EBF2-mediated degradation pathway, and that regulation of EIN3/EIL1 protein abundance in the nucleus is the key step in the response to ethylene gas.

P-719

Sub-cellular localisation of the *Arabidopsis* sphingosine kinase and sphingosine transfer protein. Cliona Hann¹, Tou Cheu Xiong¹, Carl Ng¹. ¹ University College Dublin, Dublin, Ireland

Stomata form pores on leaf surfaces that enable the plant to breathe. These pores function primarily to regulate the uptake of carbon dioxide for photosynthesis and the loss of water during transpiration under constantly changing environmental conditions. Each stomatal pore is surrounded by a pair of guard cells and changes in the turgor of the guard cell pair results in the opening and closing of the stomatal pore.

Sphingosine-1-phosphate, which results from the phosphorylation of sphingosine by sphingosine kinase (SphK), has been implicated in guard cell signalling (1, 2). The sphingosine transfer protein, ACD11, has been shown to catalyse the transfer of sphingosine between membranes in vitro (3). However, the role of ACD11 in stomatal guard cell function is unclear. We fused ACD11 to the red fluorescent protein (DsRed2) and showed, following biolistic transformation, that ACD11-DsRed2 is partitioned to discrete regions and punctate structures within the cytoplasm of onion epidermal cells. In contrast, SphK-YFP is found in both the cytoplasm and nucleus. Co-localization studies following transformation with Golgi-targeted GFP, ER-targeted GFP and mitochondria-targeted GFP suggests that ACD11 is not localized to the Golgi, ER or mitochondria. We have also analysed the expression of ACD11 and SphK and showed that both genes are highly expressed in guard cell protoplasts when compared to mesophyll protoplasts. This suggests that ACD11 may be functionally important in stomatal guard cell function.

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P-720

Secretory peptides that regulate stomatal patterning. Kenta Hara¹, Ryoko Kajita², Keiko Torii³, Dominique Bergmann⁴, Tatsuo Kakimoto¹. ¹ Osaka University, ² Osaka University, ³ University of Washington, ⁴ Stanford University

Animals use many secretory peptide mediators for control of development. Plants also use peptide mediators, such as phytosulphokine, CLV3 and CLV3-like proteins. To identify novel mediators that regulate plant development, we carried out a genome-scale screen, in which we assayed the effect on *Arabidopsis* development of individually overexpressing 153 genes predicted to encode small (<150aa) secreted peptides. Through this screen, we identified a gene EPI-DERMAL PATTERNING FACTOR 1 (EPF1) and EPF2 that decreased stomatal density when overexpressed. We show that EPF1 is expressed in stomatal cells and precursors and that controls stomatal patterning through regulation of the asymmetric cell division forming a stomatal precursor. EPF1 activity is dependent on the TOO MANY MOUTHS receptor-like protein and ERECTA-family receptor-kinases suggesting that EPF1 may provide a positional cue interpreted by these receptors. On the other hands, EPF2 regulates the density of stomatal lineage.

P-721

Methionine oxidation and protein phosphorylation: interactive partners in signaling? Shane Hardin¹, Joan Huber¹, Vanita Jain¹, Quan-Sheng Qiu¹, Man-Ho Oh², Ming Tien³, Steven Huber². ¹University of Illinois, Urbana, IL, USA, ²USDA-ARS, UIUC, Urbana, IL, USA, ³Pennsylvania State Univ, University Park, PA, USA

Signaling by reactive oxygen species (ROS) such as H₂O₂ is well established, but how these signals are sensed at the biochemical level is not clear. Our results suggest that reversible oxidation of methionine (Met) to Met sulfoxide (MetSO) may be a new mechanism to couple oxidative signals to protein phosphorylation. For example, calcium dependent protein kinases (CDPKs) and SNF1-related protein kinases (SnRK1s) target the canonical motif Φ -X-Basic-X(2)-[ST]-X(3)- Φ , where Φ is a hydrophobic residue. We observed that when Met is the essential hydrophobic residue at pS-5 or pS+4, its oxidation to MetSO strongly inhibits phosphorylation in vitro. This likely occurs because the MetSO sidechain is hydrophilic rather than hydrophobic. That this effect may also occur in vivo is suggested by the observation that transgenic *Arabidopsis* plants over expressing a peptide Met sulfoxide reductase (PMSRA3) have increased phosphorylation of numerous cellular proteins as revealed by ProQ Diamond Phosphoprotein staining of 2-DE gels. Preliminary MALDI-TOF MS analysis or immunoblotting with specific antibodies has identified hsp70, nitrate reductase, and chloroplast elongation factor EF-Tu as three of the phosphoproteins that may be sensitive to oxidative signals in vivo. In silico analysis predicts numerous proteins may be dually regulated by reversible phosphorylation and Met oxidation. We are speculating that the propensity of Met residues to be reversibly oxidized to MetSO may have been exploited during evolution to produce phosphorylation sites in certain proteins that would be responsive to oxidative signals. This general mechanism could be of broad significance as oxidative signals are generated during normal growth and also in response to most biotic and abiotic stresses.

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P-722

Genetic Linkages between Circadian Clock-Associated Components and Phytochrome-Dependent Red Light-Signal Transduction in *Arabidopsis thaliana*. Shogo Ito¹, Norihito Nakamichi¹, Yuko Nakamura¹, Yusuke Niwa¹, Takahiko Kato¹, Masaya Murakami¹, Masanori Kita¹, Tsuyoshi Mizoguchi², Kanae Niinuma², Takaumi Yamashino¹, Takeshi Mizuno¹. ¹Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Nagoya, Japan, ²Institute of Biological Sciences, University of Tsukuba, Tsukuba, Japan

The current best candidates for *Arabidopsis thaliana* clock components are CCA1 (CIRCADIAN CLOCK-ASSOCIATED 1) and its homolog LHY (LATE ELONGATED HYPOCOTYL). In addition, five members of a small family, PSEUDO-RESPONSE REGULATORS (including PRR1, PRR3, PRR5, PRR7, and PRR9), are believed to be another type of clock component. The originally described member of PRRs is TOC1 (or PRR1) (TIMING OF CAB EXPRESSION 1). Interestingly, seedlings of *A. thaliana* carrying a certain lesion (i.e., loss-of-function or misexpression) of a given clock-associated gene commonly display a characteristic phenotype of light response during early photomorphogenesis. For instance, cca1 lhy double mutant seedlings show a shorter hypocotyl length than the wild type under a given fluence rate of red light. In contrast, both toc1 single and prr7 prr5 double mutant seedlings with longer hypocotyls are hyposensitive under the same conditions. These phenotypes are indicative of linkage between the circadian clock and red light-signal transduction mechanisms. Here this issue was addressed by conducting combinatorial genetic and epistatic analyses with a large number of mutants and transgenic lines carrying lesions in clock-associated genes, including a cca1 lhy toc1 triple mutant and a cca1 lhy prr7 prr5 quadruple mutant. Taking these results together, we propose a genetic model for clock-associated red light signaling, in which CCA1 and LHY function upstream of TOC1 (PRR1) in a negative manner, in turn, TOC1 (PRR1) serves as a positive regulator. PRR7 and PRR5 also act as positive regulators, but independently from TOC1 (PRR1). It is further suggested that these signaling pathways are coordinately integrated into the phytochrome-mediated red light signal transduction pathway, in which PIF3 (PHYTOCHROME-INTERACTING FACTOR 3) functions as a negative regulator immediately downstream of phyB.

P-723

Functional analyses of an *Arabidopsis* transcription factor involved in callus formation. Akira Iwase¹, Nobutaka Mitsuda¹, Tomotsugu Koyama¹, Keiichiro Hiratsu², Takashi Arai³, Yasunori Inoue³, Masaru Ohme-Takagi¹. ¹ Gene Reg. Res. Gro., Res. Inst. Genome-based Biofac., Natl. Inst. of Sci. & Tech. (AIST), ² Dep. of Appl. Chem., Natl. Def. Acad., ³ Dep. of Appl. Biol. Sci. Facul. of Sci. and Tech., Tokyo Univ. of Science.

Callus is a mass of dedifferentiated cells and formed in wound-healing tissue. Phytohormones, namely auxin and cytokinin, are known to be key regulators for the induction of cell-dedifferentiation and callus formation. However, factors that induces and/or maintain the dedifferentiated state has not been characterized. To identify such factors, we performed comparative analyses of the gene expression profiles between *Arabidopsis* seedlings and three callus lines by DNA microarray, and found that a gene for the plant specific transcription factor of AP2/ERF family designated as *Callus Formation Factor 1* (CAF1) was upregulated in the cell lines. When CAF1 was ectopically expressed under CaMV 35S promoter, the transgenic lines induced callus in the shoot, hypocotyl and root. Surprisingly, the callus generated from the overexpressor (CAF1 callus) grew and maintained dedifferentiation state without addition of phytohormone. The seedlings of the T2 generation of the overexpressor with weak phenotype were dedifferentiated into callus with lower concentration of auxin, in which condition wild type seedlings did not form callus. To investigate whether CAF1 increase endogenous auxin concentration, auxin-responsive promoter-reporter activity was examined in the CAF1 callus. However, the promoter activity was not detected in the callus without addition of external auxin. Moreover, the concentration of endogenous free auxin was not different between the CAF1 overexpressor and wild type seedlings. These results suggest that CAF1 is not likely to increase the auxin concentration. CAF1 promoter activity was detected in wounded part of tissues and in callus. Microarray analysis showed that expression of wounding-responsive genes and cultured-cell-upregulated genes were significantly enhanced in CAF1 overexpressor. Our result suggest that transcription factor CAF1 plays an important role on the callus formation and wound-healing process.

P-724

Transcription factor-interacting proteins RCD1 and SRO1 are involved in abiotic stress tolerance. Jinja Jaspers¹, Tiina Blomster¹, Reetta Ahlfors¹, Mikael Brosch¹, Hannes Kollist¹, Kirk Overmyer¹, Aliri Lamminmäki¹, Jaakko Kangasjoki¹. ¹ Plant Biology, Dept. of Biol. & Env. Sci., University of Helsinki, Finland

We have isolated a series of *rcd*-mutants (*radical-induced cell death*) that display visible HR-like lesions coupled with increased reactive oxygen species accumulation when exposed to ozone. The *rcd1* mutant is sensitive to apoplastic ROS, but more tolerant to chloroplastic ROS, has deficiencies in abscisic acid, ethylene, and methyl jasmonate regulated gene expression, overproduces nitric oxide, has constitutively more open stomata than the wild type, is slightly insensitive to glucose and is sensitive to salt. Two members of the plant-specific RCD1 protein family, RCD1 and SRO1 (Similar to RCD One 1) contain a WWE-domain for protein-protein-interactions and canonical nuclear localization sequences. In addition RCD1 and all SRO-proteins have poly-ADP-ribosylase (PARP) core domain involved in NAD-binding, and have a highly conserved C-terminal domain that is involved in protein-protein interactions. Nuclear localization resembling the distribution of active chromatin has been shown for RCD1. Furthermore, it has been shown that under salt and oxidative stress conditions RCD1 interacts with plasma membrane proteins. Yeast two-hybrid analysis identified several RCD1 and SRO1-interacting proteins, most of which are transcription factors related to salt and osmotic stress (e.g., DREB2A), or carbohydrate-regulation of gene expression. According to yeast 2-hybrid analyses, in addition to their interaction with transcription factors, RCD1 can interact with itself, and also with SRO1. Only the *rcd1* mutant has a pleiotropic phenotype whereas no phenotypes have been found for the *sro1* mutant. However, the *rcd1 sro1* double mutant is unviable when grown in soil and can barely grow under *in vitro* conditions. Thus, both RCD1 and SRO1 seem to be involved in processes that affect interplay between hormonal signaling cascades, acclimatization to oxidative stress and salt and osmotic stress, and are required for the proper growth and development of the plant.

P-725

Glucosamine, amine-containing sugar causes repression of seedling growth by overproduction of ROS through hexokinase-mediated phosphorylation in *Arabidopsis*. Hyun-Woo Ju¹, Sun-Hee Kim¹, Gang-il Kim¹, Hojoong Lee², Suk-Whan Hong¹. ¹ Department of Plant Biotechnology and Agricultural Plant Stress Research Center, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Korea, ² Division of Life and Genetic Engineering, College of Life and Environmental Sciences, Korea University, 1, 5-ka Anam-dong, Sungbuk-ku, Seoul, Korea

Glucosamine (GlcN) is a naturally occurring amino-sugar, enzymatically synthesized by the amidation of fructose-6-phosphate. Although a number of reports have shown the biological effect of GlcN on insulin resistance and immune activity in mammalian system, little is known about its physiological roles in plants. In this study, we found that exogenous treatment of GlcN causes repression of seedling growth and hypocotyl elongation in *Arabidopsis*. The addition of glucose, fructose, 6-deoxy glucose, and 3-O-methyl glucose is capable of reversing these adverse effects of GlcN on hypocotyl elongation. Our results showed that GlcN is able to induce a significantly increase in production of reactive oxygen species and that the GlcN-mediated inhibition of hypocotyl elongation is relieved by reducing agents, such as glutathione and ascorbic acid.

Furthermore, mannoheptulose, a specific hexokinase (HXK) inhibitor, was able to restore hypocotyl elongation in the presence of GlcN and *gin2-1* mutant plants exhibited less sensitivity to GlcN, compared with wild-type plants. Whereas expression of glucose-inducible genes, encoding chalcone synthase, beta-amylase and chlorophyll binding a/b protein remained unchanged on glucosamine treatment, level of ASN1 (asparagine synthetase 1) mRNA was down-regulated by exogenous glucosamine as well as glucose. Taken together, these results provide evidence that GlcN causes oxidative stress represses through HXK-dependent mechanism and affects gene expression in *Arabidopsis thaliana*.

P-726

The GIGANTEA-Controlled MicroRNA172 Mediates Photoperiodic Flowering Independent of CONSTANS in Arabidopsis. Jae-Hoon Jung¹, Seok-Ki Kang¹, Yeon-Hee Seo¹, An-Kyo Lee¹, Eunjung Kwon¹, Jose Luis Reyes², Ju Yun¹, Nam-Hai Chua², Chung-Mo Park¹. ¹Molecular Signaling Laboratory, Department of Chemistry, Seoul National University, Seoul, 151-742, Korea,²Laboratory of Plant Molecular Biology, Rockefeller University, New York, New York 10021-3699, USA

Regulated RNA metabolism appears to be a critical component of molecular mechanisms directing flowering initiation in plants. A group of RNA-binding proteins regulate flowering time through the autonomous flowering pathway. Posttranscriptional mechanisms exerted by microRNAs (miRNAs) also play a key role in flowering time control. Here, we show that the GIGANTEA (GI)-controlled microRNA172 (miR172) defines a unique genetic pathway that regulates photoperiodic flowering by inducing FLOWERING LOCUS T (FT) independent of CONSTANS (CO). A late flowering mutant in which a miR172 target gene TOE1 is activated by the nearby insertion of the CaMV 35S enhancer normally responded to vernalization and gibberellic acid (GA) treatments. In contrast, its response to daylength changes was severely disrupted. In the mutant, FT was significantly repressed, but other flowering genes were unaffected. Notably, miR172 abundance is regulated by photoperiod via GI in a CO-independent manner. Accordingly, miR172-overproducing plants exhibit early flowering both under long days and short days, even in the absence of functional CO, indicating that miR172 promotes photoperiodic flowering by inducing FT. Therefore, it appears that GI-mediated photoperiodic flowering is governed by coordinate interaction of two distinct pathways: one mediated via CO and the other mediated via miR172 and its targets.

P-727

Global transcriptional analysis of ROP GTPase-mediated gene expression in *Arabidopsis thaliana*. Stephen Karr¹, Tongda Xu¹, Zhenbiao Yang¹. ¹U. C. Riverside, Riverside, CA, U. S. A.

There are eleven ROPs (Rho-like GTPases in plants) in Arabidopsis, which comprise a novel and diverse functioning set of genes important in many signaling pathways in plants including responses to hormones (ABA, BR and auxin), responses to biotic and abiotic stresses (e.g., hypoxia and nutrient deficiency), and developmental process like cell polarity and morphogenesis. There is strong sequence identity (>70%) and functional redundancy and overlaps among members of the ROP family, so it is important to confer multiple mutations to bring about an observable phenotype. We chose three ROPs that comprise a functionally redundant group and constructed a *rop4-1 rop6-1* double mutant into which a *rop2* RNAi construct was stably transformed, in the ecotype Wassilewskija. The triple mutant appears to have defects in seed production as well as an increase in rosette leaf senescence. We also performed Affymetrix gene chip experiments using the triple mutant to see if any of the apparent phenotypes could be explained by changes in gene expression. We found that genes associated with stress and senescence are up-regulated in the triple mutant compared to the wild-type and that these genes are primarily expressed in the adult leaves, callus, pollen and radicle. It is interesting to note that the genes up-regulated in only the adult leaves are related to defense response, stress response, signal transduction, electron transport, and secondary metabolism which may contribute to the observed triple mutant phenotype.

P-728

Identification of a calmodulin-binding NAC-like protein (CBNAC) in *Arabidopsis*. Ho Soo Kim¹, Mi Soon Jung¹, Sang Min Lee¹, Byung Oak Park¹, Hay Ju Han¹, Kyeong Eun Kim¹, Chae Oh Lim¹, Woo Sik Chung¹. ¹Division of Applied Life Science (BK21 Program), Plant Molecular Biology and Biotechnology Research Center, and Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea

Calmodulin (CaM), a ubiquitous calcium-binding protein, regulates diverse cellular functions by modulating the activity of a variety of enzymes or proteins. However, direct regulation of transcription factors by Ca²⁺/CaM has been poorly understood. By screening of the *Arabidopsis* cDNA expression library with HRP conjugated calmodulin as a probe, we identified calmodulin binding NAC-like protein, designated CBNAC. By using truncated versions of fusion proteins of CBNAC, we identified a Ca²⁺-dependent CaM binding domain (CaMBD) in the C-terminus. Specific binding of CaM to CaMBD was corroborated by site direct mutagenesis and split-ubiquitin assay. To determine the specific DNA sequence necessary for CBNAC binding, we employed a PCR-mediated random binding site selection method. This analysis showed that CBNAC binding to DNA requires the GCTT core sequence and other specific sequences immediately flanking both ends of the GCTT motif. Actually, CBNAC was able to bind the identified consensus sequence and repress the activity of transcription in *Arabidopsis* leaf protoplasts. Interestingly, the transcriptional repression mediated by CBNAC was enhanced by CaM. These results suggest that CBNAC is a CaM regulated transcriptional repressor in *Arabidopsis*.

P-729

Characterization of A Novel Calmodulin-Binding Protein (AtCBP54) that Interacts with Shikimate Kinase. Sun Ho Kim¹, Yun Hwan Kang¹, Min Chul Kim¹, Chae Oh Lim¹, Woo Sik Chung¹. ¹Division of Applied Life Science, Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju, Korea

Calmodulin (CaM), a Ca²⁺ sensor in all eukaryotes, is known to be involved in the induction of defense responses in plants. However, the molecular targets of CaM are not well known. To elucidate the components in the CaM-mediated defense signal pathway, we screened an *Arabidopsis* expression library with a horseradish-peroxidase (HRP)-conjugated CaM probe. Here we identified a CaM-binding protein containing a Toll/IL-1 receptor (TIR) homology domain, denoted AtCBP54 (for *Arabidopsis thaliana* calmodulin-binding protein 54kD). Using domain mapping and CaM overlay assay, we identified a Ca²⁺-dependent CaM binding domain in the C-terminal region of AtCBP54. The specific interaction of CaM with the CaM binding domain was confirmed by a gel mobility shift assay, a competition assay using a Ca²⁺/CaM-dependent enzyme, site-directed mutagenesis and a split ubiquitin assay system. By semi-quantitative RT-PCR, we detected AtCBP54 transcripts in rosette leaves, cauline leaves and stem, but not in flower and siliques. Interestingly, AtCBP54 was highly induced by not only virulence and avirulence bacterial pathogens, but also salicylic acid and jasmonic acid. These expression patterns of AtCBP54 suggest that AtCBP54 may play an important role in the pathogen signaling pathway. By yeast two-hybrid screening, we have identified the AtCBP54-binding protein (AtCBP54BP) that shows strong homology to shikimate kinase. We also find that the interaction of AtCBP54 with CaM induces the binding activity of AtCBP54BP with AtCBP54. Our study suggests that AtCBP54 may be involved in the pathogen signaling pathway through the interaction with shikimate kinase.

P-730

AtCTL2, A chitinase-like protein homolog, exhibits tissue-specific expression and repression by wounding. Gang-il Kim¹, Sun-Hee Kim¹, Hyun-Woo Ju¹, Hojoong Lee², Suk-Whan Hong¹. ¹ Department of Plant Biotechnology and Agricultural Plant Stress Research Center, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Korea, ²Division of Life and Genetic Engineering, College of Life and Environmental Sciences, Korea University, 1, 5-ka Anam-dong, Sungbuk-ku, Seoul, Korea

A number of genes, encoding chitinase-like protein (called the CTL group) have been identified from *Arabidopsis*, rice, cotton and pea. Members of the CTL group exhibit high similarity to those of glycoside hydrolase (GH) 19 family, in which most of plant chitinases are assigned, but the proteins have novel consensus sequences that were previously thought to be essential for chitinase activity. The genome of *Arabidopsis* is shown to contain two homologous genes, AtCTL1 and AtCTL2, sharing more than 62% similarity in the amino acid sequence level. There are also common in genomic structures between two homologues. Such as number of exon and insertional position of intron. The hot2 mutant is the result of a missense mutation in AtCTL1 and exhibits multiple defects in development and response to abiotic stresses. RNA blot analysis showed that AtCTL2 transcripts are highly abundant in stem and repressed by wounding stress. Transgenic *Arabidopsis* with the b-D-glucuronidase gene driven by AtCTL2 promoter showed preferential GUS activity in stem, anther and stigma. To determine the physiological role of AtCTL2 in development and in response to abiotic stresses in plants, we will identify a T-DNA insertion mutant of AtCTL2 and construct a double mutant of hot2-2, T-DNA insertion mutant of AtCTL2.

P-731

Overexpression of a gene, encoding 19-kD15P -Zein triggers up-regulation of GST gene and oxidative stress in *Arabidopsis*. Sun-Hee Kim¹, Hyun-Woo Ju¹, Gang-il Kim¹, Hojoong Lee², Suk-Whan Hong¹. ¹ Department of Plant Biotechnology and Agricultural Plant Stress Research Center, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Korea, ²Division of Life and Genetic Engineering, College of Life and Environmental Sciences, Korea University, 1, 5-ka Anam-dong, Sungbuk-ku, Seoul, Korea

De (Defective endosperm)-B30 is a dominant mutation in a gene, encoding 19-kD -Zein in maize that causes an opaque kernel phenotype with enhanced level of binding protein. The alteration of serine to proline at position 15 in a -Zein gene would cause a defective signal peptide cleavage and high induction of binding protein and other chaperones in the endoplasmic reticulum. To determine the effect of overexpression of De-B30 on UPR stress in *Arabidopsis*, we have constructed and performed characterization of transgenic *Arabidopsis* plants with a gene, encoding -Zein protein15P under 35S CaMV promoter. The transgenic plants exhibit defects in early growth in the light and inhibition of hypocotyl elongation in the dark. However, the immunoblotting and Northern analyses showed no induction of binding protein and calreticulin in transgenic plants, unlike as shown in De-B30 maize kernel. Several proteins including S-glutathion transferase (GST)1 and Malate/lactode dehydrogenase, were found to be up-regulated more than 2-fold in transgenic plants with overexpression of De-B30 gene versus vector control in the proteomic analysis. However, Northern blot analysis revealed that only GST1 gene was more abundantly expressed in transgenic plants. In addition to the up-regulation of S-glutathion transferase gene, the endogenous production of reactive oxygen species was also confirmed by 3,3-diaminobenzidine (DAB)-staining. Our findings suggest the possibility of involvement of ROS upon endoplasmic reticulum stress in *Arabidopsis*.

P-732

ROLE OF AN ARABIDOPSIS RAB GENE, ENCODING SMALL G-PROTEIN, IN AUXIN SIGNAL TRANSDUCTION. Eun-ji Koh¹, Ye-Rim Kwon¹, Hyun-Woo Ju², Kang-il Kim², Suk-Whan Hong², Hojoong Lee¹. ¹Korea University, Seoul, Republic of Korea, ²Chonnam National University, Gwangju, Republic of Korea

Small GTP-binding proteins serve as molecular switches to regulate different cellular processes. This study examined the effects of auxin on several mutated lines of *Arabidopsis* plants in which small G-protein genes were disrupted by T-DNA insertion. Salk mutant line was hypersensitive to auxin; however, this line contained an insertion in the RAB gene promoter. Since the Salk mutant line is not an RAB knock-out mutant, another Salk mutant line that contains a T-DNA insertion in the first exon of RAB was used for further studies. We found that RAB is responsive to auxin and plants that overexpress RAB exhibit hypersensitivity to auxin, due to the altered expression of auxin-responsive genes. Transient expression of GFP fused with RAB in onion epidermis and in transgenic plants showed that RAB was expressed in the endosomes and cytoskeletal network in cytoplasm, suggesting that RAB might play a role in the regulation of polar auxin transport. Taken together, these results show that RAB is an essential component of the pathway that couples auxin signaling to plant growth and development.

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P-733

The complexity of MAP kinase protein complexes. Justin Lee¹, Gerit Bethke¹, Tanja Feilner², Birgit Kersten³, Kai Naumann¹, Stefanie Ranf¹, Rita Schlichting¹, Claudia Spielau¹, Nicole Staroske¹, Joachim Uhrig⁴, Tino Uhthan¹, Ivy Widjaja¹, Dierk Scheel¹. ¹Leibniz Inst of Plant Biochem, Halle, Germany, ²Univ College Cork, Cork, Ireland, ³RZPD, Berlin, Germany, ⁴Univ of Cologne, Cologne, Germany

Recognition of pathogens initiates complex signaling networks which activate multicomponent defense responses. Mitogen activated protein kinases (MAPKs), which are activated upon various abiotic and biotic stresses, are important cellular defense signaling components in plants. The existence of multigene families encoding MAPK cascade elements together with the redundancy between individual elements in diverse cellular signaling pathways has further complicated functional analysis of signal-response specificities. Our studies focus primarily on AtMPK3, AtMPK4 and AtMPK6, which are involved in defense signaling. Treatment of *Arabidopsis* leaves or seedlings with the flagellin-derived flg22 peptide initiates transient increases of cytosolic calcium levels followed by activation of these 3 MAP kinases. Initial gel filtration analyses demonstrated that portions of MAP kinases exist in higher molecular weight complexes. In addition to yeast-2-hybrid screens, Tandem Affinity Purification (TAP) is being used to isolate components of MAP kinase protein complexes. TAP-tagged constructs of MAP kinase cascade elements have been generated in the respective knock-out background to eliminate competition from endogenous untagged proteins. In vivo interactions of protein partners are verified by fluorescence resonance energy transfer (FRET) analyses. FRET was first established based on known components of MAP kinase cascades and extended to candidate interactors isolated by the above-mentioned approaches. An ethylene-response-factor-like transcription factor (identified from Y2H screen) was confirmed to interact with MPK6 in vivo, in a flg22-dependent manner. In parallel, an in vitro kinase assay was performed on protein arrays with 1700 recombinant *Arabidopsis* proteins. Among the potential targets, amino-cyclopropane carboxylic acid synthase-6, a known MAPK substrate, was found to be phosphorylated specifically by AtMPK6. This shows that the in vitro screen can potentially uncover real in vivo targets of MAP kinases.

P-734

***Arabidopsis thaliana* inositol polyphosphate 6-/3-kinase gene (*Atipk2β*) is involved in iron homeostasis.** Yan Li¹, Huijun Xia¹. ¹Key Laboratory of MOE for Plant Developmental Biology, College of Life Sciences, Wuhan University, Wuhan, (Hubei), China

Iron is an essential element for respiration, photosynthesis, and many other cellular functions. Iron deficiency and sufficiency are important limiting factors for crop production. Phosphatidylinositol (PI) metabolic pathway is involved in many signaling pathways, including light, hormone, calcium homeostasis, etc. However, it remains unknown whether PI pathway is associated with iron homeostasis. Here, we demonstrate a novel role for inositol polyphosphate 6-/3-kinase gene (*Atipk2β*) in iron homeostasis. *Atipk2β* is an important kinase in PI pathway and phosphorylates IP3 to IP4. Our experiments showed that *Atipk2β* was induced by both iron-deficiency and – sufficiency stresses. T-DNA insertion lines exhibited hyper-responses to both iron-deficiency and sufficiency treatments compared to wild-type, while the transgenic lines displayed hypo-responses. Further research revealed that iron-deficiency caused more reduction in root elongation in mutant lines; in contrast, longer roots were observed in transgenic lines. Elemental analysis showed that *atipk2β* mutant lines decreased iron content, whereas the transgenic lines exhibited much higher iron content. Our work suggests a novel physiological function of *Atipk2β* in iron homeostasis. Substantial progresses will be presented and discussed.

P-736

Heterotrimeric G proteins and ABA signaling in *Arabidopsis*. Xigang Liu¹, Wei Li¹, Fangming Wu¹, Ligeng Ma¹. ¹National Institute of Biological Sciences, Beijing 102206, CHINA

Abscisic acid (ABA) is an important hormone that mediates many aspects of plant growth and development, particularly in response to the environmental stresses. Several components involved in ABA response were identified, however, the ABA signaling pathways is not well defined. Recent reports revealed that the nuclear RNA-binding protein FCA and the chloroplast protein Mg-chelatase H subunit are ABA receptors, suggesting the existence of multiple ABA receptors in *Arabidopsis*. In addition, previous report verified that the only canonical heterotrimeric G protein α subunit, GPA1, is involved in ABA-mediated seed germination, stomata opening, and inward K⁺ channel in guard cell, suggesting that GPA1 is involved in ABA signaling pathway. Heterotrimeric G proteins coupled with a plasma membrane localized receptor to transduce the extracellular signaling. In the present work, we characterized a putative G protein-coupled receptor. We found that this receptor interacts with both GPA1 and AGB1 (*Arabidopsis* Gβ subunit), and the interaction between this receptor and GPA1 is dependent on the intrinsic GTPase activity of GPA1. We also observed that mutation in this receptor leads to the defects in ABA responses from seed germination, stomata closure and opening to gene expression. This receptor specific binds to ABA, and the binding between the receptor and ABA follows receptor kinetics. Thus, our results revealed that this receptor is the plasma membrane receptor for ABA, and it mediates all major ABA response via heterotrimeric G proteins in *Arabidopsis*.

P-735

Analyses of *Arabidopsis* Mutants Defective in Multiple Receptor Genes Reveal Distinct and Unique Roles of ETR1 and ERS1 in Repressing Ethylene Responses. Qian Liu¹. ¹National Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Arabidopsis ethylene receptor genes negatively regulate ethylene responses and the loss of receptor genes leads to de-repression of ethylene responses. Due to genetic redundancy, it is challenging to dissect function of individual receptor genes. However, unique roles of ethylene receptors have been demonstrated. For example, ETR1 is required for the seedling nutation and the RTE1 function. In this study, phenotypes of mutants defective in multiple receptor genes were comprehensively compared. Our results show that the loss of ERS1 conversely resulted in repression of ethylene while the overexpression of ERS1 confers elevated ethylene responses. It is thus likely that ERS1 can also function as a positive regulator of ethylene responses. Previously it has been reported that the loss of multiple receptor genes will cause de-repression in ethylene responses. However, our study shows that the receptor identity, rather than the receptor number, is more important to degrees in the repression of ethylene responses. At last, we show that the dominant etr1-1 receptor gene can cause ethylene insensitivity in a quintuple mutant, lacking all the five wild-type receptor genes, but fails to completely restore rosette growth. This result implies that the ethylene receptors may have functions other than the repression of ethylene responses.

P-737

Phytochromes inhibit shade avoidance by triggering the degradation of growth-promoting bHLH transcription factors. Séverine Lorrain¹, Paula Duek¹, Trudie Allen², Garry Whitelam², Christian Fankhauser¹. ¹CIG, University of Lausanne, CH-1015 Lausanne, Switzerland, ²Department of biology, University of Leicester, LE1 7RH, United Kingdom

Plant growth and development is particularly sensitive to changes in the light environment and especially to vegetational shading. The shade-avoidance response is mainly controlled by the phytochrome photoreceptors. In *Arabidopsis*, recent studies have identified several related bHLH class transcription factors (the Phytochrome Interacting Factors) as important components in phytochrome signaling. In addition to a related bHLH domain, most of those PIFs share the Active Phytochrome Binding (APB) domain that mediates their interaction with light-activated phytochrome B (phyB). Here we show that PIF4 and PIF5 act early in the phytochrome signaling pathways to promote the shade avoidance response. PIF4 and PIF5 accumulate to high levels in the dark, are rapidly degraded in response to red light and rapidly re-accumulate under shade-mimicking conditions. Degradation of these transcription factors is preceded by phosphorylation, requires the APB domain and is sensitive to inhibitors of the proteasome, suggesting that PIF4 and PIF5 are degraded upon interaction with light-activated phyB. Our data suggest that in dense vegetation, which is rich in far-red, shade avoidance is triggered, at least partially, as a consequence of reduced phytochrome-mediated degradation of transcription factors such as PIF4 and PIF5. Consistent with this idea, the constitutive shade-avoidance phenotype of phyB mutants is partially reverted in the absence of PIF4.

P-738

GIBBERELLIN INSENSITIVE DWARF1 (GID1), a soluble gibberellin receptor in rice. Makoto Matsuoka¹. ¹Nagoya University Bio-Science BioTechnology Center, Nagoya, Japan

GIBBERELLIN INSENSITIVE DWARF1 (GID1) encodes a soluble GA receptor that shares sequence similarity with a hormone sensitive lipase (HSL). Previously, a yeast two hybrid (Y2H) assay revealed that the GID1-GA complex directly interacts with SLR1, a DELLA repressor protein in GA signaling. Recently, we also demonstrated, by a pull-down and Bimolecular Fluorescence Complementation (BiFC) experiments, that the GA-dependent GID1-SLR1 interaction occurs in planta. GA4 was found to have the highest affinity to GID1 in Y2H assays and is the most effective form of gibberellin in planta. Domain analysis of SLR1 by Y2H assay and gel filtration analysis revealed that the DELLA and TVHYNP domains of SLR1 are required for the GID1-SLR1 interaction. To identify the important regions of GID1 for GA- and SLR1-interactions, we used many different mutant versions of GID1, such as the spontaneous mutant GID1s, N- and C-terminal truncated GID1s, and mutagenized GID1 proteins with conserved amino acids replaced with alanine.

The amino acid residues important for SLR1-interaction overlapped the residues required for GA-binding, that were scattered throughout the GID1 molecule. When we plotted these residues on the GID1 structure predicted by analogy with HSL tertiary structure, many residues were located at regions corresponding to the substrate binding pocket and lid. Further, the GA-GID1 interaction was stabilized by SLR1. Based on these observations, we proposed a molecular model for interaction between GA, GID1, and SLR1.

P-740

Functional cross talk between two-component and phytochrome B signal transduction in *Arabidopsis*. Virtudes Mira-Rodado¹, Christopher Grefen¹, Tim Kunkel², Eberhard Schaefer², Klaus Harter¹. ¹ZMBP / Plant Physiology, University of Tuebingen, Tuebingen, Germany, ²Institute for Botany II, University of Freiburg, Freiburg, Germany

The A-type response regulator ARR4 is an element in the two-component signalling network of *Arabidopsis*. ARR4 interacts with the N-terminus of the red/far-red light photoreceptor phytochrome B (phyB) and functions as a modulator of photomorphogenesis (Sweere et al., 2001). In concert with other A-type response regulators, ARR4 also participates in the modulation of the cytokinin response pathway. We present evidence that ARR4 directly modulates the activity state of phyB in planta, not only under inductive but also under extended irradiation with red light (Mira-Rodado et al., 2007). Mutation of the phosphorylatable aspartate to asparagine within the receiver domain creates an ARR4 version that negatively affects photomorphogenesis. Additional evidence suggests that ARR4 activity is regulated by a phosphorelay mechanism that depends on the AHK family of cytokinin receptors. Accordingly, the ability of ARR4 to function on phyB is modified by exogenous application of cytokinin. These results implicate a cross talk between cytokinin and light signalling mediated by ARR4. This cross talk enables the plant to adjust light responsiveness to endogenous requirements in growth and development.

Sweere et al. (2001), *Science* 294, 1108-1111.

Mira-Rodado et al. (2007), *J. Exp. Botany*, in press.

P-739

Regulation of WRKY53 transcription factor at the onset of plant leaf senescence of *Arabidopsis thaliana*. Ying Miao^{1,2},

Ulrike Zentgraf². ¹Botanical Institute of Kiel University, Kiel, Germany,

²Center of Plant Molecular Biology of Tuebingen University, Tuebingen, Germany

The transcription factor WRKY53 plays an important role in the early stage of *Arabidopsis* senescence but almost nothing is known of how this factor is regulated. Here, we show that the WRKY53 protein exists in an active and inactive form. WRKY53 can interact with a MAP kinase kinase kinase (MEKK1) which contains a NLS and ser/thr protein kinase domain on the protein level and can be phosphorylated in vitro. The DNA binding affinity of WRKY53 is positively influenced by this phosphorylation. In addition, the expression of the WRKY53 gene is activated by MEKK1 binding directly to CA/TNTG elements of the WRKY53 promoter regulating the switch of leaf age dependent to plant age dependent expression. Microarray data and northern blot results show that MEKK1 has the same expression pattern as WRKY53 during the developmental period of *Arabidopsis* plants. The inactive WRKY53 protein interacts with UPL, a putative polyubiquitin ligase E3 which consist of a HECT domain, a leucine zipper motif and a C-type lectin motif, and can be polyubiquitinated in vitro. Induction of UPL expression in transgenic plants leads to a decrease of the WRKY53 protein level. In contrast, an UPL knock-out line shows higher WRKY53 protein levels. The results indicate that MEKK1 positively regulates senescence by directly phosphorylating the WRKY53 transcription factor through a shortcut in the MAPK signaling cascade and UPL negatively regulates senescence by targeting WRKY53 for protein degradation.

P-741

A ROLE FOR PROTEIN KINASE CK2 IN CELL DIVISION AND ARABIDOPSIS DEVELOPMENT: EVIDENCE USING A DOMINANT NEGATIVE MUTANT. Jordi Moreno-Romero¹, M. Carme Espuny¹, M. Carmen Martinez¹. ¹Universitat Aut noma de Barcelona

Protein kinase CK2 is an evolutionary conserved Ser/Thr phosphotransferase composed by two distinct subunits, alpha (catalytic), and beta (regulatory). In *Arabidopsis* each subunit is encoded by four genes that fulfill partial specific and partial redundant functions. In order to study the effects of loss-of function mutants of CK2 in plant systems we have undertaken a dominant-negative approach by stable overexpression of a CK2 kinase-inactive mutant generated by site-directed mutagenesis. A conserved lysine residue of the catalytic subunit, involved in ATP binding, was changed to Methionine, and the resulting protein was catalytically inactive. The construct was cloned downstream of an inducible promoter to avoid possible lethal effects, and stable transformed *Arabidopsis* plants and tobacco BY2 cells were obtained. We found that induction of the catalytically inactive CK2 was lethal in *Arabidopsis* plants, and that dark-grown mutants exhibited short hypocotyls but they do not express light-induced genes in the dark. In synchronized BY2 cells, induction of the transgene provoked cell arrest in G2, that was apparent both phenotypically and by the expression pattern of cell-cycle marker genes. Induction of CK2-inactive subunit in *Arabidopsis* for short times allowed plant survival but lateral root formation was completely suppressed. Our results support the idea of CK2 as a positive regulator of cell division and likely of cell expansion/elongation.

P-742

The role of ARR22 and two-component systems during *Arabidopsis* pod development. Erolid Naomab¹, Stefano Gattolin², Monica Alandete-Saez³, Zinnia Gonzalez-Carranza¹, Jeremy Roberts¹. ¹University of Nottingham, ²University of Birmingham, ³University of California, Berkeley, Department of Plant and Microbial Biology

Response regulators are part of a multi-component phosphorelay system and are implicated in playing pivotal roles in modulating plant responses to critical environmental signals such as cytokinin and ethylene. ARR22 (At3g04280) is an atypical response regulator gene, whose function in *Arabidopsis thaliana* is unknown. Time course RT-PCR and Northern blot hybridization analysis reveal transcripts of ARR22 pre-dominantly in reproductive organs (Gattolin et al., 2006). Furthermore, β -glucuronidase (GUS) reporter gene expression driven by ARR22 promoter (ARR22::GUS) is specifically localized at the seed:funiculus junction. However, GUS activity is primarily visible when pods or seeds are mechanically wounded. Promoter analyses have shown no indication that the 183bp 5'UTR intron regulates the wound inducibility of ARR22. Over-expression of ARR22 using the constitutive 35SCaMV promoter resulted in extreme dwarf transgenic phenotypes. ARR22 knock-out (KO) transgenic lines are phenotypically indistinguishable from wild type individuals under the conditions studied. Double knock-out lines of ARR22 and ARR24 are also similar to wild-type plants and whilst these two genes exhibit 66% amino acid similarity there is no evidence that they are functional homologous. Microarray approach has been followed to identify ARR22 co-regulated genes during pod development. ARRE is another gene having 75% amino-acid sequence similarity to ARR22 but which is structurally highly divergent, having two possible open reading frames. The two open reading frames were over-expressed separately using the constitutive 35SCaMV promoter. Homozygous 35S::ARRE transgenic lines have similar phenotypic characteristics to wild-type individuals.

Gattolin S, Alandete-Saez M, Elliott K, Gonzalez-Carranza Z, Naomab E, Powell C, Roberts JA (2006) Spatial and temporal expression of the response regulators ARR22 and ARR24 in *Arabidopsis thaliana*. J. Exp. Bot., 57(15): 4225 - 4233.

P-743

Reactive oxygen species (ROS) play a protective role during ER stress in *Arabidopsis*. Savitha Narendra¹, Shiyu Wang¹, Nina Fedoroff¹. ¹Penn State University, University Park, (PA), USA

ER stress occurs when the protein folding machinery is compromised and unfolded proteins accumulate within the ER lumen. It is relieved by triggering the unfolded protein response (UPR), a complex protective response that is initiated by signaling from the ER to the nucleus. We have reported that *Arabidopsis* plants homozygous for *agb1-2*, a null allele of the gene coding for the G β subunit of the heterotrimeric G protein, are resistant to tunicamycin, a protein glycosylation inhibitor that triggers ER stress and cell death. When compared with wildtype plants or *gpa1-4* homozygotes which lack the G α subunit of the heterotrimeric G protein, G β mutant plants exhibit a delayed and attenuated UPR, as judged by the induction of ER stress markers such as BiP, PDI and P58IPK and the presence of large protein aggregates, and little or no cell death. Taken together with the ER localization of a majority of the G β protein, these observations suggest that the G β complex has an important ER signaling function in UPR and UPR-associated cell death. Here we investigate the role of ROS in cell death signaling during UPR. We find that tunicamycin induces a biphasic oxidative burst that exerts a protective effect against UPR-associated cell death. Inhibition of ROS production by diphenylene iodonium (DPI), a flavin oxidase inhibitor, and treatment with the ROS scavenger N-acetyl cysteine delay the expression of UPR marker genes and increase the sensitivity of *Arabidopsis* plants to tunicamycin-induced cell death. We find that the area of dead tissue that develops in response to local subepidermal infiltration of tunicamycin is much larger in ROS-inhibited plants than in untreated plants and in *AtrbohD* homozygotes than in wildtype plants. We further observe that UPR-induced cell death is suppressed in plants carrying the bacterial *NahG* gene encoding salicylate hydroxylase. Thus ROS signaling evokes a protective response that curtails the spread of UPR-induced cell death mediated by salicylic acid. These observations thus reveal novel aspects of the operation of known cellular and intercellular stress signaling and defense networks in response to signals emanating from the ER.

P-744

Identification of signaling pathways in biotic and abiotic stresses. SAEID NAVABPOUR¹, VICKY B-WOLLASTON². ¹Dept. of Agronomy and Plant Breeding, University of Agricultural Sciences and Natural Resources, Gorgan, IRAN, ²Horticultural Research International, Wellesbourne, UK

Senescence is highly regulated process and is genetically controlled. It can be initiated by a wide variety of different factors, both internal and external. As well as being essential part of plant development, senescence in leaves is also induced prematurely by a number of different environmental stresses such as nutrient stress e. g. insufficient nitrogen, phosphorus or water. Plants that have been exposed to oxidative stress such as ozone also show senescence-like symptoms and localized cell death. This response appears to have some similarity to the hypersensitive response seen with incompatible pathogens. Infection by pathogens can result in senescence-like symptoms. Senescence and abscission of the infected leaf is a mechanism that could be useful to reduce the spread of the pathogen to the rest of the plant. Instead of causing senescence-like symptoms, infection with incompatible pathogens can result in a different type of cell death. The promoter region from "LSC54" gene has been cloned and fused in to the "GUS" gene. The seedling from the transformed "*Arabidopsis*" plants were exposed to a range of different "*Peronospora parasitica*" isolates, expression of the "GUS" gene was found to be regulated in an isolate-dependent manner.

P-745

Exploring the early events of root gravity and touch signal transduction with a proteomics approach. Carolyn O. S. Neal¹, Peizhen Yang¹, Patrick Masson¹, Elizabeth Craig¹. ¹ University of Wisconsin-Madison, Madison, (Wisconsin) United States

Our lab conducted a proteomic analysis of *Arabidopsis* root tips to identify proteins whose abundance and/or modification change early in response to mechano-stimulation. The study identified proteins with interesting temporal patterns of response to gravity and touch stimulation, and uncovered groups of differentially regulated proteins with clearly related functions that had not previously been implicated in gravity or touch signaling. We are using a combination of reverse genetics, temporal and spatial expression analysis in *Arabidopsis*, and transformation-rescue experiments in *Saccharomyces cerevisiae*, to identify and characterize the potential role of one of these proteins, which we call SKU15, in mechano-transduction.

P-747

Hormone-dependent targeting of HAVOC repressor proteins by the SCFCO11 ubiquitin ligase during jasmonate signaling. Yajie Niu¹, Bryan Thines¹, Ajin Mandaokar¹, John Browse¹. ¹ Institute of Biological Chemistry, Washington State University, Pullman WA 99163-6340, USA

Jasmonic acid (JA), a lipid-derived signaling compound, is well known for its role in flower development and plant defense. However, the molecular mechanism of JA signaling remains unclear. The F-box protein, COI1, is required for all responses to JA. COI1 is a component of SCFCO11 complex, an E3 ubiquitin ligase and therefore COI1 links JA signaling to the ubiquitin/26S proteasome pathway. Despite the important role of COI1 in JA signaling, the key components, substrates of SCFCO11, have not been identified to date. Here, we describe a new family of eight HAVOC (HVC) genes which were rapidly upregulated by JA in our previous transcription profiling experiments of JA signaling in *opr3* stamens. Our results demonstrate that HVC proteins act as repressors of JA-responses. Most interestingly, JA treatment causes HVC degradation by SCFCO11, which allows propagation of the JA signal. Our characterization of the HVC family provides the basis for understanding the broad functions of JA in defense responses and development of plants, and gives us an opportunity to further investigate the mechanism of JA signaling.

P-746

Temporal analysis of sucrose-induced phosphorylation changes in plasma membrane proteins of *Arabidopsis*. Totte Niittyiae¹, Anja Fuglsang², Wolf Frommer¹, Waltraud Schulze³. ¹ Carnegie Institution, Stanford, ²University Copenhagen, ³Max Planck Institute for Molecular Plant Physiology

Sucrose is the main product of photosynthesis and the most common transport form of carbon in plants. In addition, sucrose is a compound that serves as a signal affecting metabolic flux and development. Here we provide first results of externally induced phosphorylation changes of plasma membrane proteins in *Arabidopsis*. In an unbiased approach, seedlings were grown in liquid media with sucrose, then starved without sucrose before sucrose was resupplied. Plasma membranes were purified, phosphopeptides enriched, and subsequently analyzed quantitatively by mass spectrometry. In total, 67 phosphopeptides were identified, most of which were quantified over five time points of sucrose resupply. Among the identified phosphorylation sites, the well-described phosphorylation site at the C-terminus of plasma membrane H⁺-ATPases shows a relative increase in phosphorylation level in response to sucrose. This corresponded to a significant increase of proton pumping activity of plasma membrane vesicles from sucrose supplied seedlings. A new phosphorylation site was identified in the plasma membrane H⁺-ATPase AHA1 and/or AHA2. This phosphorylation site was shown to be crucial for ATPase activity and overrode regulation via the well-known C-terminal phosphorylation site. Novel phosphorylation sites were identified for both receptor kinases and cytosolic kinases which show rapid increases in relative intensities after short times of sucrose treatment. Seven response classes were identified including non-responsive, rapid-increase (within 3 min), slow-increase, and rapid-decrease. Relative quantification of phosphorylation changes by phosphoproteomics provides a means for identification of fast responses to external stimuli in plants as a basis for further functional characterization.

P-748

Loss of function of a novel cytochrome P450 leads to the suppressed phenotypes of *bak1* (BRI1-Assocoated Kinase1) mutant in *Arabidopsis*. You-Jin Oh¹, Yu Jeong Jeoung², Young Hee Bae¹, Myeong Min Lee³, June Seung Lee², Kyoung Hee Nam¹. ¹ Division of Biological Science, Sookmyung Women's University, Seoul, 140-742, Korea, ²Department of Biological Science, Ewha Women's University, Seoul, 120-750, Korea, ³Department of Biology, Yonsei University, Seoul, 140-749, Korea

Brassinosteroids signaling is initiated by the activation of BR-receptor complex consisting of two receptor-like serine/threonine kinases, BRI1 and BAK1 upon BL binding to the extracellular domain of BRI1. We isolated a putative *bak1* suppressor from the *bak1* transformed with activation-tagging vector construct. As found in the T2 generation, it is loss-of-function T-DNA insertional mutant showing longer leaf and petiole than *bak1* mutant. We performed TAIL-PCR analysis and identified the T-DNA insertional site in the gene encoding one of the cytochrome P450 oxidases. This belongs to the CYP705 family in the 71 clan. So far, it has been reported 62 CYP families in plants. Among them CYP 705 family consisting of 25 individual members and 8 pseudogenes is specific in Brassicaceae. We searched the single mutant for this and observed any morphological differences. No noticeable phenotypic changes were occurred in single mutant compared with background ecotype, Ler. However, double mutant between this candidate gene and *bak1* gave similar phenotypes appeared in original suppressor of *bak1*, confirmed that the suppressed phenotype of *bak1* resulted from the loss of this CYP gene. We also did genetic crosses between putative suppressors with *bri1-9* mutant allele and analyzed F2 plants segregated. Few triple mutants which have two T-DNA insertions in *bak1* and suppressor genes, respectively and *bri1-9* mutation showed much weaker *bri1-301* phenotypes, indicating the lack of this suppressor gene make the severe *bri1* plants partially rescued. Based on the GUS reporter gene expression, the expression of gene for *bak1* suppressor was found in the root tip just above the columella, vascular strands, and hypocotyls of the very young seedlings. No positive signal was detected in the leaf primordia which is the sites for the highest expression of BAK1.

P-749

A Protein Phosphatase (DsPTP1) Regulates the Activities of MPK Isoforms through the Interaction with Calmodulin. Byung Ouk Park¹, Kyung Eun Kim¹, Eun Hyeon Song¹, Ho Soo Kim¹, Mi Soon Jung¹, Man Soo Choi¹, Sung Cheol Koo¹, Sang Min Lee¹, Hay Ju Han¹, Woo Sik Chung¹. ¹Division of Applied Life Science (BK21 Program), and Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Korea

Mitogen-activated protein kinases (MPKs) control many kinds of signal transduction in all eukaryotic organisms. MPKs are known to be inactivated by dual specificity MPK phosphatases (DsPTPs). We previously isolated and reported a CaM-binding dual-specificity protein phosphatase1 (DsPTP1). Dephosphorylation of pNPP, a common synthetic protein phosphatase substrate, by DsPTP1 was increased in the presence of CaM. However, tyrosine dephosphorylation activity of DsPTP1 on phosphorylated MBP was inhibited by CaM (J. Biol. Chem. 279: 848-858. 2004). In this study, we found that DsPTP1 directly bound to MPK3, MPK4, and MPK6 using yeast two-hybrid analysis and in vitro binding assay. DsPTP1 dephosphorylated and inactivated MPK3, MPK4, and MPK6 phosphorylated by MEK1 and MEK2 in an enzyme concentration-dependent manner. Interestingly, the activity of DsPTP1 on phospho-Tyr was inhibited by the addition of CaM, whereas activity on phospho-Ser/Thr was not affected against MPK3, MPK4, and MPK6 substrates phosphorylated by MEK1 and MEK2. This result implies that Ca2+ mediated signaling pathway can cross-talk with MPKs signal pathway via DsPTP1 regulation.

P-751

SCF-SKP2A complex regulates cell division. Juan C. del Pozo¹, Silvia Jurado¹, Zamira Abraham¹, Concepción Manzano¹. ¹Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). Dpto. Biotecnología, Ctra. de la Coruña Km 7,5 28040 Madrid. España.

Selective turnover of proteins through the ubiquitin-proteasome pathway regulates diverse processes during plant development in response to internal and / or external stimuli. Protein targeting with ubiquitin (Ub) requires the sequential activity of the E1, the E2 and the ubiquitin-ligase or E3 enzymes. The SCF is an E3 multisubunit complex composed of four proteins, Cullin1, RBX, ASK1 and an F-box protein. The F-box proteins are the subunits responsible for specific recognition of the target. In silico analyses have identified more than seven hundred F-box proteins in *Arabidopsis*, although it is still unclear whether all of them are part of functional SCF complexes. In this work we show that SKP2A, an F-box protein forms an active SCF in vivo that has E3 ubiquitin ligase activity. SKP2A gene is regulated at the transcriptional level and the SKP2A protein is degraded through the ubiquitin pathway. Interestingly, this proteolysis is accelerated in response to auxin. We also found that SKP2A participates in the control of cell division, regulating the activity of transcription factor E2FC-DPB. Overexpression of SKP2A increased the number of dividing cells in root and shoot meristems and also the number of lateral roots, likely by reducing the levels of E2FC and DPB proteins, which acts as a repressor of cell division.

In an attempt to identify more SCF-SKP2A targets, we are conducting a proteomic approach. We are carrying out 2D liquid electrophoresis and comparing wt and SKP2A-overexpressing protein extracts in the presence or absence of the proteasome inhibitor MG132. So far, we have identified several proteins that are up-regulated in the overexpressor plants and several that are more represented in wt plants. This lower amount in wt plants might be the consequence of a down-regulation of gene expression or a faster degradation of the proteins by the SCF-SKP2A. At present, we are trying to get the identity of these proteins by LC-MS and determine whether or not they are targets of the SCF-SKP2A complex. These and further results will be presented at the meeting.

P-750

CPSF gene regulates the normal growth through involving in brassinosteroid signal transduction in *Arabidopsis thaliana*. Gayoung Park¹, Dilli P. Paudyal¹, Hyeonsook Cheong¹. ¹Department of Biotechnology Chosun University, Gwangju, Korea

Brassinosteroids (BRs) are a special class of plant steroid hormones that are essential for normal growth and development processes. Assessment of genes involvement in BRs biosynthesis and perception reveals that there are numbers of genes showing their role in BRs signaling. We have screened the activation tagged mutant that having remarkable phenotypic difference and responding with an active BRs biosynthetic inhibitor, brassinazole (Brz). The mutant was taller, late flowering, dark green, containing rounded leaves and had fewer seeds. TAIL-PCR, genotyping PCR and segregation ratio of T2 plants had shown a single T-DNA insertion at the first exon of Cleavage Polyadenylation Specific Factor (CPSF) gene in chromosome III that encodes a 130 kD protein having functional homologue with the specific mRNA splicing factor exhibiting a Brz resistant mutant. Real Time RT-PCR results showed that there was no increase in expression level of neighboring genes of the T-DNA insertion. Hypocotyls length of Brz treated *cpsf* mutant seedlings under dark measured nearly two fold longer than Columbia 0. The study unveiled that the CPSF is involved in BRs signaling in *arabidopsis*.

P-752

Bikinin, a chemical inhibitor of plant GSK-3 kinase activity, unleashes downstream brassinosteroid signalling. Bert De Rybel¹, Dominique Audenaert¹, Grégoire Verl², Wilfried Rozhon³, Silvie Coutuer¹, Tinneke Denayer⁴, Leentje Jansen¹, Claudia Jonak³, Kris Vleminckx⁴, Joanne Chory⁵, Dirk Inz¹, Eugenia Russinova¹, Tom Beeckman¹. ¹Department of Plant Systems Biology, VIB UGent, B-9052 Ghent, Belgium, ² Biochimie & Physiologie Moléculaire des Plantes, CNRS, Institut de Biologie Intégrative des Plantes, 34060 Montpellier, France, ³Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna Biocenter, A-1030 Vienna, Austria, ⁴ Department of Molecular Biomedical Research, VIB UGent, B-9052 Ghent, Belgium, ⁵Plant Biology Laboratory and Howard Hughes Medical Institute, The Salk Institute for Biological Studies, La Jolla, California 92037, USA

Brassinosteroids (BRs) are plant hormones that bind to the plasma-membrane localised receptor BRI1 and consequently activate an intracellular signalling cascade. Central in BR signalling, a GSK-3-like kinase (BIN2) and a Ser/Thr phosphatase (BSU1) modulate the phosphorylation state of transcription factors, which in turn regulate BR-responsive gene expression. Over the past decade, important players in BR signalling have been identified. However, the link between BRI1-activation and the inhibition of BIN2 activity and the regulatory mechanism for BIN2 inhibition itself remain unsolved. Using a chemical genetics approach, we identified a small non-steroidal molecule that induces constitutive BR-responses in planta similar to the action of BRs. We provide genetic and biochemical evidence that application of this compound activates the signalling pathway downstream of BRI1 by inhibiting BIN2 kinase activity, hence the name bikinin for BIN2 KInase INhibitor.

Bikinin targets specific subgroups of *Arabidopsis thaliana* GSK-3-like kinases, while it has no effects on other plant Ser/Thr protein kinases. Also, the inhibitory effect of bikinin is much weaker on human GSK-3beta kinase activity. Furthermore, in *Xenopus laevis*, bikinin had no effects on embryonic development, even though GSK-3beta is essential in body patterning throughout development. In summary, our study identified the first plant-specific inhibitor of GSK-3-like kinases and demonstrated the existence of non-overlapping GSK-3 inhibition mechanisms in plants and animals.

P-753

Functional Complementation of *Arabidopsis* hexokinase I (*AtHXK1*) by the Cyanobacterial Glucokinase (SII0593) from the *Synechocystis* sp. PCC 6803Jee-Youn Ryu¹, Suk Won Jeong¹, Soo Youn Kim¹, Youn-II Park¹. ¹Chungnam National University

In *Arabidopsis*, differential regulation of genes involved in the photosynthesis and carbon metabolism is sensed by AtHXK1. Though photosynthetic prokaryotic cyanobacterium *Synechocystis* sp. PCC 6803 does also show glucose-induced differential expression of genes involved in bioenergetics and carbon metabolism similar to those of *Arabidopsis*, glucose signaling is not sensed by a glucokinase SII0593, instead by unknown sensor downstream glucose phosphorylation. This difference could be due to either the inability of cyanobacterial SII0593 to function as a glucose sensor or lacking certain interacting factor(s) present in the *Arabidopsis*, but not in *Synechocystis*. In order to answer this question, the SII0593 was introduced into the glucose insensitive *Arabidopsis* mutant, gin2-1. *Arabidopsis* SII0593 overexpressing transgenics regained their sensitivity to glucose treatment with respect to seedling growth and development along with transcript levels of genes known responsive to glucose. Based on these results, we suggest that inability for SII0593 to sense glucose in *Synechocystis* is attributable to lacking of interacting protein factors. To exploit proteins interacting with both AtHXK1 and SII0593, we conducted yeast two hybrid analyses, resulting in several candidates. Currently, functional characterization of these proteins is under way.

P-754

Understanding of Light signalling through photoreceptor-interacting proteins in *Arabidopsis*. Jong Sang Ryu¹, Sung Hyun Hong¹, Hyunmo Choi¹, Suyeong Jeong¹. ¹ POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Light is a crucial environment signal that controls many photomorphogenic and circadian responses in plants. This light information is first perceived various photoreceptors, which transmit the light information to light-dependent downstream responses. In higher plants, three photoreceptor systems have been reported : the red and far-red light-absorbing phytochromes, the UV-A/blue light-absorbing cryptochromes / phototrophins, and the as-yet unidentified UV-B light-absorbing receptors. To transmit the responsive light information properly, plants have evolved a set of signaling components that assess and relay the nature of the incident signals to the changes of gene expression that control responses in growth and development. Since protein-protein interactions are fundamental to all cellular signaling processes, we tried to find photoreceptor-interacting proteins using various methods, and identified several authentic proteins.

We identified PAPP5 by yeast two-hybrid screening of phytochrome-interacting proteins. It specifically dephosphorylates Pfr-phytochromes and enhances phytochrome-mediated photoresponses. Depending on the specific serine residues dephosphorylated by PAPP5, phytochrome stability and affinity for a downstream signal transducer, NDK2, were enhanced. Another intriguing protein is BIT1. It activates blue light induced gene expression through Cryptochrome1. CRY1 directly bind to BIT1 and controls the transcriptional activity of BIT1. And we isolated the 149 phyB-interactomes that form during different light conditions in a phyB-overexpressing transgenic *Arabidopsis* line by a one-step co-IP method. we study the possibility of these proteins function as the immediate controller, transducer and responder in the light signaling pathway.

P-755

LAF1 involves in plant development as a positive regulator of auxin signaling. Wan Gyu Sang¹, Bong Soo Park¹, Song Yion Yeu², Ga Hyun Son¹, Yeon Jeong Kim¹, Jong Tae Song³, Hak Soo Seo¹. ¹Department of Plant Science, Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea, ²School of Agricultural Biotechnology, Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea, ³School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Korea

Auxin affects a large number of important growth and developmental processes including of shoot and root branching in higher plants. Several recent results have shown that the polar auxin transport inhibitors such as N-1-naphthylphthalamic acid, 9-hydroxyfluorene-9-carboxylic acid, and 2,3,5-triiodobenzoic acid affect leaf growth and leaf vein morphology. To investigate possible role of LAF1 (Long After Far-red light 1), a R2R3 Myb transcription factor and a signal transducer of far-red light, in auxin transport, we examined vein patterning of *laf1* mutant. Analyses of leaf growth and leaf vein pattern of *laf1* mutant showed that length of rosette leaf is shorter than wild type and mid-vein is much broader than wild type. In addition, number of secondary veins was decreased in *laf1* mutant. Besides, GUS assay revealed that LAF1 is specifically expressed in root, in particular in main root tip and lateral root, and its expression is induced by auxin NAA in roots. Moreover, transcript level of PIN3 (PIN-FORMED3) encoding auxin carrier protein is decreased in *laf1* mutant compared to that in wild type, which suggest that LAF1 may positively regulate expression of genes relating to auxin signaling through its transcriptional activity and auxin transport might be significantly blocked in *laf1* mutant. Taken together, our results indicate that LAF1 involves in leaf and root developments as a positive regulator of auxin signaling.

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P-756

bZIP10-LSD1 antagonism modulates basal defense and cell death in *Arabidopsis* following infection. Katia Schuetze¹, Hiromi Kaminaka², Petra Epple², Christina Chaban¹, Thomas Merkle³, Jeff Dangl², Klaus Harter¹. ¹ZMBP, University Tuebingen, Germany, ²Dept of Biology, University of North Carolina, NC USA, ³Biology III, University Bielefeld, Germany

Plants use sophisticated strategies to balance responses to oxidative stress. Programmed cell death, including the hypersensitive response (HR) associated with successful pathogen recognition, is one cellular response regulated by reactive oxygen in various cellular contexts. The *Arabidopsis* basic leucine zipper (bZIP) transcription factor At-bZIP10 shuttles between the nucleus and the cytoplasm and binds consensus G- and C-box DNA sequences. Surprisingly, AtbZIP10 can be retained outside the nucleus by LSD1, a protein that protects *Arabidopsis* cells from death in the face of oxidative stress signals. We demonstrate that AtbZIP10 is a positive mediator of the uncontrolled cell death observed in *lsd1* mutants. AtbZIP10 and LSD1 act antagonistically in both pathogen-induced HR and basal defense responses. LSD1 likely functions as a cellular hub, where its interaction with At-bZIP10 and additional, as yet unidentified, proteins contributes significantly to plant oxidative stress responses.

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P-757

***Arabidopsis* phytochrome A interacts with FHY1 and plays an important role in FHY1 phosphorylation upon red light exposure.** Yunping Shen^{1,2}, Zhenzhen Zhou^{1,3}, Suhua Feng¹, Haiyang Wang⁴, Li-Jia Qu², Xing Wang Deng^{1,2}. ¹Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8104, ²Peking-Yale Joint Center of Plant Molecular Genetics and Agrobiotechnology, College of Life Sciences, Peking University, Beijing 100871, China, ³Department of Biological Sciences, State University of New York at Binghamton, Binghamton, New York, 13902-6000, ⁴Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York 14853

The phytochrome family of red/far-red photoreceptors regulates a variety of developmental processes throughout the life cycle of plants. Among the five phytochromes in *Arabidopsis*, phytochrome A (phyA) is the key photoreceptor for response to high irradiance far-red (FR) light. FHY1 (far-red elongated hypocotyl 1), which is a positive regulator in phyA pathway, has been shown to interact with phyA by *in vitro* pull-down assays. Here we report that FHY1 protein becomes phosphorylated rapidly after exposure of the *Arabidopsis* seedlings to red light; and this process is dependent on the Pfr form of phyA. Furthermore, we demonstrate the FHY1-phyA interaction by both bimolecular fluorescence complementation (BiFC) assays in living onion cells and co-immunoprecipitation experiments in *Arabidopsis* seedlings. FHL, an FHY1 homolog protein that shares overlapping functions with FHY1, was also shown to interact with phyA *in vivo* in a similar manner. Therefore, our results suggest that interaction between phyA and FHY1 as well as phyA-induced phosphorylation of FHY1 are important molecular events in mediating light signaling process.

P-758

MAP kinases in guard cell ABA signaling. Charlotte Song¹, Sangmee Lee¹, Nathalie Leonhardt², Michael Djaoui³, Caroline Sirichandra³, Jeffrey Leung³, Sylvain Merlot³, June M Kwak¹. ¹University of Maryland, College Park, MD 20742, USA, ²CNRS-CEA-Université Aix-Marseille II, St Paul Lez Durance, France, ³ISV-CNRS, Gif sur Yvette, France

Drought causes severe damage and reduced yields for crops. The phytohormone, 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 are responsible for controlling CO₂ uptake and water loss by regulating the size of stomatal pores. During drought stress, a rapid response of a plant is to close stomatal pores. This process is mediated by ABA. In stomatal guard cells, reactive oxygen species (ROS) have been suggested to function in 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 GC-MAPK4, 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. Interestingly, 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 a strong ABA-insensitive response in stomatal movement assays, whereas mutants carrying a mutation in one of these genes did not show any altered 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. Currently, we are trying to further determine functional contribution of these genes to the ABA/ROS signaling and stress response network.

P-759

Genetic characterization of a long hypocotyl mutant, insensitive to light 1. Xiaodong Sun¹, Min Ni¹. ¹University of Minnesota, Twin Cities, St. Paul, MN, USA

Light has a profound effect on plant growth and development, and plants have evolved three families of photoreceptors to sense their ambient light conditions, including the red and far-red light-absorbing phytochromes and the blue and UV-A light-absorbing cryptochromes and phototropins. The photoreceptors, after being excited by light photons of various wavelengths, initiate downstream signaling cascades and regulate very similar set of plant responses such as de-etiolation, photoperiodic flowering, and circadian rhythm. The coordination and integration among the different signaling pathways have been documented extensively. We have screened an *Arabidopsis* T-DNA insertion population for mutants defective in their de-etiolation responses under red light condition, and identified one long hypocotyl mutant, *itl1* for insensitive to light 1. Genetic studies have revealed that the *itl1* mutation is recessive. Like many other mutants involved in light signaling, the *itl1* mutation also caused petiole and flowering phenotypes. Although isolated from a T-DNA insertional population, the *itl1* mutant was kanamycin-sensitive. We have therefore initiated efforts to clone the mutated gene through a map-based strategy. Preliminary studies have mapped the *itl1* mutation to a 100 KB region on chromosome 4. Future studies will be directed to complement the mutant phenotype with genomic contigs and to identify a second mutant allele from the public T-DNA knockout collections.

P-761

AtGluRS functions as a positive regulator in the ABA signal pathway. Yulin Tang¹, Erwin Grill². ¹College of Life Science, Shenzhen University, Shenzhen 518060, China, ²Technische Universitaet Muenchen, Freising D-85354, Germany

Abscisic acid (ABA) plays crucial roles in various aspects of plant growth and development, as well as in adaptation to adverse environmental stresses. A series ABA signal components have been characterized. The key component of ABA signaling ABI1 regulates several ABA responses. As a target of the PP2C ABI1, the *Arabidopsis thaliana* homeobox protein AthB6 functions down-stream of ABI1 and acts as a negative regulator in ABA signal pathway (Himmelbach et al., 2002).

To identify more ABA signaling components, the screening for the interaction partners of AthB6 was performed. And the AtGluRS (*Arabidopsis thaliana* glutamyl-tRNA synthetase) were identified through screening of the *arabidopsis* cDNA libraries in a yeast two-hybrid system by using the N-terminal AthB6 as the bait. Further studies in yeast three-hybrid system and the *in vivo* co-immunoprecipitation revealed that the AtGluRS interacts not only with AthB6 but also with ABI1. It suggested that the complicated interaction presented among AthB6, AtGluRS and ABI1.

The function of the interaction between AtGluRS, AthB6 and ABI1 was surveyed using maize protoplast transient expression system. The co-expression of ABI1 with AthB6 elevated two fold the AthB6-activated gene expression, while the AtGluRS exerted an inhibition in the AthB6-activated expression of the reporter gene. Results demonstrate a positive regulatory role of ABI1 and a suppression effect of AtGluRS upon the AthB6 function. A basis for explaining these findings was provided by the protein localization studies, which revealed that the AtGluRS-GUS was strictly localized in the cytoplasm, and the ectopic expressed AthB6-GUS was in the nucleus, while the co-expression of both AtGluRS and AthB6-GUS resulted in both nuclear and cytoplasmic localization of AthB6-GUS. Thus, the AtGluRS is postulated to interact with AthB6 in cytoplasm and reduce the nuclear compartmentation of AthB6, consequently the function of AthB6 as the transcriptional regulator is suppressed. The results unravel a new function of AtGluRS as a positive regulator in ABA signal pathway through inhibiting the function of the ABA negative regulator AthB6.

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The MAP Kinase Cascade MKK3-MPK6 Is an Important Part of the Jasmonate Signal Transduction Pathway in *Arabidopsis*. Fuminori Takahashi¹, Riichiro Yoshida², Kazuya Ichimura³, Tsuyoshi Mizoguchi², Shigemi Seo⁴, Masahiro Yonezawa¹, Kyonoshin Maruyama⁵, Kazuko Yamaguchi-Shinozaki^{5,6}, Kazuo Shinozaki^{1,2,3}. ¹RIKEN PSC, Tsukuba, Japan, ²Tsukuba University, Tsukuba, Japan, ³RIKEN PSC, Yokohama, Japan, ⁴NIAST, Tsukuba, Japan, ⁵JIRCAS, Tsukuba, Japan, ⁶The University of Tokyo, Tokyo, Japan

The plant hormone jasmonic acid (JA) plays a key role in the environmental stress responses and developmental processes of plants. Although ATMYC2/JIN1 is a major positive regulator of JA-inducible gene expression and essential for JA-dependent developmental processes in *Arabidopsis*, molecular mechanisms underlying the control of ATMYC2/JIN1 expression remain largely unknown. Here, we identify a Mitogen-Activated Protein kinase (MAPK) cascade, MAPK kinase 3 (MKK3)-MAPK 6 (MPK6), which is activated by JA in *Arabidopsis*. We also show that JA negatively controls ATMYC2/JIN1 expression, based on quantitative RT-PCR and genetic analyses using mutants of gain-of-function and loss-of-function of the MKK3-MPK6 cascade. These results indicate that this kinase unit plays a key role in JA-dependent negative regulation of ATMYC2/JIN1 expression. Both positive and negative regulation by JA may be used for fine-tuning ATMYC2/JIN1 expression to control JA signaling. Moreover, JA-regulated root growth inhibition is affected by mutations in the MKK3-MPK6 cascade, which indicates important roles in JA signaling. We provide a model explaining how MPK6 can convert three distinct signals, JA, pathogen, and cold/salt stress, into three different sets of responses in *Arabidopsis*.

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Heterotrimeric G protein γ Subunits Provide Functional Selectivity in G $\beta\gamma$ Dimer Signaling in Arabidopsis. Yuri Trusov¹, James Rookes¹, Kimberley Tilbrook¹, David Chakravorty¹, Michael Mason¹, David Anderson¹, Jin-Gui Chen², Alan Jones², Jimmy Bo-tella¹. ¹University of Queensland, Brisbane, Australia, ²University of North Carolina, Chapel Hill, USA

The heterotrimeric G protein complex is encoded by single canonical G α and G β subunit genes and two G γ subunit genes in Arabidopsis (AGG1 and AGG2) raising the possibility that the two potential G protein complexes mediate different cellular processes. Analysis of mutants with reduced expression of one or both G γ genes revealed specialized roles for each G γ subunit. AGG1-, but not AGG2-deficient mutants showed impaired resistance against necrotrophic pathogens, reduced induction of the plant defensin gene PDF1.2 and decreased sensitivity to methyl jasmonate. In contrast, both AGG1- and AGG2-deficient mutants were hypersensitive to auxin-mediated induction of lateral roots suggesting that G $\beta\gamma$ 1 and G $\beta\gamma$ 2 synergistically inhibit auxin-dependent lateral root initiation. However, the involvement of each individual G γ subunit in this root response differ, with G $\beta\gamma$ 1 acting within the central cylinder attenuating acropetally transported auxin signaling, while G $\beta\gamma$ 2 affects the action of basipetal auxin and graviresponsiveness within the epidermis and/or cortex, consistent with their gene expression patterns. This selectivity also operates in the hypocotyl. Selectivity in G $\beta\gamma$ signaling was also found in other known AGB1-mediated pathways. agg1 mutants were hypersensitive to glucose and the osmotic agent mannitol during seed germination, while agg2 mutants were only affected by glucose. Our results show that both G γ subunits form functional G $\beta\gamma$ dimers and each provide functional selectivity to the plant heterotrimeric G proteins, revealing a mechanism underlying the complexity of G protein mediated signaling in plants.

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NOVEL OXYLIPIN SIGNALING CASCADES. Tamara Vellosillo¹, Marta Martinez¹, Miguel Angel Lopez¹, Jorge Vicente¹, Tomas Cascon¹, Liam Dolan², Mats Hamberg³, Carmen Castresana¹. ¹Centro Nacional de Biotecnología CSIC, Madrid, Spain, ²John Innes Centre Norwich UK, ³Karolinska Institutet, Stockholm, Sweden

Oxylipins, lipid derivatives generated by oxygenation of fatty acids, function in signaling pathways related to various physiological and pathological responses in both plants and animals. It is known that a variety of plant oxylipins have antimicrobial effects, stimulate plant defense gene expression, and regulate plant growth and development. The most well-characterized oxylipin in plants is the phytohormone jasmonic acid. This and other plant oxylipins are formed from the oxygenation of fatty acids, mainly linoleic acid and linolenic acid, by the action of lipoxygenases or a-dioxygenases, followed by various secondary transformations. The expression of genes encoding these enzyme activities is specifically induced upon inoculation with plant pathogens, and alterations in the synthesis of oxylipins in mutants and transgenic lines have been shown to modify the plant response to pathogen infection.

We used a collection of pure oxylipins and an in vitro seedling assay to study the functionality of compounds in Arabidopsis. Seedlings grown in the presence of oxylipins showed three distinct phenotypic alterations: root waving with lateral root arrest, growth arrest with loss of root apical dominance, and overall decrease of root elongation. Results from studies with mutants impaired in these responses revealed that the root phenotypes observed were mediated by at least three distinct signalling pathways.

Further characterization of the response to 9-hydroxyoctadecatrienoic acid (9-HOT), the strongest inducer of root waving, suggested a role for 9-HOT, or a closely related 9-lipoxygenase product, in the formation of lateral roots and defense against pathogens via effects on gene expression, callose and pectin deposition, and the production of reactive oxygen species.

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THE REGULATORY MECHANISM OF BRASSINOSTEROID RECEPTOR BRI1 SIGNALING BY BKI1 IN ARABIDOPSIS. Xuelu Wang^{1,2}, Shanshan Zhang¹, Niyan Wang¹, Ying Wei¹, Yiming Yu¹, Joanne Chory². ¹The State Key Lab of Genetic Engineering, The School of Life Sciences, Fudan University, Shanghai, China, ²HHMI, The Salk Institute for Biological Studies, La Jolla, CA, USA

Brassinosteroids (BRs) play essential roles in regulating many physiological and developmental processes in plants. BRs are perceived at cell surface by a leucine-rich repeat (LRR) receptor serine/threonine kinase, BRI1. BRs bind to the extracellular domain of BRI1 and induce the phosphorylation of the carboxy-terminus and other domains of the pre-existing BRI1 homodimers. This leads to an enhanced affinity of BRI1 for BAK1, a second LRR-receptor kinase. A newly identified protein, BKI1 specifically interacts with BRI1 and keeps the receptor in an inactive state by preventing the interaction of BRI1 with its positive interactors, such as BAK1. The phenotypes of Arabidopsis that over- or under-express BKI1 and the phosphorylation status of BES1 suggest that BKI1 is a negative regulator of BR signaling. BRs induce a rapid dissociation of BKI1-YFP from the plasma membrane in a BRI1-dependent manner and release the inhibitory effect. A myristoylated BKI1 can constantly associate with plasma membrane and strongly inhibits plant growth. In addition, BKI1 is a phosphoprotein in vivo and can be phosphorylated by BRI1 kinase in vitro, suggesting that BRI1-dependent phosphorylation of BKI1 may be essential for its dissociation from plasma membrane. We have also been identifying factors that interact with BKI1 and investigating the biochemical roles of BKI1 in BRI1 signaling. A model of BKI1's function in BR signaling is proposed.

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Identification of a Lipid Transfer Protein Binding site on Rice Plasma Membrane. Xiaofeng Wang¹, Kaiming Cao¹, Xiaochun Ge¹. ¹Fudan University.

Nonspecific lipid transfer proteins are a type of widely distributed small proteins in plants. It is cysteine-rich, basic and extracellular. A rice nonspecific lipid transfer protein LTP144 was shown to be located in the cell wall and associated with the cell membrane simultaneously when visualized by the fluorescence of GFP in fusion with the protein. The cell membrane association mechanism was studied further using E. Coli expressed active protein Trx-nsLTP144 as binding ligand in the membrane affinity-binding assay. The radioactive 125I-labeled Trx-nsLTP144 can bind to rice plasma membranes with an apparent Kd of 16nM and Bmax of 190fmol/mg proteins. The competition experiment results indicated that the membrane binding activity of Trx-nsLTP144 was specific, which can be competed by cold Trx-nsLTP, but cannot be competed by the fusion tag Thioredoxin. Protease treatment of the plasma membranes can abolish the binding but glycosidase cannot, suggesting that nsLTP144 can bind to a membrane protein. Cross-linking agent BS3 could cross-link 125I-nsLTP144 with the plasma membrane receptor. After SDS-polyacrylamide gel electrophoresis and autoradiography, one putative protein receptor on the rice plasma membrane with the molecular mass around 60 kDa was identified. The in vivo function of nsLTP144 was speculated based on the experiments.

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Mechano-sensitive ATP release in the touch response of *Arabidopsis* roots. Ravisha Weerasinghe¹, Sarah Swanson², Michele Garrett¹, Seiko Okada³, Richard Boucher³, Simon Gilroy², Alan Jones¹. ¹ Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA, ²Department of Biology, Pennsylvania State University, University Park, PA16802, USA, ³Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Perception and response to mechanical stimuli are important to the fitness of organisms. In animal cells, mechanosensing involves the release of ATP and the perception of extracellular ATP by cell surface G-protein coupled receptors. Plant roots are mechanostimulated as they circumnavigate obstacles during their gravity vector mediated downward growth and mechanostimulation of roots causes ATP release. Plant roots lacking a heterotrimeric G-protein complex have a normal gravity-oriented growth response but they poorly circumnavigate barriers indicating that the single G protein complex in *Arabidopsis* is involved in mechanosensing. We dynamically profiled the spatio-temporal changes in ATP and calcium and show that when roots reach a barrier, they utilize a mechanically-stimulated release of ATP distal to the touch position. Touch and ATP induce changes in cytosolic calcium. Roots lacking a heterotrimeric G protein display touch-induced increase in extracellular ATP but their ATP response is diminished. We propose that ATP acts as an extracellular signal released by mechanostimulation in roots and that ATP binds a cell surface receptor that is coupled to calcium mobilization via the heterotrimeric G protein complex.

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Identification of a New Component and Unique Roles of ETR1 and ERS1 in the *Arabidopsis* Ethylene Signal Transduction. Chi-Kuang Wen¹, Fang Xie¹, Xin Zhou¹, Qian Liu¹, Li-Ping Qiu¹. ¹National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China

The ethylene signal transduction in *Arabidopsis* is mediated through a linear pathway involving five ethylene receptors, downstream components, and several regulatory factors. In order to further understand how ethylene responses would be regulated, we focused on the identification of new regulatory components and the study on the receptor signal output. Here we reported that altered expression of a Ser/Thr kinase causes constitutive ethylene responses. On the contrary, the overexpression of the kinase domain leads to ethylene insensitivity. The kinase region is being ectopically expressed in ethylene response mutants to further identify its roles in the regulation of ethylene responses. In an attempt to elucidate how the receptor signal is mediated to downstream components, the ETR1 N-terminal signaling was studied. Previously it is reported that the wild-type ETR1 N terminus cannot repress ethylene responses. However, we found that "etr1-1-349" restored the growth of "etr1-7 ers1-2", but not that of "etr1-7 ers1-3", implying that the wild-type ETR1 N terminus can mediate receptor signal output depending on subfamily I receptors. Besides, the ETR1 N terminus is essential to the function of RTE1, a negative regulator of ethylene responses, while an RTE1 N portion can be dispensable to the etr1-2 function. Specific interactions of "ETR1" and "RTE1" were demonstrated by genetic and transformation studies. Physical interaction between ETR1 and RTE1 was further supported by co-immunoprecipitation. Subcellular localization of RTE1 was studied and possible roles of "RTE1" in the repression of ethylene responses are discussed. Because subfamily I receptors are essential to the ETR1 N terminal signaling while the "RTE1" function is "ETR1" dependent, we further dissected unique function of ETR1 and ERS1. Unexpectedly, genetic analyses reveal that "ERS1" can function as a positive regulator of ethylene responses.

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Application and optimization of Bioluminescence Resonance Energy Transfer (BRET) for real-time detection of protein-protein interactions in transgenic *Arabidopsis* *in vivo*. Jongchan Woo¹, Chitra Subramanian¹, Jamie Light¹, Albrecht von Arnim¹. ¹University of Tennessee, Department of Biochemistry, Cellular and Molecular Biology, Knoxville, TN 37996-0840 USA

Bioluminescence resonance energy transfer (BRET) is a biological phenomenon in some marine organisms such as *Renilla reniformis* and jellyfish where resonance energy from the enzymatic decarboxylation of coelenterazine is transferred from a luciferase to a spectrally compatible fluorescent protein. The distance between the energy donor, for example *Renilla* luciferase (RLUC), to the energy acceptor, for example yellow fluorescent protein (YFP) should be ~5nm. In a typical experiment, a candidate protein is genetically fused to RLUC, and its putative interaction partner is fused to YFP. Although BRET has already been applied successfully to monitor and even image *in vivo* protein-protein interactions in real time, there is room for optimization of RLUCs enzymatic activity. Homology modeling predicted a hydrophobic binding pocket coordinated by 24 amino acids. A gateway composed of residues I163, M174, F180, and T184 is connected to a catalytic triad composed of Asp120, Glu144, and His285. Site-directed and random mutagenesis, as well as pharmacological tests were carried out on RLUC expressed in *E. coli* to test the model of the RLUC structure and its mechanism. Based on the mutagenesis result, we generated a new triple mutant which showed increased luciferase activity and increased half-life, and was more resistant to inhibition by high substrate concentration.

Our results provide enzymatic characteristics of RLUC and, furthermore, suggest that the RLUC triple mutant may possess advantageous properties for BRET assays and BRET imaging of protein-protein interactions in *Arabidopsis*.

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The ETR1 N Terminus Is Essential to the RTE1 Function in the Repression of Ethylene Responses Cross Endomembranes. Zou Xin¹, Qian Liu¹, Fang xie¹, Chi-Kuang Wen¹. ¹National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, PR China

Arabidopsis RTE1 encodes a membrane protein and functions as a negative regulator of ethylene responses. Through genetic and transformation studies, here we show that the function of RTE1 is primarily dependent on ETR1 dosages and can be independent of the other receptors. To narrow down possible regions that are essential to the function of ETR1 and RTE1, truncated etr1 and rte1 were each expressed ectopically. The ETR1 N terminus is essential to the RTE1 function and ectopic expression of ETR1 N terminus restored ethylene insensitivity in 35S::gRTE1 etr1-7. N terminal deletions in RTE1 also restored ethylene insensitivity in etr1-2 rte1-2. These data suggest that ETR1 and RTE1 may each function through specific domains.

Possible interaction between ETR1 and RTE1 was examined by co-immunoprecipitation and our result shows that RTE1 can associate with ETR1 but not ERS1. The RTE1 transcript accumulates upon ethylene treatment but its promoter activity was not ethylene-inducible, implying post-transcriptional regulation of the RTE1 transcript. Subcellular localization of RTE1 was studied using GFP-RTE1 fusion and RTE1 could function in Golgi. Possible mechanisms by which ethylene responses are repressed by RTE1 and ETR1 is discussed.

P-770**The sphingolipid-signalling system in stomatal guard cells.**

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Stomata form pores on leaves and function to regulate the uptake of carbon dioxide for photosynthesis and the loss of water during transpiration under changing environmental conditions. These two physiological processes are of central importance to plant growth and development, contributing to overall carbon assimilation and nutrient uptake. To do this, stomata open or close in response to various environmental stimuli, including light, humidity, atmospheric CO₂ and plant hormones. The guard cell pair that surrounds the stomatal pore control stomatal aperture by regulated changes in cell turgor. Sphingosine-1-phosphate (S1P) has been shown to be an important lipid mediator of stomatal guard cell responses to the drought hormone, abscisic acid (1,2).

Additionally, there is evidence to suggest that Phyto-S1P also regulates changes in stomatal apertures (3). It is likely that the availability of sphingoid bases (sphingosine and phytosphingosine, and hence S1P and Phyto-S1P, respectively) is regulated by the concerted action of both delta-4-desaturases and sphingolipid C4-hydroxylases. We have used fluorescent protein-fusions to demonstrate that the delta-4-desaturase and sphingolipid C4-hydroxylases are localized to the endoplasmic reticulum. Additionally, we observed that the sphingolipid C4-hydroxylases are highly expressed in guard cell protoplasts, relative to mesophyll protoplasts. This suggests the importance of sphingolipid metabolism to stomatal guard cell function.

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P-771**Regulation of growth and mRNA translation by the *Arabidopsis* TOR (target of rapamycin) kinase.**

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The modulation of growth rate in response to environmental cues such as nutrient availability or stresses is essential for survival. The central function of the conserved eukaryotic TOR kinase is to promote cell growth and repress autophagy of cellular constituents in response to growth factors or favorable conditions. In animal and yeast cells, the TOR signalling pathway is now starting to be well known and has links with mitogens and growth-promoting factors signalling pathways. Plants, unlike animals, have a plastic and undetermined organ growth which is tightly controlled by exogenous information. However, little is known so far on how this information is perceived and transduced into coherent growth and developmental decisions. The *Arabidopsis* TOR kinase (AtTOR) is essential for embryo development but its precise role remains to be established. Here we show that plant vegetative and reproductive growth is positively correlated with the level of expression of the AtTOR kinase. AtTOR kinase regulates plant cell size and cell number and the accumulation of biomass. The level of expression of the AtTOR gene is also correlated with the plant tolerance to osmotic stresses. Downregulation of AtTOR by constitutive or inducible RNAi led to a post-germinative halt in plant growth, to reduced growth in the vegetative phase and to early senescence. Plants with partial silenced AtTOR expression also showed a marked reduction in the amount of polysomes. We conclude that the AtTOR kinase is one of the main contributors to the link between environmental cues and growth processes in plants. Therefore the manipulation of the AtTOR expression and signalling pathway could be of importance for improving plant biomass production.

§ coauthor

P-772**The Isolation of Mutants that Enhance or Suppress a Weak ctr1 Mutation.**

wei zhang¹, chan xu¹, Chi-Kuang Wen^{1,1} National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences

The seedling triple-response phenotype has been used for the analysis of ethylene responses in *Arabidopsis* and for the isolation of ethylene response mutants. However, it appears that no more new mutant can be isolated based on the seedling triple-response phenotype, probably due to the saturation of genetic screen. Here we report that using a weak ctr1 (Constitutive Triple Response 1) mutant, we were able to isolate mutants that enhance or suppress the ctr1 mutant phenotype. The ecr (Enhancer of CTR1) mutation elevates the ctr1 mutant phenotype and the ecr ctr1 double mutant exhibits severe inhibition in the hypocotyl elongation in etiolated seedling and the rosette growth is largely inhibited when grown in the air. The rcr (Reverser of CTR1) mutation substantially restores the ctr1 mutant growth and the rcr ctr1 double mutant exhibits a long seedling hypocotyl and root growth in the air. In the adult stage, crc ctr1 phenotypically resembles weak ctr1 mutant. When germinated in ethylene, the rcr ctr1 seedling is short but exhibits root growth and has no apical hook. Genetic analyses for the ecr and rcr mutants are now in progress. Preliminary data suggest that the ecr mutant phenotype is dependent on the ctr1 mutation, implying an ethylene signal transduction pathway in parallel to or independent of CTR1.

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An Arabidopsis CDPK Functions in Calcium Regulation of Pollen Inward Potassium Channels and Pollen Tube Growth. Wen-Zheng Zhang¹, Wei Zhang¹, Lian-Fen Song¹, Jun-Jie Zou¹, Li Yao¹, Xiao-Ling Ren¹, Li-Na Zhao¹, Wei-Hua Wu¹. ¹State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, National Plant Gene Research Centre (Beijing), China Agricultural University, Beijing 100094, China

Cytosolic free Ca²⁺ plays crucial roles in the regulation of pollen germination and tube growth, but little is known about the downstream regulatory components of Ca²⁺ signaling pathways. K⁺-uptake by a growing pollen tube is essential for pollen tube growth. However, molecular mechanism of K⁺-channel regulation during pollen germination and tube growth remains unknown. Here, we show that an Arabidopsis CDPK regulates pollen tube growth and K⁺ influx during pollen germination and tube growth. Overexpression of this AtCPK significantly inhibited pollen tube growth, whereas pollen tubes of T-DNA insertion mutant grew faster than that of wild type plants. By conducting patch-clamp whole-cell recording with pollen and pollen tube protoplasts, we observed that increase of cytoplasmic [Ca²⁺] from 10 nM to 10 mM significantly inhibited the inward K⁺ currents for wild type plants, whereas increase of cytoplasmic [Ca²⁺] had no effect on the inward K⁺ currents for this AtCPK knock-out mutants. The results demonstrated that this AtCPK, may mediate regulation of K⁺ influx by cytoplasmic [Ca²⁺] changes and consequently regulates pollen germination and tube growth.

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Arabidopsis IQM1 Encodes an IQ Motif-Containing Protein That is Involved in Calmodulin, Light and Auxin Signaling *. Yiping Zhou¹, Xiaolan Wang¹, Takahiro Fujibe², Huizen Cheng¹, Kotaro T. Yamamoto², Chang-en Tian^{1,3}, ¹Research Center for Genomics Function and Biological Microarray, Guangzhou University, Guangzhou 510405 China,²Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo, 060-0810 Japan,³ School of Biological Science, Guangzhou University, Guangzhou 510006 China

Calmodulin (CaM), acting as a kind of receptor of Ca²⁺, plays a central role in the Ca²⁺ signal transduction. Through its downstream target, such as calmodulin-binding protein (CaMBP), CaM regulates cell physiology. Therefore functional characterization of CaMBP is essential to reveal CaM signaling. We have been carrying out phenotypic characterization of two T-DNA insertion lines of the IQM1 gene available in the Salk T-DNA library. The IQM1 gene consists of 9 exons, and its product contains an IQ motif to which CaM possibly binds in the absence of calcium ion. Using the yeast two-hybrid system, IQM1 was demonstrated to bind with CaM2, which is one of 9 typical CaMs in Arabidopsis. RT-PCR analyses showed that the IQM1 mRNA was not transcribed downstream of the T-DNA insertion site in either mutant. Higher concentrations of auxin inhibit root hair formation in wild type. However, root hair formation was not inhibited in iqm1 as readily as in wild type. Staining pattern of the IQM1 promoter-GUS lines showed that IQM1 strongly expressed in the cortex of the differentiation zone of roots. The GUS staining was also observed in guard cells and trichomes of leaves. Both RT-PCR analysis and the GUS staining showed that IQM1 was a light-inducible gene. In addition, IQM1 was strongly induced by treatment with dehydration or NaCl when Arabidopsis was grown in the dark condition. The present results suggest that IQM1 is involved in adaptation to the environmental changes as well as differentiation of epidermal cells, both of which may require CaM signaling.

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These authors contribute to this work equally.

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Involvements of the SPX and EXS domains of SHB1 in Light Signaling. Yun Zhou¹, Min Ni². ¹Department of Plant Biology, University of Minnesota, Saint Paul, MN, USA,²Department of Plant Biology, University of Minnesota, Saint Paul, MN, USA

The phy- and cry-mediated red, far-red, and blue light perception and signaling regulate plant photomorphogenesis. We have identified an Arabidopsis knock-out mutant shb1 or short hypocotyl under blue 1. However, shb1-D, a dominant gain-of-function allele, exhibited a long hypocotyl phenotype under red, far-red, and blue light. Therefore, SHB1 is involved in cry-mediated blue light signaling but over-expression of SHB1 may expand its signaling activity to red and far-red light. SHB1 is homologous to SYG1 protein family, and contains a N-terminal SPX domain and a C-terminal EXS domain. In order to investigate the structure and function of SHB1 in light signaling, we have conducted transgenic deletion analysis and forward genetic screen for intragenic or extragenic suppressors. The transgenic plants that overexpress the N-terminal 520 amino acids phenocopied shb1-D with a long hypocotyl phenotype under red, far-red, and blue light. In contrast, the transgenic plants that overexpress three C-terminal truncations showed a short hypocotyl under blue light similar to shb1. The phenotypes may be created through a dominant negative mechanism, and all three truncations encompass the putative EXS domain. Forward genetic suppressor screens of shb1-D have identified 58 suppressors. Among them, 10 have been characterized as intragenic suppressors and the rest are extragenic. The intragenic suppressors all contained mis-sense mutations in the SHB1 gene and caused several amino acids alterations in its N-terminal SPX domain. In summary, we demonstrated that the SPX domain is important for SHB1 signaling, whereas the EXS domain may be involved in the formation of a homodimer or an interaction of SHB1 with other protein molecules.

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Molecular control of S-RNase-based self-incompatibility. Lan Zhao¹, Jian Huang¹, Zhonghua Zhao¹, Qun Li¹, Yongbiao Xue¹. ¹Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

The self-incompatibility (SI) response occurs widely in flowering plants as a means of preventing self-fertilization. In these self/non-self discrimination systems, plant pistils reject self or genetically related pollen. In the most widespread SI system, pistil-secreted S-RNases enter the pollen cytoplasm and function as cytotoxins to specifically inhibit growth of the cognate pollen tube. However, the precise role of the S-locus F-box protein, the pollen determinant, is largely unknown. We provide evidence showing that SLF-interacting SKP1-like1 (SSK1) protein is a specific adaptor in an SCFSLF complex and that SSK1 is essential for pollen to overcome S-RNase cytotoxicity. This pollen-specific SSK1-SLF interaction occurs in Petunia and Antirrhinum, two species from the Solanaceae and Scrophulariaceae, respectively, indicating that this novel SCFSLF complex is conserved in the two different families with the S-RNase-based SI system. Significant reduction of SSK1 expression level does not impair the pollen tube growth in SI-defective styles, but results in pollen inhibition in cross-pollinations of functional SI-styles. This general incompatibility suggests that the pollen determinant contributes to inhibiting, rather than maintaining the S-RNase activity, at least in solanaceous plants.

Aceituno Felipe	S-28	Chabannes Matthieu	P-648	Ent Sjoerd Van der	S-46
Adachi Sumiko	S-77	Chakravorty David	P-702	Escobar-Restrepo Juan-Miguel	S-2
Aggarwal Pooja	P-183	Chandler John	P-202	Espunya M. Carme	P-653
Aguilar-Martínez José Antonio	P-184	Chandra-Shekara A. C.	P-703	Estavillo Gonzalo	P-214
Aida Mitsuhiro	P-185	Chandra-Shekara A. C.	S-67	Estelle Mark	P-714
Airoldi Chiara A	P-186	Chang Caren	P-704	Estelle Mark	S-57
Aker Jose	P-698	Chao Xu	P-535	Fan Jun	P-654
AKI Shiori	P-530	Chapple Clint	P-492	Farhoudi rozbeh	P-655
Altamura Maria Maddalena	P-187	Chapple Clint	S-61	Feldmann Kenneth	S-69
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Bao Dongping	P-189	Chen Xin	P-128	Fujiwara Makoto	P-133
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Bartel Bonnie	S-26	Chen Yanmei	P-707	Gabiati Massimo	P-550
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Beale Michael	P-488	Chen Zhongying	P-536	Ganagappa Sreeramaiah N.	P-715
Beckers Gerold	P-532	Che Ping	P-203	Gang Gee-Sook	P-551
Beemster Gerrit	P-192	Chini Andrea	P-709	Gan Yinbo	P-91
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Bem Sylwia	P-123	Cho Daeshik	P-710	Gao Ming-Jun	P-218
Benfey Philip	S-9	Choi Kyuha	P-207	Gao Ming-Jun	P-219
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