



21ST INTERNATIONAL CONFERENCE ON ARABIDOPSIS RESEARCH



Yokohama, Japan
June 6-10, 2010
Pacifico Yokohama

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21st International Conference on Arabidopsis Research

Program & Abstracts

June 6 – 10, 2010
Pacifico Yokohama
YOKOHAMA, JAPAN

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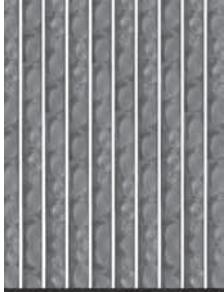
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Kazuko Yamaguchi-Shinozaki (The University of Tokyo / JIRCAS, Japan)

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ARABIDOPSIS

2011

JUNE 22-25 • UNIVERSITY OF WISCONSIN-MADISON WISCONSIN, USA



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WELCOME BACK!

Contact: Joanna Friesner, NAASC Coordinator: jdfriesner@gmail.com
<http://www.union.wisc.edu/arabidopsis/>

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SESSION OVERVIEW

Sunday, June 6, 2010 15:30 - 15:40 15:40 - 17:40 18:00 - 20:00	Opening Remark Keynote Lectures Welcome Drink
Monday, June 7, 2010 09:00 - 10:30 11:00 - 12:30 12:45 - 13:45 14:00 - 15:45 16:15 - 18:00 18:30 - 19:45 18:45 - 20:45	Plenary 1: Plant Hormone Regulation Plenary 2: Cell Biology MASC Phenome Subcommittee Forum MASC Proteomics Subcommittee Forum Concurrent 1: Regeneration Concurrent 2: Biotic Responses Concurrent 3: Abiotic Responses Concurrent 4: Epigenetic and RNA Regulation Workshop 1 - 3 Poster Session 1: Odd-numbers
Tuesday, June 8, 2010 09:00 - 10:30 11:00 - 12:30 12:30 - 18:30 13:00 - 15:00 18:30 - 19:45 18:45 - 20:45	Plenary 3: Environmental Responses Plenary 4: Epigenomics and RNA Regulation Free Afternoon "NSF 2010 and Beyond" Session Workshop 4 - 6 Poster Session 2: Even-numbers
Wednesday, June 9, 2010 09:00 - 10:30 11:00 - 12:30 14:30 - 16:15 16:45 - 18:30 19:00 - 21:00	Plenary 5: Crop Genomics Plenary 6: Systems Biology and Metabolism Concurrent 5: Novel Function of Peptides in Intercellular Signaling Concurrent 6: Metabolism and Systems Biology Concurrent 7: Developmental Regulation Concurrent 8: Research Tools and Resources Banquet
Thursday, June 10, 2010 09:00 - 10:30 11:00 - 12:30 12:30 - 12:40	Plenary 7: Evolution and Natural Variations Plenary 8: Development Closing Remark

MEETING SPONSORS

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The Japanese Society of Plant Physiologists

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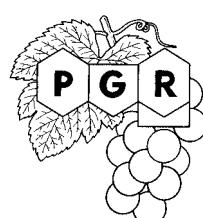


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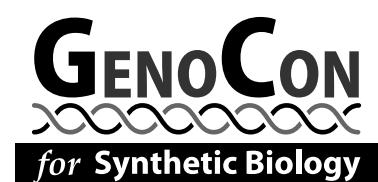
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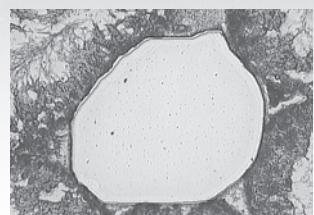
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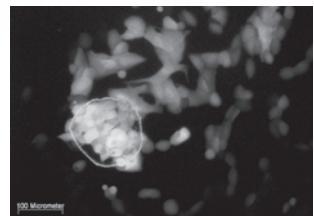
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Boulin's paraffin embedded Mouse testis, stained with Hematoxylin and Eosin.



Boulin's paraffin embedded Mouse testis, stained with Hematoxylin and Eosin.



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PROGRAM OVERVIEW

Poster Schedule

All posters are displayed throughout the conference in the Exhibition Hall A. Exhibition Hall A will be opened on the following schedule:

June 7 (Day 2) 8:30 - 22:00
June 8 (Day 3) 8:30 - 22:00
June 9 (Day 4) 8:30 - 21:00
June 10 (Day 5) 8:30 - 12:00

The posters are classified by category number. Poster abstracts are available in the abstract book or online web site of this conference. Posters of late submission are available only in the web site. Each poster board (W90 X H210 cm) is numbered. Poster presenters can mount their posters on the board according to their poster abstract number from 8:30 of June 7 (Day 2). Poster presenters are requested to stand in front of their posters in the following time:

Odd-numbers: June 7 (Day 2) 18:45 - 20:45
Even-numbers: June 8 (Day 3) 18:45 - 20:45

All posters have to be removed by the noon of June 10 (Day 5). At the lunch time of June 10, all remaining posters will be removed by the conference organizers.

Sunday 6th June 2010

14:00 - 20:00	Registration	2F Counter
15:30 - 15:40	Opening Remark Kazuo Shinozaki	1F Main Hall
15:40 - 17:40	Keynote Lecture Chairs - Kazuo Shinozaki and Kiyotaka Okada	1F Main Hall
<hr/> <p>Abstract K001: Maarten Koornneef, Max Planck Institute for Plant Breeding Research, Germany – <i>Arabidopsis natural variation: from genes to evolutionary ecology</i></p>		
<hr/> <p>Abstract K002: Elliot M. Meyerowitz, California Institute of Technology, USA – <i>Plant Computational Morphodynamics: Live Imaging and Computational Models of Pattern Formation at the Shoot Apex</i></p>		
<hr/> <p><i>These lectures are sponsored by Japan Society for the Promotion of Science (JSPS).</i> http://www.jsps.go.jp/english/index.html</p>		
18:00 - 20:00	Welcome Drink	3F 301-304

Monday 7th June 2010

08:30 - 18:30	Registration	2F Counter
08:30 - 22:00	Posters Open	Exhibition Hall A
09:00 - 10:30	Plenary Session 1: <i>Plant Hormone Regulation</i> Chair - Hitoshi Sakakibara	1F Main Hall
	09:00-09:30 Abstract PL101: Peter McCourt, University of Toronto, Canada — "Think globally act locally" Using systems biology to understand ABA signaling	
	09:30-10:00 Abstract PL102: Shinjiro Yamaguchi, RIKEN Plant Science Center, Japan — Regulation of shoot branching by strigolactones	
	10:00-10:30 Abstract PL103: Makoto Matsuoka, Nagoya University, Bioscience and Biotechnology Center, JAPAN — Molecular Interaction of Gibberellin Signaling Components, GID1, SLR1, and GID2	
	<i>The session is sponsored by The Plant Journal/Wiley-Balckwell.</i> http://www3.interscience.wiley.com/journal/118488398/home	
10:30 - 11:00	Refreshment Break	
11:00 - 12:30	Plenary Session 2: <i>Cell Biology</i> Chair - Akira Nagatani	1F Main Hall
	11:00-11:30 Abstract PL201: Kathryn M. Barton, Department of Plant Biology, Carnegie Institution for Science, Stanford, USA — HDZIP SOUP: Untangling the network that controls axis formation in <i>Arabidopsis</i>	
	11:30-12:00 Abstract PL202: Ikuko Hara-Nishimura, Kyoto University, Japan — A membrane fusion-mediated plant defense strategy against bacterial pathogens	
	12:00-12:30 Abstract PL203: Inhwan Hwang, Pohang University of Science and Technology, Korea — Distribution of newly synthesized organellar proteins in plant cells	
	<i>This session is sponsored by Plant and Cell Physiology/Oxford University Press.</i> http://pcp.oxfordjournals.org/	
12:30 - 14:00	Lunch	
12:45 - 13:45	MASC Phenome Subcommittee Forum Organizers - Eva Huala and Minami Matsui	3F 313-314
12:45 - 13:45	MASC Proteomics Subcommittee Forum Organizers - Joshua L. Heazlewood, Alex Jones and Hirofumi Nakagami	3F 315
14:00 - 15:45	Concurrent Session 1: <i>Regeneration</i> Chair - Mitsuyasu Hasebe	1F Main Hall
	14:00-14:25 Abstract C101: Kenneth D. Birnbaum, New York University, USA — Plant Regeneration and Root Meristem Organization	

14:25-14:50 Abstract C102: Elliot M. Meyerowitz, California Institute of Technology, USA — *Callus Formation and Shoot Regeneration in Arabidopsis*

14:50-15:15 Abstract C103: Mitsuyasu Hasebe, Japan Science and Technology Agency / National Institute for Basic Biology, Japan — *Molecular mechanisms of cell-fate change from a differentiated cell to a stem cell in the moss Physcomitrella patens*

15:15-15:30 Abstract C104: Akira Iwase, Plant Science Center, RIKEN, Japan — *A Wound Responsive Transcription Factor WIND1 Promotes Cell Dedifferentiation in Arabidopsis*

15:30-15:45 Abstract C105: Munetaka Sugiyama, The University of Tokyo, Japan — *Requirement for the RID3-dependent negative control of cell proliferation in de novo morphogenesis*

This session is sponsored by The Hasebe Reprogramming Evolution of ERATO-JST.
http://www.jst.go.jp/erato/project/hbz_P/hbz_P.html

14:00 - 15:45 Concurrent Session 2: *Biotic Responses* 3F 301-302
Chair - Ken Shirasu

14:00-14:25 Abstract C201: Xinnian Dong, Duke University, USA — *Cellular responses in plant immunity*

14:25-14:50 Abstract C202: Scott C. Peck, Department of Biochemistry, University of Missouri, USA — *MAP kinase phosphatase 1 is a negative regulator of PAMP responses and resistance against Pseudomonas syringae pv tomato*

14:50-15:15 Abstract C203: Jian-Min Zhou, National Institute of Biological Sciences, Beijing, China — *Pseudomonas syringae effector proteins modulate MAP kinases to enhance host susceptibility*

15:15-15:30 Abstract C204: Jane Glazebrook, University of Minnesota, United States — *Multiple routes to activation of salicylic acid signaling - A key role of CBP60 proteins*

15:30-15:45 Abstract C205: Thorsten Nuernberger, University of Tuebingen, Center of Plant Molecular Biology, Germany — *The peptidoglycan pattern recognition receptor LYM3 is crucial for Arabidopsis immunity to bacterial infection*

This session is sponsored by The North American Arabidopsis Steering Committee (NAASC).
http://www.arabidopsis.org/portals/masc/countries/NAASC_Info.jsp

15:45 - 16:15 Refreshment Break

16:15 - 18:00 Concurrent Session 3: *Abiotic Responses* 1F Main Hall
Chair - Kazuko Yamaguchi-Shinozaki

16:15-16:40 Abstract C301: Zhizhong Gong, China Agricultural University, China — *ABA Regulates Root Development*

16:40-17:05 Abstract C302: Erwin Grill, Technische Universität München, Germany — *Plant's long and short distance signalling for controlling the water status*

17:05-17:30 Abstract C303: Kazuko Yamaguchi-Shinozaki, The University of Tokyo, Japan — *Regulatory networks of gene expression in abiotic stress response in Arabidopsis*

17:30-17:45 Abstract C304: Laurent Nussaume, CEA, FRANCE — *Molecular dissection of local and systemic responses to phosphate starvation in Arabidopsis thaliana*

17:45-18:00 Abstract C305: Takashi Kuromori, RIKEN Plant Science Center, Japan — *ABC transporter AtABCG25 involved in ABA transport and responses*

This session is co-sponsored by Target Proteins Research Program of MEXT and Plant Physiology/American Society of Plant Biologists (ASPB).
http://www.tanpaku.org/e_index.php
<http://www.plantphysiol.org/>

16:15 - 18:00 Concurrent Session 4: *Epigenetic and RNA Regulation* 3F 301-302
Chairs - Yuichiro Watanabe and Tetsuji Kakutani

16:15-16:40 Abstract C401: Marjori Matzke, Gregor Mendel Institute of Molecular Plant Biology, Austria — *RNA-directed DNA methylation*

16:40-17:05 Abstract C402: Vincent Colot, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), FRANCE — *Mechanisms and phenotypic consequences of DNA methylation in Arabidopsis*

17:05-17:30 Abstract C403: Daniel Zilberman, University of California, Berkeley, USA — *DNA methylation in rice seed*

17:30-17:45 Abstract C404: Ho-Ming Chen, Institute of Plant and Microbial Biology, Academia Sinica, Taiwan — *22-nucleotide RNA triggers secondary siRNA biogenesis in plants*

17:45-18:00 Abstract C405: Filipe Borges, Instituto Gulbenkian de Ciencia, Portugal — *The Role of miRNAs During Germ Cell Specification in Arabidopsis Pollen*

This session is sponsored by National Institute of Genetics (NIG).
<http://www.nig.ac.jp/index-e.html>

18:00 - 18:30 Refreshment Break

18:30 - 19:45 Workshop 1: *Polypliody and Hybrid Vigor* 3F 301-302
Organizer - Z. Jeffrey Chen

Workshop 2: *Future of Arabidopsis Informatics* 3F 303-304
Organizer - Ruth Bastow

Workshop 3: *Plant Senescence and the Associated Cell Death* 3F 315
Organizers - Hong Gil Nam and Pyung Ok Lim

18:45 - 20:45 Poster Session 1: *Odd-numbers* Exhibition Hall A

This session is sponsored by Bayer CropScience K.K.
<http://www.bayercropscience.com/>

Tuesday 8th June 2010

08:30 - 18:30	Registration	2F Counter
08:30 - 22:00	Posters Open	Exhibition Hall A
09:00 - 10:30	Plenary Session 3: <i>Environmental Responses</i> Chair - Takashi Araki	3F 301-304
	09:00-09:30 Abstract PL301: Steve A. Kay, University of California, San Diego, USA — <i>Large Scale Approaches to Deconvolving Arabidopsis Circadian Networks</i>	
	09:30-10:00 Abstract PL302: Chentao Lin, University of California, Los Angeles, USA — <i>The signaling mechanisms of Arabidopsis blue light receptors cryptochromes</i>	
	10:00-10:30 Abstract PL303: Koh Iba, Kyushu University, Japan — <i>Analysis of Arabidopsis CO₂ response mutants by thermal imaging</i>	
	<i>This session is co-sponsored by RIKEN Plant Science Center and BioResource Center.</i> http://www.psc.riken.go.jp/english/index.html http://www.brc.riken.go.jp/inf/en/index.shtml	
10:30 - 11:00	Refreshment Break	
11:00 - 12:30	Plenary Session 4: <i>Epigenomics and RNA Regulation</i> Chair - Motoaki Seki	3F 301-304
	11:00-11:30 Abstract PL401: David C. Baulcombe, University of Cambridge, United Kingdom — <i>The diverse roles of small, non coding RNA in plants</i>	
	11:30-12:00 Abstract PL402: Tetsuji Kakutani, National Institute of Genetics, Japan — <i>Genetics of DNA methylation in genes and transposons</i>	
	12:00-12:30 Abstract PL403: Joseph R. Ecker, The Salk Institute, USA — <i>Sequencing Epigenomes of Plants and People</i>	
	<i>This session is sponsored by RIKEN Plant Science Center.</i> http://www.psc.riken.go.jp/english/index.html	
12:30 - 14:00	Lunch	
12:30 - 18:30	Free Afternoon	
13:00 - 15:00	"NSF 2010 and Beyond" Session Chair - Mary Lou Guerinot Machi Dilworth - <i>Opening Remark</i> Andrew Millar - <i>From Genetics to Systems Biology with GARNet</i> Klaus Harter - <i>AFGN: A success story of basic research in and around Germany</i> Mary Lou Guerinot - <i>From the Ionome to the Genome: Identifying the gene networks involved in regulating ion homeostasis in plants</i> Kazuo Shinozaki - <i>Arabidopsis Functional Genomics in Japan</i>	3F 301-304

18:30 - 19:45	Workshop 4: <i>Brassicaceae Map Alignment Project: An International Consortium to Generate High Quality Phylogenetically Representative Reference Genomes across the Brassicaceae</i> Organizers - Rod A. Wing, Detlef Weigel and Chris J. Pires	3F 301-302
	Workshop 5: <i>Cell separation processes in plants</i> Organizers - Zinnia H. Gonzalez-Carranza, Reidunn Aalen and Melinka Butenko	3F 303-304
	Workshop 6: <i>TAIR workshop: Making best use of TAIR tools and datasets</i> Organizer - Eva Huala	3F 315
18:45 - 20:45	Poster Session 2: <i>Even-numbers</i>	Exhibition Hall A

This session is co-sponsored by American Society of Plant Biologists (ASPB) and The North American Arabidopsis Steering Committee (NAASC)
<http://www.aspб.org/>
http://www.arabidopsis.org/portals/masc/countries/NAASC_Info.jsp

Wednesday 9th June 2010

08:30 - 18:30	Registration	2F Counter
08:30 - 21:00	Posters Open	Exhibition Hall A
09:00 - 10:30	Plenary Session 5: <i>Crop Genomics</i> Chair - Minami Matsui	3F 301-304
	09:00-09:30 Abstract PL501: Satoshi Tabata, Kazusa DNA Research Institute, Japan — <i>Development of a basis for marker-assisted selection in breeding in crop plants</i>	
	09:30-10:00 Abstract PL502: Qifa Zhang, Huazhong Agricultural University, China — <i>Progresses of rice functional genomics research and the implications in crop genetic improvement</i>	
	10:00-10:30 Abstract PL503: Michael W. Bevan, John Innes Centre, UK — <i>Progress in Crop Plant Genomics</i>	
	<i>This session is sponsored by Kazusa DNA Research Institute.</i> http://www.kazusa.or.jp/e/index.html	
10:30 - 11:00	Refreshment Break	
11:00 - 12:30	Plenary Session 6: <i>Systems Biology and Metabolism</i> Chair - Kazuki Saito	3F 301-304
	11:00-11:30 Abstract PL601: Dirk Inzé, VIB; UGent, Belgium — <i>Systems biology of leaf growth</i>	
	11:30-12:00 Abstract PL602: Seung Yon Rhee, Carnegie Institution for Science, Arabidopsis Metabolomics Consortium, USA — <i>Integration of Metabolomics, Metabolic Network and Gene Function Network for Systematic Gene Function Identification</i>	
	12:00-12:30 Abstract PL603: Kazuki Saito, RIKEN Plant Science Center; Graduate School of Pharmaceutical Sciences, Chiba University, Japan — <i>Plant metabolomics for systems biology - from Arabidopsis to crops</i>	
	<i>This session is sponsored by RIKEN Plant Science Center.</i> http://www.psc.riken.go.jp/english/index.html	
12:30 - 14:30	Lunch	
14:30 - 16:15	Concurrent Session 5: <i>Novel Function of Peptides in Intercellular Signaling</i> Chair - Hiroo Fukuda	3F 301-302
	14:30-14:55 Abstract C501: Rüdiger Simon, Heinrich-Heine University, Germany — <i>Peptide signalling in plant primary meristems</i>	
	14:55-15:20 Abstract C502: Hiroo Fukuda, University of Tokyo, Japan — <i>CLE Peptides Regulating Vascular Stem Cell Fates</i>	

15:20-15:45 Abstract C503: Tetsuya Higashiyama, Nagoya University, Japan — *Pollen tube guidance by attractant peptides*

15:45-16:00 Abstract C504: Melinka A. Butenko, University of Oslo, Norway — *A Transcription Factor Suppressing Floral Abscission Downstream in the IDA-HAE/HSL2 Signaling Pathway*

16:00-16:15 Abstract C505: Tomoo Shimada, Graduate School of Science, Kyoto University, Japan — *Stomagen is a mesophyll-derived positive regulator of stomatal density*

This session is co-sponsored by The Fukuda Plant Peptides Project of BRAIN and The Plant Cell/American Society of Plant Biologists (ASPB).
<http://brain.naro.affrc.go.jp/index-e.html>
<http://www.plantcell.org/>

14:30 - 16:15 Concurrent Session 6: *Metabolism and Systems Biology* 3F 303-304
Chairs - Masami Yokota Hirai, Tetsuro Mimura and Shuichi Yanagisawa

14:30-14:55 Abstract C601: Tetsuro Mimura, Kobe University, Graduate School of Science, JST CREST, Japan — *Metabolome researches in plant metabolic regulation*

14:55-15:20 Abstract C602: Stanislav Kopriva, John Innes Centre, Norwich, UK — *The importance of having sulfur*

15:20-15:45 Abstract C603: Robert L. Last, Michigan State University, USA — *Forward and Reverse Genetics Meet: The Chloroplast 2010 Project*

15:45-16:00 Abstract C604: Wolf B Frommer, Carnegie Institution for Science, USA — *High throughput analysis of membrane/signaling protein interactions in Arabidopsis*

16:00-16:15 Abstract C605: Fumiaki Katagiri, University of Minnesota, USA — *Network modeling reveals prevalent negative regulatory relationships between signaling sectors in Arabidopsis immune signaling.*

This session is sponsored by The Metabolomics Project of CREST-JST.
<http://www.jst.go.jp/kisoken/crest/en/category/area03-5.html>

Organizers (Masami Yokota Hirai, Tetsuro Mimura and Shuichi Yanagisawa) dedicate the present session to a Professor Emeritus at the University of Tokyo, Koichi Suzuki. Prof. Suzuki was a significant research supervisor for CREST metabolome research groups.

16:15 - 16:45 Refreshment Break

16:45 - 18:30 Concurrent Session 7: *Developmental Regulation* 3F 301-302
Chairs - Yasunori Machida and Hidehiro Fukaki

16:45-17:10 Abstract C701: Gerd Jürgens, University of Tübingen, Germany — *Cell fate specification in early embryogenesis*

17:10-17:35 Abstract C702: Mary Byrne, John Innes Centre, United Kingdom — *A role for the ribosome in organ patterning*

17:35-18:00 Abstract C703: Malcolm J. Bennett, Centre for Plant Integrative Biology (CPIB), University of Nottingham, UK — *Systems Analysis of Lateral Root Development: An Emerging Story*

18:00-18:10 Abstract C704: Fred Sack, University of British Columbia, Canada — *Regulation of stomatal lineage cell proliferation by the Arabidopsis MYB FOUR LIPS via direct targeting of core cell cycle genes*

18:10-18:20 Abstract C705: Toshiro Ito, Temasek Life Sciences laboratory, Singapore — *Cell cycle-dependent developmental timing control of floral stem cells*

18:20-18:30 Abstract C706: Ykä Helariutta, FIN-00014 University of Helsinki, Finland — *Analysis of cell signalling during vascular morphogenesis in Arabidopsis*

This session is sponsored by The Machida Plant Meristems Project of JSPS.
http://www.bio.nagoya-u.ac.jp/~yas/tokutei_plant_meristems/0903tokutei/index.html
<http://www.jsps.go.jp/english/e-grants/grants.html>

16:45 - 18:30 Concurrent Session 8: *Research Tools and Resources* 3F 303-304
Chairs - Masatomo Kobayashi and Minami Matsui

16:45-17:10 Abstract C801: Masatomo Kobayashi, RIKEN BioResource Center, Japan; Minami Matsui, RIKEN Plant Science Center, Japan — *Japanese resource project on Arabidopsis – outlines and introduction of FOX lines*

17:10-17:35 Abstract C802: Erich Grotewold, The Ohio State University, USA — *The Arabidopsis Biological Resource Center (ABRC)*

17:35-18:00 Abstract C803: Eva Huala, Carnegie Institution for Science, Stanford, CA, USA — *New data and tools at TAIR (the Arabidopsis Information Resource)*

18:00-18:15 Abstract C804: Geert De Jaeger, VIB-Ghent University, Belgium — *Protein complex purification for gene network building*

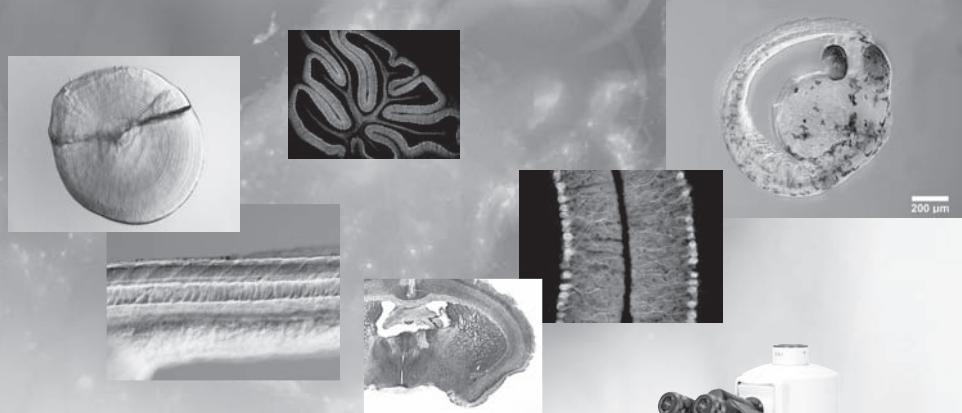
18:15-18:30 Abstract C805: David Somers, Ohio State University/POSTECH, USA/South Korea — *Rapid assessment of gene function using artificial microRNA in Arabidopsis mesophyll protoplasts*

This session is sponsored by RIKEN BioResource Center.
<http://www.brc.riken.go.jp/lab/epd/Eng/>

19:00 - 21:00 Banquet Ballroom,
InterContinental Yokohama The Grand Hotel 3F

Thursday 10th June 2010

08:30 - 12:30	Registration	2F Counter
08:30 - 12:00	Posters Open	Exhibition Hall A
09:00 - 10:30	Plenary Session 7: <i>Evolution and Natural Variations</i> Chair - Hirokazu Tsukaya	3F 301-304
<hr/>		
	09:00-09:30 Abstract PL701: Olivier Loudet, INRA, France – <i>Natural variation for growth and the interaction with the environment in Arabidopsis thaliana</i>	
	09:30-10:00 Abstract PL702: Detlef Weigel, Max Planck Institute for Developmental Biology, Germany – <i>Next-generation genetics in Arabidopsis thaliana: Evolutionary tradeoffs, immunity and speciation</i>	
	10:00-10:30 Abstract PL703: Motoyuki Ashikari, Nagoya University, Japan – <i>Rice breeding by QTL pyramiding</i>	
<hr/>		
	<i>This session is sponsored by Journal of Plant Research/Springer Japan KK</i> <i>http://www.springerlink.com/content/j248h8566528/?p=3d31d16c42454af99f2ba381f36b1462&pi=0</i>	
10:30 - 11:00	Refreshment Break	
11:00 - 12:30	Plenary Session 8: <i>Development</i> Chair - Kiyotaka Okada	3F 301-304
	11:00-11:30 Abstract PL801: Keiko U. Torii, University of Washington, USA – <i>Receptor signaling in Stomatal Patterning: Complexity, Challenges, and New Insights</i>	
	11:30-12:00 Abstract PL802: Philip N. Benfey, Duke University, USA – <i>Development rooted in interwoven networks</i>	
	12:00-12:30 Abstract PL803: Cris Kuhlemeier, University of Bern, Switzerland – <i>Leaf development: molecular genetics of the golden angle</i>	
<hr/>		
	<i>This session is sponsored by National Institute for Basic Biology (NIBB).</i> <i>http://www.nibb.ac.jp/en/index.php</i>	
12:30 - 12:40	Closing Remark Kiyotaka Okada	3F 301-304

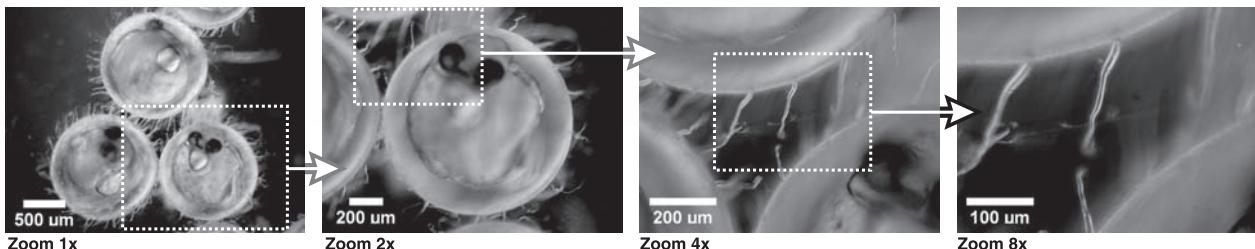


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WORKSHOP DESCRIPTIONS

Workshop 1

June 7 (Monday) Rm301-302 18:30-19:45

Organizer: Z. Jeffrey Chen (The University of Texas at Austin, USA)

Polyplody and Hybrid Vigor

Hybrid plants are formed between different varieties, ecotypes and species and often grow stronger and more vigorously than the parents. The phenomenon is known as hybrid vigor or heterosis. The degree of hybrid vigor is usually correlated with the genetic distance of the two parents if they can form hybrids. Doubling the chromosomes in the interspecific hybrids lead to the formation of allopolyploids that permanently fix the heterozygosity and hybrid vigor. However, many hybrids and allopolyploids cannot produce offspring, a phenomenon known as hybrid lethality. The modern iteration of Dobzhansky, Muller and Bateson model suggests that hybrid incompatibilities are caused by allelic interactions between genes and RNAs that have functionally diverged in the respective hybridizing species. This workshop will highlight recent discoveries in hybrid vigor and incompatibility using *Arabidopsis* and rice as model systems.

Program

- 18:30 **Thomas Altmann** (IPK Gatersleben, Germany)
Molecular and genetic analysis of biomass-heterosis in *Arabidopsis thaliana* (10012)
- 18:50 **Z. Jeffrey Chen** (The University of Texas at Austin, USA)
Polyplody and hybrid vigor in *Arabidopsis* (17011)
- 19:10 **Detlef Weigel** (Max Planck Institute for Developmental Biology, Germany)
Hybrid incompatibility in *Arabidopsis thaliana* (17012)
- 19:30 **Qifa Zhang** (Huazhong Agricultural University, China)
The genetic basis of heterosis in a highly heterotic rice hybrid (17007)
- 19:50 **Tsutomu Kuboyama** (Ibaraki University, Japan)
Epistatic interaction between an allele of a bacterial blight-resistance gene Xa1 and an allele of a putative transcriptional repressor HWC1 causes hybrid weakness of rice (10006)

Workshop 2

June 7 (Monday) Rm303-304 18:30-19:45

Organizer: Ruth Bastow (GARNet / MASC)

Future of Arabidopsis Informatics

Over the past 20 years *Arabidopsis thaliana* has emerged as the reference organism for plant research, underpinning breakthroughs in both basic and applied plant biology and beyond. This success has been driven by significant international investment and a fully annotated genome, which has led to a plethora of data, tools and resources.

Over the past ten years the amount of data available to Arabidopsis researchers has grown exponentially in amount and complexity. Consequently the informatics needs of the community are rapidly evolving and coordinated infrastructure and data management will be required if researchers are to be able to deal with this 'data deluge' and effectively turn it into useful information and scientific knowledge.

To help map out the future needs of the community MASC and NAASC have undertaken a number of activities in 2009 and 2010 including a series of workshops and questionnaires.

This workshop will look at the problems the community is currently facing, highlight some of the potential solutions for the future and provide an open forum for discussion.

Program

18:30 **Nick Provart** (University of Toronto, Canada)
Community Perspective

18:45 **Eva Huala** (Carnegie Institution, USA)
TAIR Perspective

19:00 **Tetsuro Toyoda** (RIKEN BASE, Japan)
Looking to the Cloud

19:15 **Blake Meyers** (Delaware Biotechnology Institute, USA)
Keith Lindsey (Durham University, UK)
A vision of the future

19:30 Open Forum/Discussions

Workshop 3

June 7 (Monday) Rm315 18:30-19:45

Organizers: Hong Gil Nam (POSTECH, Pohang, Korea)
Pyung Ok Lim (Jeju National University, Jeju, Korea)

Plant Senescence and the Associated Cell Death

Plant senescence and the associated cell death are developmentally programmed processes that occur in an age-dependent manner, integrating multiple developmental and environmental signals. Recently genetic and molecular analyses have led to identification of loci required to regulate this process and have established a signaling framework in part. Additionally, the structure and dynamics of cellular and organismal function have begun to be analyzed in the context of senescence and the associated PCD.

Our goal is to bring together people using multi-disciplinary approaches to study of plant senescence and the associated PCD. The mix of researchers including molecular geneticists, cell biologists and physiologists in this field will facilitate exchange of expertise and reinforce the concept that multidisciplinary approaches are required to understand, and manipulate plant senescence and PCD.

18:30 **Ja Choon Koo** (Chonbuk National University, Korea)

A membrane-bound receptor kinase, RPK1, positively controls age-dependent leaf senescence and cell death in *Arabidopsis*. (11007)

18:45 **Ying Miao** (Institute of Botany, Christian-Albrechts-university of Kiel, Germany)

The dual-targeted protein Whirly1 regulates WRKY53-mediated leaf senescence in *Arabidopsis thaliana* (11005)

19:00 **Kohki Yoshimoto** (RIKEN Plant Science Center, Japan)

Plant autophagy negatively regulates cell death by controlling salicylic acid signaling during senescence and the innate immune response (11033)

19:15 **Vicky Buchanan-Wollaston** (Horticulture Research International, University of Warwick, UK)

Systems biology as a means to understand *Arabidopsis* leaf senescence (11006)

19:35 **Hong Gil Nam** (POSTECH, Pohang, Korea)

Open Discussion for Future Issues or Directions

Workshop 4

June 8 (Tuesday) Rm301-302 18:30-19:45

Organizers: **Rod A. Wing (University of Arizona, Tucson, USA)**
Detlef Weigel (Max Planck Institute for Developmental Biology, Tübingen, Germany)
Chris J. Pires (University of Missouri, Colombia, USA)

Brassicaceae Map Alignment Project: An International Consortium to Generate High Quality Phylogenetically Representative Reference Genomes across the Brassicaceae

Because closely related genomes can be unambiguously aligned, their comparison offers exciting opportunities for both functional annotation based on sequence conservation and for understanding genome evolution. This has already been aptly demonstrated with the "12 *Drosophila* Genomes" project as well as the ongoing *Oryza* Map Alignment Project OMAP (<http://OMAP.org>).

Arabidopsis thaliana was one of the first eukaryotic species and the first plant for which a high quality reference genome sequence became available. Through various sources, including the Joint Genome Institute of the US Department of Energy, Genome Canada, BGI Shenzhen and others, several additional genome sequences from the family Brassicaceae, to which *Arabidopsis* belongs, are being produced.

The purpose of this workshop is to establish an international collaborative network that will formulate a roadmap for the generation of a comparative genomics platform for the Brassicaceae. It is the third in a series that includes two previous workshops held in San Diego and Tübingen earlier this year.

The workshop will have three 15 minute talks followed by a discussions focusing on:

- 1) Identification of a set of grand challenge questions that need to be addressed in the Brassicaceae.
- 2) Establishment of a prioritized list of species to be sequenced. Emphasis will be placed on the generation of high-quality reference genome sequences for key species that can subsequently be used as resequencing templates for more closely related species/populations.
- 3) Discussion of plans to develop a white paper for use by the international community obtain funding to support this project.

Program

18:30 **Detlef Weigel & Rod A. Wing** (Max Planck Institute for Developmental Biology, Germany)
Welcome/Introduction – BMAP concept – 3 meetings, consensus building, white paper, grant proposals.

18:35 **Rod A. Wing** (Arizona Genomics Institute, University of Arizona, Tucson, USA)
The International *Oryza* Map Alignment Project (I-OMAP): A golden path to unlock the genetic potential of the wild relatives of rice. (17018)

18:50 **Detlef Weigel** (Max Planck Institute for Developmental Biology, Tübingen, Germany)
The basis of rapid genome size change in *Arabidopsis*. (17019)

19:05 **Eric Schranz** (University of Amsterdam, The Netherlands)
Comparative analysis of polyploidy, gene loss and chromosomal rearrangements in the sister-families Brassicaceae and Cleomaceae. (17020)

19:20 **Detlef Weigel & Rod A. Wing** (Max Planck Institute for Developmental Biology, Germany)
BMAP Discussion – Grand challenge questions, Draft list of species selections, Database needs, Community input, Time table.

Workshop 5

June 8 (Tuesday) Rm303-304 18:30-19:45

**Organizers: Zinnia Gonzalez-Carranza (The University of Nottingham)
Reidunn Aalen and Melinka Butenko (University of Oslo)**

Cell Separation Processes in Plants

Cell separation is a fundamental process in plant development. It enables lateral roots to emerge through the cortex and epidermis of the primary root; leaves, floral organs and fruits to be shed, through the activation of specific abscission zones; and the dehiscence of anthers and pods to allow for the dispersal of pollen and seeds. Other processes that involve cell separation include aerenchyma production, stomatal development, hydathode formation, hypocotyl elongation and growth of fibres in woody species.

In this workshop signalling pathways regulating cell separation in different tissues will be examined as well as some strategies to study the process. The importance of understanding how cell separation is regulated will be discussed with respect to optimisation of crop growth and development.

18:30 **Zinnia H. Gonzalez-Carranza** (The University of Nottingham Sutton Bonington Campus, United Kingdom)

Overview of cell separation processes- Introduction.

18:35 **Reidunn B. Aalen** (University of Oslo, Norway)

Signalling and regulation of cell separation processes- Components of the same ligand-receptor signalling system control cell separation both during floral organ abscission and lateral root emergence (17009)

18:50 **Thorsten Hamann** (Imperial College London, UK)

Regulation of cell wall separation- The plant response to cell wall damage is regulated through the interaction of ROS and JA mediated processes (12008)

19:04 **Zinnia H. Gonzalez-Carranza** (The University of Nottingham Sutton Bonington Campus, United Kingdom)

Strategies to study cell separation processes; Abscission- A novel approach to dissect the abscission process in Arabidopsis: single cell analysis (13007)

19:17 **Charles Anderson** (University of California Berkeley, United States)

Strategies to study cell separation processes; cell expansion- Identification of new genes involved in cell wall expansion using activation tag Screening (13002)

19:31 **Hardy Hall** (Botany Department, University of British Columbia, Canada)

Strategies to study cell separation processes- Fibre growth patterns within the Arabidopsis inflorescence stem dissected through integration of growth kinetic profiling, laser scanning confocal microscopy and laser capture microdissection (08059)

Workshop 6

June 8 (Tuesday) Rm315 18:30-19:45

Organizer: Eva Huala (TAIR, Stanford, CA)

TAIR Workshop: Making Best Use of TAIR Tools and Datasets

This workshop is designed for users who wish to learn more about the various curated data sets and software resources provided by TAIR. We will address curation of two major data types at TAIR: gene structure and gene function. In the 'Gene Structure Annotation' section, we will provide details on the TAIR10 genome release. In particular, we are going to explain how we used new types of experimental data such as proteomics (from mass spec) and RNAseq data to add new genes and updates splice variants. We will talk about upcoming projects aimed to further improve existing annotations and add missing genes. An overview of how to search for gene structure related data in TAIR will also be given. In the 'Gene Function Annotation' section, we will describe the process of annotating from the literature using GO and PO controlled vocabularies, and then demonstrate how controlled vocabularies allow for standardization of annotation, assist in comparative genomics and can be used to classify large data sets. Curators will also demonstrate how to search the TAIR database for functional information.

In the second half of this workshop we are going to demonstrate how to use the bioinformatics tools provided on the TAIR website site. Special emphasis will be placed on our two newest tools: Gbrowse_syn, a synteny viewer, allows researchers to study and analyze syntenic regions, homologous genes and other conserved elements between the *Arabidopsis thaliana* genome and the *Arabidopsis lyrata* or *Populus trichocarpa* genomes. N-Browse, a protein-protein interaction viewer, allows the user to search for protein-protein interactions found in the literature. These interactions are visualized as an interactive protein interaction network that can be customized by either expanding certain nodes or by selecting for specific types of edges. Hands-on examples of how to use these tools will be provided at this workshop.

18:30 **Philippe Lamesch** (Carnegie Institution for Science, Stanford, CA, USA)
Introduction

18:35 **Philippe Lamesch** (Carnegie Institution for Science, Stanford, CA, USA)
Gene structure curation at TAIR. Description of the TAIR10 Arabidopsis genome release.
Overview on how to find gene structure related data in TAIR.

19:00 **Donghui Li** (Carnegie Institution for Science, Stanford, CA, USA)
Gene function annotation at TAIR. Description of the curatorial process of functional annotation using GO and PO controlled vocabulary terms.

19:25 **Philippe Lamesch** (Carnegie Institution for Science, Stanford, CA, USA)
New tools at TAIR. Overview and demo of the newest analysis tools at TAIR including N-Browse, Gbrowse_Syn and GBrowse.

MASC Phenome Subcommittee Forum

June 7 (Monday) Rm 313-314 12:45-13:45

Organizers: **Eva Huala (Stanford University)**
Minami Matsui (RIKEN Plant Science Center)

Recent progress of high-through-put phenotyping makes possible to monitor plant growth and development more accurately and systematically.

There are several innovations to make these measurements possible and these technologies can be applied not only for basic science but also for various plant species to monitor biomass increase and metabolite changes. In this Phenome Subcommittee Forum we will discuss recent progress on phenome studies developed by several pioneers.

12:45-13:00	Robert L. Last (Michigan State Univ. US) <i>Approaches to Accurate Tracking and Analysis of Data from a Large Functional Genomics Project</i> (18004)
13:00-13:15	Eli Kaminuma (National Institute of Genetics, Japan) <i>Quantification of spatio-temporal gene expression of <i>Arabidopsis</i> LucTag line using digital image sequence analysis</i> (18005)
13:15-13:30	Barry Pogson (The Australian National University, Australia) <i>Non-invasive phenotyping of <i>Arabidopsis</i> at the Australian Plant Phenomics Facility</i> (18006)
13:30-13:45	Olivier Loudet (INRA, National Institute for Agricultural Research Versailles, France) <i>High-throughput phenotyping of <i>Arabidopsis</i> shoot growth under different water deficit treatments</i> (18007)

MASC Proteomics Subcommittee Forum

June 7 (Monday) Rm 315 12:45-13:45

Organizers: **Joshua L. Heazlewood** (Lawrence Berkeley National Laboratory)
Alex Jones (The Sainsbury Laboratory)
Hirofumi Nakagami (RIKEN Plant Science Center)

Proteomics is a rapidly growing field that has been widely adopted by plant researchers especially in model systems such as Arabidopsis. The proteomics subcommittee of the Multinational Arabidopsis Steering Committee (MASCP) was established to assist in the coordination of international research in Arabidopsis in the area of proteomics.

12:45-12:50	Joshua L. Heazlewood (Lawrence Berkeley National Laboratory, USA) <i>Introduction to MASCP</i>
12:50-13:05	Alex Jones (The Sainsbury Laboratory, UK) <i>Focusing on Phosphorylation</i> (18001)
13:05-13:20	Hirofumi Nakagami (RIKEN Plant Science Center, Japan) <i>Phosphoproteomics in plants</i> (18002)
13:20-13:35	Hiren Joshi (Lawrence Berkeley National Laboratory, USA) <i>An online Arabidopsis proteomics data aggregator</i> (18003)
13:35-13:45	General Discussion



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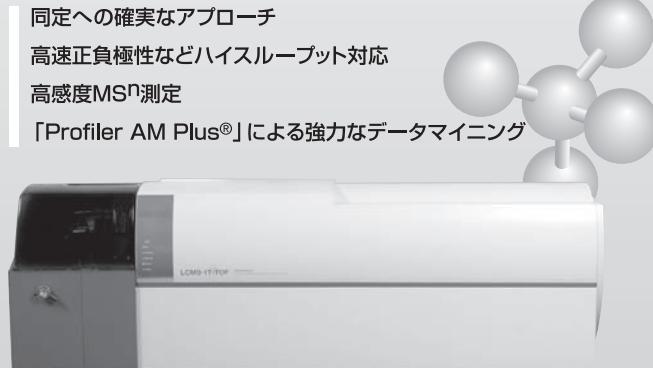
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検索

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KEYNOTE LECTURE ABSTRACT

K001

ARABIDOPSIS NATURAL VARIATION: FROM GENES TO EVOLUTIONARY ECOLOGY

*Maarten Koornneef (Max Planck Institute for Plant Breeding Research, Germany), Ruben Alcazar (Max Planck Institute for Plant Breeding Research, Germany), Leonie Bentsink (Wageningen University, Netherlands), Kathleen Donohue (Duke University, USA), Juliette de Meaux (Max Planck Institute for Plant Breeding Research, Germany), Matthieu Reymond (Max Planck Institute for Plant Breeding Research, Germany)

koornnee@mpiz-koeln.mpg.de

Natural variation in plants, even in model species such as *Arabidopsis thaliana*, represents genetic variation that might reflect adaptation to local environments, although some of this variation results from the demographic history, e.g. genetic drift or founder effects. A QTL (Quantitative Trait Loci) approach is commonly used to decipher genetic differences between accessions. Thereafter, the genetic polymorphisms underlying the effects of the detected QTL can be identified at the sequence level. To address whether the genes identified are under natural selection, molecular population genetic as well as ecological experiments can be carried out. Following this approach, novel genetic components in the seed dormancy pathway could be identified. We also showed how genetic variation controlling the trait influences the response to natural selection on the timing of germination. Another example concerns the molecular genetic basis of lines with reduced fitness segregating in specific mapping populations at low temperature. We have revealed that some allele combinations of genes involved in plant immunity underlie the observed phenotypes and identified signatures of local adaptation at one of the loci involved. Different alleles at this locus provide contrasted thresholds for pathogen resistance, which suggests that the adaptive fine-tuning of adequate defense responses has the potential to promote the emergence of incompatibilities which can lead to reproductive isolation barriers.

K002

PLANT COMPUTATIONAL MORPHODYNAMICS: LIVE IMAGING AND COMPUTATIONAL MODELS OF PATTERN FORMATION AT THE SHOOT APEX

*Elliot M. Meyerowitz (California Institute of Technology, USA)

meyerow@caltech.edu

To develop a predictive theory of plant development, of how to get from genome to phenotype, my laboratory and others have been developing Computational Morphodynamics: the use of live imaging and computational models to capture and model the causal mechanisms of development at the cellular and tissue level. By live image analysis of shoot apical meristems, the stem cell populations in flowering plants for all above ground tissues, we have found that chemical and physical signals interact to lead to pattern. Chemical signals in the shoot meristem include secretion of signaling peptides that are ligands for receptor kinases, diffusion of small molecules such as cytokinins, and regulated transport of other small molecules, auxins. Physical signals are also involved in signaling. The cytoskeleton of meristem cells responds directionally to stress, and work in progress indicates that stress also may control auxin flow. Therefore both chemical and physical processes interact in producing the phyllotactic pattern, the spiral pattern of leaves and flowers around the stem, and may play key roles in other patterning processes as well, such as the patterns and planes of cell division. Computational models of phyllotaxis and primordial growth at the shoot apex show how local interactions of cells can lead to complex global patterns.

PLENARY SESSION ABSTRACT

PL101

"THINK GLOBALLY ACT LOCALLY" USING SYSTEMS BIOLOGY TO UNDERSTAND ABA SIGNALING
Shelley Lumba (University of Toronto, Canada), Shegeo Toh (University of Toronto, Canada), Alan Moses (University of Toronto, Canada), Darrell Desveaux (University of Toronto, Canada), *Peter McCourt (University of Toronto, Canada)
peter.mccourt@utoronto.ca

The success of the genomics revolution to construct a genetic architecture of a variety of model organisms has put functional biologists under pressure to show what each individual gene does *in vivo*. Traditionally this task has fallen on geneticists who systematically perturb gene function and study the consequences. Often, however, genetic redundancy and issues of lethality limit genetic analysis as a functional tool. In this presentation I will show how systems biology can be used in combination with traditional genetic analysis to unravel complex hormone signaling pathways. We have applied a systems biology approach to furthering our understanding of the plant hormone abscisic acid (ABA). Using the premise that proteins of co-expressed genes more often interact with each other to signal we have constructed a focused ABA orfeome of co-regulated ABA response genes. This interactome has allows the development of development of computational and functional hypotheses to test questions of ABA signaling.

PL102

REGULATION OF SHOOT BRANCHING BY STRIGOLACTONES

*Shinjiro Yamaguchi (RIKEN Plant Science Center, Japan)
shinjiro@postman.riken.jp

Shoot branching is a major determinant of plant architecture and is highly regulated by endogenous and environmental signals. We have recently shown that strigolactones (SLs), a group of terpenoid lactones, act as hormones or their biosynthetic precursors that regulate shoot branching by inhibiting axillary bud outgrowth. SLs were first discovered in root exudates as seed germination stimulants of root parasitic plants, such as *Striga* and *Orobanche* species. More recently, they were shown to have a function in symbiotic interaction with arbuscular mycorrhizal fungi, which facilitate the uptake of inorganic nutrients by plants. Thus, SLs act as an endogenous growth regulator as well as being a chemical signal in symbiosis and parasitism in the rhizosphere. Our recent experiments using rice mutants indicated that SLs play a role in minimizing shoot branching under phosphate-deficient conditions, suggesting that the dual role of SL as an endogenous hormone and a rhizosphere signal might be closely related to adaptive responses to inorganic nutrient availability.

PL103

MOLECULAR INTERACTION OF GIBBERELLIN SIGNALING COMPONENTS, GID1, SLR1, AND GID2

*Makoto Matsuoka (Nagoya University, Bioscience and Biotechnology Center, JAPAN)
makoto@agr.nagoya-u.ac.jp

Through the genetical and biochemical analyses using rice gibberellin (GA)-related mutants, we have elucidated the nature of GA signaling during the past decade. Now, GA perception has been considered as follows; the binding of GA to GID1 induces the formation of a GID1-GA-DELLA complex and, following the degradation of DELLA with aid of F box protein, GID2, via 26S proteasome pathway, results in various GA-triggered actions. Our recent study on the structure of rice GID1 protein revealed that 1) GID1 resembles hormone sensitive lipase (HSL) and interacts with GA in its binding pocket, 2) bioactive GA is held in its pocket by specific polar and non-polar amino acids via hydrogen bonds and hydrophobic interaction, 3) GID1 evolved by replacement of some important amino acids that were refined for high affinity and specificity to the currently active higher plant GAs. Then, the next question is why the formation of GID1-GA-DELLA complex induces the interaction between DELLA and GID2. We produced various mutated DELLA and GID2 to determine the regions of DELLA essential for GID2 binding and GID2 for DELLA binding by Y3H. We also examined the effect of these mutations in planta using transgenic plants.

PL201

HDZIP SOUP: UNTANGLING THE NETWORK THAT CONTROLS AXIS FORMATION IN ARABIDOPSIS

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Class III HDZIP transcription factors control the development of ad/abaxial and apical/basal axes in the plant. Our latest work has focused on the mechanism through which these factors effect development and the regulatory environment they operate within. Our findings expand the network controlling ad/abaxial development to include members of other HDZIP families and reveal interactions of HDZIPIII proteins with the growth regulators auxin and brassinolide.

PL202

A MEMBRANE FUSION-MEDIATED PLANT DEFENSE STRATEGY AGAINST BACTERIAL PATHOGENS

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Because plants do not have immune cells, each cell has to provide its own defense against invading pathogens. A common plant defense strategy involves programmed cell death (PCD) at the infection site, but how the PCD-associated immunity is executed in plants is not fully understood. In this symposium, we provide a novel mechanism underlying cell-autonomous immunity, which involves the fusion of membranes of a large-central-vacuole with the plasma membrane, resulting in the discharge of vacuolar antibacterial proteins to the outside of the cells where bacteria proliferate (1,2). We found that a defect in proteasome function abolished the membrane fusion associated with both disease resistance and PCD in response to avirulent bacterial strains but not to a virulent strain. Furthermore, RNAi plants with a defective proteasome subunit PBA1 have reduced DEVDase activity, which is an activity associated with caspase-3, one of the executors of animal apoptosis. The plant counterpart of caspase-3 has not yet been identified. Our results suggest that PBA1 acts as a plant caspase-3-like enzyme. Thus, this novel defense strategy through proteasome-regulating membrane fusion of the vacuolar and plasma membranes provides plants with a mechanism for attacking intercellular bacterial pathogens that invade from stomata of the leaves. The immune system complements another vacuolar defense mechanism in which viral propagation inside the cell is checked by vacuolar collapse (3,4). Our findings suggest that plants have evolved a cell-autonomous immune system based on membrane fusion to inhibit proliferation of bacterial pathogens and a vacuolar-collapse system to be unable to spread systemically viral pathogens.

(1) Hatsugai et al. (2009) Genes & Dev., 23: 2496-2506.

(2) Pajerowska-Mukhtar et al. (2009) 23: 2449-2454.

(3) Hara-Nishimura et al. (2005) Curr. Opin. Plant Biol., 8: 404-408.

(4) Hatsugai et al. (2004) Science, 305: 855-858.

PL203

DISTRIBUTION OF NEWLY SYNTHESIZED ORGANELLAR PROTEINS IN PLANT CELLS

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In eukaryotic cells, a major portion of cellular activities is performed by organelles that are surrounded by lipid membranes. In doing so, one major challenge is how to provide proteins to these organelles since they do not have or have very limited protein synthetic capacity. In eukaryotic cells, organellar proteins are distributed by two different modes, protein targeting and protein trafficking, depending on individual organelles. Proteins destined to the plastid, mitochondrion, nucleus, peroxisome and ER are targeted to these organelles directly from the cytosol whereas proteins destined to the Golgi apparatus, endosomes, plasma membrane and vacuoles are delivered by protein trafficking after initial targeting to the ER. In general, the mechanism of protein trafficking is conserved in all eukaryotic cells. However, plant cells have a unique situation in the mode of the protein targeting to organelles since they have plastid and mitochondrion derived from endosymbiotic bacteria. The mechanisms of protein targeting to these organelles appear to be similar to each other and at the same time display a high degree of specificity. We investigated the nature of the signal sequences that direct proteins to these organelles and also proteins that recognize these signal sequences. At the talk, I will describe our findings on the nature of sequence motifs for protein targeting to chloroplasts and mitochondria and also for specificity determination between chloroplasts and mitochondria. In addition, I will talk about the mechanisms of unimported plastid precursor response controlled by Hsc70-4 and targeting of membrane proteins to chloroplast outer envelope membranes by AKR2.

PL301**LARGE SCALE APPROACHES TO DECONVOLVING ARABIDIOPSIS CIRCADIAN NETWORKS**

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Our laboratory is undertaking systems level approaches to understanding circadian clock function in plants. The long-term goal is to understand the circuitry required to generate robust, physiologically relevant rhythms, as well as using a comparative approach to understand the evolution of circadian clocks and the underlying design principles. We combine forward genetics with cell-based assays and whole-genome transcriptome approaches in an attempt to understand the network of circuits that are required for the core clock, and how the clock exerts its outputs upon the cell. These outputs include the rhythmic control of a substantial proportion of the transcriptome, and thus understanding the hierarchy of factors that must be required to achieve phase-specific expression of large numbers of genes is also of interest to us. We are beginning to discover that circadian clocks of plants are composed not of a single autoregulatory loop or limit cycle, but rather of multiple positive and negative interlocking feedback loops. We propose that this complex network architecture provides robustness (i.e. resistance to stochastic perturbation), multiple opportunities for output control and several pathways for controlling inputs or environmental entrainment of the oscillator(s).

PL302**THE SIGNALING MECHANISMS OF ARABIDOPSIS BLUE LIGHT RECEPTORS CRYPTOCHROMES**

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Cryptochromes are photolyase-like blue-light receptors that mediate light responses in plants and animals. The Arabidopsis genome encodes three cryptochromes, CRY1, CRY2, and CRY3. CRY1 and CRY2 mediate blue light control of seedling development and floral initiation, respectively, whereas CRY3 acts as an organellar DNA photolyase. It is known that CRY1 and CRY2 mediate blue-light modulation of genome-wide gene expression changes to affect growth and development, but the exact molecular mechanism underlying cryptochrome regulation of gene expression is not fully understood. We have identified and investigated proteins that specifically interact with Arabidopsis cryptochromes in response to blue light. Cryptochromes interact with RING-Finger proteins (such as SPA1, SPA2, etc) in a blue light-dependent manner to confer blue-light suppression of the COP1-dependent E3 ubiquitin ligase complex (unpublished results). Moreover, cryptochromes also interact with transcription factors (such as CIB1, CIB4, CIB5, etc) in response to blue light to directly affect transcription (Science 322: 1535-1539, and unpublished results). The CRY signaling processes are also modulated by the actions of other photoreceptors to achieve a coordinated system network for light regulation of gene expression. For example, The CRY2-interacting CIB proteins are degraded in the absence of blue light, via the ubiquitin/proteasome pathway, whereas blue light specifically suppresses CIB degradation (unpublished results). However, neither cryptochromes nor the COP1 E3 ligase complex is directly involved in the blue light suppression of CIB degradation. How other photoreceptors may mediate blue light suppression of CIB degradation to modulate cryptochrome activity will be discussed.

PL303

ANALYSIS OF ARABIDOPSIS CO₂ RESPONSE MUTANTS BY THERMAL IMAGING

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Currently, CO₂ concentration in the global atmosphere is continuing its rapid rise, and is forecast to reach approximately twice the level it had before the industrial revolution in the second half of our century. As a greenhouse gas, CO₂ is thought to contribute to global warming. It is also an important environmental factor as it is the basic carbon source for plants to perform photosynthesis. Stomatal pores in the epidermis of plants enable gas exchange between plants and the atmosphere. Pairs of specialized guard cells surround and control stomatal apertures. It is possible that guard cells monitor CO₂ concentrations and possess signal transduction pathways which transmit such signals, but currently we do not understand the details and molecular mechanisms. We have used a forward genetic approach to isolate key genes involved in CO₂ responses in guard cells, by using high-throughput leaf thermal imaging to screen for mutants with altered CO₂ sensitivity. The isolated mutants are divided into 2 groups according to their phenotypes: (1) impaired in stomatal opening under low CO₂ concentrations (*high leaf temperature* mutants: *ht*) or impaired in stomatal closing under high CO₂ concentrations (*low leaf temperature* mutants: *lot*); (2) impaired in stomatal movement, even if CO₂ concentration is changed (*carbon dioxide insensitive* mutants: *cdi*). Characterization of these mutants has begun to yield insights into the mechanisms by which stomatal CO₂ responses are controlled. I will discuss the current status of the functional analysis of identified CO₂ regulators and future prospects.

PL401

THE DIVERSE ROLES OF SMALL, NON CODING RNA IN PLANTS

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Eukaryotes contain small regulatory RNAs that have been referred to as the dark matter of genetics. They are typically 21-24 nucleotides long, associated with Argonaut or Piwi proteins. Some of these small RNAs guide the Argonaut/Piwi protein to a complementary RNA and they are negative regulators of gene expression acting at the level of messenger RNA turnover or translation. Others participate in more complex regulatory systems affecting chromatin or they act as part of an RNA signal that moves between cells.

In plants the small RNA silencing systems are fundamentally for defense. I will describe how in virus infected plants their role is more subtle than simple suppression of virus accumulation — there is a spatial factor such that the block on virus accumulation is in the meristem and growing point of the infected plants and how the mobile silencing signal is associated with this spatial effect .

I will also describe genetic evidence that the RNA silencing signal is linked to mechanisms affecting chromatin modification in a process that may be linked to defense against invading DNA either from viruses or transposable elements.

Finally I shall describe how the defense capacity of RNA silencing may operate on a genome-wide basis so that there is an effect on interactions between genomes of hybrid plants. Some of these interactions in the endosperm are based on maternal-specific expression of small RNAs but other interactions in vegetative tissues may be biparental. I shall describe how these RNA silencing systems may influence non additive phenotypes in hybrids.

PL402

GENETICS OF DNA METHYLATION IN GENES AND TRANSPOSONS

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DNA methylation is an epigenetic modification of genomic DNA, conserved among vertebrates, some fungi, bacteria, and plants. In the plant genome, most of the methylation is found in repeats and transposons, and the methylation level is much lower in active genes. To understand control and function of DNA methylation, we are taking genetic approaches using mutants of *Arabidopsis*. An *Arabidopsis* chromatin remodeling protein DDM1 (decrease in DNA methylation) is necessary for methylating repeats and transposons. On the other hand, jmjC-domain-containing protein IBM1 (increase in BONSAI methylation) is necessary for not methylating genes. In mutants of genes encoding these proteins, several types of developmental abnormalities were induced. I am going to talk about our genetic and genomic approaches to understand the impact of these mutations.

PL403

SEQUENCING EPIGENOMES OF PLANTS AND PEOPLE

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The development of DNA sequencing technologies that produce vast amounts of sequence information has triggered a paradigm shift in biology, enabling massively parallel surveying of complex nucleic acid populations. The diversity of applications to which these technologies have already been applied demonstrates the immense range of cellular processes and properties that can now be studied at the single-base resolution. These applications include, but are not limited to, the sequencing of genomes to uncover nucleotide polymorphisms and structural variation, as well as epigenomes to reveal sites of DNA-protein interaction and cytosine methylation, allowing an assessment of how their dynamic interactions impact the transcriptome. Approaches that utilize new sequencing technologies will be described to address questions about epigenomic variation from plants and humans.

PL501

DEVELOPMENT OF A BASIS FOR MARKER-ASSISTED SELECTION IN BREEDING IN CROP PLANTS

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Rapidly accumulating genomic information and material resources for a wide variety of crop plants, combined with genomics technologies, allow us to improve strategies for molecular breeding. Marker-assisted selection in breeding (MAS) facilitated by a large number of genome-wide DNA markers and investigation of genomic diversity followed by identification of genes responsible for traits of agronomic importance would be such examples. We have been focusing on development of a platform for utilization of DNA markers for molecular breeding, which includes generation of DNA markers and development of tools for data analyses.

Among various DNA markers, genome- or EST-derived microsatellite markers are popular because of their multi-allelic and co-dominant characteristics. We have been developing microsatellite markers for soybean, Azuki bean, peanut, red clover, white clover, tomato, watermelon, Japanese radish, taro potato, *Jatropha curcas*, *Eucalyptus camaldulensis*, turf grasses, carnation, and laver (*Polyphyla yezoensis*). When higher degree of polymorphisms is required, single nucleotide polymorphism markers are developed with the aid of the second generation DNA sequencers. Using these markers and resulting high-density genetic linkage maps, genetic analysis was performed to detect quantitative trait loci (QTLs) responsible for biotic- and abiotic-stress resistances, and morphological characteristics.

Many complex traits of agronomic importance, such as crop yield and stress resistances, are controlled by multiple QTLs. In order to utilize the accumulating DNA markers for dissection of QTLs toward breeding, we have developed a new QTL mapping approach named Genotype Matrix Mapping (GMM). GMM searches for QTL interactions not only in family data but also in various genetic backgrounds. Feasibility examination of GMM is in progress.

PL502

PROGRESSES OF RICE FUNCTIONAL GENOMICS RESEARCH AND THE IMPLICATIONS IN CROP GENETIC IMPROVEMENT

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There has been a large global effort in rice functional genomics research aiming at characterization of the full complement of the rice genes. The Chinese national program on rice functional genomic research is composed of the following components: (1) development of technological platforms, (2) functional genomics of agriculturally important traits, (3) molecular cloning of important genes and, (4) gene discovery by resequencing natural diversity of the rice species. The traits targeted for functional genomic studies include yield, grain quality, stress tolerance, disease and insect resistances, and nutrient use efficiency. Major progress has been made in a number of fronts. Totally 270,000 independent transformants have been generated for the T-DNA insertion mutant library and are now being screened for mutations of important traits. Over 50000 flanking sequences have been isolated, and their analyses identified a number of interesting features of nonrandom distributions of the T-DNA insertions in the rice genome. A large number of mutants have now been targeted for gene isolation. For genome-wide expression profiling, data have been collected from more than 40 tissues covering the whole life cycle of the rice plants and under various conditions. Map-based cloning has been applied to isolate genes of agronomic importance, including dozens of genes for yield, grain quality, fertility restoration, resistances to biotic and abiotic stresses. Hundreds of accessions of rice germplasm have been resequenced using new-generation technology. The implications of these developments in crop genetic improvement will be discussed in the presentation.

PL503

PROGRESS IN CROP PLANT GENOMICS

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Access to a complete and accurate record of all the genes in their genomic location has revolutionised biology. In plants genome sequences has facilitated the rapid acceleration of new knowledge acquisition of gene function in model systems, primarily *Arabidopsis*, within a whole systems context.

The genome sequences of several other plants has not been generated and provides the opportunity for understanding the mechanisms of genome change at a sequence level, and for understanding how evolution and domestication have shaped genomes.

In my presentation I will review progress in the field of plant genomics and emphasise the use of new genome sequencing methods to generate reference genome sequences from large and complex genomes.

PL601

SYSTEMS BIOLOGY OF LEAF GROWTH

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Understanding the mechanisms that govern tissue, organ and organism size are amongst the most mysterious and fascinating open questions in biology. Our long term goal is to unravel the molecular pathways that govern leaf size and biomass production in *Arabidopsis*. One of our approaches is based on studying the action mechanisms of genes which, when mutated or overexpressed, enlarge leaf size (hereafter called “intrinsic yield genes (IYG)). Such analysis is likely to shed light on the various instructor systems governing leaf size. Currently, we have confirmed the positive effect of 13 IYGs operating in seemingly unrelated pathways on *Arabidopsis* leaf size. In all cases examined so far enlarged leaf size and increased biomass production results from an increased cell number without any significant effect on cell size. Our results indicate that, by a yet unknown mechanism, the instructor network must affect the developmental timing of cell division. Cell cycle control genes are likely targets for the instructor genes. Various approaches are now being used to decipher how leaf size is determined. Combining IYGs by crossing lines overexpressing single genes yielded unexpected synergistic effects and different ‘omics’ technologies are also being applied to determine the molecular networks governing enhanced organ growth. The technical bottlenecks, such as the small size of the growth zones, and challenges in front of us will be illustrated with specific examples. The long-term goal is to develop computational models describing the molecular basis of organ size and to use these models to improve biomass production and crop yield.

PL602

INTEGRATION OF METABOLOMICS, METABOLIC NETWORK AND GENE FUNCTION NETWORK FOR SYSTEMATIC GENE FUNCTION IDENTIFICATION

He Kun (Carnegie Institution for Science, Arabidopsis Metabolomics Consortium, USA), Insuk Lee (Yonsei University, South Korea), Preeti Bais (Arabidopsis Metabolomics Consortium), Kate Dreher (Carnegie Institution for Science, USA), Julie Dickerson (Arabidopsis Metabolomics Consortium), Phillip Dixon (Arabidopsis Metabolomics Consortium), Oliver Fiehn (Arabidopsis Metabolomics Consortium), B. Markus Lange (Arabidopsis Metabolomics Consortium), Edward Marcotte (University of Texas at Austin), Stephanie Moon (Arabidopsis Metabolomics Consortium), Basil J. Nikolau (Arabidopsis Metabolomics Consortium), Mary Roth (Arabidopsis Metabolomics Consortium), Vladimir Shulaev (Arabidopsis Metabolomics Consortium), Lloyd Sumner (Arabidopsis Metabolomics Consortium), Ruth Welti (Arabidopsis Metabolomics Consortium), *Seung Yon Rhee (Carnegie Institution for Science, Arabidopsis Metabolomics Consortium, USA)

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After a decade of the uncovering of the Arabidopsis genome, many of the genes remain uncharacterized and their functions unknown, even based on sequence similarity. To generate specific hypotheses about possible functions for these genes, we attempted a systematic approach by combining computational modeling, literature curation, genetic and metabolomics approaches. First, we built a genome-wide functional association network of Arabidopsis genes using over 24 datasets from functional genomics experiments in plant and animal model organisms. This network, called AraNet, has been used to predict candidate genes involved in small molecule metabolism using well-characterized genes involved in specific metabolic pathways as bait. Second, knock-out mutants of predicted candidate genes were subjected to metabolite profiling on 11 analytical platforms, which have the combined ability to generate relative abundance data of nearly 3100 Arabidopsis metabolites/analytes. Third, we identified metabolites that were significantly altered in these mutants, and used them to delineate reactions and domains of the metabolic network that are significantly altered by using AraCyc, a metabolic network of Arabidopsis that has been curated with experimental information from the literature. Preliminary results from these analyses will be presented.

PL603

PLANT METABOLOMICS FOR SYSTEMS BIOLOGY - FROM ARABIDOPSIS TO CROPS

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Metabolomics plays a major role in plant systems biology. This is based on the fact that plant cellular function is achieved through the networking with multiple-layers' components such as genes, transcripts, proteins and metabolites. Systems understanding of genetic and metabolomics networks is urgently required for plant biology leading to its biotechnology application to sustain and improve our lives. Using *Arabidopsis thaliana*, an integrated analysis of transcriptome and metabolome led to prediction of gene-to-metabolite relations. Co-regulation framework models of genes and metabolites in the pathways of flavonoid, sulfur compound and lipid, suggested the specific involvement of co-expressed genes in the synthesis and accumulation of the metabolites in the pathways. Reverse genetic and reverse biochemistry confirmed those delimited genes' functions in the pathways. The *Arabidopsis* tissue-specific metabolite accumulation database, AtMetExpress development, was constructed. AtMetExpress is designed to be compatible with AtGenExpress to allow the efficient elucidation of metabolite-transcript networks during tissue development of *A. thaliana*. Metabolomics developed in *Arabidopsis* is further applicable to crops and medicinal plants to decipher the genes' functions and to improve the agronomical and food-chemical traits. In this presentation, the crucial roles of metabolomics in plant systems biology and application to crop biotechnology will be discussed.

PL701

NATURAL VARIATION FOR GROWTH AND THE INTERACTION WITH THE ENVIRONMENT IN ARABIDOPSIS THALIANA

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Following a long history of quantitative genetics in crop plants, it now becomes feasible to use naturally-occurring variation contained in *Arabidopsis thaliana* accessions as the source of quantitative genomics approaches, designed to map QTLs and resolve them at the gene level. Apart from being able to exploit -in multiple genetic backgrounds- allelic variation that cannot be easily retrieved from classical mutagenesis, the (relatively few) success of the QTL studies has often been because of the use of quantitative phenotyping, as opposed to the qualitative gauges used in typical mutant screens. The objective of my work is to apply genome-wide quantitative molecular genetics to both, a very integrative and classical quantitative trait (growth in interaction with the environment) and a molecular trait a priori more directly linked to the source of variation (gene expression under cis-regulation). We are using a combination of our unique high-throughput phenotyping robot (Phenoscope), fine-mapping, complementation approaches and association genetics to pinpoint a significant number of QTLs and eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity. Exploiting these strategies at an unprecedented scale should finally allow to resolve enough quantitative loci and pay a significant contribution to drawing a general picture as to how and where in the pathways adaptation is shaping natural variation and improve our understanding of the transcriptional cis-regulatory code.

I will present recent and unpublished results obtained when trying to decipher the genetic architecture of shoot growth response to the environment, as well as genetic incompatibilities, typical projects that will be scaled-up after my lab has been granted one of the 'Starting Independent Researcher Grants' by the European Research Council.

PL702

NEXT-GENERATION GENETICS IN ARABIDOPSIS THALIANA: EVOLUTIONARY TRADEOFFS, IMMUNITY AND SPECIATION

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We are addressing three core questions in evolution:

- How, and how frequently, do new genetic variants arise?
- Why do some variants increase in frequency?
- Why are some combinations of new variants incompatible?

These correspond to the fundamental evolutionary processes of mutation, selection and speciation, which we are studying using both bottom-up and top-down approaches. I will first demonstrate the power of second-generation sequencing, both in support of forward genetics (Schneeberger et al., Nat. Methods 2009), and in determining the rate and spectrum of spontaneous mutations (Ossowski et al., Science 2010). Based on our experience with short-read sequencing (Ossowski et al., Genome Res. 2008), we have been advocating a 1001 Genomes project for *A. thaliana* (<http://1001genomes.org>), and we have already sequenced 84 wild strains from this species. Interpretation of within-species polymorphism data is greatly facilitated by outgroup information from *A. lyrata*.

Next, I will discuss a fitness trade-off we recently discovered. Allelic variants beneficial in one setting might be detrimental under different circumstances. Plants vary greatly in their responses to pathogens, and this is thought to reflect fitness costs in the absence of pathogens or predators. We have found that allelic diversity at a single locus, ACCELERATED CELL DEATH 6 (*ACD6*) , underpins dramatic variation in both vegetative growth and resistance to microbial infection. *ACD6* is also a causal factor for an autoimmune-like response that behaves as expected for Dobzhansky-Muller incompatibilities, which are often thought of underlying speciation events. Together with other discoveries made by my group (Bomblies et al., PLoS Biol., 2007), this implicates the extreme allelic diversity of disease resistance genes (Clark et al., Science 2007), presumably due to pathogen pressures, as potential causes for the evolution of gene-flow barriers in plants.

PL703

RICE BREEDING BY QTL PYRAMIDING

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Many agronomically important traits are governed by a number of genes known as quantitative trait loci (QTLs). Identification of important QTL-controlled agricultural traits has been difficult because of their complex inheritance; however, completion of the rice genomic sequence facilitates QTLs cloning and their pyramiding for practical breeding. Since QTLs are derived from natural variation, the use of a wider range of variations such as would be found in wild species is important. Additionally, Introgression Lines (ILs) developed from wild species in combination with Marker Assisted Selection (MAS) will facilitate efficient gene identification. I present in detail recent developments in rice QTL analysis including mapping, cloning and pyramiding QTL for breeding.

PL801

RECEPTOR SIGNALING IN STOMATAL PATTERNING: COMPLEXITY, CHALLENGES, AND NEW INSIGHTS

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Efficient gas exchange between a plant and the atmosphere requires coordinated spacing of stomata, turgor-driven valves on the plant epidermis. Recent progress by our group and others has led to the discovery of key molecules and pathways controlling stomatal patterning and differentiation. For instance, molecular signals that enforce proper stomatal patterning have recently been identified as the EPIDERMAL PATTERNING FACTOR-LIKE (EPFL) family of small, secreted cysteine-rich peptides. Two founding members, EPF1 and EPF2, are expressed in the late and early stages of stomatal cell-lineages, respectively. EPF2 restricts the specification of cells undergoing the initial asymmetric cell division that creates stomatal precursor cells. On the other hand, EPF1 enforces stomatal spacing. Four putative receptors, TOO MANY MOUTHS (TMM) and three ERECTA-family LRR-RLK, ERECTA, ERL1, and ERL2, exhibit synergistic as well as contrasting effects on stomatal patterning. ERECTA is expressed in the entire epidermal tissues while ERLs and TMM are expressed in the stomatal-lineage cells. ERECTA-family RLKs are also expressed in the internal tissues. Genetic studies revealed that ERL1 and TMM have opposite functions in specifying meristemoid differentiation. Therefore, important questions are whether these putative receptors physically associate with each other, whether they comprise the receptor complex for EPFLs, and how combinatorial activities of the ligands and receptors create diversity of outputs. I will present our latest research findings and discuss about the complexity, challenges, and new insight into our understanding of receptor-mediated cell signaling during plant tissue patterning.

PL802

DEVELOPMENT ROOTED IN INTERWOVEN NETWORKS

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Specification and maintenance of cell identity are central processes of development. In an effort to understand the regulatory networks that control cell identity, we have profiled all cell types and developmental stages within a single organ, the *Arabidopsis* root. To acquire global expression profiles we developed technology that uses sorted marked populations of cells with subsequent hybridization of the labeled RNA to microarrays. We are using computational methods to infer networks functioning within different cell types and developmental stages and have begun to test the hypothesized relationships. Another key process in the development of multicellular organisms is precise regulation of asymmetric cell division to generate diverse cell types. The molecular mechanisms responsible for this process are still poorly understood, in particular how developmental pathways trigger asymmetric divisions. Asymmetric divisions in the *Arabidopsis* root are controlled by a finely orchestrated interplay between the transcription factors SHORTROOT (SHR) and SCARECROW (SCR). Our studies provide evidence for a direct mechanistic link between development and cell cycle progression as well as between organ patterning mechanisms and asymmetric cell division. In plant roots the radial tissue organization is highly conserved with a central vascular cylinder in which two water conducting cell types, protoxylem and metaxylem, are patterned centripetally. We have evidence that this patterning occurs through crosstalk between the vascular cylinder and the surrounding endodermis mediated by cell-to-cell movement of a transcription factor in one direction and a microRNA in the other.

PL803

LEAF DEVELOPMENT: MOLECULAR GENETICS OF THE GOLDEN ANGLE

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Phyllotaxis, the regular arrangement of leaves or flowers around a plant stem, is an example of developmental pattern formation and organogenesis. Phyllotaxis is characterized by the divergence angles between the organs, the most common angle being 137.5°, the golden angle. This quantitative aspect makes phyllotaxis an unusual developmental problem. It has traditionally attracted the interest of physicists, mathematicians and computer scientists, who have constructed a wide variety of quantitative models. To the biologist it is surprising that these models can correctly and robustly recreate phyllotactic patterns even though they make only minimal assumptions about the underlying molecular mechanisms. The challenge is to construct quantitative models that integrate the experimental data from the past 10 years. Such models can direct and be tested by further experiments.

Mechanistic models of phyllotaxis must explain its de novo establishment in the radially symmetric embryo, the stable maintenance of regular phyllotactic patterns and the transitions between these patterns. Most importantly, they must explain the specific divergence angles of 180°, 90°, 137.5°, and in rarer cases other angles as well. I will describe the relevant experiments and how they form the basis for experiment-based computer simulations. In these models, the central regulator of phyllotaxis consists of a positive feedback loop between auxin and its transporter, the PIN1 protein. This regulatory loop generates regularly spaced auxin maxima within the shoot apical meristem, which cause differential gene expression, localized growth, and organ development. Guided by the models, we investigate the role of other auxin transporters, mechanical signals and environmental inputs.

CONCURRENT SESSION ABSTRACT

C101

PLANT REGENERATION AND ROOT MERISTEM ORGANIZATION

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It is well known that plants have a high capacity to regenerate their organs. This can happen from callus or without the need to pass through callus. For example, we study regeneration of the root tip after it has been severed in *Arabidopsis*. We approach this problem through both high resolution confocal microscopy of regenerating roots and transcriptional profiling of the the regenerating organ and cells. The critical question we ask is how the plant organizes the reformation of pattern and cell identities. I will report progress on recent work in which we follow a single cell type as it traverses fate from one cell type to another. The system permits us to order a cell-type specific transcriptional cascade to piece together the dynamic assembly of quiescent center cell identity. This work shows that auxin triggers a transcriptional cascade that appears to directly instruct certain cell fates that may be pre-patterned to respond to auxin. Whether these cells form an organizer that instructs other fates or are part of a larger organizing system is still not clear. Several models of organ regeneration and how they relate to meristem function have emerged from recent work in regeneration.

C102

CALLUS FORMATION AND SHOOT REGENERATION IN ARABIDOPSIS

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We have studied three aspects of shoot regeneration. The first is the origin of callus from organ explants, the second the state of cellular differentiation and gene expression in callus, and the third the early patterning of the de novo formation of shoot apical meristems on the callus surface. We have studied all three using live imaging. It is known that root- and hypocotyl-derived callus originates (as do lateral root primordia) from pericycle cells. Our observations show that callus from cotyledons and from petals also originates from perivascular cells that express pericycle markers. Our studies of callus show that it resembles root tip meristem in its global patterns of gene expression, and that the spatial patterns of gene expression of root-specific genes resemble the patterns found in lateral root primordia. This is the case regardless of the origin of the callus; whether from roots, or from aerial organs such as cotyledon and petal that do not ordinarily form lateral roots. Mutant plants that do not initiate lateral roots also do not form callus. Once callus is established, a change in the hormone concentrations of the growth medium leads to de novo formation of new shoot apical meristems. Live imaging shows that the new shoot meristems in root-derived callus derive from a small number of progenitor cells through a stereotyped series of gene expression and protein localization changes, beginning with broad activation of the *WUSCHEL* gene, and followed by activation of the *PIN-FORMED1* auxin efflux carrier in the cells at the surface and transcription of *SHOOT MERISTEMLESS* in a ring of cells surrounding the future meristem. A series of additional stereotyped changes then leads to a typical meristem organization, with *CLAVATA3* activation at the apex and internal expression of *WUSCHEL*.

C103

MOLECULAR MECHANISMS OF CELL-FATE CHANGE FROM A DIFFERENTIATED CELL TO A STEM CELL IN THE MOSS *PHYSCOMITRELLA PATENS*

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Differentiated cells can be reprogrammed to be pluripotent stem cells that are undifferentiated type of cells with abilities for both self-renewing and giving rise to most cell types in the organism. An induction of reprogramming is more easily manipulated in plants than in animals, although genetic and molecular bases of

the difference are mostly unexplored. The moss *Physcomitrella patens* has a high ability of reprogramming. Cells in a dissected leaf segment can be reprogrammed in water without any exogenous chemicals within 24 hours. We found that the reprogramming is induced by wounding and light signaling, which is mediated by *squamosa* promoter binding proteins, *bZIP* transcription factors, AP2 domain proteins, cold shock-domain proteins, and auxin signaling pathways. Dynamic change of small RNA population and chromatin modification are also involved in the process. Spatial pattern of the reprogramming in the dissected leaf is regulated by an unknown inhibitory factor initiated from leaf cells under reprogramming. I will discuss on their regulatory networks and the connection to cell cycle regulators.

C104

A WOUND RESPONSIVE TRANSCRIPTION FACTOR WIND1 PROMOTES CELL DEDIFFERENTIATION IN *ARABIDOPSIS*

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Many multicellular organisms have a remarkable capability to regenerate new organs after wounding. Cellular dedifferentiation in adult somatic tissues serves as a first key step in organ regeneration but the mechanisms underlying this process remain unknown in plants. Here we show that a novel transcription factor WOUND INDUCED DEDIFFERENTIATION 1 (*WIND1*) functions as one of the core regulators of cell dedifferentiation in *Arabidopsis*. *WIND1* is induced at the site of wounding and is required for the wound-induced cell dedifferentiation accompanied by formation of callus in which cells undergo unorganized proliferation. We demonstrate that constitutive overexpression of *WIND1* is sufficient to establish and maintain the dedifferentiation status of somatic cells without auxin and cytokinin — two plant hormones that strongly influence the plant cell fate. This activity of *WIND1* is associated with its ability to activate cytokinin cellular response but *WIND1* has minimum effects on auxin. Consistently, *WIND1* does not induce cell dedifferentiation in plants with mutations in cytokinin signaling. Together, our data indicate that wound-induced cell dedifferentiation in *Arabidopsis* employs local activation of *WIND1* which then modulates endogenous hormone response.

C105

REQUIREMENT FOR THE RID3-DEPENDENT NEGATIVE CONTROL OF CELL PROLIFERATION IN DE NOVO MORPHOGENESIS

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Recently we identified two regulatory genes, *RGD3* and *RID3*, through genetic analysis of shoot regeneration with temperature-sensitive mutants of *Arabidopsis*. Positional cloning revealed that *RGD3* and *RID3* encode a TBP-associated factor, BTAF1, and an evolutionarily conserved, uncharacterized WD40-repeat protein, respectively. Expression patterns of these genes were examined during shoot regeneration from callus. Before the induction of shoot regeneration, both *RGD3* and *RID3* were expressed uniformly throughout the callus. After shoot induction, however, *RGD3* expression was restricted to the regions of pre-meristematic cell mound formation at the callus surface while *RID3* expression was lost from these regions. The *rgd3* mutation inhibited cell mound formation and shoot regeneration-associated expression of *CUC1* and *STM*. In contrast, the *rid3* mutation caused a disorganized overgrowth of cell mounds and a high-level diffused expression of *CUC1* and *STM*.

We have been further analyzing physiological roles of *RID3* in several aspects of development.

Overexpression of *RID3* under the 35S promoter resulted in a shoot meristem-less phenotype and a marked reduction of expression of *CUC1* and *STM*. *RID3* overexpression also interfered with shoot regeneration variously in tissue culture. In severe cases, callus formation was inhibited by *RID3* overexpression. To test the requirement for the *RID3* function in embryogenesis, developing embryos of the *rid3* mutant were exposed to the restrictive temperature. As a result, abnormal morphology accompanied by expanded expression of *STM* was induced in the mutant embryos. Our findings suggest that de novo morphogenesis generally requires the *RID3*-dependent negative control of cell proliferation, which might be partly attributable to the down-regulation of the CUC-STM pathway.

C201

CELLULAR RESPONSES IN PLANT IMMUNITY

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Induction of plant immune responses involves significant transcriptional programming, which leads to drastic changes in cellular functions. Using transcription profiling, we discovered that ER-resident genes (ER genes) are coordinately upregulated after treatment by the defense signal salicylic acid. Genetic experiments showed that induction of these ER genes is required for proper folding and secretion of the antimicrobial pathogenesis-related (PR) proteins. This "ER-gene transcriptome" is controlled by a novel cis-element TL1. Using bioinformatics and a modified Y1H, we identified the TL1 binding transcription factor (TBF1) that specifically binds to the TL1 element and regulates genes with this element. Characterization of the *tbf1* mutant showed that ER function is required for multiple immune responses. Another cellular response that is activated during plant immune responses involves DNA damage repair (DDR). Using a genetic screen, we found multiple key players in DDR to be involved in defense gene transcription in a signal-specific and gene-specific manner. These DDR proteins may be recruited to defense gene promoters to remodel the chromatin and to prevent transcription-associated DNA damage.

C202

MAP KINASE PHOSPHATASE 1 IS A NEGATIVE REGULATOR OF PAMP RESPONSES AND RESISTANCE AGAINST *PSEUDOMONAS SYRINGAE* PV TOMATO

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For protection against potential microbial pathogens, plants have developed effective defense mechanisms initiated by extracellular detection of conserved microbial features intrinsic to the lifecycle of the potential pathogen. Recognition of these pathogen-associated molecular patterns (PAMPs) results in the activation of phosphorylation cascades resulting in enhanced resistance against bacteria. We have identified a loss-of-function mutant for *MAP Kinase Phosphatase 1* (*MKP1*) in the Wassilewskija ecotype [*mkp1(Ws)*] that displays enhanced resistance against the normally pathogenic bacteria, *Pseudomonas syringae* pv tomato DC3000. However, untreated *mkp1(Ws)* plants do not show stunted growth or spontaneous necrotic lesions usually associated with constitutive resistance responses, indicating that *mkp1(Ws)* plants may have stronger PAMP responses leading to faster and/or more robust defense responses. In response to PAMPs, the *mkp1* mutant displays enhanced activation of MAP kinases, enhanced expression of some but not all transcription factors examined, and enhanced seedling growth inhibition. Mutations in MPK6 but not MPK3, two MAPKs that are activated during PAMP responses, suppress the *mkp1* phenotypes, indicating that MKP1 is a negative regulator of MPK6-mediated PAMP signaling pathway(s). Therefore, the *mkp1(Ws)* mutant provides a unique genotype for performing experiments to dissect MPK6-dependent PAMP signaling pathways as this mutant potentiates a MPK6-specific response. Analysis of the *mkp1* mutant will define

signaling pathways leading to specific PAMP responses and provide new insights into how MKP1 and the signaling pathways it negatively regulates can enhance resistance against bacteria without detrimental growth phenotypes.

C203

PSEUDOMONAS SYRINGAE EFFECTOR PROTEINS MODULATE MAP KINASES TO ENHANCE HOST SUSCEPTIBILITY

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Plants are equipped to sense pathogen/microbe-associated molecular patterns (PAMPs/MAMPs), which are molecular signatures conserved among microbes. The perception of PAMPs rapidly activates mitogen-activated protein kinases MPK3, MPK4 and MPK6 in Arabidopsis. These MPKs are thought to represent two distinct MAPK cascades regulating plant immunity. MPK4, its upstream MAP kinase kinases MKK1 and MKK2, and the MAP kinase kinase kinase MEKK1 form a cascade that negatively regulates defenses in Arabidopsis. In contrast, MPK3, MPK6, MKK4 and MKK5 are thought to form a cascade that positively regulates plant defenses. The bacterial pathogen *Pseudomonas syringae* secretes a large repertoire of effector proteins into the plant cell to enhance virulence. We previously showed that the *P. syringae* effector HopAI1 impairs PAMP-induced defenses by inactivating MPK3 and MPK6, rendering plants highly susceptible to nonpathogenic *P. syringae* bacteria. Our recent analyses showed that another *P. syringae* effector protein, AvrB, interacts with and stimulates the activity of MPK4 to enhance plant susceptibility in the absence of cognate R proteins RPM1 and TAO1. This process is assisted by the molecular chaperone HSP90 and its co-chaperones SGT1 and RAR1. Interestingly, the AvrB-interacting protein RIN4 can be phosphorylated by MPK4 in vitro and is required for AvrB to induce plant susceptibility to *P. syringae*. It remains to be determined if the phosphorylation of RIN4 by MPK4 plays a role in regulating plant immunity. In addition, we found a third effector that can target MKKs, likely through an ADP-ribosyltransferase activity, to block the PAMP-induced activation of MPKs and defenses. Together, these findings illustrate a variety of strategies used by *P. syringae* to modulate host MAPK cascades during the infection process, re-enforcing the importance of MPKs in PAMP-triggered immunity.

C204

MULTIPLE ROUTES TO ACTIVATION OF SALICYLIC ACID SIGNALING - A KEY ROLE OF CBP60 PROTEINS

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Salicylic acid (SA) signaling is important for resistance to biotrophic and hemi-biotrophic pathogens such as the bacterium *Pseudomonas syringae* pv. *maculicola* strain *Psm* ES4326. It can be activated by recognition of Microbe Associated Molecular Patterns (MAMPs) such as flg22, a fragment of bacterial flagellin (Pattern Triggered Immunity, PTI), by recognition of pathogen effectors (Effector Triggered Immunity, ETI), or other responses to pathogen attack. In published work, we showed that Calmodulin Binding Protein 60g (CBP60g) contributes to activation of SA production during PTI, is required for wild-type levels of resistance to *Psm* ES4326, and requires the ability to bind calmodulin (CaM) in order to function (Wang et al., PLoS Pathogens 5(12): e1000772 2009). Another family member, CBP60h, is also required for resistance to *Psm* ES4326, but does not bind CaM. SA levels in *cbp60h* mutants are normal early during PTI, but reduced at later times and during infection by *Psm* ES4326. Double *cbp60g,h* mutants have severely reduced SA levels under all conditions tested and extreme susceptibility to *Psm* ES4326. Thus, these two proteins perform a critical and partially-redundant function in activation of SA production during defense responses.

Expression profiling revealed that the CBP60g,h node lies between the EDS1/PAD4 and SA nodes in the defense signaling network, and suggests that none of *eds1*, *pad4*, or *cbp60g,h* result in complete loss of function of their respective nodes. Our results suggest a model in which CBP60g responds to Ca⁺² flux early during PTI and activates SA signaling. As the Ca⁺² flux wanes, CBP60h assumes primary responsibility for promotion of SA production.

C205

THE PEPTIDOGLYCAN PATTERN RECOGNITION RECEPTOR LYM3 IS CRUCIAL FOR ARABIDOPSIS IMMUNITY TO BACTERIAL INFECTION

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Host pattern recognition receptor-mediated perception of microbe-associated molecular patterns (MAMP) is a prerequisite for the initiation of antimicrobial defenses in all multicellular organisms including plants. Peptidoglycan (PGN) fractions from Gram-negative (*Xanthomonas*, *Pseudomonas*, *Agrobacterium*, *Escherichia*) and Gram-positive (*Staphylococcus*) bacterial genera have been shown to trigger immunity-associated responses in *Arabidopsis thaliana*. In addition, muropeptide fragments released by enzymatic digestion of intact PGN activated similar defenses. By reverse genetics, AtLYM3 has been implicated in peptidoglycan perception and immunity to bacterial infection. LYM3 constitutes a secreted protein that is tethered to the outer plant plasma membrane protein through a GPI-anchor. The extracellular domain of LYM3 harbours two LysM domains thereby resembling bacterial and plant LysM proteins involved in carbohydrate ligand perception. Recombinant LYM3 binds PGN whereas other members of the LYM protein family do not show affinity to this ligand. Importantly, other complex carbohydrate ligands, such as cellulose, chitin, lipo-chitooligosaccharide (nod factor), did not bind to LYM3. Genetic inactivation of LYM3 abolished all peptidoglycan-induced plant responses, including posttranslational activation of MAPK activity and immune response gene expression. Most importantly, different lym3 alleles were found to be super-susceptible to bacterial infections by *Pseudomonas syringae* strains pv. tomato DC3000 (Pto DC3000), Pto DC3000 lacking AvrPto/PtoB, and Pto DC3000 hrcC, suggesting that PGN recognition contributes significantly to basal immunity in *Arabidopsis*. Whereas PGN is a weak inducer of plant immunity-associated defenses, flagellin is a strong and effective inducer. Here we discuss the potential role of PGN as a primer for MAMP-triggered defenses and propose a model of different layers of inducible defenses underlying basal plant immunity.

C301

ABA REGULATES ROOT DEVELOPMENT

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Root architecture is a determined agronomic trait which influences both crop yields and adaptation to environmental stress. Plant root growth is regulated by both different environmental factors and various plant hormones. Under drought or osmotic stress, the accumulated ABA inhibits root growth. However, the key factors for ABA regulating root development are largely unknown. Screening for abscisic acid (ABA) overly sensitive mutants in *Arabidopsis* has identified several mutants which show enhanced ABA sensitivity in root growth. We have identified several genes from these mutants. Genetic and molecular analyses

suggest that the signals from mitochondria and crosstalk between auxin, brassinosteroids, ethylene, and ABA exist for ABA-mediated root development.

C302

PLANT'S LONG AND SHORT DISTANCE SIGNALLING FOR CONTROLLING THE WATER STATUS

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Photosynthesis and biomass production of plants are controlled by the soil water status. Plants experiencing a limiting water supply respond by inducing ABA biosynthesis and adjusting leaf transpiration. Our analyses reveal a hydraulic signal mediating the root to shoot communication of plant water status. The physical signal is subsequently converted into the chemical signal ABA for short distance signaling. Cytosolic receptors for ABA consist of a heteromeric complex between a protein phosphatase 2C (PP2C) and a regulatory component (RCAR/PYR/PYL) that binds ABA. Binding of ABA to the receptor complex inactivates the PP2Cs, thereby activating the large variety of physiological processes regulated by ABA. The *Arabidopsis* genome encodes thirteen homologues of RCAR1 and approximately 80 PP2Cs, of which six in clade A including the prototypes ABI1 and ABI2 have been identified as negative regulators of ABA responses. Biochemical analyses revealed a combinatorial assembly of PP2C/RCAR complexes that differ in the sensitivity of ABA-mediated regulation and in the selectivity for ABA and ABA analogues. Crystal structure analyses of the RCAR/PYR/PYL and of two heteromeric ABA holo-receptors by several laboratories revealed a ligand-binding pocket of RCAR/PYR/PYL that is closed in response to ABA binding. The ABA-induced conformational change stabilizes complex formation of RCAR/PYR/PYL with the PP2C, which blocks the active site of the protein phosphatase. Future studies have to address the role of distinct ABA receptor complexes in plant responses. First findings point to a selective function of ABA receptors for targeting different downstream signaling components and for adjustment of ABA-signaling to strongly variable ABA levels.

C303

REGULATORY NETWORKS OF GENE EXPRESSION IN ABIOTIC STRESS RESPONSE IN *ARABIDOPSIS*

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Drought, high salinity, and freezing are environmental conditions that cause adverse effects on the growth of plants and the productivity of crops. Expression of a variety of genes is induced by these stresses in a variety of plants. Their gene products function in stress tolerance and response. We have been carrying out global analysis of expression profiles of drought-inducible genes, and functional analysis of their products in stress tolerance in *Arabidopsis*. We have identified several different regulatory systems in stress-responsive gene expression, two are ABA-dependent and two are ABA-independent. In one of the ABA-independent pathways, a cis-acting element (DRE/CRT) and its binding proteins, DREB2 with a AP2/ERF domain, are important in stress-responsive gene expression. ABA regulates diverse plant processes, including stress tolerance, seed development and germination. bZIP-type transcription factors, AREB/ABFs are involved in ABA-dependent pathways under water stress conditions. The AREB proteins were phosphorylated *in vitro* by ABA-activated SNF1-related protein kinase 2s such as SRK2D/SnRK2.2, SRK2E/SnRK2.6, and SRK2I/SnRK2.3 (SRK2D/E/I). *Srk 2d/e/i* triple mutants exhibit greatly reduced tolerance to drought stress, viviparity, and highly enhanced insensitivity to ABA. In the triple mutants, ABA- and water stress-dependent gene expression is globally and drastically impaired under water stress conditions. We showed that these three protein kinases function as main positive regulators and reciprocal modulation of SRK2s and Group A PP2Cs controls ABA signaling in plants. Moreover, these kinases were shown to be essential for the control of seed development and dormancy through the extensive control of gene expression in seeds.

C304

MOLECULAR DISSECTION OF LOCAL AND SYSTEMIC RESPONSES TO PHOSPHATE STARVATION IN ARABIDOPSIS THALIANA

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Phosphate (Pi) is a crucial and often limiting nutrient for plant growth. It is also a very insoluble ion heterogeneously distributed in soil. To cope with such a situation, plants have evolved a complex network of morphological and biochemical processes (1) controlled by various regulatory systems. These signalling pathways are triggered either by Pi concentration in the growth medium (external Pi; 2,3,4), or by plant cells internal Pi. A split-root assay performed to mimic an heterogeneous environment combined with a transcriptomic analysis was used to identify clusters of genes locally or systemically regulated by Pi starvation. In addition, the combination of genetic tools and physiological analysis revealed distinct regulatory roles for the internal and the external Pi. They also pointed out a central role of the transcription factor PHR1 for genes systemically controlled by low Pi.

In addition, results obtained with a set of tools including chemical genetics and manipulations of the entire family of the high affinity Pi transporters will be presented. These tools offer novel powerful approaches to dissect Pi perception pathways.

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C305

ABC TRANSPORTER ATABCG25 INVOLVED IN ABA TRANSPORT AND RESPONSES

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Many molecules related to ABA signaling or ABA recognition have been identified to date. In particular, multiple receptors that recognize ABA were recently reported, based on various phenomenal characterizations. In addition to intra-cellular regulation triggered by ABA receptors, inter-cellular functioning of ABA is predicted to exist in plants; for example, ABA is predominantly produced in vascular tissues, but acts in stomatal responses of distant guard cells. However, the molecular basis of ABA transport remains to be investigated. Here, we present evidence that one of ATP-binding cassette (ABC) transporter genes, *AtABCG25*, encodes a protein that is responsible for ABA transport and responses in *Arabidopsis*. We isolated *atabcg25* mutants by genetically screening in transposon tagged lines for ABA sensitivity. *AtABCG25* was expressed mainly in vascular tissues. The fluorescent protein-fused *AtABCG25* was localized at the plasma membrane in plant cells. In membrane vesicles derived from *AtABCG25*-expressing insect cells, *AtABCG25* exhibited ATP-dependent ABA transport. The *AtABCG25*-overexpressing plants showed higher leaf temperature, implying an influence on stomatal regulation. These results suggest that *AtABCG25* is an exporter of ABA through plasma membranes and is involved in the inter-cellular ABA signaling pathway. ABC transporters are broadly conserved from prokaryotes to higher eukaryotes. Especially, plants have more expanded families in their genomes. It is consistent that some of ABC transporters developed to have plant-specific and important functions for plant development or responses.

C401

RNA-DIRECTED DNA METHYLATION

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RNA-directed DNA methylation is a small RNA-mediated epigenetic modification that is highly developed in flowering plants (1). To understand the mechanism and functions of RNA-directed DNA methylation, we are carrying out a forward genetic screen using a transgene silencing system that exploits an enhancer active in shoot and root meristem regions. The silencing system also features the production of secondary siRNAs that foster the spread of methylation beyond the originally targeted region (2,3). Recent data on new mutants identified in the screen and secondary siRNA biogenesis and function will be presented.

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C402

MECHANISMS AND PHENOTYPIC CONSEQUENCES OF DNA METHYLATION IN ARABIDOPSIS

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DNA methylation plays key roles in the control of genome activity in plants and mammals. It is critical for the stable silencing of repeat elements and is also involved in the epigenetic regulation of some genes. Despite similarities in the controlling functions of DNA methylation, its dynamics and deposition patterns differ in several respects between plants and mammals. One of the most striking differences is that plants tend to propagate pre-existing DNA methylation states across generations, whereas mammals re-establish them genome wide at every generation. Our recent findings on the transgenerational stability of DNA methylation patterns in Arabidopsis will be presented. The role of RNAi in the incremental methylation and silencing of repeat elements over successive generations and in the preservation of normal expression of neighboring genes will be highlighted.

C403

DNA METHYLATION IN RICE SEED

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Cytosine methylation silences transposable elements in plants, vertebrates and fungi, but also regulates gene expression. Plant methylation is catalyzed by three families of enzymes, each with a preferred sequence context: CG, CHG (H = A, C or T) and CHH, with CHH methylation targeted by the RNA interference (RNAi) pathway. We have obtained high-resolutions maps of DNA methylation in rice seeds and control seedling tissues, as well as corresponding datasets for gene expression. Unlike Arabidopsis, reduction of CG methylation in rice endosperm is local, while non-CG methylation, which is high in Arabidopsis endosperm, is greatly reduced. The wild type methylation patterns of rice endosperm closely resemble those of Arabidopsis plants with a mutation in the DEMETER demethylase, indicating that global endosperm demethylation is indeed conserved between monocots and dicots, but differences in methylation patterning have been driven by evolution of the DEMETER family.

C404

22-NUCLEOTIDE RNA TRIGGERS SECONDARY SIRNA BIOGENESIS IN PLANTS

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The effect of RNA silencing in plants can be amplified if secondary small interfering RNA (siRNA) production is triggered by the interaction of microRNAs (miRNAs) or siRNAs with a long target RNA. However, miRNA and siRNA interactions are not all equivalent because most of them do not trigger secondary siRNA production. Here we use bioinformatics to show that the secondary siRNA triggers are miRNAs and trans-acting siRNA of predominantly 22 nt, rather than the more typical 21-nt length. *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana* confirmed that the siRNA-initiating miRNAs, miR173 and miR828, were only effective as triggers if they were expressed in a 22-nt form and, conversely, that increasing the length of miR319 from 21 to 22 nt converted it into an siRNA trigger. We also predicted and validated that the 22-nt miR771 is a new siRNA trigger. Our data demonstrates that the function of small RNAs is influenced by size and that the length 22 nt is necessary for triggering secondary siRNA production.

C405

THE ROLE OF MIRNAS DURING GERM CELL SPECIFICATION IN ARABIDOPSIS POLLEN

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Plant meiocytes undergo subsequent mitotic divisions to form the gametes, which must rapidly reprogramme their epigenome before fertilization. In *Arabidopsis*, the male germline differentiates by asymmetric division of haploid uninucleated microspores, giving rise to a vegetative cell enclosing a smaller generative cell that divides before anthesis to originate two sperm cells. The vegetative nucleus (VN) retains a somatic nature, orchestrates pollen tube growth and does not contribute with genetic material to the next generation. However, recent observations indicated that DNA demethylation and expression of particular transposable element (TE) loci occurs in the VN, producing siRNAs that might reinforce epigenetic silencing of TE activity in the gametes (1).

Transcriptional profiling of FACS-purified mature pollen and sperm cells has shown that transcripts involved in small RNA biogenesis and RNA-directed DNA methylation are enriched in sperm cells (2), suggesting active epigenetic reprogramming as well as post-transcriptional regulation of gene expression. Our deep sequencing analysis of small RNA libraries from pollen and sperm cells revealed that 49 known miRNA families are enriched in the male gametes. We could predict 31 potentially novel miRNAs in sperm cells and show cleavage of some of their predicted target transcripts in pollen samples. Thus our comparative sRNA sequencing coupled with the transcriptome data and pollen 5'RACE analysis suggest that miRNA pathways are active during sperm cell specification. Moreover, we are testing the hypothesis that some miRNAs accumulate in the male gametes to be delivered to the female gametes upon fertilization and only play a role during early embryonic development.

1. Slotkin, R.K. et al. *Cell* 136, 461-472 (2009).
 2. Borges, F. et al. *Plant Physiology* 148, 1168-1181 (2008).
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C501

PEPTIDE SIGNALLING IN PLANT PRIMARY MERISTEMS

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Primary plant meristems are the shoot and root meristems that are initiated at opposite poles of the plant

embryo. They contain stem cells, which remain undifferentiated, and supply new cells for growth and the formation of tissues. The maintenance of a long-lasting stem cell population in meristems is achieved by signal exchange between organizing regions and the stem cells, and also by feedback signals emanating from differentiating cells. I will discuss the role of peptide signals that make use of different receptor classes to control the stem cell populations in both meristem types by regulating evolutionarily conserved homeodomain transcription factors.

C502

CLE PEPTIDES REGULATING VASCULAR STEM CELL FATES

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Twelve or thirteen-amino acids CLE peptides play crucial roles in plant development. In Arabidopsis, there are 32 CLE genes, which can produce at least 26 different peptides. We have analyzed the function of CLE peptides in vascular development. Previously, we identified TDIF (Tracheary element Differentiation Inhibitory Factor), which was a CLE peptide modified by hydroxylation but not by glycosylation (Ito et al., 2006). TDIF was secreted from the phloem and both suppressed tracheary element differentiation and promoted proliferation of procambial cells in hypocotyls and leaves (Hirakawa et al., 2008). Recently we also found that some other CLE peptides including CLE10 prevented protoxylem vessel formation in roots.

In this paper, we report the results from further investigation on TDIF and CLE10 signaling pathway in vascular development. We found that a leucine-rich repeat receptor-like kinase XI, TDR (TDIF receptor)/PXY is a receptor for TDIF (Hirakawa et al., 2008). Next, we searched downstream regulatory factors of the TDIF/TDR signaling, and found the WOX4 transcription factor as such a factor. The detailed analysis revealed that WOX4 functions in the promotion of procambial cell proliferation but not in the suppression of differentiation of procambial cells into xylem cells, suggesting that the two processes were separately regulated downstream of the TDIF/TDR signaling. Furthermore the detailed analysis of the CLE10 signaling revealed that CLE10 suppresses protoxylem vessel formation through the activation of cytokinin signal in roots. Based on these data, we discuss how CLE peptides regulate the fate of procambial cells.

*Ito et al. Science 313, 842-845, 2006

*Hirakawa et al., PNAS 105, 15208-15213, 2008

C503

POLLEN TUBE GUIDANCE BY ATTRACTANT PEPTIDES

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The concept of a pollen tube attractant was proposed in the nineteenth century when pollen tubes were observed to grow toward excised ovules on the medium. Since then, for more than 140 years, plant biologists tried to identify a pollen tube attractant(s) derived the ovule. However, no molecule had been convincingly demonstrated to be the true attractant that actually controls the navigation of pollen tubes to the ovule. We developed the *in vitro* *Torenia* system, whereby pollen tubes growing through a cut style are attracted to a protruding embryo sac and cause double fertilization. By using this system, the synergid cell was shown to emit some diffusible attractant (Higashiyama et al., Science, 2001). The attractant molecule was species preferential even in closely relating species, implying that the molecule had rapidly evolved (Higashiyama et al., Plant Physiol., 2006). We investigated genes expressed in the synergid cell of *Torenia*, by collecting isolated synergid cells. We found that cysteine-rich polypeptides (CRPs) were abundantly expressed in the synergid cell. Among the CRPs, at least two defensin-like polypeptides, named as LUREs, showed strong activity to attract pollen tubes. By developing a laser-assisted thermal-expansion microinjector, LUREs were finally identified as true attractants derived from the synergid cell (Okuda et al., Nature, 2009). One of our goals is to clarify the mechanism of directional control of pollen-tube growth by LUREs. In this talk, we will show our recent progress, including results based on visualization of LURE molecules.

C504

A TRANSCRIPTION FACTOR SUPPRESSING FLORAL ABSCISSION DOWNSTREAM IN THE IDA-HAE/HSL2 SIGNALING PATHWAY

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Small peptides play an important role in the coordination of plant growth and development. (*IDA*) encodes a putative peptide ligand necessary for the regulation of floral organ abscission in *Arabidopsis*. *IDA* is dependent on the two receptor-like kinases (RLK) *HAESA* (*HAE*) and *HAESA-LIKE 2* (*HSL2*) to exert its function (Stenvik, 2008; Cho, 2008). Recent studies in *N. benthamiana* suggest that a short C-terminal conserved region of the *IDA* protein is capable of interacting with one of the RLKs. We therefore propose that this peptide-receptor system induces a signaling cascade that regulates cell-cell separation in floral abscission zones (AZs). To identify proteins acting downstream of the *IDA* signaling pathway, a revertant screen was performed on the *ida* mutant. Here we present data from one line (line49), which in addition to rescuing the abscission defect of *ida*, shows some of the same floral phenotypes as plants overexpressing *IDA*. Similar phenotypes are observed in the *bp3* mutant, an allele of line 49 in the Col background. Both alleles have a mutation in the *KNAT1/BP* homeodomain transcription factor. We have shown that the double mutant *bop1bop2*, which does not differentiate morphologically distinct AZs (McKim, 2008), is epistatic to *bp3*. We therefore propose that the enlarged AZs of *bp3* are not re-differentiated AZs but rather a result of increased cell division. In addition, the *bp3* mutant is capable of rescuing the floral abscission defect of *ida* and *haehsl2*, indicating that *KNAT1* acts as a suppressor of floral organ separation in the *IDA-HAE/HSL2* signaling pathway. By using a promoter::GUS construct we show that *pKNAT1::GUS*, *pKNAT1::GUS ida* and *pKNAT1::GUS haehsl2* plants show similar levels of expression during the abscission process, indicating that the regulation of *KNAT1* by *IDA* is at the protein rather than transcriptional level.

C505

STOMAGEN IS A MESOPHYLL-DERIVED POSITIVE REGULATOR OF STOMATAL DENSITY

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The two-dimensional pattern and density of stomata in the leaf epidermis are genetically and environmentally regulated to optimize gas exchange. Two putative intercellular signaling factors, EPIDERMAL PATTERNING FACTOR 1 (EPF1) and EPF2, function as negative regulators of stomatal development in *Arabidopsis*, possibly by interacting with the receptor-like protein TOO MANY MOUTHS (TMM). Here we identified a novel secretory peptide, designated stomagen, with stomata-inducing properties. Overexpression of *STOMAGEN* increased stomatal density, whereas knockdown of *STOMAGEN* decreased it. Stomagen is a 45-amino-acid, cysteine-rich peptide that is conserved among vascular plants. A semi-in-vitro analysis with recombinant and chemically synthesized stomagen peptides showed that stomagen has stomata-inducing activity in a dose-dependent manner. A genetic analysis showed that *TMM* is epistatic to *STOMAGEN*, suggesting that stomatal development is regulated by competitive binding of positive and negative regulators to the same receptor. Notably, *STOMAGEN* is expressed in inner tissues (mesophyll) of young leaves but not in the epidermal tissues where stomata develop. Our findings provide a conceptual advancement in understanding stomatal development: inner photosynthetic tissues optimize their function by regulating stomatal density in the epidermis for efficient uptake of CO₂.

Sugano SS et al., Nature 463: 241-244 (2010)

C601

METABOLOME RESEARCHES IN PLANT METABOLIC REGULATION

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Plant metabolism has been analyzed in detail for a long time. Network of metabolic reactions, enzyme characteristics, and substrate levels etc. have been well studied. However, we have still many issues to be resolved in plant metabolism. Recent technical progress for plant metabolism using transcriptome, proteome and/or metabolome, allow the analysis of complicated regulation systems in the plant metabolism.

Our research groups are performing analysis of plant metabolism under support of CREST of JST.

We are applying transcriptome, proteome and metabolome analyses with transgenic Arabidopsis to screening of regulatory molecules for carbon and nitrogen metabolisms (Yanagisawa group), co-expression/co-accumulation analysis based on transcriptome/metabolome data for secondary metabolism and amino acid metabolism (Hirai group) and combination of proteome and metabolome analyses of isolated intact vacuoles to identify the role of vacuolar proteins in regulating cellular metabolism (Mimura group).

In the present report, by integrating these three independent approaches for analysis of plant metabolism, we would like to show the new aspect of plant metabolism researches.

C602

THE IMPORTANCE OF HAVING SULFUR

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Sulfur is an essential nutrient for all organisms. Plants take up most sulfur as inorganic sulfate, reduce it and incorporate it into cysteine in primary sulfate assimilation. However, part of the sulfate is partitioned into secondary metabolism to synthesise a variety of sulfated compounds. The two pathways of sulfate utilisation branch after the activation of sulfate to adenosine 5'-phosphosulfate (APS). To dissect the control of sulfur partitioning between primary and secondary metabolism we analysed plants in which activities of enzymes utilising APS were increased or reduced. These manipulations indeed affect concentration of multiple sulfur-containing compounds. Measuring flux through sulfate assimilation using [35S]sulfate confirmed the larger flow of sulfur through primary assimilation when APS kinase activity was reduced. Interestingly, the effects of the genetic manipulations were greater on the flux through the pathway than on the metabolite concentrations. This clearly demonstrates the importance of measuring of metabolic fluxes for a full understanding of control of plant metabolism.

C603

FORWARD AND REVERSE GENETICS MEET: THE CHLOROPLAST 2010 PROJECT

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Rational engineering of plants for improved productivity, environmental stress tolerance and increased healthfulness will require a predictive understanding of plant metabolism. The plant chloroplast is the master chemist of the plant cell, and an attractive target for metabolic engineering. In addition to serving as the site of carbon sequestration through photosynthesis, this organelle produces many nutrients essential to the human diet, including amino acids, different antioxidant vitamin classes and lipids. The Chloroplast

2010 Collaborative (www.plastid.msu.edu) is using the extensive functional genomics resources available in Arabidopsis to speed up the process of discovering new genes that contribute to chloroplast function. The long-term goal of this project is a predictive understanding of the structure and function of the Arabidopsis thaliana chloroplast. The centerpiece of this approach is the testing of thousands of knock-out mutant lines for large numbers of metabolic and developmental phenotypes. The unique features and novel results of this 'matrix genetics' approach will be discussed, as will possible biotechnological outcomes from the project. This work is funded by the US National Science Foundation 2010 Project.

C604

HIGH THROUGHPUT ANALYSIS OF MEMBRANE/SIGNALING PROTEIN INTERACTIONS IN ARABIDOPSIS

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Despite the importance of membrane proteins (which represent 20-30% of the Arabidopsis proteome), little is known about their interactions with each other or with other proteins. The goal of the project is to expand our knowledge of the Arabidopsis plasma membrane protein interactome and its interface with key signaling proteins. We are planning a screen for interactions of ca. 8600 genes comprising 5769 membranes proteins using the mating-based split-ubiquitin system (mbSUS).

The concept of mbSUS relies on the release of an artificial membrane-bound transcription factor from the membrane if two membrane proteins interact. mbSUS uses a ubiquitin split into two halves: The N-terminal domain of ubiquitin (NubG) can reconstitute a functional ubiquitin when co-expressed with its C-terminal half (Cub). When two proteins fused to NubG and Cub moieties interact, the two halves reconstitute a functional ubiquitin triggering the action of ubiquitin-specific proteases which release the transcription factor. Diffusion of transcription factor into the nucleus activates transcription of marker genes (ADE2, HIS3 and LacZ). 3800 Arabidopsis genes have been cloned of which 2400 have been mobilized into the mbSUS destination vectors. A first set of genes was screened for interactions using the Ade2 and His3 markers in a 1536-format. 288 Cub clones were pre-screened to remove false-positive and false-negative clones reducing interacting partners to 168. The Cub clones were mated individually to 467 Nub clones resulting in 105 potential interactions. 500 interaction pairs were further tested using the third marker LacZ. This small network will be presented. A second larger screen of 3800 genes is ongoing with a throughput of 72,000 pairs per week and first data will be presented here.

C605

NETWORK MODELING REVEALS PREVALENT NEGATIVE REGULATORY RELATIONSHIPS BETWEEN SIGNALING SECTORS IN ARABIDOPSIS IMMUNE SIGNALING.

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With a complex signaling network, in which components are highly interconnected, elucidating structural features of the network that govern its behavior is a challenging task. Here, we demonstrate that use of mRNA profiling to collect and analyze detailed descriptions of changes in the network state resulting from specific network perturbations is a powerful and economical strategy to elucidate regulatory relationships among the components of a complex signaling network. mRNA profiles of 22 Arabidopsis immunity mutants and wild type were collected 6 hours after inoculation with the bacterial pathogen *Pseudomonas syringae* expressing the effector protein AvrRpt2. This bacterial strain feeds multiple inputs into the signaling network, allowing many parts of the network to be activated at once. Regulatory relationships among the genes corresponding to the mutations were inferred by recursively applying a non-linear dimensionality reduction procedure to the mRNA profile data. The resulting network model accurately predicted 22 of 24 regulatory relationships reported in the literature, suggesting that predictions of novel regulatory relationships are also accurate. The network model revealed two striking features: (i) the components of

the network are highly interconnected; (ii) negative regulatory relationships are common between signaling sectors. Complex regulatory relationships, including a novel negative regulatory relationship between the early microbe-associated molecular pattern-triggered signaling sectors and the salicylic acid sector, were validated experimentally. We propose that prevalent negative regulatory relationships among the signaling sectors make the plant immune signaling network a “sector-switching” network, which effectively balances two apparently conflicting demands, robustness against pathogenic perturbations and minimization of negative impacts of immune responses on plant fitness.

C701

CELL FATE SPECIFICATION IN EARLY EMBRYOGENESIS

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The basic body organisation of *Arabidopsis* is established by position-dependent cell fate specification during early embryogenesis. Initially, the zygote divides asymmetrically to produce a small apical cell and a large basal cell. The apical cell will give rise to the embryo except for that part of the primary root meristem that is derived from the basal cell, which also and primarily generates an extra-embryonic suspensor. A major focus of our research is the establishment of apical versus basal cell fate as well as the interaction between the two cell lineages that results in the initiation of the primary root meristem.

C702

A ROLE FOR THE RIBOSOME IN ORGAN PATTERNING

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In plants, two primary meristems and their corresponding stem cells are established in the embryo, one at the apex and another at the base of the embryo. The meristem at the base of the embryo gives rise to the root system, whereas the meristem at the apex of the embryo is maintained throughout plant growth as a shoot meristem, which gives rise to all aerial portions of the plant including leaves and stems, and secondary meristems that form branches and flowers. In the shoot meristem, stem cells in the centre of the meristem give rise to peripheral region cells that are recruited to form lateral organs such as leaves. Leaves establish polar growth early in development, and this depends on signalling from the meristem to the initiating leaf, as well as concerted interactions between adaxial (dorsal) and abaxial (ventral) domains of the leaf. We are interested in understanding how these patterning processes are regulated. To this end we have identified a number of mutants in ribosomal protein genes in *Arabidopsis*, called *piggyback* (*pgy*), that are required for leaf adaxial fate and shoot meristem function. In the shoot, mutations in these ribosomal protein genes result in subtle leaf shape changes but condition adaxial ectopic leaf lamina outgrowths when combined with mutations in the MYB domain transcription factor *ASYMMETRIC LEAVES1* (*AS1*). This reflects a role for *PGY* genes in leaf adaxial fate, which occurs via a regulatory network involving the adaxial transcription factor Class III HD-ZIP genes and the abaxial transcription factor KANADI genes. Ribosomal protein genes also play a role in patterning in the embryo and we demonstrate that ribosomal protein RPL27aC is required for correct temporal establishment of the embryo shoot meristem and cotyledons. Our analysis indicates the ribosome and translation are important components controlling in patterning events during plant development.

C703

SYSTEMS ANALYSIS OF LATERAL ROOT DEVELOPMENT: AN EMERGING STORY

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The degree of root branching determines the efficiency of water uptake and acquisition of nutrients in crops. Understanding the regulation of root branching is of vital agronomic importance. Lateral root branching can be divided into 2 distinct, yet inter-connected, developmental processes; primordium initiation and emergence (reviewed in Peret et al, 2009). To date, research has focused largely on lateral root (LR) initiation. However, recent evidence suggests that LR emergence is an important checkpoint for root branching which is regulated by nutrients such as nitrate and phosphate and hormone signals such as auxin.

Lateral root primordia (LRP) originate exclusively from pericycle cells located deep within the parental root, necessitating that new primordia emerge through several overlaying tissues. We recently described how LRP emergence is a highly regulated process involving the active participation of cells in both new lateral root primordia and the parental root (Swarup et al, 2008; Peret et al, 2009). The hormone auxin originating from the developing lateral root acts as a local inductive signal which reprograms adjacent cells.

I will describe how systems biology approaches are being employed at CPIB to study how auxin regulates a transcellular gene regulatory network, the downstream target genes and biomechanical processes associated with lateral root emergence. Our systems approach involves creating increasingly sophisticated multi-cellular models of the lateral root emergence network, populating them with experimental data, and then testing the in-silico models predictive ability. I will describe several examples that illustrate how a multiscale modelling approach provides an essential tool for experimentalists to study complex non-linear systems and obtain new biological insights.

Swarup et al [2008] Nature Cell Biology 10: 625-628

Peret et al [2009] Trends in Plant Science 14: 399-408.

C704 (08004)

REGULATION OF STOMATAL LINEAGE CELL PROLIFERATION BY THE ARABIDOPSIS MYB FOUR LIPS VIA DIRECT TARGETING OF CORE CELL CYCLE GENES

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Developmental programs coordinate cell proliferation with differentiation but the underlying mechanisms are incompletely understood. Stomata, which are epidermal pores surrounded by two guard cells, develop from a specialized stem cell lineage and function in shoot gas exchange. The Arabidopsis FOUR LIPS (FLP) and MYB88 genes encode closely related and atypical two-MYB-repeat proteins, which when mutated induce excess divisions and abnormal groups of stomata in contact. Consistent with a role in transcription, we show here that FLP and MYB88 are nuclear proteins with DNA-binding preferences distinct from other known MYBs. To identify possible FLP/MYB88 transcriptional targets, we used chromatin immunoprecipitation (ChIP) followed by hybridization to Arabidopsis whole genome tiling arrays. These ChIP-chip data indicate that FLP/MYB88 target the upstream regions especially of cell cycle genes including cyclins, cyclin dependent kinases (CDKs), and components of the pre-replication complex. In particular we show that FLP represses the expression of the mitosis-inducing factor CDKB1;1 which, along with CDKB1;2, we find is specifically required both for the last division in the stomatal pathway and for cell over-proliferation in *flp* mutants. FLP recognizes a distinct cis-regulatory element that overlaps with that of the cell cycle activator E2F-DP in the CDKB1;1 promoter suggesting that these MYBs may also modulate E2F-DP pathways. We propose that FLP and MYB88 together integrate patterning with the control of cell cycle progression and terminal differentiation through multiple and direct cell cycle targets.

C705

CELL CYCLE-DEPENDENT DEVELOPMENTAL TIMING CONTROL OF FLORAL STEM CELLS

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In plant reproduction, floral stem cells are regulated by well-coordinated interplay of cell-cell signaling and transcriptional events. The maintenance of stem cells in the floral meristem is terminated after the production of a fixed number of floral organ primordia. The *Arabidopsis* floral homeotic protein AGAMOUS (AG) integrates stem cell regulation with floral patterning events. AG is induced by the stem cell determinant WUSCHEL (WUS) in early floral primordia, and then AG specifies reproductive organ identity. About 2 days after the induction of AG, AG represses *WUS* expression to terminate floral stem cells. This *WUS*-AG negative feedback pathway at the transcriptional levels plays a major part in floral stem cell regulation, but the repression mechanism was not clear. Here we show that KNUCKLES (KNU), a C2H2-type zinc finger protein mediates the repression of *WUS* as a target of AG, and the timing of *KNU* induction is key in balancing proliferation and differentiation in flower development. Delayed *KNU* expression results in an indeterminate meristem, whereas ectopic *KNU* expression prematurely terminates the floral meristem. We further show that repressive histone modification H3K27me3 at the *KNU* locus has a regulatory function in the timing control of *KNU* induction and that the induction timing is linked with cell cycle progression. We propose a model on how the transcription factor AG is involved in the changes of histone modification at the *KNU* locus in cell-cycle dependent manner.

C706

ANALYSIS OF CELL SIGNALLING DURING VASCULAR MORPHOGENESIS IN ARABIDOPSIS

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

C801

JAPANESE RESOURCE PROJECT ON ARABIDOPSIS - OUTLINES AND INTRODUCTION OF FOX LINES

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RIKEN has developed various *Arabidopsis* resources such as RIKEN *Arabidopsis* full-length cDNA (RAFL) clones and RIKEN *Arabidopsis* Transposon Mutant (RATM) lines. These valuable resources are distributed from RIKEN BioResource Center (BRC) which was established in 2001 in Tsukuba. The RIKEN BRC has joined National BioResource Project (NBRP) in 2002, and through the project, collection, preservation and distribution of mammal, plant and microbiological resources are carried out. The *Arabidopsis* project in RIKEN BRC is featured by the quality of resources. All materials mentioned above are well examined before distribution, and the results of examination are informed to the users. Another feature of the project is the wide range collection of plant full-length cDNA (fl-cDNA). Not only *Arabidopsis* but also tobacco, poplar, cassava and moss fl-cDNA clones are preserved and distributed. The number of laboratories that were distributed with our resources exceeds 1,300.

One of the plant resources unique to RIKEN is the *Arabidopsis* FOX line. For the systematic gain-of-function approach fl-cDNAs were used for ectopic expression. We mixed fl-cDNA with equal molar ratio to prevent the bias of expression level differences. About 13,000 independent *Arabidopsis* fl-cDNAs were transformed into *Arabidopsis* to generate *Arabidopsis* FOX lines. Each FOX line has extra expression of transgenes and using this transgenic pool various screening was conducted. This FOX approach was also used to identify useful traits of rice. 10,000 rice fl-cDNAs were introduced into *Arabidopsis* or rice and these transgenic pools were screened for several useful traits. Rice FOX *Arabidopsis* lines were screened with morphology, photosynthesis, pigment accumulation, element accumulation, heat tolerance, salt tolerance, bacteria and fungal resistances. These results were summarized as a database.

C802

THE ARABIDOPSIS BIOLOGICAL RESOURCE CENTER (ABRC)

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The ABRC has been collecting, preserving and distributing seed and DNA stocks of *Arabidopsis* since 1991. The collection reached nearly 1 million accessions in 2009, highlighted by ~29,000 T-DNA homozygous lines representing 18,000 genes; almost 5,000 characterized mutant, transgenic, and RNAi lines; ca. 1,000 unique natural accessions; 27 recombinant inbred populations; a large number of clones in entry and expression vectors; and ~8,000 artificial miRNAs (amiRNAs). In 2009, the Center conducted a very successful donation campaign for high-quality mutant stocks. Future campaigns are likely to focus on filling in gaps in the collection. For example, out of ~2,000 transcription factors present in *Arabidopsis*, the Center harbors DNA and/or seeds stocks for about 87% of them. Hence, no stocks are available for ~250, highlighting a need to focus on enriching the collection in underrepresented genes. Following the historical upward trend, the distribution of seed and DNA stocks exceeded 90,000 in 2009. The Center has also been anticipating the needs that the *Arabidopsis* community may have in the post-genomic era. Accordingly, during 2009, the ABRC started distributing *Arabidopsis* cells in culture, expanded the collection on other Brassicas for comparative genomics. We also initiated the allotment of protein chips generated by S. P. Dinesh-Kumar (Yale Univ.) containing more than 5,000 proteins. As always, the Center will be happy to explore the distribution of additional resources that benefit the broad *Arabidopsis* community.

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are those of the author(s) and do not necessarily reflect the views of the NSF.

C803

NEW DATA AND TOOLS AT TAIR (THE ARABIDOPSIS INFORMATION RESOURCE)

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TAIR (<http://arabidopsis.org>) is a worldwide resource for Arabidopsis data and a leader in the field of biological data curation. TAIR's team of professional curators and software developers organizes, integrates, curates and provides access to the most complete body of experimental data and biological resources available for any plant species. Incorporation of RNA-seq and proteomics data to generate a new Arabidopsis genome release (TAIR10) will be discussed, along with updates on other new tools and datasets available from TAIR. A brief update on the TAIR funding crisis will also be included.

C804

PROTEIN COMPLEX PURIFICATION FOR GENE NETWORK BUILDING

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The mapping of protein interactions is a popular method for gene discovery, functional gene analysis or the study of gene networks. Yeast two-hybrid or protein complementation assays are used to map binary interactions, but the emergence of powerful, ultrasensitive high-throughput mass spectrometry (MS), together with the availability of comprehensive protein sequence repertoires, has favored the development of methods relying on *in situ* affinity purification of protein complexes. Especially, the tandem affinity purification (TAP) approach, based on the expression of a bait protein fused to a double affinity tag, has proven to be of great value. We developed a high throughput TAP technology platform for complex isolation from *Arabidopsis* cell suspension cultures (1,2) and applied it to map the core complex machinery at the heart of the *Arabidopsis* cell cycle control. The resulting network offers for the first time a comprehensive view on CDK/cyclin complexes of a higher plant and contains over 100 new candidate cell cycle proteins. Besides a central regulatory network of core complexes, we distinguished a peripheral network that links the core machinery to up- and downstream pathways. Furthermore, we could demonstrate that plants have evolved a combinatorial toolkit comprising at least a hundred different CDK/cyclin complex variants, which strongly underscores the functional diversification among the large family of cyclins and reflects the pivotal role of cell cycle regulation in the developmental plasticity of plants.

- (1) Van Leene et al. (2007) *Mol. & Cell. Proteomics* 6, 1226-1238
 - (2) Van Leene et al. (2008) *Trends in Plant Sc.* 13, 517-520
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C805

RAPID ASSESSMENT OF GENE FUNCTION USING ARTIFICIAL MICRORNA IN ARABIDOPSIS

MESOPHYLL PROTOPLASTS

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Rapid determination of the effect of reduced levels of gene products is often a bottleneck in deciding how to proceed with an interesting gene candidate. Additionally, gene families with closely related members can confound determination of the role of even a single one of the group. We describe here an *in vivo* method to rapidly assess gene function using transient expression of artificial microRNAs (amiRNAs) in *Arabidopsis* mesophyll protoplasts. We use a luciferase-based reporter of circadian clock activity to optimize and validate this system. Protoplasts transiently co-transfected with promoter-luciferase and gene-specific amiRNA plasmids sustain free-running rhythms of bioluminescence for more than six days. Using both amiRNA plasmids available through the ABRC, as well as custom-design of constructs using the Weigel amiRNA design algorithm, we show that transient knockdown of known clock genes recapitulates the same circadian phenotypes reported in the literature for loss-of-function mutant plants. We additionally show that amiRNA designed to knockdown expression of the casein kinase 2 beta subunit (CK2B) gene family lengthens period, consistent with previous reports of a short period in CK2B overexpressors. Our results demonstrate that this system can facilitate a rapid analysis of gene function by obviating the need to initially establish stably transformed transgenics to assess the phenotype of gene knockdowns. This approach will be useful in a wide range of plant disciplines when an endogenous cell-based phenotype is observable or can be devised, as done here using a luciferase reporter.

POSTER SESSION ABSTRACT

Posters

Each poster has a five-digit number. The first and second numbers show your session as below.

- 01. Regeneration
- 02. Biotic Responses
- 03. Abiotic Responses
- 04. Epigenetic and RNA Regulation
- 05. Novel Function of Peptides
- 06. Metabolism
- 07. Systems Biology
- 08. Developmental Regulation
- 09. Research Tools and Resources
- 10. Evolution and Natural Variation
- 11. Cell Biology
- 12. Hormone Responses
- 13. Cell Wall
- 14. Plant Genomics
- 15. Plant Biotechnology
- 16. Bioinformatics
- 17. Others
- 18. Subcommittee Forum

01001

A BZIP TRANSCRIPTION FACTOR IS A PUTATIVE PROMOTING FACTOR FOR REPROGRAMMING FROM A DIFFERENTIATED LEAF CELL TO A PLURIPOTENT STEM CELL IN THE MOSS PHYSCOMITRELLA PATENS
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Reprogramming from a differentiated cell to a pluripotent stem cell is frequently observed in plants, although animals exhibit the rigidity of differentiated states. Once leaves of *Physcomitrella patens* were excised and exposed to the light, both apical cell growth and cell division started within 24 hours in the cut ends. Ultimately, they grew up to complete moss bodies. To explore the factors that promote reprogramming, we first obtained a time-course profile of gene expression by using custom-microarrays, and identified 29 transcription factors (TFs) that were significantly up-regulated within 12 hours in excised leaves. Next, we investigated their dominant repressive effects on reprogramming by conditionally overexpressing chimeric genes harboring the TF fused with a modified EAR-motif repression domain (SRDX). Among them, we found overexpression of a bZIP:SRDX fusion protein severely inhibited the reprogramming, and that the induction of D-type cyclin, a cell cycle marker, was severely inhibited. To know the gain-of-function phenotypes, we examined the moss expressing the bZIP fused to the VP16 transcriptional activation domain. Ectopic reprogramming was observed in leaves of bZIP:VP16 lines without excision stimuli. Furthermore, time-lapse observation of citrine knock-in moss revealed high-level accumulation of bZIP:citrine protein in the reprogrammed cells which were located in the cut edge. These results suggest that the bZIP TF is a promoting factor of reprogramming.

01002

METHYL JASMONATE IS INVOLVED IN THE REGULATION OF ADVENTITIOUS ROOTING *IN PLANTA* AND *IN VITRO* CULTURED THIN CELL LAYERS OF *ARABIDOPSIS THALIANA*

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Auxin stimulates adventitious root (AR) formation *in planta* and in *in vitro* cultured explants. In *Arabidopsis* plantlets grown *in vitro* ARs can be induced on the hypocotyl, without exogenous auxin, by etiolating seedlings in the dark [Takahashi et al, J Plant Res (2003); Sorin et al, Plant Cell (2005)]. In *Arabidopsis* thin cell layer (TCL) system, the stem cortical tissues are re-programmed by exogenous auxin in combination with low cytokinin under darkness to regenerate ARs [Falasca et al, Plant Cell Rep (2004)]. AR formation is also a stress induced response and it was previously shown that jasmonates can be involved in the regulation of adventitious rooting [Akhami et al, New Phytol (2009); Fattorini et al, Planta (2009)]. In addition it is known that jasmonates positively affect auxin biosynthesis in *Arabidopsis* [Sun et al, Plant Cell (2009)]. In order to better understand the role of jasmonates, we investigated the effect of MeJA, and its interaction with auxin, during AR formation *in planta* and in TCLs of *Arabidopsis*. Various MeJA concentrations were applied under hormone-free conditions and in the presence of IBA and Kinetin. Two mutants affected in jasmonic acid biosynthesis (*dde2-2* and *opr3*), and the mutant *sur2-1* known to spontaneously produce ARs due to an increased endogenous auxin level [Delarue et al, Plant J (1998)] were used for the purpose. *AtGH3-3*, *AtGH3-5*, and *AtGH3-6* are auxin-inducible genes whose protein level correlates positively with AR development *in planta*, and are up-regulated in *sur2-1* [Sorin et al, Plant Physiol (2006)]. Their expression was monitored during AR formation *in planta* and in TCLs, in the presence/absence of MeJA. All together, our results suggest an interaction between jasmonates and auxin during AR formation, in *Arabidopsis*.

01003

ARABIDOPSIS CALLUS FORMATION REQUIRES THE ACTIVATION OF THE LATERAL ROOT INITIATION PROGRAM

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Unlike most animal cells, plant cells can easily regenerate new tissues from a wide variety of organs when properly cultured. The common elements that provide varied plant cells with their remarkable regeneration ability are still largely unknown. We characterized the initial process of *Arabidopsis* *in vitro* regeneration, where a pluripotent cell mass termed callus is induced. Using live imaging and whole-genome microarray analysis, we found that callus is not an undifferentiated tissue but a somewhat differentiated tissue similar to the tip part of a root meristem, even if it is derived from aerial organs such as petals, which clearly shows that callus formation is not a simple reprogramming process backwards to an undifferentiated state as widely believed. Furthermore, callus formation in roots, cotyledons and petals is blocked in mutant plants incapable of initial cell division of pericycle cells, a layer of tissue surrounding the vasculature whose division is an early step in formation of a lateral root. The expression pattern of pericycle markers that characterize lateral root initiation was observed in the callus-forming region of roots, cotyledons and petals. It thus appears that the ectopic activation of a lateral root development program in pericycle-like cells is a common mechanism in callus formation from multiple organs. We also tested various media containing different levels of plant hormones and confirmed that the conclusions drawn above apply to a wide range of medium conditions.

01004

WUSCHEL PROMOTES CELL DIVISION BOTH IN EMBRYO STEM DURING EARLY EMBRYO AND IN HYPOCOTYL AT POSTEMBRYO DEVELOPMENT

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WUS plays a vital role in maintaining plant stem cell homeostasis of shoot apical meristem (SAM). A *WUS* gain-of-function mutant, *wus-1D*, previously isolated from an activation tagging genetic screen displays a drastically elongated hypocotyl phenotype, whereas a loss-of-function mutant, *wus-1*, exhibits a shortened hypocotyl phenotype. Analysis of cell number in embryo stem and hypocotyl of seedling showed that variable number of cells, but not cell length result in aberrant hypocotyls. Cell number is increased both in embryogenesis and postembryogenesis in *wus-1D*. Analysis of cell division rate revealed that cells in *wus-1D* embryo stem divide faster, whereas *wus-1*

embryo stem cells divide slower than wild-type plants during the early stage. Several genes related to cell division are up-regulated in early embryo and seedling in *wus-1D* and almost not detected in that of *wus-1*. *CYCB1;1::GUS* is ectopically expressed in the hypocotyl of *wus-1D* which suggests ectopic cell division in hypocotyl. Taking together, our data suggested that *WUS* might play an important role in cell division and affected cell number of embryo stem and hypocotyl in seedling. Our findings might unravel an underlying linkage between stem cell maintenance genes regulation and cell division genes regulation.

01005

THE *PHYSCOMITRELLA CYCLIN-DEPENDENT KINASE A* LINKS CELL CYCLE REACTIVATION AND CELLULAR CHARACTERIZATION IN STEM CELL REGENERATION

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Differentiated plant cells retain the capacity to change to pluripotent stem cells during regular development and regeneration. This capacity is associated with both cell cycle reactivation and the acquisition of specific cellular characters. However, the factors that coordinate the two functions have not been determined. Excised leaf cells of the moss *Physcomitrella patens* are readily reprogrammed to pluripotent chloronema apical cells. Here, we report a link between cell cycle reactivation and the acquisition of chloronema characters by the activity of the mammalian cyclin-dependent kinase 1 (CDK1) ortholog A-type CDK (CDKA;1). Leaf excision induced CDK activity, which reactivated the cell cycle from the G1 resting phase. A CDK inhibitory agent, roscovitine, and kinase-negative form CDKA;1 inhibited cell cycle progression and unexpectedly also inhibited the acquisition of apical growth, which is specific to moss stem cells, although inhibition at the S phase by aphidicolin did not prohibit apical growth. These results indicate that CDKA;1 activation coordinates cell cycle reactivation and cellular changes in the G1 phase during stem cell formation.

01006

ANALYSIS ON THE INHIBITORY ACTION OF 5-BROMO-2'-DEOXYURIDINE AGAINST DEDIFFERENTIATION OF ARABIDOPSIS THALIANA.

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In *Arabidopsis*, shoot redifferentiation can be induced from hypocotyl and root explants by a two-step culture method, in which explants are precultured on callus-inducing medium and then transferred onto shoot-inducing medium. With this culture system, we found tissue-, time-, and dose-dependent effects of a thymidine (dT) analog, 5-bromo-2'-deoxyuridine (BrdU). One of the notable findings was that shoot redifferentiation from hypocotyl explants is inhibited by treatment with BrdU at high concentrations during preculture while shoot redifferentiation from root explants is not inhibited by the same BrdU treatment. Our previous analysis of this BrdU action suggested that BrdU interferes with the acquisition of cell proliferation competence during dedifferentiation.

In an attempt to identify a molecular event involved in dedifferentiation as a BrdU target, we isolated four BrdU-resistant mutants, *bromodeoxyuridine-resistant organogenesis* (*bro*) 1 to 4, by screening with shoot redifferentiation as an index phenotype. A wide spectrum of normally BrdU-sensitive phenomena, including root growth and callus growth, were also resistant to BrdU in these mutants. For further characterization of *bro* mutants, we examined the sensitivity of these mutants to 5-fluorodeoxyuridine (FdU). As compared with the wild type, *bro2* and *bro4* were resistant to FdU as well as BrdU in the tissue culture test. In contrast, *bro1* and *bro3* were hypersensitive to FdU. This result reflects that different mechanisms are responsible for BrdU resistance in these mutants.

By chromosome mapping and DNA sequencing, mutations that probably confer BrdU resistance were found within a gene encoding UBA1a in *bro1* and within a gene encoding a dT kinase in *bro2*. Since UBA1a was reported to bind U-rich RNA and regulate the stability of mRNAs, the possibility has arisen that BrdU targets some mRNA metabolisms.

01007

IDENTIFICATION AND CHARACTERIZATION OF CHEMICAL INHIBITORS OF REPROGRAMMING PROCESSES IN *PHYSCOMITRELLA PATENS* AND *ARABIDOPSIS THALIANA*

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In the moss *Physcomitrella patens*, differentiated leaf cells of an excised leaf change to protonemal apical stem cells without exogenous phytohormones within a few days, which provides an outstanding experimental system for the study of molecular basis of reprogramming process in plants. To clarify this mechanism, we tried to screen the chemical compounds inhibiting the reprogramming process from a leaf cell to an apical stem cell. Of 12,000 chemicals, 4 chemicals were isolated as the reprogramming inhibitors (RINs), which all show (1) inhibition of the stem cell formation from a leaf cell, (2) an insignificant effect on the protonemal growth, and (3) inhibition of expression of the reprogramming marker gene. Further characterization revealed that RIN16B, one of RINs, also disturbs the lateral root development in *Arabidopsis thaliana*. Based on this effect, RIN16B-resistant lines were screened by utilizing RIKEN Arabidopsis FOX (Full-length cDNA over-expressor) lines. As a result, we isolated a transgenic line overexpressing the PHD finger gene named *RIN16B RESISTANT 1* (*AtRIR1*), in which the lateral-root developmental defect is suppressed in the presence of RIN16B. Our database analysis identified two *P. patens* genes homologous to *AtRIR1*. Here, we report the phenotypic analyses of conditional overexpression lines and loss-of-function mutants on the *PpRIR* genes and discuss the possible common mechanism for stem cell establishment involving the *RIR* genes both in *P. patens* and *A. thaliana*.

02001

CHARACTERIZATION OF NONHOST DISEASE RESISTANCE OF ARABIDOPSIS TO BACTERIAL PATHOGENS

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Nonhost disease resistance is the most common plant defense mechanism which protects plants from various potential pathogens.

Despite the important role of nonhost resistance in plant defense, the molecular mechanisms underlying plant nonhost resistance has not been fully understood. Recently, we identified genes involved in nonhost resistance against bacterial pathogens using virus-induced gene silencing (VIGS) in *N. benthamiana*. To further characterize the function of these genes, we searched the gene homologs from the model plant species, *Arabidopsis*. Five genes were initially selected for further analyses in *Arabidopsis*. The *Arabidopsis* knockout mutants for these genes were susceptible to several nonhost bacterial pathogens and supported more bacterial growth. To further uncover the molecular mechanisms and signal pathways underlying nonhost resistance in *Arabidopsis*, we are currently taking several approaches like gene expression profiling, subcellular protein localization, yeast-two-hybrid analyses to identify interacting proteins, etc. The results from these studies will provide significant insight to the basis of molecular signal networks during nonhost disease resistance of *Arabidopsis*. We will present the studies on the identification of genes involved in nonhost resistance in *N. benthamiana* and the functional characterization of the genes in *Arabidopsis* to dissect their role of nonhost resistance.

02002

PHOSPHOLIPASE D ISOFORMS IN DEFENCE RESPONSE OF ARABIDOPSIS THALIANA TO PSEUDOMONAS SYRINGAE.

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In plants, the attack of pathogen leads to the accumulation of salicylic acid (SA) in the infected tissue and could lead to the development of systemic acquired resistance (SAR) against pathogen. SA triggers a complicated cascade of signalling events resulting in the expression of PR (Pathogenesis-Related) genes enabling the plant to cope with pathogen. The molecular mechanism of the early events in signalling pathway leading to SAR development is largely unknown but increasing evidence points to phospholipid signalling as important component of this pathway. Recently, we brought the evidence that phospholipase D and its product phosphatidic acid (PA) is involved in a rapid response of *Arabidopsis* suspension cells to the SA treatment (1) The same effect could be seen in wild type plants. In comparison to other eukaryots, plant phospholipase D represents numerous gene family (12 genes in *Arabidopsis thaliana*) divided to 5 groups differing significantly in their biochemical characteristics. This fact arouses the question whether and what are their specific roles in the plant response. We show on *Arabidopsis* T-DNA mutant lines that multiple isoforms of phospholipase D are involved in response to SA and virulent pathogen *Pseudomonas syringae* pv *maculicola*. Corresponding changes in the level of distinct PA species determined by mass spectrometry will be also presented.

- (1) Krinke O, Flemr M, Vergnolle Ch, Collin S, Renou J-P, Teconnat L, Yu A, Burketova L, Valentova O, Zachowski A and Ruelland E (2009) Phospholipase D Activation Is an Early Component of the Salicylic Acid Signaling Pathway in *Arabidopsis* Cell Suspensions. *Plant Physiology* 150, 424-436.

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02003

ENDOSOME-ASSOCIATED CRT1 FUNCTIONS EARLY IN R (RESISTANCE) GENE-MEDIATED DEFENSE SIGNALING

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Resistance gene-mediated immunity confers protection against pathogen infection in a wide range of plants. A genetic screen for *Arabidopsis* mutants compromised for recognition of Turnip Crinkle Virus previously identified CRT1, a member of the GHKL ATPase/Kinase superfamily. Here we demonstrate that CRT1 interacts with various resistance proteins from different structural classes, and this interaction is disrupted when these resistance proteins are activated. The *Arabidopsis* mutant crt1-2 crh1-1, which lacks CRT1 and its closest homologue, displayed compromised resistance to avirulent *Pseudomonas syringae* and *Hyaloperonospora arabidopsis*. Additionally, resistance-associated hypersensitive cell death was suppressed in *Nicotiana benthamiana* silenced for expression of CRT1 homologue(s). Thus, CRT1 appears to be a general factor for *resistance* gene-mediated immunity. Since elevation of cytosolic calcium triggered by avirulent *P. syringae* was compromised in crt1-2 crh1-1 plants, but cell death triggered by NtMEK2^{DD} was unaffected in CRT1-silenced *N. benthamiana*, CRT1 likely functions at an early step in this pathway. Genome-wide transcriptome analysis led to identification of *CRT1*-Associated genes, many of which are associated with transport processes, responses to (a)biotic stress and the endomembrane system. Confocal microscopy and subcellular fractionation revealed that CRT1 localizes to endosome-like vesicles, suggesting a key process in resistance protein activation/signaling occurs in this subcellular compartment.

02004

XOPR, A TYPE III EFFECTOR SECRETED BY XANTHOMONAS ORYZAE PV. ORYZAE, INHIBITS BASAL DEFENSE RESPONSES IN ARABIDOPSIS

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XopR (XOO4134) is an effector protein secreted into rice cells through the type III secretion apparatus of *Xanthomonas oryzae* pv. *oryzae* strain MAFF 311018, the causal agent of bacterial blight of rice (Furutani et al., MPMI 22(1): 96-106 2009). In order to clarify the effects of XopR on plants, we have developed a transgenic *Arabidopsis* plant (Col-0 background) which expresses XopR under steroid hormone dexamethasone (DEX) treatment. Expression patterns of known pathogen-associated molecular pattern (PAMP)-responsive genes were analyzed by qRT-PCR followed by inoculation with *Xanthomonas campestris* pv. *campestris* ATCC33913 HrcC⁻ (Xcc HrcC⁻) mutant which lacks the type III secretion apparatus. Although no significant visible phenotype was shown after DEX treatment, expression of PAMP-responsive genes such as At2g17740, At2g46400 (WRKY46), At2g19190 (FRK1) and At5g57220 (CYP81F2) in DEX-treated plants was distinctly suppressed compared with those of DEX-untreated ones. Indicating XopR inhibits plant basal defense responses caused by Xcc HrcC⁻.

02005

IDENTIFICATION OF HOST TARGETS OF XANTHOMONAS ORYZAE EFFECTORS.

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Plant bacterial pathogens equipped with the type III secretion system (TTSS) generally deliver different TTSS effector proteins into plant cells. These TTSS effector proteins modulate the function of crucial host regulatory molecules and allow bacteria to invade plant cells. So far, we have isolated 16 TTSS effectors from *Xanthomonas oryzae* pv. *oryzae* (*Xoo*; Furutani *et al.*). To identify *Xoo* TTSS effectors that inhibit host immune responses, we generated transgenic rice plants expressing each of 10 *Xoo* effectors. These transgenic plants were inoculated with the TTSS-deficient *hrpX* mutant of *Xoo*. Transgenic plants expressing *Xoo* effectors showed different levels of susceptibility to the *hrpX* mutant, indicating that these effectors have the abilities to inhibit PAMPs-triggered immunity (PTI) inside plant cells. Especially, transgenic plants expressing four of 10 effectors developed severe disease lesions of the *hrpX* mutant, suggesting that these four effectors may block the important steps in PTI. We identified host targets of these effectors by two-hybrid system, which include receptor-like kinase and transcription factor. We also found that one of these effectors interacts with BAK1, which is supported by the fact that its over-expressor exhibits brassinosteroid insensitive phenotype. The roles of target factors in PTI will be discussed.

02006

EDM2, A NOVEL TRANSCRIPTIONAL REGULATOR CONTROLLING IMMUNE RESPONSES AND DEVELOPMENTAL PROCESSES IN ARABIDOPSIS THALIANA

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The *Arabidopsis thaliana* defense regulator EDM2 was previously shown to be specifically required for immunity mediated by the disease resistance (*R*)-gene *RPP7* against the Hik1 isolate of the biotrophic oomycete *Hyaloperonospora arabidopsis* (*Hpa*). Unlike many other plant defense mechanisms against biotrophs EDM2/RPP7-mediated immunity is independent from the defense hormone salicylic acid. EDM2 bears typical features of transcription factors and epigenetic regulators, but does not belong to any established class of plant proteins controlling transcription. EDM2 most likely contributes to disease resistance by promoting transcription of *RPP7*. New observations also suggest that EDM2 affects the transcript levels and activity of two additional *R* genes. Furthermore, we found EDM2 to have a promoting effect on several distinct developmental processes including the floral transition. EDM2 interacts in nuclei with the WNK8 protein kinase that phosphorylates EDM2. WNK8, however, seems not to play a role in defense, but to act upstream from EDM2 in flowering time control. In addition, EDM2 interacts in nuclei with several other regulatory proteins, some of which contribute to the immune function of EDM2. We are currently characterizing these new components of the plant immune system. The involvement of EDM2 in both developmental processes and gene-for-gene resistance is intriguing and an important question is, if these roles of EDM2 are unrelated or if there is a biologically significant connection between these different types of roles. New results on the molecular functions of EDM2 at the interface between defense and developmental programs will be presented at the meeting.

02007

INCREASED EARLY CALLOSE DEPOSITION LEADS TO COMPLETE RESISTANCE TO POWDERY MILDEW

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In plant-fungal interaction, callose is deposited at sites of fungal penetration as the most common chemical constituent in papillae. The cell wall thickenings are thought to slow down pathogen invasion. In *Arabidopsis*, stress-induced callose deposition is dependent on the activity of the callose synthase PMR4 (=GSL5). Interestingly, *pmr4* mutants without stress-induced callose deposition reveal a higher resistance to powdery mildew than wild-type plants. Further analysis linked the higher resistance to an increased induction of salicylic acid synthesis.

We could show that constitutive expression of *PMR4* under the CaMV35S promoter leads to a complete resistance to the powdery mildew *Golovinomyces cichoracearum*. In contrast to *pmr4* mutants and wild-type, no fungal penetration, haustorium, and mycelium formation was observed on leaves after *G. cichoracearum* inoculation of the 35S::*PMR4* lines. Real-time PCR analysis revealed that salicylic acid synthesis is not induced in these lines. However, 6 hours post-inoculation, increased callose synthase activity was measured that directly correlated with an extended callose deposition at sites of attempted fungal penetration. Callose synthase activity in leave tissue was 5 times higher in 35S::*PMR4* lines than in wild-type, and the diameter of callose plagues was 3 times higher. A C-terminal fusion of GFP to PMR4 enabled the localization of PMR4 within epidermal cells. Confocal laser-scanning microscopy revealed that PMR4 accumulates at sites of attempted penetration. A direct correlation of the PMR4-GFP signal pattern and the pattern of the callose plagues was evident. In control plants, a GFP-signal could not be detected even though western blot analysis showed the presence of PMR4. These results conclude that increased early callose deposition confers to strong fungal resistance, and that translocation is important for regulation of stress-induced callose synthesis.

02008

PHOSPHORYLATION EVENTS IN PLANT-PATHOGEN INTERACTIONS

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A mechanistic understanding of signalling pathways requires the identification of kinases and their targets. Identification of the site(s) of phosphorylation on a protein is only part of the information required to understand their function. The dynamic interplay of phosphorylation and dephosphorylation has important consequences for signalling, as does the stoichiometry of phosphorylation. I will discuss the identification and quantification of differential phosphorylation events in plant-pathogen interactions and present data on a receptor-like kinase (CERK1), a kinase involved in resistance signalling (Pto) and a effector kinase from *Phytophthora infestans*.

02009

PAMP-TRIGGERED IMMUNITY AGAINST BACTERIA IS POSITIVELY REGULATED BY A LEGUME-LIKE LECTIN RECEPTOR KINASE IN ARABIDOPSIS

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Plants initially sense microbial pathogens through perception of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors located on the cell surface. Recognition leads to the activation of PAMP-triggered immunity (PTI). This work describes the functional characterization of a lectin receptor kinase (LecRK) in the *Arabidopsis* defense response to microbial pathogens. Dip-inoculated

lecRK T-DNA insertion knock-out (KO) mutant lines were found to be more susceptible to *Pseudomonas syringae* and *Erwinia carotovora* bacteria. However, no differences in susceptibility were observed after infiltration inoculation. This observation suggests this LecRK acts during the early defense response to bacteria. We thus analyzed the activation of the PTI response in *lecRK* loss-of-function mutants after bacterial infection and bacterial PAMPs treatment. Reduced up-regulation of PTI marker genes such as *FRK1* and *WRKY53*, and impaired callose deposition were observed. By contrast *lecRK* KO lines demonstrated a normal PTI response after treatment with the fungal PAMP chitin and a normal resistance to the fungus *Botrytis cinerea*. These data indicate this LecRK may not play a critical role in fungal PTI. Finally, we show functional complementation of the *lecRK* KO mutant phenotype by constitutive expression of *LecRK*. Complemented lines with high *LecRK* expression levels demonstrated a constitutive up-regulation of *FRK1*, *WRKY53*, the salicylic acid-dependent *PR1* gene and high resistance to *Pst* DC3000. We propose that the legume-like LecRK under study is a positive regulator of bacterial-induced PTI.

02010

DIFFERENT NETWORK PROPERTIES DEFINE THE DIFFERENCE BETWEEN PATTERN- AND EFFECTOR-TRIGGERED IMMUNITY

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Two modes of plant immunity against biotrophs, Pattern-Triggered Immunity (PTI) and Effector Triggered Immunity (ETI), are triggered by recognition of conserved microbial features called Microbe-Associated Molecular Patterns (MAMPs) and specific pathogen effectors, respectively. Previously, we had reported that *PR1* was induced during the PTI triggered by the MAMP flg22 (flg22-PTI) in a salicylic acid (SA)-dependent manner. We found that *PR1* was induced during the ETI triggered by the effector AvrRpt2 (AvrRpt2-ETI) in an SA-independent manner. MAP kinases, MPK3/6, are activated during PTI and ETI. However, the timing of activation is different: rather transient in PTI; prolonged in ETI. We found that prolonged MPK3/6 activation by inducible expression of a constitutively active form of the corresponding MAPKK, MKK4DD, led to induction of *PR1* in an SA-independent manner. These results suggest that the prolonged MPK3/6 activation during ETI but not transient activation during PTI triggers SA-independent *PR1* induction. We have recently reported that the network defined by the four genes *DDE2*, *EIN2*, *PAD4* and *SID2* accounts for most of flg22-PTI and AvrRpt2-ETI against *Pseudomonas syringae*, indicating that these cases of PTI and ETI extensively share signaling machinery. We estimated the effects of the wild-type genes and their interactions on immunity using the data from all combinatorial mutants and wild type. This allocation analysis revealed that the common signaling machinery was used differently in PTI and ETI: synergistic relationships among the signaling sectors are evident in PTI, which may amplify the signal; compensatory relationships among the sectors dominate in ETI, explaining the robustness of ETI against genetic and pathogenic perturbations. Taken together, these results suggest that what differentiates PTI and ETI signaling is not a difference in the signaling machinery but a difference in how it is used.

02011

A PHOSPHO-MIMIC MUTANT OF RIN4 IS SUFFICIENT TO TRIGGER RPM1-MEDIATED DISEASE RESISTANCE

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RPM1, an Arabidopsis NB-LRR protein, mediates disease resistance in response to the bacterial type III effector protein AvrB and AvrRpm1. We proposed that a host target of both effectors, RIN4, is guarded by RPM1. Modification of RIN4 induced by effectors, potentially via phosphorylation, is perceived as modified self by RPM1, leading to suppression of bacterial growth and a hypersensitive response (HR). Based on the characterized co-crystal structure of AvrB and RIN4¹⁴²⁻¹⁷⁶, RIN4 point mutants in the AvrB-binding site (BBS) was generated: T166A, T166D, H167A, I168A, F169A and an HIF-AAA mutant. The yeast-two-hybrid verified the interaction between RIN4 BBS mutants and AvrB. We reconstructed RPM1-mediated HR with transiently expressed RIN4 and either AvrB or AvrRpm1 in *Nicotiana benthamiana*. Wild-type RIN4, T166D and H167A supported AvrB-triggered HR with RPM1. The phospho-mimic mutants of RIN4 (T166D and T166E) caused effector-independent HR with RPM1. No HR was observed with T166D and RPM1 G205E (p-loop dead) mutant. The AvrRpm1-RIN4-RPM1 reconstruction displayed the same BBS mutant results as shown in AvrB-RIN4-RPM1 reconstruction except that AvrRpm1 activated RPM1-dependent HR with RIN4 T166A. We infected transgenic Arabidopsis expressing RIN4 BBS mutants with *Pseudomonas syringae* pv. *tomato* (*Pto*) DC3000 containing *AvrB* or *AvrRpm1*. The H167A fully complemented RPM1-mediated HR and suppression for both strains, but I168A, F169A and HIF-AAA mutant did not. The RIN4 T166A mutant showed loss-of-function for RPM1-mediated HR by *Pto* DC3000 *AvrB* and partial complementation to *Pto* DC3000 *AvrRpm1*. Our data suggest that RIN4 T166 is necessary for AvrB-triggered, RPM1-mediated disease resistance and enhances AvrRpm1-triggered, RPM1-dependent disease resistance. We conclude that AvrB-dependent phosphorylation (or ampylation) of RIN4 T166 activates RPM1.

02012

USE OF ENHANCER TRAPPING TO IDENTIFY PATHOGEN-INDUCED REGULATORY EVENTS SPATIALLY RESTRICTED TO PLANT-MICROBE INTERACTION SITES

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Plant genes differentially expressed during plant/pathogen interactions can be important for host immunity or contribute to pathogen virulence. Microarray analyses and other large scale transcript profiling methods have revealed the identity of many pathogen-responsive plant genes as well as the timing of their expression changes. However, they do typically not provide any information regarding the spatial patterns of transcriptional reprogramming. Furthermore, these methods may not allow for the identification of genes that exhibit only weak transcriptional changes or respond only in a small number of plant cells. We utilized enhancer trapping to identify local events of differential gene expression triggered by virulent isolates of the oomycete *Hyaloperonospora arabidopsis* (*Hpa*) in Arabidopsis. Screening 11,300 Arabidopsis lines containing the pD991 enhancer trap vector (Campisi et al. 1999, Plant J. 17: 699) we identified several individuals exhibiting reporter gene activity in plant tissue closely associated with *Hpa* structures. We isolated the respective insertion loci by ligation-mediated PCR. An enhancer trap, which resides in an exon of *Hyphae-Associated Response Gene (HARG)1*, responds strongly in plant cells directly surrounding *Hpa* hyphae and significantly reduces the formation of *Hpa* spores. Two additional *harg1*T-DNA mutants exhibit the same phenotype. As its mutants seem not to exhibit constitutive activation of defense responses, *HARG1* may serve a virulence function for *Hpa*. In microarray experiments with whole Arabidopsis seedlings, *HARG1* transcript levels showed only a weak (~1.3-fold), yet significant, up-regulation upon infection with *Hpa*. Thus, this local gene-regulatory event, which seems to be of functional importance for the outcome of Arabidopsis/*Hpa* interactions, could have easily been missed. We are currently investigating the role of *HARG1* in *Hpa* virulence.

02013

SUSTAINED ACTIVATION OF PATTERN RECOGNITION RECEPTOR-MEDIATED SIGNALING FOR ROBUST IMMUNITY

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In plants and animals, recognition of microbe-associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs) leads to an enhanced state of immunity, termed MAMP-triggered immunity (MTI). However, the presence of MAMPs essentially in all microbes raises an important, as-yet-unsolved question of how hosts distinguish pathogens from non-pathogens to selectively mount effective defenses against the former. The *Arabidopsis* Leu-rich repeat receptor kinases (LRR-RKs) EFR and FLS2 act as PRRs that recognize the bacterial elongation factor (EF)-Tu epitope elf18 and the flagellin epitope flg22, respectively. We revealed a series of *Arabidopsis* priority in sweet life (psl) mutants that show defects in EFR accumulation and signaling, but not of FLS2, due to the improper quality control of EFR in the endoplasmic reticulum (ER). We notice that EFR signaling is partially and differentially impaired in weakly dysfunctional psl alleles without a significant decrease of the receptor steady-state levels. Remarkably, in an allele of ER-resident glucosidase II A subunit, designated rsw3, EFR-triggered immunity collapses against the bacterial phytopathogen *Pseudomonas syringae*, despite nearly intact co-activation of MAPKs, reactive oxygen species, ethylene biosynthesis, and callose deposition by the PRR. However, rsw3 plants fail to maintain high transcript levels of defense-promoting WRKY, PR1 and PR2 genes at late time points (4 to 24 hours) after elf18 elicitation. This strongly suggests the importance of sustained activation of PRR signaling for extensive transcriptional reprogramming as a key step in the establishment of effective MTI. We will discuss potential mechanisms that couple initial MAMP detection to robust defense activation upon pathogen recognition, with a focus on molecular processes affected in rsw3 plants.

02014

ATEB1S INTERACT WITH ATBZIP10 AT MICROTUBULE PLUS-ENDS AND ARE REQUIRED FOR CELL DEATH CONTROLLED BY LSD1
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Programmed cell death (PCD), including the hypersensitive response associated with the successful immune responses, results from various cellular responses to oxidative stresses in plants. To clarify how plants induce PCD upon pathogen's challenge, we have focused on the function of LSD1 (Lesion Simulating Disease resistance 1), a negative regulator of cell death, in *Arabidopsis*. LSD1 seems to control the cell death-related transcriptional activity of the bZIP transcription factor AtbZIP10 by altering its intracellular partitioning via direct binding in the cytosol (Kaminaka et al., EMBO J., 2006). Since the molecular mechanism for induction of cell death mediated by the function and interaction of LSD1 and AtbZIP10 is still not clear, we carried out yeast two-hybrid (Y2H) screening, using AtbZIP10 as the bait protein. As a result, we identified AtEB1 (End-Binding 1), which is one of the +TIPs (microtubule plus-end tracking proteins), as a novel AtbZIP10-interacting protein. We determined that AtEB1 recognizes the IP motif present in AtbZIP10. The IP motif is a highly conserved motif identified in mammalian +TIPs. Of the three EB1s in *Arabidopsis*, AtbZIP10 specifically interacted with AtEB1a and AtEB1b, but not AtEB1c. In addition, *in planta* bio-imaging analysis demonstrated that AtbZIP10 co-localized and interacted with AtEB1a located on microtubules, particularly at their plus-ends. The *lsd1 ateb1a ateb1b ateb1c* quadruple mutant displayed significant reduction of the uncontrolled cell death induced by benzothiadiazole compared to that of the *lsd1* single mutant. The level of cell death induced by mycotoxin fumonisin B1 was also reduced in *ateb1a ateb1b ateb1c* triple mutant plants, whereas it was increased in *35S::AtEB1a* plants. These results indicate that AtEB1 participates in cell death induced by mycotoxin as well as controlled by LSD1, perhaps through its interaction with AtbZIP10 at microtubule plus-ends.

02015

INVOLVEMENT OF TOBACCO TRANSCRIPTION FACTOR DOF PROTEINS IN THE PROMOTER ACTIVATION OF THE RESISTANCE GENE *N* AGAINST TOBACCO MOSAIC VIRUS
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Nicotiana tabacum cv. Samsun NN is a tobacco cultivar that carries a resistance gene *N* against *Tobacco mosaic virus* (TMV). Upon TMV infection, *Nicotiana* species with the *N* gene show hypersensitive response (HR) leading to programmed cell death at infection sites, which result in the inhibition of systemic virus spread. The helicase domain (p50) of the virus replicase is known to be an elicitor to interact with the *N* gene product. The transcription of the *N* gene has been reported to be upregulated prior to HR induction by TMV infection. Previously, we showed a 269-bp DNA fragment as a minimum promoter sequence located upstream of the translational initiation codon of the *N* gene. Through a transient assay with GFP reporter gene, coexpression of p50 or N protein was shown to cause about 3-fold increase in the promoter activity. Furthermore, the coexpression of both p50 and N protein synergistically stimulated the *N* promoter activity. Thus, the resistance gene product N functions as an transcriptional activator.

Deletion analysis of the 269-bp sequence identified a novel 20-bp sequence from -269 to -250 required for both p50 and the N protein to enhance the *N* promoter activity. This 20-bp sequence contained two AAAG motifs that are reported to be binding sites for Dof transcription factors. Fusion of the 20-bp sequence to a truncated version of the *Cauliflower mosaic virus* promoter resulted in an elicitor-dependent transcriptional activation. Mutational analysis indicated that the two Dof-binding motifs in the 20-bp sequence played an important role for transcriptional upregulation. We have cloned the tobacco Dof cDNAs and investigated the functional involvement of Dof proteins in the elicitor- and N protein-dependent transcriptional activation and HR induction.

02016

S-NITROSOTHIOLS REGULATE HYPERSENSITIVE CELL DEATH DURING THE PLANT DISEASE RESISTANCE RESPONSE

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Nitric oxide (NO) is a fundamental regulator of both eukaryotic and prokaryotic redox signalling networks and its biology impinges upon a plethora of key physiological processes. S-nitrosylation, the addition of a NO moiety to a reactive cysteine thiol, to form a nitrosothiol (SNO), has recently emerged as an important mechanism to convey NO bioactivity. Despite its potential significance, however, the roles of

this post-translational modification in plant biology remain largely unexplored. Recently, we provided the first genetic evidence consistent with a key role for S-nitrosylation in plants. *Arabidopsis thaliana* S-nitrosoglutathione reductase 1 (AtGSNOR1), was demonstrated to control cellular SNO levels in both naïve and pathogen challenged plants. Furthermore, we have identified SNOs as pivotal regulators of plant hypersensitive cell death (HCD) in response to attempted pathogen infection. Collectively, our data implies that the kinetics of NO and ROI accumulation are inversely controlled through changes in SNO formation and turnover, which ultimately modulates the rate and magnitude of HCD development. Thus, AtGSNOR1 functions as a binary control switch at the centre of HCD regulation. Here, we will discuss the molecular mechanism by which S-nitrosylation of specific target proteins modulates HCD and pathogen resistance.

02017

PAMP PERCEPTION MEDIATES RECRUITMENT OF THE ARABIDOPSIS PEN3 ABC TRANSPORTER TO SITES OF PATHOGEN DETECTION AND ACTIVATION OF PEN3 DEFENSE FUNCTION.

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The *Arabidopsis* PEN3 ABC transporter is required for full penetration resistance to the barley powdery mildew *Blumeria graminis* f. sp. *hordei* and also participates in resistance to the necrotrophic fungus *Plectosphaerella cucumerina* and the oomycete *Phytophthora infestans* (Stein et al., 2006). The PEN3 transporter is hypothesized to export antimicrobial compounds derived from indole glucosinolates (Bednarek et al., 2009; Clay et al., 2009). PEN3 resides in the plasma membrane and is recruited to sites of attempted penetration by invading fungal appressoria (Stein et al., 2006).

We report that recruitment of the PEN3 transporter to sites of cell wall reinforcement, known as papillae, is triggered by perception of PAMPs such as flagellin and chitin and requires the corresponding pattern recognition receptors (PRRs). Recruitment of PEN3, but not the PEN1 syntaxin, to sites of papilla deposition requires intact actin filaments, but is not affected by inhibitors of microtubule polymerization or vesicle trafficking. Additionally, PAMP-induced recruitment of PEN3 to sites of papilla deposition is unaltered in the presence of inhibitors of PRR endocytosis and downstream signaling such as wortmannin and K252A or in the absence of the BAK1 kinase that interacts with FLS2 and is required for full responsiveness to flagellin. PEN3 was previously found to be phosphorylated in response to PAMP perception (Benschop et al., 2007; Nuhse et al., 2004; Nuhse et al., 2007). We generated alanine substitution variants at sites of PAMP-induced PEN3 phosphorylation and found that phosphorylation of PEN3 is required for its defense function, but does not affect recruitment of the transporter to sites of papilla deposition. Current work is aimed toward understanding the mechanisms through which PRRs initiate recruitment of PEN3 and other defense proteins to sites of papilla deposition and elucidating the significance of PEN3 phosphorylation for its defense function.

02018

DAD1 AND DALS: LIPASES REQUIRED FOR JASMONIC ACID BIOSYNTHESIS AFTER WOUNDING

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Jasmonic acid (JA) is a multifunctional plant hormone involved in a resistance to insect and pathogen attacks and in developmental events such as flower opening and pollen maturation. Although the biosynthetic pathway of JA is almost unveiled, the mechanism that activates JA biosynthesis in response to insect attacks or wounding is largely unknown. The first step of JA biosynthesis is a release of free linolenic acid from chloroplastic polar lipids catalyzed by lipases such as DEFECTIVE IN ANTER DEHISCENCE1 (DAD1) and DAD1-LIKE LIPASE6 (DAL6)/DONGLE (DGL) in *Arabidopsis*. The *DAD1* gene is activated by wounding but not by exogenously applied JA, suggesting that the *DAD1* expression is directly regulated by wounding and not by a feedback of accumulated JA. By a promoter analysis using GUS reporter gene, we identified a *cis*-regulatory element responsible for the wound induction of *DAD1*.

We established sextuple mutants *dad1 dal1 dal2 dal3 dal4 dal6/dgl* (*dd12346*), in which all active genes for chloroplastic DAD1-like lipases are disrupted, and found that the sextuple mutants still produced nearly a half amount of JA in wounded leaves in comparison to the wild type. However, a quantitative RT-PCR experiment revealed that the expression level of *VSP2* gene in wounded *dd12346* leaves was decreased to 4% of that in wild type. Similarly, the wound induction of many genes involved in metabolism of glucosinolate and isothiocyanate, a class of defense compounds, was highly repressed in the *dd12346* mutants. From these data, we concluded that the DAD1 and DALs are responsible for production of a half amount of JA after wounding, but this half is more important for defense against insect attacks.

02019

ARABIDOPSIS SYNAPTOTAGMIN SYTA HAS A KEY ROLE IN REGULATING THE CELL-TO-CELL MOVEMENT OF DIVERSE PLANT VIRUSES

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Synaptotagmins are a large gene family, which have been characterized in animals due to their role in regulating calcium-dependent exocytosis to release neurotransmitter. They have also been shown to have a role in endocytosis to recapture membrane and maintain homeostasis. Thought to be exclusive to animals, synaptotagmins have recently been characterized in *Arabidopsis thaliana*, in which they comprise a five gene family (SYT A, B, C, D and E). Using infectivity and leaf-based functional assays, we have shown that *Arabidopsis SYTA* regulates endocytosis and movement protein (MP)-mediated trafficking of *Cabbage leaf curl virus* (CaLCuV, a *Geminivirus*) and *Tobacco mosaic virus* (TMV, a *Tombamovirus*) genomes through plasmodesmata. To determine if SYTA has a central role in regulating the cell-to-cell trafficking of most, if not all, plant viruses, we have extended our studies to examine the role of SYTA in the cell-to-cell movement of a *Potyvirus* (TuMV) and a *Caulimovirus* (CaMV), as well as of a *Tombamovirus* that infects *Arabidopsis* (TVCV). Using an *Arabidopsis* T-DNA *syta* knockdown line, we find that TVCV, TuMV and TVCV systemic infection is delayed, and the cell-to-cell trafficking of the MPs encoded by each of these viruses is inhibited, when compared to wild type *Arabidopsis* Col-0 plants. Our findings suggest that SYTA plays a key role in regulating the cell-to-cell movement of diverse plant viruses, which have been reported to employ distinct modes of movement via plasmodesmata. A model for how these diverse virus movement proteins transport their cargos to plasmodesmata for cell-to-cell spread via a SYTA-dependnet endosome recycling pathway will be presented.

02020

INVOLVEMENT OF ARABIDOPSIS HOS15 IN HISTONE DEACETYLATION AND SALICYLIC ACID-MEDIATED INNATE IMMUNITY

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In eukaryotic cells, chromatin modifications play a key role in gene regulation in many cellular processes. To elucidate functions of chromatin modification in biotic stress response in plants, we characterized the function of HOS15, a WD40-repeat protein which shares high sequence similarity with human transducin-beta like protein (TBL), a component of a repressor protein complex involved in histone deacetylation. Mutations in the hos15 gene generated phenotypes characterized by spontaneous cell death, and smaller size than wildtype. Further, hos15 plants exhibit constitutive systemic acquired resistance (SAR). This phenotype is characterized by elevated accumulation of salicylic acid, increased resistance to the bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 and constitutive expression of pathogenesis-related (PR) genes. Also observed are increased callose deposition, visualized by trypan blue stain, and ROS accumulation observed after DAB staining of hos15 mutant plants. In addition, microarray and RT-PCR analyses revealed systemic high expression of pathogenesis-inducible genes in hos15 mutant plants compared to control plants. Together, the results point to a regulatory function of HOS15 in orchestrating SA-mediated innate immunity in *Arabidopsis*.

02021

PLANT SUGAR EFLUX TRANSPORTERS FOR NUTRITION OF PATHOGENS

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Plants require sugar efflux transporters to feed seed, pollen, produce nectar and to nurture beneficial microorganisms in the rhizosphere. The identity of these efflux transporters remained elusive. A novel class of sugar transporters (GLUE, glucose effluxer) was identified using FRET glucose sensors in combination with a mammalian expression system. *Arabidopsis* GLUE1 and 8 as uniporters can serve in import and export of sugars. Fungal and bacterial infection altered the mRNA abundance of GLUEs indicating that pathogens modulate GLUE expression to alter glucose transport. Expression of homologs in nectaries and root nodules suggests roles in feeding pollinators and symbionts. Understanding GLUE function may provide new tools to redirect sugar flux in plants to prevent pathogen infection.

02022

TZS IS INVOLVED IN AGROBACTERIUM VIRULENCE AND GROWTH

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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, two *tzs* deletion mutants and three *tzs* frame-shift mutants were generated and characterized. High performance liquid chromatography (HPLC) analyses demonstrated the *tzs* deletion and frame-shift mutants produce no *trans*-zeatin under AS inductions. Both *tzs*-deletion and frame-shift mutants reduce stable and transient transformation efficiency in *Arabidopsis* roots, suggesting that *Tzs* is likely involved in step(s) prior to T-DNA integrations. The exogenous applications of cytokinin during infections also restored the transient transformation efficiencies in the *tzs* mutants, suggesting that the cytokinin is responsible for the efficient transformation on *Arabidopsis* roots. The *tzs* mutants were able to enhance transformation efficiency on green pepper and cowpea, reduce transformation efficiency on white radish and other plant species. These data strongly suggest that *Tzs*, likely via synthesizing *trans*-zeatin at early stage(s) of infection process, is involved in the transformation efficiency of *A. tumefaciens* and may play different roles in different host plants.

02023

A GENETIC SCREEN FOR SUPPRESSORS OF *NPR1*-MEDIATED SALICYLIC ACID TOXICITY IDENTIFIES A NOVEL POSITIVE REGULATOR OF PLANT IMMUNITY

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Plants have evolved inducible immune responses to pathogen infection. Pathogen-induced, isochorismate synthase-dependent salicylic acid (SA) biosynthesis promotes immunity to biotrophic pathogens, partially through the activation of *NPR1* (non-expressor of pathogenesis-related genes 1). During infection, *NPR1* also prevents harmful SA hyperaccumulation and SA cytotoxicity through unknown mechanisms. In a genetic screen for suppressors of *npr1*-mediated SA cytotoxicity, we isolated the *snt1* (suppressor of *npr1*-mediated SA cytotoxicity 1) mutant. *snt1* also suppressed SA hyperaccumulation in *npr1*, and enhanced pathogen susceptibility. Our results suggest *SNT1* promotes both ICS1-dependent SA biosynthesis and SA-inducible pathogenesis-related (PR) gene expression. The molecular characterization of *snt1* is presented, and a working model is proposed whereby *SNT1* functions cooperatively with *NPR1* to regulate transcriptional reprogramming during the immune response.

02024

PSEUDOMONAS SYRINGAE EFFECTOR PROTEIN AVR-B PERTURBS ARABIDOPSIS HORMONE SIGNALING BY ACTIVATING MAP KINASE 4

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Pathogenic microbes often perturb phytohormone physiology in the host to their advantage. We previously showed that the *Pseudomonas*

syringae effector protein AvrB perturbs hormone signaling exemplified by the up-regulation of jasmonic acid (JA) response gene expression and enhances plant susceptibility. Here we show that AvrB interacts with the Arabidopsis mitogen activated protein kinase MAP KINASE 4 (MPK4) and HSP90 chaperone components to render plants more susceptible to the bacterium. AvrB induces MPK4 activation and this is directly promoted by the HSP90 chaperone. A previously identified AvrB-interacting protein, RIN4, also is required for AvrB to perturb hormone signaling and induce plant susceptibility, likely by acting down-stream of MPK4. These findings uncover a novel pathway by which a bacterial effector protein modulates plant hormone signaling to the benefit of the *P. syringae* bacterium.

02025

FUNCTIONAL CHARACTERIZATION OF RNA-BINDING PROTEINS AND MICRORNAs IN PLANT DEFENSE AGAINST DIVERSE PATHOGENS

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RNA-binding proteins (RBPs) and microRNAs (miRNAs) are being recognized as central regulators of posttranscriptional response of plants to diverse pathogens. Here, the pathogen-responsive expression patterns and the role of RBPs and miRNAs in defense response were investigated in *Arabidopsis thaliana* and tobacco. *Arabidopsis* glycine-rich RNA-binding protein 7 (AtGRP7), the RBP of which is ADP-ribosylated by HopU1 and quells the host immunity, conferred defense against fungus and virus as well as bacteria. ADP-ribosylation of AtGRP7 by HopU1 abolished RNA chaperone activity of AtGRP7, thereby eliminating AtGRP7's role in host defense. Analysis of loss-of-function mutants of pathogen-regulated RBPs demonstrated that a specific type of RBP confers defense against diverse pathogens including bacteria and fungi. To understand the involvement and roles of miRNAs in plant defense against pathogens, the expression patterns of miRNAs in *Arabidopsis* challenged with pathogens or elicitors were analyzed. Comprehensive analysis of miRNAs upon pathogen or elicitor treatment showed that specific miRNA family members are modulated by pathogen infection. Analysis of transgenic plants overexpressing a particular miRNA demonstrated the involvement of miRNAs in plant defense against diverse pathogens. Collectively, these results point to the importance and potential roles of RBPs and miRNAs as posttranscriptional regulators in plant defense response. [Supported by grants from APSRC (R11-2001-092-04002-0) and WCU (R32-2008-000-20047-0)]

02026

ARABIDOPSIS ENHANCED DISEASE RESISTANCE 1 IS REQUIRED FOR PATHOGEN-INDUCED EXPRESSION OF PLANT DEFENSINS IN NONHOST RESISTANCE

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In nonhost interaction with fungal pathogens including *Colletotrichum* species, *Arabidopsis* exhibits durable pre-invasive resistance that terminates fungal entry trial at cell periphery. PEN2-related indole glucosinolate pathway is broadly involved in entry control against non-adapted fungi. Here, we represent that *ENHANCED DISEASE RESISTANCE 1* (*EDR1*) encoding a protein kinase acts as a critical factor for novel defense layer in this cell-periphery resistance. Non-adapted *Colletotrichum gloeosporioides* increases the entry rates in the *edr1* mutants, which is contrast with enhanced resistance of the *edr1* mutant against an adapted powdery mildew. Analysis of the *edr1* *pen2* double mutant indicates that EDR1 functions in a defense pathway uncoupled with PEN2. To understand EDR1 function in detail, we investigated the transcript profiles of the *edr1* mutant during nonhost defense response by DNA microarray. The analysis revealed that the expression of four plant defensin genes upon the pathogen inoculation is impaired in the *edr1* mutant, indicating requirement of EDR1 for pathogen-induced expression of the antifungal proteins. GFP-based analysis suggested that EDR1 localizes in nucleus as well as in cytosol. It has been shown that the MYC2-encoded transcription factor negatively regulates the defensin expression. Inactivation of MYC2 fully restored the defensin expression in the *edr1* mutant with the restored pre-invasive defense, suggesting that EDR1 might interfere MYC2 to cancel the repression of the defensin expression in the entry control of *C. gloeosporioides*. Furthermore, constitutive expression of the plant defensin partially recovered pre-invasive resistance in the *edr1* mutant. These results indicate that EDR1 plays a critical role in nonhost resistance in part by inducing the antifungal protein expression.

02027

ATNUDX6, AN ADP-RIBOSE/NADH PYROPHOSPHOHYDROLASE IN ARABIDOPSIS, POSITIVELY REGULATES NPR1-DEPENDENT SALICYLIC ACID SIGNALING

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We have previously reported that, among cytosolic Nudix hydrolases having the pyrophosphohydrolase activities toward either ADP-ribose or NADH (AtNUDX2, 6, and 7), AtNUDX2 and 7 contribute to keeping the energy and redox homeostasis, and/or modulating defense responses to both biotic and abiotic stress (*Plant J.* 2009, 57: 289-301, *Plant Physiol.* 2009, 151: 741-54). Here, we assessed the physiological function of AtNUDX6 using its overexpressor (*Pro_{35S}:AtNUDX6*) or disruptant (*KO-nudx6*). Judging from the pyrophosphohydrolase activity toward ADP-ribose and NADH, and the intracellular levels of those molecules, it was clear that AtNUDX6 prefers NADH to ADP-ribose as a physiological substrate. The expression level of *AtNUDX6* and the activity of NADH pyrophosphohydrolase in the control plants, but not in the *KO-nudx6* plants, were increased by the treatment with salicylic acid (SA), but not by various stressful conditions. The expression of SA-induced genes (SAIGs) depending on Nonexpresser of Pathogenesis-Related genes 1 (NPR1), a key component required for pathogen resistance, was significantly suppressed and enhanced in the *KO-nudx6* and *Pro_{35S}:AtNUDX6* plants, respectively, under the treatment with SA. Induction of *thioredoxin 5* (*TRX-h5*) expression, which catalyzes an SA-induced NPR1 activation, was suppressed and accelerated in the *KO-nudx6* and *Pro_{35S}:AtNUDX6* plants, respectively. The expression of *isochorismate synthase 1* required for regulation of SA synthesis through the NPR1-mediated feedback loop was decreased and increased in the *KO-nudx6* and *Pro_{35S}:AtNUDX6* plants, respectively. These findings indicated that the regulation of NADH metabolism by AtNUDX6 significantly impacts the plant immune response as a positive regulator of NPR1-dependent SA signaling pathways through modulation of *TRX-h5* expression.

02028

ALLELIC MINING OF PATHOGENESIS-RELATED GENES FROM WILD RICE (*ORYZA GRANDIGLUMIS*)

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The multiple techniques were used to identify the genes differentially expressed at wounding treated wild rice (*Oryza grandigumis*). These techniques were suppression subtractive hybridization, SMART cDNA synthesis and RACE-PCR. Here we report the cDNA cloning and expression analysis of pathogenesis-related protein genes PR1, PR10, class IV chitinase, UBC. The deduced amino acid sequence of *OgPR1* has a high level of identity with rice acidic PR1 protein. The *OgPR1* gene induction was light-, time-, and dose-dependent manner by jasmonic acid (JA), cantharidin (CN), endothall (EN), and completely inhibited by cycloheximide, but not by tetracycline. Sequence analysis of *OgPR10* revealed that significant homology at the amino acid level exists between the predicted PBZ1 protein and pathogenesis-related (PR10) proteins. The *OgPR10* gene was examined after treatment with probenazole, which induces disease resistance in rice against rice blast fungus. Dduced amino acid sequences of class IV chitinases (*OgChitIV* and *OgChitIVa*) have a high level of identity with rice class IV chitinase (*OsChitIV* and *OsChitIVa*). It was shown that overexpression of *OgChitIV* and *OgChitIVa* in Arabidopsis resulted in mild resistance against the fungal pathogen, *Botrytis cinerea*. The *OgUBC* gene induced by wounding, fungal elicitor, jasmonic acid (JA), salicylic acid (SA), protein phosphatase inhibitors cantharidin (CN) and endothall (EN) as well as UV-B. The survival rate of *OgUBC* transgenic plants under UV exposure greatly increased and damage level of transgenic plants was lower compared to the wild type.

02029

A MULTIPLEX REAL TIME PCR DIAGNOSTIC TOOL FOR THE PIERCE'S DISEASE OF GRAPEVINE

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To develop an effective diagnostic system, potential Pierce's disease (PD) up-regulated genes in grapevines were identified by *in silico* analysis of the grape genomic database. Reverse transcription PCR further confirmed four genes up-regulated upon PD infection. These selected plant genes were used, in combination with a *Xylella fastidiosa* marker gene, for the development of a sensitive and reliable multiplex real-time PCR-based dual PD detection tool by which can detect both pathogen and plant marker genes simultaneously. This method was optimized for conventional reverse transcription PCR as well as real-time PCR and tested for the relationship between PD and water deficit. Different levels of water deficit imposed did not result in considerable differences in gene expression level while the expression of the selected four genes was highly specific in diseased plants. These results are starting to shed some light on the specificity on the diagnosis of PD and the differentiation between drought and PD in plants at the molecular level. We further anticipate that the diagnostic system developed in this study will provide a powerful tool for the understanding of the grape-*Xylella* interaction, and that it can be incorporated into disease prevention and eradication systems as well as breeding programs for selection of PD-resistant varieties.

02030

ENHANCED RESISTANCE TO *BOTRYTIS CINEREA* MEDIATED BY TRANSGENIC EXPRESSION OF THE SPIDER CHITINASE GENE *AVCHIT* IN ARABIDOPSIS

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The *AvChit* gene encodes for a chitinase from the spider, *Araneus ventricosus*. This spider, *A. ventricosus*, is an abundant species in Korea. *A. ventricosus* chitinase (*AvChit*) is 1515 bp and contains an ORF encoding a 431 amino acid polypeptide. *AvChit* possesses the chitinase family 18 active site signature and one N-glycosylation site. The deduced amino acid sequence of *AvChit* cDNA showed 43% identity to both *Glossina morsitans morsitans* chitinase and a human chitotriosidase. *AvChit* cDNA showed 30-40% identity to some insect chitinases which lack both the serine/threonine and chitin binding domains. *Arabidopsis thaliana* plants were transformed with the *AvChit* gene using *Agrobacterium tumefaciens*. Thirteen transgenic lines expressing the *AvChit* gene were obtained. Functional expression of the *AvChit* gene in transgenic *Arabidopsis* was confirmed by Southern, northern and western blot analysis. The *AvChit* cDNA was expressed as a 61 kDa polypeptide in baculovirus-infected insect Sf9cells. *AvChit* protein extracted from transgenic *Arabidopsis* exhibited high levels of chitinase activity. Phytopathological tests showed that two transgenic *Arabidopsis* lines expressing the *AvChit* gene displayed high levels of resistance to gray mold disease (*Botrytis cinerea*).

02031

GENOME-WIDE REDIRECTION OF GRAPEVINE'S GENE EXPRESSION IN RESPONSE TO THE PIERCE'S DISEASE PATHOGEN, *XYLELLA FASTIDIOSA*, AND WATER DEFICIT

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Grapevines, *Vitis vinifera*, respond to *Xylella* infection with a massive redirection of gene transcription. This transcriptional response is characterized by increased transcripts for phenylpropanoid and flavonoid biosynthesis, ethylene production, adaptation to oxidative stress, and homologs of pathogenesis related (PR) proteins, and decreased transcripts for genes related to photosynthesis. In addition to highlighting potential metabolic and biochemical changes that are correlated with disease, the results suggest that susceptible genotypes respond to *Xylella* infection by induction of limited, but inadequate, defense response. A long-standing hypothesis states that Pierce's disease results from pathogen-induced drought stress, with the consequent development of disease symptoms. To test this hypothesis, we compared the transcriptional and physiological response of plants treated by pathogen infection, low or moderate water deficit, or a combination of pathogen infection and water deficit. Although the transcriptional response of plants to *Xylella* infection was distinct from the response of healthy plants to moderate water stress, we observed synergy between water stress and disease, such that water stressed plants exhibit a stronger transcriptional response to the pathogen. This interaction was mirrored at the physiological level for aspects of water relations and photosynthesis, and in terms of the severity of disease symptoms and pathogen colonization, providing a molecular correlation of the classical concept with the disease triangle.

02032

FLAGELLIN-TRIGGERED INDUCTION OF NOVEL SMALL OPEN READING FRAMES

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PAMP-triggered immunity (PTI) is activated upon the perception of pathogen associated molecular patterns (PAMPS) by receptors at the plant cell membrane. PTI operates to prevent the entry of pathogens into the plant cell, and is characterized by the production of reactive oxygen species, transcription of defence-related genes, stomatal closure and callose deposition on the cell walls. Although there has been recent progress in our understanding of PTI, it is likely that key signaling and receptor proteins, and host-derived danger associated molecular patterns (DAMPs) remain undiscovered. Since the completion of genome sequencing projects, small open reading frames (sORFs) in the so-called intergenic regions have been shown to be transcribed in various organisms. These potentially novel protein encoding transcripts are short (90–300 nt) and may have escaped annotation as coding sequences due to the conservative nature of the gene prediction models used. In *Arabidopsis thaliana*, at least 3000 ORFs are predicted to be novel protein coding genes. To test whether sORFs in the Arabidopsis genome might regulate plant immunity, we used a custom microarray to compare the expression of ~6000 unannotated sORFs in Arabidopsis Col-0 with the flagellin-insensitive mutant *fis2*, following treatment with the PAMP flg22. 3279 sORF transcripts were detected in the microarray, and 48 were differentially upregulated in Col (Col > 5 fold induction; *fis2* < 2 fold induction) by 60 mins post flg22 treatment compared to the mock treatment. The flg22-triggered differential induction of 14 candidate sORFs, chosen based on their expression profiles and homology with plant proteins, was verified by qRT-PCR using biological replicates. Overexpressor lines are now being produced to analyse the role of the candidate sORFs in PTI and plant defence.

02033

BACTERIAL VOLATILES AS TRIGGERS OF PLANT DEFENSE AND GROWTH PROMOTION

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Some plant growth-promoting rhizobacteria (PGPR), in the absence of physical contact with plants, stimulate plant growth and elicit induced systemic resistance (ISR) via volatile organic compound (VOC) emissions. Gas chromatographic analysis of VOCs collected from the PGPR strains *Bacillus subtilis* strain GB03 and *B. amyloliquefaciens* strain IN937a reveals consistent patterns in VOC emissions. The two most abundant compounds, 2,3-butanediol and 3-hydroxy-2-butanone, are consistently emitted from GB03 and IN937a while these metabolites are not released from DH5a. Of several *Arabidopsis* mutant lines tested for regulatory control of ISR against *Erwinia carotovora* subsp. *carotovora*, only the ethylene-insensitive line (*ein2*) did not exhibit an amelioration of disease symptoms when *Arabidopsis* plants were pre-treated with GB03 volatiles. Cytokinin was revealed to have a critical role in bacterial VOC-elicited plant growth promotion. To assess potential utilization of PGPR VOCs, volatile blends from GB03 and IN937a were applied to plant roots. The survival capacity of 2,3-butanediol null bacterial mutants was significantly reduced in proximity with plant roots. These reduced bacterial survival rates suggest that in addition to bacterial VOCs' triggering plant growth and ISR in plants, such chemicals provide protection for PGPR via chemical signaling within the host plant. Our results suggest that 2,3-butanediol produced by *B. subtilis* may serve in dual functions to elicit indirectly ISR on the foliar parts and directly the production of plant antimicrobial compounds on the root system and to act as a protecting agent for bacterial cells against the compounds.

02034

INDUCTION OF SYSTEMIC RESISTANCE BY BACTERIAL VOLATILES REQUIRES CYTOKININ SIGNALING IN ARABIDOPSIS

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Plants are continuously responded to attack by nonhost pathogens and saprophytes with extensive changes in gene expression that lead to induced systemic resistance (ISR) phenomena. We previously discovered elicitation of systemic resistance by bacterial volatiles in *Arabidopsis*. In *Arabidopsis* seedlings exposed to bacterial volatile blends from a rhizobacterium *Bacillus subtilis* GB03, disease resistance against a soft rot pathogen *Erwinia carotovora* subsp. *carotovora* was significantly augmented when compared with control. In this study, we evaluated cytokinin signaling as an important battery for ISR. To obtain direct evidences, the role of the cytokinin receptors AHK2, AHK3 and CRE1/AHK4 that activate a multistep phosphorelay at the plasma membrane was assessed. The *ahk3* null mutant plant was impaired bacterial volatile elicited-ISR indicating that the AHK2 receptor or its downstream can be involved. The ISR capacity by the volatiles did not be compromised in *Arabidopsis* mutants of type B- ARABIDOPSIS RESPONSE REGULATORS that were known as positive regulators of cytokinin signaling. In contrast, the transcriptional expression of the type A-ARRs including ARR4 and ARR6 genes and the cytokinin oxidase 2 (CKX2) and CKX4 genes was significantly down-regulated by the emission of bacterial volatiles. Collectively, our results suggest that the increased endogenous cytokinin contents by the decreased expression of negative regulators ARR 4 and ARR6 and of cytokinin degrading enzymes CKX2 and CKX4 directly involved in ISR. The further analysis with examining expression of *PR1*, *PR5*, and *PDF7.2* revealed that salicylic acid and jasmonate/ethylene signaling was implicated in this ISR machinery. Overall, our data indicate that bacterial volatile elicit ISR through orchestrating several plant hormone signaling mainly by cytokinin signaling and its cross-talking with major plant defense hormones.

02035

ARABIDOPSIS PLANTS ARE SUSCEPTIBLE TO FUSARIUM SPOROTRICHIOIDES PRODUCING TYPE A TRICHOTHECENE PHYTOTOXINS.

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Type A highly toxic trichothecene mycotoxins often contaminated with cereal grains throughout the world. Some *Fusarium* species such as *F. sporotrichioides* are known to produce type A trichothecenes such as T-2 toxin. However, little is known the interaction between type A-producing *Fusarium* and host plants. Although *F. sporotrichioides* could not infect with *Arabidopsis* leaves by spray inoculation, their invasive hyphae were observed in intracellular spaces of *Arabidopsis* leaves by infiltration inoculation. During these interactions, defense genes are significantly induced in these *Fusarium*-infected host tissues. Trichothecene biosynthetic genes were also expressed in these tissues, resulting in T-2 toxin accumulation. Then, proteomic analysis was carried out in *F. sporotrichioides*-inoculated *Arabidopsis* leaves using 2-D DIGE assays. The accumulations of several defense related-proteins such as glutathione S-transferases (GSTs) and an ascorbate peroxidase (APX) were enhanced by inoculation of *F. sporotrichioides*. Up-regulation of these proteins was also reported in type B-producing *F. graminearum*-infected wheat flowers. Therefore, GSTs emerge as candidates for trichothecene-detoxifying enzymes regardless of their molecular species in host plants.

02036

PROTEOME ANALYSIS REVEALED INDUCTION OF PHOTOSYNTHESIS, ETHYLENE BIOSYNTHESIS AND ANTIOXIDANT-RELATED PROTEINS ON THE EXPOSURE OF ARABIDOPSIS TO BACTERIAL VOLATILES

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Plants acquire self-defense against a variety of plant pathogens. Previous our study provided that plant root-associated bacteria induced systemic resistance against foliar and root pathogens. Among multi-bacterial determinants involving in such the induction of systemic resistance (ISR), we reported that bacterial volatiles elicited ISR via ethylene (ET)-dependent manner in Arabidopsis. To exploit global de novo expression of plant proteins, proteomics was employed on Arabidopsis leaf to exposure of a rhizobacterium *Bacillus subtilis* GB03. Differential protein expressions were validated by repeated experiments and the related signal pathway proteins. In the line of our previous finding, ET biosynthesis enzymes were significantly up-regulated indicating that bacterial volatile induce directly the production of exogenous ethylene compounds. Through the assessment of the transcriptional expression of ET synthesis-related genes, *ACO2*, *SAM2*, *ACS4*, and *ACS12* and ET-response genes, *ERF1* and *PDF1.2*, we confirmed that ET signaling play a critical role on bacterial volatile elicited-ISR. In addition to ET biosynthesis proteins, antioxidant proteins were also provided an evidence of active defense response to elicit ISR by bacteria volatiles. Our results suggest that bacterial volatile-mediated ISR occurred by orchestrated ethylene synthesis enzymes and reactive oxygen species generations.

02037

SIGMA S AS A REGULATOR FOR BACTERIAL VIRULENCE AND SURVIVAL IN *RALSTONIA SOLANACEARUM*

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Ralstonia solanacearum causes lethal wilt of the many solanaceous plants and Arabidopsis, and the bacterium survives over seasons in soil and water ecosystem. Sigma S, which is a component of RNA polymerase complex, has been shown to regulate gene expression during stationary phase or in response to stress conditions in bacteria. In this study, we investigated the role of sigma S in *R. solanacearum* under different stress conditions for bacterial survival and virulence. The *rpoS* gene encoding sigma S was disrupted by marker exchange mutagenesis from *R. solanacearum*. Bacterial growth rate and production of a key virulence factor of *R. solanacearum*, extracellular polysaccharide, were not different upon comparison between *rpoS* mutant and the wild type strain. Both wild type and the mutant strain retained the same activity of pectin degradation, while the *rpoS* mutant exhibited reduced cellulase activity. RT-PCR analysis revealed that expression of two endoglucanase gene was down-regulated in the *rpoS* mutant. Furthermore, the virulence of the mutant decreased significantly upon infection of the tomato plant and Arabidopsis plants. Complete loss of virulence by soil soaking inoculation, much delayed virulence by petiole inoculation, and loss of cellulase activity of *rpoS* mutant suggest that sigma S may be involved in early infection of *R. solanacearum*. Under stress conditions, wild type strain was more sensitive to starvation but less sensitive to hydrogen peroxide exposure than *rpoS* mutant. Complementation of the mutant with original *rpoS* gene provided in trans restored the original phenotypes of *R. solanacearum*. *RpoS* mutant survived long during extended incubation under low-nutrient condition compared to its wild type and the result suggested that sigma S is a regulator to control cell death during starvation.

02038

FUNCTIONAL STUDIES ON MACPF-CONTAINING PROTEIN NSL2 INVOLVED IN PLANT IMMUNITY OF ARABIDOPSIS

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The Arabidopsis mutant *nsl2* (*necrotic spotted lesion 2 = cad1*) shows a phenotype of hypersensitive response-like cell death and restriction of pathogenic bacterial growth, indicating that the NSL2 protein negatively controls the SA-mediated pathway in plant immunity. The NSL2 protein contains a domain with significant homology to the MACPF domain of complement components and perforin proteins that are involved in innate immunity in animals (Plant Cell Physiol, 2005, 46: 902; Plant Sci. 2008, 175: 604). The *nsl2* showed accelerated senescence under dark conditions, suggesting that the NSL2 is also associated to chlorophyll degradation. Moreover, dark treatment leaded to rapid decrease in photosynthetic proteins in the mutant. Characters of NSL2-mediated senescence and chlorophyll degradation will be reported.

02039

MOLECULAR CHARACTERIZATION OF SYSTEMIC ACQUIRED RESISTANCE IN *ARABIDOPSIS THALIANA*

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Systemic acquired resistance (SAR) is a long-lasting and broad-spectrum disease resistance induced at the whole plant level upon a locally restricted pathogen inoculation. SAR is associated with a *de novo* synthesis of salicylic acid (SA), locally at the site of infection and systemically, in non-infected tissue. This in turn induces systemic expression of a specific set of pathogenesis-related (*PR*) genes. We have previously shown that *FLAVIN-DEPENDENT MONOOXYGENASE 1* (*FMO1*) is an essential component of biologically induced SAR in Arabidopsis. Our working model includes the existence of an amplification mechanism in which *FMO1* and other defense regulators participate to amplify incoming SAR long-distance signals and thus realize defense responses at the systemic level. Recent microarray analyses indicate that the SAR-induced state is associated with the systemic up- and down-regulation of 560 and 150 genes, respectively. This massive switch in gene expression is completely lost in *fmo1* mutant plants. The critical function of *FMO1* during SAR is presumably based on its ability to catalyse the oxidation of an N- or S-containing metabolite. We have expressed the *FMO1* protein with different

solubility tags in *E. coli*, and are currently testing the enzymatic activities of the recombinant fusion proteins to different candidate substrates. One possible albeit exogenous substrate might be β -amino butyric acid (BABA). Exogenous application of BABA enhances plant resistance through a mechanism similar to SAR, and this resistance induction is compromised in *fmo1* mutant plants. Current experiments are testing whether FMO1 is able to convert BABA into a resistance-enhancing compound. Subcellular localization studies of FMO::GFP fusion proteins transiently expressed in onion epidermal cells indicate that FMO1 might exert its resistance-enhancing function in the nucleus.

02040

OVEREXPRESSION OF ATCAF1, CCR4-ASSOCIATED FACTOR1 HOMOLOGUE IN ARABIDOPSIS THALIANA, NEGATIVELY REGULATES WOUNDING-MEDIATED DISEASE RESISTANCE

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The CCR4-CAF1-NOT complex, a major cytoplasmic deadenylase complex in eukaryotes, regulates the degradation of polyadenylated mRNA. This mRNA degradation was recently recognized as an important mechanism for gene regulation for the efficient response to invading pathogens in animals and plants. Using knockout mutants and transgenic plants overexpressing AtCAF1, Arabidopsis thaliana CAF1 homologue, we herein examined the role of AtCAF1 in the innate immune responses of plants mediated by the pattern-recognition receptor like kinases. Our results showed that overexpression of AtCAF1 significantly down-regulated the transcript level of ERF but not FLS2 and BRI1, as well as abolished up-regulated expression pattern of ERF in response to wounding. Consistently, Agrobacterium-mediated transient expression of GUS was highly enhanced in the transgenic plants overexpressing AtCAF. Furthermore, jasmonic acid (JA) responsive genes were down-regulated by overexpression of AtCAF, which was consistent with result that the transgenic plants overexpressing AtCAF were more susceptible to necrotrophic fungal pathogen, Botrytis cinerea. However, overexpression of AtCAF1 did not affect the salicylic acid (SA)-mediated defense response that is a major signaling pathway for innate immune responses against biotrophic pathogens in plants, resulting in similar growth levels of virulent or avirulent *Pseudomonas syringae* strains on Arabodpsis plants that express different levels of AtCAF1. Together, these results suggest that the CCR4-CAF1-NOT complex may regulate mRNA degradation of wound-response genes specifically.

02041

USING SMALL MOLECULES TO UNRAVEL IMMUNITY SIGNALLING PATHWAYS IN PLANTS

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In plants the signalling events that link the perception of pathogen molecules by cell-surface and intra-cellular receptors, such as resistance (R) proteins, to defence outcomes are not well characterised. We are using small molecules to identify novel components of plant signalling pathways as a way of gaining insight into the molecular events necessary for generating immunity. From a screen of 10,000 small organic compounds, 27 candidate molecules were identified that alter (inhibit or potentiate) the cell death response of *Arabidopsis thaliana* cells to treatment with the incompatible pathogen *Pseudomonas syringae* pv. *tomato* DC3000 expressing the effector proteinavrRpm1 (Pst avrRpm1). *In planta* tests of the candidate inhibitors identified several compounds that reduce the size of hypersensitive cell death lesions caused by Pst avrRpm1, when co-infiltrated into *A. thaliana* leaves with the pathogen, and alter both resistance and susceptibility to bacterial infection. We are now investigating the effect of these compounds on other defence responses and will describe our progress in identifying the *in vivo* targets of the most promising compounds using genetic and biochemical approaches.

02042

CHARACTERIZATION OF ARABIDOPSIS MEKK1-INTERACTING E3 UBIQUITIN LIGASES

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A mitogen-activated protein kinase (MAPK) cascade has an important role in innate immunity in plants. Arabidopsis MEKK1, a MAPK kinase kinase (MAPKKK) is an important factor of innate immunity signaling in the upstream of MPK4 which has at least two functions, negative regulation of SA-mediated defense responses and positive regulation of JA-induced gene expression. MEKK1 is essential for flg22- and H₂O₂-induced MPK4 activation, however regulatory mechanism of MEKK1 remains to be elucidated.

To identify possible regulator of MEKK1, we performed yeast two-hybrid screening using MEKK1 protein as bait. We found two novel MEKK1-binding proteins which contain U-box domain, named PUB25 and PUB26. These proteins specifically bind to N-terminal regulatory domain of MEKK1 in yeast. Protein harboring U-box domain is thought to be function as an ubiquitin E3 ligase. To check this possibility, we performed *in vitro* ubiquitylation assay, which revealed that these proteins are functional E3 ligases. To analyze function of PUB25 and PUB26 in planta, we obtained *pub25* and *pub26* mutants and produced *pub25/26* double mutant. Immune-complex kinase assay showed reduced MPK4 and MPK6 activity in the *pub25/26*. These results suggest that PUB25 and PUB26 positively contribute MEKK1 activity rather than simple degradation of MEKK1.

02043

REPRESSION OF BIP, AN ER-RESIDENT PROTEIN, PREVENTS R-GENE-MEDIATED HYPERSENSITIVE RESPONSE BUT ENHANCES NON-HOST PATHOGEN-INDUCED NECROSIS IN *N. BENTHAMIANA*

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HRT, encoded a classic leucine zipper/nucleotide binding site/leucine rich repeat-containing protein, is required for HR development and resistance after turnip crinkle virus infection. In tobacco leaves, transient co-expression of the HRT and its elicitor, the TCV coat protein (CP), results in rapid cell death. To isolate genes involved in HRT/CP-mediated cell death in *Nicotiana benthamiana*, we analyzed pepper cDNA microarray. Based on virus-induced gene silencing (VIGS) analysis, we identified 10 genes that were responsive to the HRT/CP-mediated cell death in *N. benthamiana*. Silencing of one of genes, encoding a luminal binding protein 5 precursor (BiP5-ER resident heat shock protein 70), showed the inhibition of plant growth and development. Moreover, VIGS of BiP5 resulted in the abolishment of R-gene mediated HR signaling including HRT and Rx, which confer resistance to turnip crinkle virus (TCV), potato virus X (PVX), respectively, and

cell death by transient expression of Bax which encoded a protein that promotes apoptosis in animal system. However, cell death induced by non-host pathogen, *Pseudomonas syringae* pv. *syringae* strain 61, was more rapidly progressed in BiP5-silenced plants. These results suggest that BiP5 might play an opposite role in cell death between host pathogen and non-host pathogen. In mammals, BiP is known as negative regulator of signal transducer of unfolded protein response (UPR) following ER stress. To investigate the involvement of the UPR pathway during plant-pathogen interactions, we are testing whether a typical UPR, triggered by treatment with tunicamycin, will affect the HRT/CP-induced cell death.

02044

SCREENING FOR AND CHARACTERIZATION OF GENES FOR RESISTANCE TO BOTH BACTERIAL AND FUNGAL PATHOGENS IN 21K RICE-ARABIDOPSIS FOX LINES

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Cross resistance, the ability of a gene to confer resistance against at least two different pathogens, is a desirable trait. Approximately 21,000 of the Rice-Arabidopsis FOX (Full-length cDNA Over-eXpressor) transgenic lines, which overexpress 13,000 rice full-length cDNAs in Arabidopsis, have been screened for disease resistance to a bacterial pathogen, *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000), and a fungal pathogen, *Colletotrichum higginsianum* (*C. higginsianum*) by dip inoculation. *C. higginsianum* infects Arabidopsis by forming appressoria and penetration pegs, a process similar to that by which *Magnaporthe grisea* infects rice. Totals of 84 and 112 Rice-Arabidopsis FOX hunting lines were selected by screening for resistance to *Pst* DC3000 and *C. higginsianum*, respectively. Of these, 18 lines showed high resistance to both *Pst* DC3000 and *C. higginsianum*. In addition, when screening for moderate resistance were performed by using a lower concentration of these pathogens, six out of 84 lines showed high resistance to *Pst* DC3000 and moderate resistance to *C. higginsianum*. 24 out of 112 lines showed high resistance to *C. higginsianum* and moderate resistance to *Pst* DC3000. The rice cDNAs in the resistant lines were identified and some of these genes have been re-introduced into rice for overexpression to evaluate the disease resistance phenotype. In the T1 generation, at least one of the genes, when overexpressed in rice, enabled high resistance to both *Xanthomonas oryzae*, the bacterial pathogen that causes rice leaf blight, and *Magnaporthe grisea*, the fungal pathogen that causes rice blast.

02045

THE ROLE OF CHLOROPLAST PROTEIN CAS IN PLANT INNATE IMMUNITY

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Chloroplasts are the site of photosynthesis in plant cells, and also involved in the synthesis of stress-induced hormones (jasmonic acid, salicylic acid, abscisic acid) and the generation of reactive oxygen species (ROS). However, unlike mitochondria which act as central regulators of apoptosis in mammalian cells, very little is known about the role of chloroplasts in a network of intracellular signaling pathways of plant cells. Recently, we found that pathogen elicitors (flg22 and chitin) evoke a rapid and transient increase in stromal Ca^{2+} concentration in chloroplasts, suggesting that pathogen signals are relayed quickly to chloroplasts in plant cells (Komori et al., ICAR 2008). Here, we show that flg22 (elicitor)-induced stromal Ca^{2+} oscillation was partially dependent on thylakoid bound Ca^{2+} -binding protein CAS. Furthermore, we demonstrate that chloroplast protein CAS mediates a wide range of plant innate immune responses, including elicitor-induced stomatal closure, expression of PR genes and the R gene-mediated hypersensitive cell death in *Arabidopsis* and *Nicotiana benthamiana*. Consequently, *cas-1* knock out mutants show low-level resistance against *Pseudomonas syringae* pv. *tomato* DC3000. Interestingly, H_2O_2 accumulation induced by pathogen infection was significantly reduced in *cas-1*. Overall these results should provide insights into how chloroplasts mediate plant innate immunity.

02046

A MEMBRANE-BOUND TRANSCRIPTION FACTOR BZIP60 MEDIATES TWO DIFFERENT SIGNALING PATHWAYS

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AtbZIP60 is an *Arabidopsis* bZIP type transcription factor which localizes to the ER membrane with a transmembrane domain (TMD). Tunicamycin, an inhibitor of N-linked glycosylation, which induces the ER stress response, causes transcriptional induction and proteolysis of AtbZIP60. After processing, N-terminal portion of AtbZIP60 translocates to the nucleus where it activates transcription of target genes such as BiP genes. A T-DNA knockout mutant of AtbZIP60 showed loss of transcriptional induction of AtBiP3 suggesting AtBiP3 is prominently regulated by AtbZIP60. On the other hand, little change was observed in induction profile of AtBiP1/2 indicating involvement of additional transcription factor in regulation of the ER stress response. As a candidate of alternative transcription factor, we identified AtbZIP28 which also has TMD and is released from the ER membrane by ER stress. Activation of AtbZIP60 was observed when *Arabidopsis* was treated by fumonisin B1 (FB1), a toxin produced by several species of *Fusarium*. FB1 did not induce expression of genes enhanced by tunicamycin such as BiPs. Thus, the effect of FB1 was considered to be independent to the ER stress response. FB1 has been known to induce program cell death in *Arabidopsis*. When effect of FB1 was compared between wild type and KO mutant of AtbZIP60, the KO mutant is more sensitive to FB1. Microarray analysis identified several genes of which induction by FB1 was dismissed in the KO mutant. Promoter of AtPDR12, one of those genes, was activated by FB1 in transient assay. Activation of AtPDR12 promoter was also observed by co-expression of active form of AtbZIP60. These results suggested that bZIP60 is activated by FB1 and regulates expression of AtPDR12 by ER stress-independent manner.

02047

BORON-INDUCED DEGRADATION OF THE BORATE TRANSPORTER BOR1 IS REGULATED BY UBIQUITINATION IN ARABIDOPSIS

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Boron (B) is essential micronutrients for plants but is toxic in large doses. BOR1, an arabidopsis borate transporter, plays a key role facilitating aggressive B uptake in the root system under the B limiting environment, and meanwhile, is proteolytically degraded in the endocytic pathway to avoid excess B uptake when high B is applied. Molecular mechanism of the sensing of B availability that regulates BOR1 proteolysis is poorly understood. Here, we show that ubiquitination of BOR1 is fundamental for the B-induced degradation. We found that a mutation K590A in BOR1 blocked the degradation. BOR1 was mono- or di-ubiquitinated by application of high B and K590A mutant was not ubiquitinated. Furthermore, the K590A mutation abolished transport of BOR1 to the lytic vacuole but did not abolish Brefeldin A-induced BOR1 internalization. Our results show that ubiquitination of BOR1 responded to high B is not required for endocytosis from plasma membrane but crucial for sorting of internalized BOR1 to the vacuole.

02048

PLANT PATHOGEN-RESPONSIVE PROTEIN, ATDABB1 EXHIBITS THE ANTIFUNGAL ACTIVITY IN ARABIDOPSIS THALIANA

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A plant antifungal protein was purified from *Arabidopsis thaliana* leaves by using a typical procedure consisting of anion exchange chromatography and high-performance liquid chromatography. We determined the amino acid sequence of the purified protein using MALDI-TOF/MS analysis, and found that the sequence matched that of a hypothetical *Arabidopsis* protein in GenBank (accession number NP_175547).

We designated the protein as AtDabb1. After the cDNA encoding the AtDabb1 gene was cloned from an *Arabidopsis* leaf cDNA library, the recombinant protein was expressed in *Escherichia coli* and found to significantly inhibit cell growth of various pathogenic fungal strains. mRNA expression of the AtDabb1 gene was induced by pathogen-related signaling molecules including salicylic acid and jasmonic acid. These results suggest that AtDabb1 may contribute to the induced plant defense mechanism against diverse pathogenic fungi.

02049

CHINESE CABBAGE CYCLOPHILIN POSSESSES THE ANTIFUNGAL ACTIVITY.

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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.

02050

PROTEOMIC ANALYSIS OF SALICYLIC-ACID-RESPONSIVE PROTEINS IN *BETULA PLATYPHYLLA* VAR. *JAPONICA* NO.8 PLANTLET

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Salicylic acid (SA) is a signal molecule to induce systemic acquired resistance (SAR) in plants. It is considered that resistance against pathogens are activated by increase in SA-responsive pathogenesis-related proteins and by other pathogen-responsive proteins in many plants. However, there are less researches on SAR induction by SA in woody plants. Hence, we investigated SA-responsive proteins to clarify the mechanisms of SA functions in birch plantlets. One μ L of 0.5 mM SA aqueous solution was injected to the sterile Japanese birch (*Betula platyphylla* var. *japonica*) No.8 plantlets. Intact, and wounded and ultra-pure-water-injected plantlets were also prepared as controls. The proteins were extracted from the plantlets after 2 days of the treatments. The protein samples were subjected to two-dimensional electrophoresis, and the gels were stained with CBB. The gel images were incorporated to a personal computer and were analyzed with an image analysis software. Specifically expressed and 2-fold increased proteins by SA treatment were cut off from the gels, in-gel-digested with trypsin, and then were analyzed with MALDI-TOF-MS. Protein identification was performed by peptide mass finger printing (PMF) with using MASCOT software. The number of the specifically expressed proteins and 2-fold increased proteins were 20 and 13, respectively, and out of them respective 16 and 9 proteins were analyzed with MALDI-TOF-MS. AS the results, 8 proteins could be almost identified by PMF: NPR1-like protein, MADS-domain transcription factor, *Ceratodon purpureus* phytochrome photoreceptor CERPU; PHYO; 2, oxygen enhancer protein 2, and 4 unknown proteins. Based on the results obtained, the Japanese birch No.8 plantlet is considered to have ability inducing SAR by SA treatment.

02051

MECHANISMS OF *NIP5;1*, A BORON DEFICIENCY INDUCIBLE GENE, REGULATION IN RESPONSE TO BORON IN *ARABIDOPSIS THALIANA*

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NIP5;1, an aquaporin-like gene, is a boron (B) channel required for normal plant growth under low B conditions and its transcript accumulation is upregulated under low B conditions (Takano et al., 2006).

To elucidate mechanisms of *NIP5;1* regulation in response to B, *NIP5;1* fused N terminally to GFP reporter gene was expressed under the control of the promoter *NIP5;1* with or without 5'UTR. The GFP expression analysis pattern suggests that 5' untranslated region (5'UTR) (312 bp) is involved in *NIP5;1* transcript accumulation in response to low B. To reveal the region of *NIP5;1* promoter involved in *NIP5;1* transcript accumulation in response to B, a series of truncated 5' UTR were generated, were fused to the GUS reporter gene under the control of cauliflower mosaic virus 35S RNA promoter and were introduced into *Arabidopsis thaliana*. The GUS activity inserted 312 bp 5'UTR in transgenic plants is higher in low B condition than in high B condition. On the other hand, the GUS activity inserted 5'UTR but not including +184-+197(13bp) in transgenic plants is high in both low and high B conditions. These results suggested that at least +184-+197 regions of 5'UTR are important for *NIP5;1* transcript accumulation in response to low B.

02052

CHEMICAL BIOLOGY TOWARD UNDERSTANDING HYPERSENSITIVE CELL DEATH IN ARABIDOPSIS

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We employed a chemical biology approach to investigate *Arabidopsis R*-gene mediated defense responses to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 *avrRpm1*. A high-throughput quantitative assay system for HR cell death, an indicator of defense responses, was established using *Arabidopsis* suspension cells. Seven compounds which potentiate HR in a concentration dependent manner were identified from the screening of a commercial library of 10,000 diverse chemicals. Application of these potentiators without pathogen induced *PR1* gene expression, indicating that they act on plant cells. Since these compounds enhanced disease resistance in *Arabidopsis* plants, they have possibilities to be developed as plant protecting agents. The *PR1* inductions of 5 compounds were diminished in *sid2* mutant which lacks accumulation of salicylic acid (SA) after pathogen infection. Furthermore, most of the SA-dependent potentiators induced production of SA in suspension cells. Based on the effects of these potentiators, their putative action points were categorized into three groups: 1. Activation of SA biosynthesis, 2. SA mimics and 3. Inhibition of the SA-inactivating pathway. We found that three of the SA-dependent potentiators, CB_8, CB_9 and CB_11 inhibit activity of SA glycosyltransferase (SAGT), an SA-inactivating enzyme. The concentration ranges of them for SAGT inhibition coincided with those for HR enhancement, suggesting that their HR activation effects would be accounted for the inhibitory effects on SAGT. We analyzed the enzyme activity of UGT74F1, one of the *Arabidopsis* SAGTs, and the inhibitory effect of the HR potentiators. An inhibition mechanism will be discussed together with the reaction kinetics of UGT74F1 and the inhibition constants of the potentiators. A chemical genetics approach for the target identification of the other potentiators will be introduced as well.

02053

EFFECT OF ENDOPHYTIC COLONIZATION WITH AZOSPIRILLUM SP. ON THE GROWTH OF ARABIDOPSIS

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Azospirillum, free-living nitrogen fixing rhizobacteria found in close association with plant roots and stems, is a well-studied genus of plant growth promoting bacteria (PGPB) that have beneficial effects on plants, such as increasing crop yield. Recently, we clarified the effect of a bacterial endophyte, *Azospirillum* sp. B510, on enhanced-disease resistance in host rice plants and *Arabidopsis*. In this study, we analyze the effects of broth filtrate of *Azospirillum* sp. B510 on plant growth in *Arabidopsis*. *Arabidopsis thaliana* (ecotype Columbia-0) plants were grown on 1/2 MS salt medium, with 0.8% agar when the plants were grown on plates with a horizontal orientation. In this study, treatment of *Arabidopsis* with *Azospirillum* sp. B510 was found to stimulate the root elongation and the number of lateral root. The broth filtrate has same activity. The stable active substance was detected in the ethyl acetate extraction of the broth filtrate. This data suggest the possibility that IAA is active substance of broth filtrate.

02054

REGULATION OF MICROBE-ASSOCIATED MOLECULAR PATTERN-INDUCED DEFENSE RESPONSES BY CALCINEURIN B-LIKE PROTEIN-INTERACTING PROTEIN KINASES

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Though cytosolic free Ca^{2+} mobilization induced by microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) is postulated to play a pivotal role in innate immunity in plants, the molecular links between Ca^{2+} and downstream defense responses still remain largely unknown. Calcineurin B-like proteins (CBLs) act as Ca^{2+} sensors to activate specific protein kinases, CBL-interacting protein kinases (CIPKs). We have identified CIPKs rapidly induced by MAMPs, including chitooligosaccharides and xylanase (TxV/EIX) in suspension-cultured rice cells (Kurusu et al. *Plant Physiol.* 2010). The CIPKs interacted with several CBLs through the FISL/NAF-motif in yeast cells and showed the strongest interaction with OsCBL4. The recombinant CIPK proteins showed Mn^{2+} -dependent protein kinase activity, which was enhanced both by deletion of their FISL/NAF-motifs and by combination with OsCBL4. The CIPKs-suppressed RNAi transgenic cell lines showed reduced sensitivity to TxV/EIX for the induction of a wide range of defense responses, including hypersensitive cell death, mitochondrial dysfunction, phytoalexin biosynthesis, and pathogenesis-related (PR) gene expression. On the other hand, TxV/EIX-induced cell death was enhanced in the CIPK overexpressing lines. The present results suggest that the CIPKs play a crucial role in MAMP-induced defense signaling pathway.

02055

A CAM BINDING RECEPTOR-LIKE PROTEIN KINASE (CBRLK1) FUNCTIONS AS A NEGATIVE REGULATOR IN PLANT DEFENSE RESPONSES

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Calmodulin-regulated protein phosphorylation plays a pivotal role in amplifying and diversifying the action of calcium ion. In this study, we identified a calmodulin-binding receptor-like protein kinase (CBRLK1) that was classified into an S-locus RLK family. The plasma membrane localization was determined by the localization of CBRLK1 tagged with a green fluorescence protein. Calmodulin bound specifically to a Ca^{2+} -dependent calmodulin binding domain in the C-terminus of CBRLK1. The bacterially expressed CBRLK1 kinase domain could autophosphorylate and phosphorylates general kinase substrates, such as myelin basic proteins. The autophosphorylation sites of CBRLK1 were identified by mass spectrometric analysis of phosphopeptides. In Arabidopsis, the receptor-like protein kinase (RLK) gene family contains more than 600 members, and some of these are induced by pathogen infection, suggesting a possible role in plant defense responses. CBRLK1 mutant and CBRLK1-overexpressing transgenic plants showed enhanced and reduced resistance against a virulent bacterial pathogen, respectively. The altered pathogen resistances of the mutant and overexpressing transgenic plants were associated with increased and reduced induction of the pathogenesis-related gene *PR1*, respectively. These results suggest that CBRLK1 plays a negative role in the disease resistance signaling pathway in Arabidopsis.

02056

STUDIES ON MECHANISM OF DISEASE RESISTANCE INDUCED BY HIGH TEMPERATURE TREATMENT

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Systemic acquired resistance (SAR), one of plant defense systems, is induced by pathogen infection through salicylic acid (SA) accumulation. SAR has been well characterized and set of pathogenesis-related (*PR*) genes has been identified as SAR marker genes. The induction of disease resistance by high temperature treatment has been reported in cucumber, in which SA is likely to take part. To investigate the detailed mechanism of this resistance, effects of high temperature treatment on Arabidopsis were investigated. The high temperature treatment induced expression of PR genes and disease resistance to *Pseudomonas syringae* pv. *tomato* DC3000 in *Arabidopsis*. The treatment induced accumulation of SA and the expression of the *Isochorismate synthase 1* (*ICS1*) gene, encoding an SA biosynthetic enzyme. Analyses using the *sid2* (*salicylic acid induction deficient 2*) mutant defective in *ICS1* and NahG transgenic plants expressing SA degrading enzyme indicated that high temperature-induced disease resistance required SA biosynthesis. SAR induction has been known to be suppressed by abscisic acid (ABA) mediated signaling activated by environmental stress. Treatment with ABA suppressed high temperature-induced expression of SAR marker genes. Thus, these results indicated that high temperature-induced disease resistance is similar to SAR.

03001

FOUR TRANSCRIPTION FACTORS ARE INVOLVED IN THE EXPRESSION OF MULTI-STRESS RESPONSIBLE A. THALIANA ATGST11 GENE

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Plants always change expression of many genes to adapt to various environmental conditions. As Arabidopsis AtGST11 (glutathione S-transferase, At1g02920.1) gene is induced by Aluminum (Al) stress, heavy metal stress and oxidative stress, we believe that this is a good model system to characterize the common response mechanism for these stresses. Isolation and characterization of transcription factors (TF) related to expression of AtGST11 are performed in this study.

The cDNA clones encoding TF proteins which can bind to promoter region of the AtGST11 gene were isolated by two methods, One-hybrid method and Bio-panning. DNA sequence analysis indicated that a putative bZIP transcription factor (#11-1-1, At2g21230), Ethylene response element binding factor 2 (#11-1-3, At5g47220), Putative RING zinc finger (#13, At1g63900) and Homeobox protein 6 (#43, At2g22430) were included in the obtained clones. These candidates were confirmed their DNA binding capability to the promoter of AtGST11 by gel-shift assay. It was indicated that each candidate connected to the different part of this promoter. Furthermore, AtGST11 promoter activity assay based on a dual Luciferase assay was performed using tobacco culture cells to estimate the effect of the 4 clones in the gene-expression. Two candidates (#11-1-1 and #11-1-3) showed about 2 times higher activities than the control condition (without effectors) and the others (#13 and #43) showed about half. These results suggested the former two candidates are activators and the latter are repressors. Furthermore, each candidate was analyzed its expression pattern in Al stress by RT-PCR. Expression of three TFs, #11-1-1, #13 and #43, were increased after 4 h, but #11-1-3 was not. The former TFs (#11-1-1, #13 and #43) may be involved in the AtGST11 gene response to Al stress of. The #11-1-3 may be related to the expression of the multi stress responsible AtGST11 gene for other stress.

03002

EXTRA-NUCLEAR PROTECTION OF CHROMOSOMAL DNA FROM OXIDATIVE STRESS

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Eukaryotic organisms evolved under aerobic conditions, subjecting nuclear DNA to damage provoked by reactive oxygen species (ROS). Although ROS are thought to be a major cause of DNA damage, implicated in many human pathologies, little is known about how nuclear DNA is protected from ROS. Here we show that protection of nuclear DNA requires a coordinated function of ROS scavenging pathways in the cytosol and other cellular compartments, demonstrating that nuclear ROS scavengers are insufficient to safeguard DNA integrity. Deficiency in cytosolic and peroxisomal ROS scavenging proteins in Arabidopsis thaliana is also shown to trigger a network of DNA repair, cell cycle control, and endoplasmic reticulum cell death pathways, rendering cells highly tolerant to oxidative stress, and demonstrating the existence of novel ROS protection pathways.

03003

ENHANCEMENT OF ICE1 ACTIVITY FOR COLD TOLERANCE BY SUBSTITUTION OF SERINE 403, WHICH REGULATES UBIQUITYLATION OF THE TRANSCRIPTION FACTOR

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ICE1, a MYC-type transcription factor, has an important role in the induction of *CBF3/DREB1A* to regulate cold signaling and tolerance. In this study, we reveal that serine 403 of ICE1 is involved in regulating the transactivation and ubiquitylation of the ICE1 protein. Substitution of serine 403 to alanine enhanced the transactivation activity of ICE1 in Arabidopsis protoplasts. Overexpression of the *ICE1(S403A)* conferred more freezing tolerance than that of *ICE1(WT)* in Arabidopsis. Expression of cold-regulated genes such as *CBF3/DREB1A*, *COR47*, and *KIN1* was enhanced in *ICE1(S403A)*-overexpressing plants. Furthermore, the ICE1(S403A) protein level was not changed after cold treatment, whereas the ICE1(WT) protein level was reduced. Interestingly, polyubiquitylation of ICE1 protein in vivo was apparently blocked in *ICE1(S403A)*-overexpressing plants. These results demonstrate that serine 403 of ICE1 has roles in both transactivation and cold-induced degradation of ICE1 via the ubiquitin/26S proteasome pathway, suggesting that the serine 403 is a key residue for attenuation of cold stress responses by HOS1 mediated-degradation of ICE1.

03004

THE ARABIDOPSIS MUTANT LACKING ACYL-COA-BINDING PROTEIN ACBP1 IS FREEZING TOLERANT

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A family of six genes, designated *ACBP1* to *ACBP6*, encodes Arabidopsis acyl-coenzyme A-binding proteins (ACBPs). ACBP1 contains an *N*-terminal transmembrane domain that targets it to the plasma membrane and the endoplasmic reticulum (ER), and *C*-terminal ankyrin repeats. To establish ACBP1 function, transgenic Arabidopsis plants overexpressing ACBP1 were subject to lipid analysis. ACBP1 overexpressors showed reduction in several species of diunsaturated phosphatidylcholine (PC), prompting us to examine if they were altered in response to freezing stress. Results revealed that ACBP1 overexpressors showed increased freezing sensitivity accompanied by a decrease in PC and an increase in phosphatidic acid (PA). In contrast, *acbp1* mutant plants demonstrated an enhanced freezing tolerance associated with PC accumulation and PA reduction. Since phospholipase D α 1 is a major enzyme that promotes the hydrolysis of PC to PA, *PLD α 1* expression was investigated and was observed to be higher in ACBP1 overexpressors than *acbp1* mutants. However, the expression of PLD δ , a positive regulator in freezing tolerance, had declined in ACBP1 overexpressors and increased in *acbp1* mutants. Given that the ACBP1-mediated response is independent of osmolyte accumulation, ACBP1, which can bind phospholipids, could regulate the pool of membrane-associated phospholipids at the ER and plasma membrane.

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03005

CHARACTERIZATION OF THE INITIAL PHASE OF ARABIDOPSIS HEAT STRESS RESPONSE

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The initial phase of the heat stress (HS) response is aimed to prevention of the acute damage. It was characterized by transient expression of heat stress factor *HSFA2* in apex and leaves, coinciding with expression of transcription factor *DREB2B* (15–45 min after HS initiation). Simultaneously, transient increase of stomata conductance was found, which indicated stimulation of leaf transpiration rate. Stomata opening is positively affected by plant hormones cytokinins (CKs). HS response was associated with a mild, temporary increase in levels of CK biosynthetic precursors (CK phosphates) in apex and leaves, with maximum after 15 min. Bioactive CKs showed broader maximum (15–45 min), in spite of the fact that expression of genes for CK biosynthetic enzymes isopentenyltransferases (especially *IPT3*) in these tissues started decreasing immediately at HS. Elevation of CK content in apex and leaves coincided with up-regulation of CK signalling, indicated by stimulation of expression of genes for CK receptors (*AHK3*, 2 and 4), as well as for positive regulators of CK signal transduction, type-B response regulators (*ARR10* and *ARR12*), in apex (around 15 min) and to lower extent in leaves. Transcripts for CK degrading enzymes - cytokinin oxidases/dehydrogenases decreased, reaching the minimum after 30 min of HS. Fast decrease was observed also in case of negative regulators, type-A response regulators (*ARR8*, *ARR9*). CK signalling exhibited the second maximum after 2 h, which might indicate plant adaptation to supraoptimal temperature. Response in roots differed significantly depending whether only leaves or the whole plants were exposed to HS. In the former case, transient stimulation of CK biosynthesis and signalling was observed in roots after 30–60 min. Our data indicate an important physiological role of CKs in the HS response. This work was supported by GA CR, project no. 206/09/2062 and MEYS project no. LC06034.

03006

INVOLVEMENT OF AN AP2/ERF TRANSCRIPTION FACTOR HIF1 IN MODULATING ETHYLENE RESPONSES DURING HYPOXIA IN ARABIDOPSIS

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In recent years, many *AP2/ERF* genes have been shown to function in abiotic and biotic stress responses and these genes are often induced by multiple stresses. We report here characterization of an *AP2/ERF* gene, designated HIF1 (hypoxia-inducible factor-1), which is specifically induced during hypoxia but not by cold, drought or dehydration. We showed that under normoxic conditions the expression of *HIF1* could be induced by an addition of exogenous ACC and that a combination of hypoxia and ACC resulted in hyper induction of *HIF1*. In addition, hypoxia induction of *HIF1* is reduced, but not completely abolished, in *ein2-5* and *etr1-1* mutants. These results suggest that, in addition to ethylene, an ethylene-independent signal is also required to mediate hypoxia induction of *HIF1*.

To assess the role of HIF1, we generated 3 independent RNA-knock down lines (*hif1-ko*) of *HIF1*. Under normoxic conditions, the *hif1-ko* seedlings have increased ethylene sensitivity and exaggerated triple responses, indicating that HIF1 might play a negative regulatory role in modulating ethylene responses. Quantitative real-time PCR analyses showed that hypoxia inducible genes could be affected by *hif1-ko* mutations in two different ways: hypoxia induction of *ADH*, *PDC1*, *PDC2*, *SUS1*, and *SUS4* was reduced, whereas induction of a number of peroxidase and cytochrome P450 genes was increased. Taken together, our results showed that HIF1 is involved in modulating ethylene responses under both normoxia and hypoxia.

03007

IDENTIFICATION OF SALT TOLERANCE GENES FROM THE MANGROVE PLANT *BRUGUIERA GYMNORHIZA* BY FUNCTIONAL ANALYSIS IN TRANSGENIC ARABIDOPSIS.

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To identify key genes in the regulation of salt tolerance in the mangrove plant *Bruguiera gymnorhiza*, we have performed two experiments. One is the gene expression profiling under salt-stress (500mM NaCl) using a microarray, followed by functional analysis of the salt-responsive genes. Expression vectors for selected salt responsive genes were constructed and transformed in *A. tumefaciens*, and then screened for salt tolerance using Agrobacterium and Arabidopsis as hosts. Second is the comprehensive functional screening of the Agrobacterium libraries expressing the mangrove cDNAs. We have already reported identification of 5 salt tolerance genes, genes for lipid transfer, ankyrin repeat, zinc finger, Bg70 and cyc02-like proteins, using these methods. Here, we report the identification of additional 2 salt tolerance genes from *B. gymnorhiza* by the same methods. Arabidopsis plants transformed with a blight associated protein gene homolog, which was selected by microarray profiling. Transgenic Arabidopsis plants expressing the gene exhibited better growth on 1/2MS medium supplemented with 150mM NaCl. Screening of the Agrobacterium libraries on medium supplemented with 300mM NaCl identified a salt tolerance clone, in which metallothioneine cDNA was cloned. Transgenic Arabidopsis plants expressing the cDNA for metallothioneine exhibited better growth on 1/2MS medium supplemented with 150mM NaCl. As metallothioneine is a metal-binding protein, we examined heavy metal tolerance of the transgenic lines. The metallothioneine transformants were transplanted on 1/2MS medium supplemented with Zn, Mn, Co, Cu, Cd, Mo, Fe, or Ni. The transformants showed better growth on medium supplemented with 100 µM Cu as compared with WT. Preliminary experiment showed Cu content in the transgenic lines on Cu-containing medium was similar with that in WT. Further analysis on the transgenic plants is in progress.

03008

AUXIN RESPONSE IN *ARABIDOPSIS* UNDER COLD STRESS: UNDERLYING MOLECULAR MECHANISMS

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Plants respond to environmental stresses in multiple ways including changing the hormonal responses. Although the plant hormone auxin controls every aspect of plant growth and development, little is known about the effect of cold environment on its response. To understand the mechanistic basis of cold temperature stress and auxin response, we characterized root growth and gravity response of *Arabidopsis thaliana* at 23°C after pre-incubating the seedlings at 4°C. The time course assay revealed that 8- to 12 h pre-incubation at 4°C inhibited root growth and the gravity response by approximately fifty percent compared to that of untreated controls. The auxin-signaling mutants *axr1* and *tir1*, which show a reduced gravity response, responded to cold treatment like wild-type, suggesting that cold stress affects auxin transport rather than auxin signaling. Consistently, expression analyses of an auxin responsive marker, *IAA2-GUS*, as well as a direct transport assay further confirmed that cold inhibits root basipetal (shoot-ward) auxin transport. Microscopy of living cell revealed that trafficking of the auxin efflux carrier PIN2, which plays an important role in basipetal auxin transport, was dramatically reduced by cold. The lateral relocalization of PIN3, which has been suggested to mediate the early phase of root gravity response, was also inhibited by cold stress. Additionally, cold differentially affected various protein trafficking pathways. Furthermore, the inhibition of protein trafficking by cold is independent of cellular actin organization and membrane fluidity. Taken together, these results suggest that the effect of cold stress on auxin is linked to the inhibition of intracellular trafficking of auxin efflux carriers.

03009

CROSSTALK BETWEEN PHOTOPERIODIC FLOWERING AND STRESS SIGNALING MEDIATED BY *GIGANTEA* (*GI*) IN *ARABIDOPSIS*

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Timing of the transition from juvenile to adult state to synchronize in favorable season of the year is critical for successful sexual reproduction in many plants. This phase change is irreversible and regulation of its timing must be tightly controlled for sufficient and good health. It has been observed that plants tend to induce flower under biotic and/or abiotic stress conditions. Although this phenomenon has been known as a physiological state, the molecular basis of this regulatory mechanism is still unidentified.

GI is a key regulator of the photoperiodic response of *Arabidopsis*. The plants which carrying mutations in this gene, show no longer flower rapidly in response to long day. Since the role of *GI* in promoting flowering was first identified by mutant analysis, *GI* has been shown to have other distinct function such as an internal oscillator that regulates daily rhythms of 24 hours' duration.

gi-3 exhibited high sodium sensitivity and less accumulation of sodium in roots. Also, it showed lower gene expression of Na^+/H^+ antiporters, which have functions in ion homeostasis. Twenty-one percentage of salt treated plants represented early flowering phenotype in wild type and this phenotype was enhanced in *gi-3* (76%). To identify the integrator of stress-induced-flowering pathway, we examined the gene expression of *SOC1* and *FT* at different developmental stages in wild type and *gi-3*. *SOC1* gene expression was induced in leaves instead of *FT* under salt conditions in both genotypes.

Taken together, we indicated that *GI* mediates the sodium stress response via induction in gene expression of Na^+/H^+ antiporters as a result of circadian clock regulation. We also found that *GI* suppresses the newly identified stress-induced-flowering pathway, which induces *SOC1* expression as an integrator for flowering.

03010

FUNCTIONAL ANALYSIS OF *ARABIDOPSIS THALIANA* PUTATIVE SELENIUM BINDING PROTEIN (SBP) IN RESPONSE TO STRESS

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The function of SBP that are present among diverse species and kingdoms has not been established yet. Their highly conserved sequences suggest that they share a fundamental biological role. In mammals, selenium (Se) is an essential nutrient incorporated in the selenoaminoacid, Se-Cys, required for the translation of numerous proteins having a critical role in cell defence and hormone regulation. Mammals SBP1, that shows ability to bind Se, may contribute to Se transport. To date Se has not been demonstrated to be essential in land plants. We will present advances made in our laboratory in understanding the function of a putative SBP in *Arabidopsis thaliana*. SBP1 was identified in a proteomic analysis designed to highlight perturbations associated with cadmium stress in *Arabidopsis* cells (Sarry et al., Proteomic, 2006, 6(7), 2180-98). When overexpressed in *Arabidopsis* SBP1 enhanced tolerance to Cd suggesting that SBP1 represents a new detoxification mechanism that plants use to face the metal toxicity possibly through direct binding to Cd (Dutilleul et al., Plant Physiol, 2008, 147(1), 239-51). *Arabidopsis* plants that express the LUCIFERASE reporter gene under the control of SBP1

promoter showed that SBP1 expression was specifically enhanced in response to stress provoking a cellular sulfur demand. Correlated to these results, SBP1 expression was enhanced in response to sulfur starvation and regulated by glutathione (GSH) level. SBP1 function/activity may therefore be linked to GSH because SBP1 over-expression enhanced tolerance to stresses that require GSH for tolerance (Hugouvieux et al., Plant Physiology, 2009, 151(2), 768-81). Plants with lower level of SBP protein were recently generated using the artificial microRNA technology. Preliminary data will be as well presented on the characterization of these lines and the ability of SBP1 to bind to metals/metalloids.

03011

FORMATION OF HIGHLY REACTIVE ALDEHYDES FROM POLYUNSATURATED FATTY ACIDS IN CHLOROPLASTS

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Lipid peroxide-derived aldehydes and ketones (carbonyls) act as defense signals in plant stress responses and also exert cytotoxicity if they accumulate under severe stress. In order to obtain insight into their formation mechanism in plants, we here established a comprehensive analysis method and estimated their basal levels. Carbonyls were extracted from plant tissues, derivatized with 2,4-dinitrophenylhydrazine and separated by reverse-phase HPLC equipped with an FT-ICR-MS analyzer. Mass data were filtered to pick up the hydrazine-derivatives. Carbonyls were identified via comparison of their retention time and the MS/MS fragment profile with those of authentic standards. In unidentified carbonyls, aldehydes were distinguished from ketones based on a specific marker fragment in the MS/MS spectrum. Content of each carbonyl was determined on the absorbance chromatogram. In *Arabidopsis thaliana* Col-0 leaves, aldehydes and ketones of C1-C7 with various levels of unsaturation and oxygenation were detected at 10-70 nmol/g fresh weight level, including the reactive species malondialdehyde, acrolein and (E)-2-pentenal and less reactive species such as acetone, propionaldehyde, acetaldehyde, 3-pantanone, formaldehyde and n-pentanal. Carbonyl content in the Col-0 leaves was compared with that in the mutant *fad7-1fad8*, which is deficit of the trienoic fatty acid biosynthesis in plastids and contains less linolenic acid and more linoleic acid than Col-0. Col-0 contained significantly higher amounts of malondialdehyde, acrolein and (E)-2-pentenal than the mutant, while lower amounts of acetone, 3-pantanone, and n-hexanal, indicating the formers were derived from linolenic acid and latters from linoleic acid in the chloroplast. Thus, polyunsaturated fatty acids in the chloroplast are constitutively oxidized by reactive oxygen species, to form highly reactive aldehydes even under 'normal' conditions.

03012

PHOSPHORYLATION-MEDIATED REGULATION OF CALCINEURIN B-LIKE CALCIUM SENSOR PROTEINS

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Calcium signaling has been implicated in various responses to abiotic and biotic stimuli. However, the underlying mechanisms how calcium signals achieve appropriate transduction of such diverse stimuli remain largely unclear. In higher plants, calcineurin B-like (CBL) proteins and CBL-interacting protein kinases (CIPKs) play important roles in translating cellular calcium signals into protein phosphorylation. It is assumed that, in response to calcium signals, CBLs interact specifically with the regulatory domain of CIPKs and activate their kinase activity towards substrate proteins. The *Arabidopsis thaliana* genome encodes 10 CBL proteins and 26 CIPK proteins. Preferential complex formation of individual CBLs with defined subsets of CIPKs has been found to contribute to generating specificity in signaling networks. Interestingly, recent studies revealed a new aspect that CIPKs phosphorylate CBLs to enhance the CBL-CIPK interaction. In this report we found that CIPK24 phosphorylates CBL1, CBL4 and CBL10 *in vitro*. Moreover, CBL1 was phosphorylated by CIPK1 and CIPK23 as well, suggesting that the regulation is rather common in CBL-CIPK complexes. A truncated CIPK24 protein, which contains only the kinase domain, failed to phosphorylate those CBL proteins. In addition, CBL7 that does not interact with CIPK24 *in planta* was not phosphorylated. It is most likely that the phosphorylation requires specific interaction of CBLs with the regulatory domain of CIPKs. Furthermore, we identified a conserved phosphorylation site on CBL proteins and found that the phosphorylation could not only positively but also negatively affect CBL-CIPK interaction. This additional complexity may contribute to more diversity in the CBL/CIPK network.

03013

CHEMICAL GENETIC APPROACH REVEALS NEGATIVE REGULATION OF ABA AND Ca^{2+} SIGNALING NETWORKS BY PLANT IMMUNE SIGNALING PATHWAY

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Elaborate regulation of protection mechanisms against environmental abiotic stress and pathogen attack is essential for plant adaptation and survival. To address redundancy and robustness of the ABA signaling network, we have developed a chemical genetics approach. Microarray experiments allowed identification of a strong ABA-inducible promoter that functions at multiple developmental stages. By screening a chemical library using GFP reporter transgenic lines under the control of this strong ABA-inducible promoter as readout, we identified small molecules inhibiting ABA-induced gene expression. One particular small compound, DFPM was characterized that specifically blocks ABA-dependent gene expression and also inhibits ABA- and Ca^{2+} -induced stomatal closure. Transcriptome analyses confirm that DFPM inhibits ABA induction of more than 50% of the ABA-inducible genes. While DFPM does not interfere with early ABA PYR/RCAR receptor signaling mechanisms, DFPM affects cytosolic Ca^{2+} signals and downstream anion channel activation. Notably, transcriptome analyses also established that DFPM stimulates expression of genes related to plant immune signaling responses. Analysis of mutants showed that some major regulators of race-specific disease resistance pathways including *EDS1*, *PAD4*, *RAR1*, and *SGT1B* are required for the DFPM-inhibition of ABA signal transduction whereas components of salicylic acid and jasmonic acid signaling are not necessary for this response. Findings will be presented showing that activation of an NB-LRR (Nucleotide Binding-Leucine Rich Repeat) plant immune signaling pathway mediates control of ABA and Ca^{2+} signal transduction. Mechanisms and functions of signaling network interactions during biotic and abiotic signal transductions will be discussed.

03014

CHARACTERIZING NOVEL CIRCADIAN CLOCK COMPONENTS IN ARABIDOPSIS

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The *Arabidopsis* circadian clock plays a crucial role in co-ordinating plant metabolic function with predictable environmental variables such as dusk and dawn whilst also modulating adaptive responses to biotic and abiotic challenges. This internal timekeeper additionally acts to permit the co-ordination of longer-term developmental processes such as flowering time. The central role of the circadian clock in a broad range of biochemical and developmental processes has provided the impetus for extensive work to identify components of this molecular oscillator, primarily through the use of medium-throughput mutant screens. Whilst these analyses have been highly effective at identifying core clock components it is becoming clear that we are reaching the sensitivity limits of this method, with recent screens identifying multiple alleles of previously characterized circadian genes.

In order to uncover novel clock components we used publicly available microarray data to identify genes co-expressed with central core components. This analysis revealed that a putative JUMONJI-class histone demethylase (*JMJ*) is regulated in concert with the known clock gene *TOC1*. *TOC1* is necessary for circadian clock oscillation but has unknown biochemical function. Given that the plant circadian clock is primarily regulated by a transcriptional feedback loop this uncharacterized histone demethylase may act in parallel with *TOC1*. In support of this notion *jmj toc1* double mutant plants have a mild circadian defect whilst *jmj toc1* double mutant lines have a stronger mutant phenotype than either mutation alone. Recent progress in the characterization of this novel clock component will be discussed.

03015

CONTROL MECHANISM OF OSMOTIC STRESS RESPONSE AND PLANT GROWTH BY POTASSIUM TRANSPORTER IN ARABIDOPSIS

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A potassium transporter, *KUP6* belongs to *KUP/HAK/KT* family, which has been found in bacteria, fungi, and plants and presumably functions as K^+/H^+ symporters. *KUP6* is induced by drought and salinity stresses, and abscisic acid (ABA) in *Arabidopsis*. GUS activity of the *KUP6pro:GUS* transgenic plants was detected mainly in vascular tissues of the root. The localization of the *KUP6-GFP* protein to plasma membrane was observed in *35S:KUP6-GFP* transgenic plants by confocal microscope. We generated *kup6 kup8* double mutant plants and found that the disruption of *KUP6* and its homolog, *KUP8*, in *Arabidopsis* resulted in increased formation of the lateral roots and root hairs. Furthermore, the *kup6 kup8* double mutant showed increased auxin (indole-3-acetic acid; IAA) sensitivity and decreased sensitivity to an auxin transport inhibitor, naphthalphthalamic acid (NPA), and ABA in the lateral root growth. *KUP6*-overexpressing transgenic plants under the control of the *35SCaMV* promoter showed less transpirational water loss and increased tolerance to drought stress. ABA response in the stomata closing in *kup6 kup8* was decreased and the water loss rates of the *kup6 kup8* leaves were enhanced. These data suggested that *KUP6* controls both plant growth and osmotic stress responses in *Arabidopsis*.

03016

A CASCADE OF TRANSCRIPTION FACTOR DREB2C AND HSFA3 REGULATES THE OXIDATIVE STRESS TOLERANCE OF ARABIDOPSIS

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Dehydration-responsive element binding protein (DREB) family is an important in regulating plant responses to different stresses. *DREB2C* is one of the *Arabidopsis* class 2 *DREB*, and the *DREB2C* transcripts were induced by nonlethal heat treatment. Here, the *DREB2C* transcripts were induced by a superoxide anion propagator, methyl viologen (MV) and a reactive oxygen species, H_2O_2 . The degree of oxidative tolerance of *DREB2C* over-expressing transgenic plants was found to be significantly greater than that of wild-type plants as measured by ion leakage and chlorophyll fluorescence value under the light condition. The several ascorbate peroxidase (APX) transcripts and an APX isoform (APX^c) activity were highly induced in the transgenic plants. Additionally, the level of H_2O_2 in the transgenic plants was lower than that in the wild-type plants under oxidative stress conditions. The electrophoretic mobility shift assay (EMSA) and dual activator-reporter assay showed that the expression of APX2 was regulated by HsfA3 and the HsfA3 is transcriptionally controlled by DREB2C. Our results suggest that *DREB2C* have an important role in improving oxidative stress endurance in *Arabidopsis*.

03017

AREB1, AREB2, AND ABF3 ARE MASTER TRANSCRIPTION FACTORS THAT COOPERATIVELY REGULATE ABRE-DEPENDENT ABA SIGNALING INVOLVED IN DROUGHT STRESS TOLERANCE AND REQUIRE ABA FOR FULL ACTIVATION

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Abscisic acid (ABA) is a phytohormone that regulates diverse plant processes including seed germination and dormancy, stomatal closure, and alteration of gene expression in response to drought stress. Many drought stress-inducible genes are activated by ABA, and in the promoter regions of such ABA-regulated genes, conserved cis-elements, designated ABA responsive elements (ABREs), control gene expression via bZIP-type AREB/ABF transcription factors. Although all three members of the AREB/ABF subfamily, AREB1, AREB2, and ABF3, are upregulated by ABA and water stress, it remains unclear whether these are functional homologs. In this study, we report that all three AREB/ABF transcription factors require ABA for full activation, can form hetero- or homodimers to function in nuclei, and can interact with SRK2D/SnRK2.2, an SnRK2 protein kinase that was identified as a regulator of AREB1. Along with the tissue-specific expression patterns of these genes and the subcellular localization of their encoded proteins, these findings clearly indicate that AREB1, AREB2, and ABF3 have largely overlapping functions. To elucidate the role of these AREB/ABF transcription factors, we generated an areb1 areb2 abf3 triple mutant. Large-scale transcriptome analysis, which showed that stress-responsive gene expression is remarkably impaired in the triple mutant, revealed novel AREB/ABF downstream genes in response to water stress, including many LEA class and group-Ab PP2C genes and transcription factors. The areb1 areb2 abf3 triple mutant is more resistant to ABA than are the other single and double mutants with respect to primary root growth, and it displays reduced drought tolerance. Thus, these results indicate that AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent gene expression for ABA signaling under conditions of water stress.

03018

PIF7 NEGATIVELY REGULATES DREB1 EXPRESSION UNDER CIRCADIAN CONTROL IN ARABIDOPSIS

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Transcription factors of the DREB1/CBF family specifically interact with a *cis*-acting dehydration-responsive element/C-repeat (DRE/CRT) involved in low-temperature stress-responsive gene expression in *Arabidopsis*. Expression of the *DREB1* genes is regulated by circadian control, and induced rapidly and significantly by low temperature. Promoter sequences of *DREB1*s contain six conserved motifs, Boxes I to VI. We analyzed the promoter region of *DREB1C* using transgenic plants and showed that a 65-bp region of the promoter including Boxes V and VI responds not only to low temperature but also to the circadian clock. We found that Box V with the G-box sequence negatively regulates *DREB1C* expression under circadian control and the region around Box VI contains positive regulatory elements for low-temperature-induced expression of *DREB1C*. Using yeast one-hybrid screens, we isolated cDNA encoding the transcriptional factor PIF7, which specifically binds to the G-box of the *DREB1* promoter. Transactivation experiments using *Arabidopsis* protoplasts indicated that PIF7 functions as a transcriptional repressor for *DREB1C* expression and that its activity is regulated by PIF7-interacting factors TOC1 and PhyB, which are components of the circadian oscillator and the red light photoreceptor, respectively. Moreover, in the *pif7* mutant, expression of *DREB1B* and *DREB1C* was not repressed under light conditions, indicating that PIF7 functions as a transcriptional repressor for the expression of *DREB1B* and *DREB1C* under circadian control. This negative regulation of *DREB1* expression may be important for avoiding plant growth retardation by the accumulation of DREB1 proteins under unstressed conditions.

03019

THE ABIOTIC STRESS-RESPONSIVE NAC-TYPE TRANSCRIPTION FACTOR OSNAC5 REGULATES STRESS-INDUCIBLE GENES AND STRESS TOLERANCE IN RICE

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The plant-specific NAC transcription factors regulate stress responses and development. The transcription factor OsNAC5 is a member of the stress-related NAC family in rice. Expression of *OsNAC5* is induced by abiotic stresses, such as drought, cold, high salinity, ABA and MeJA. By transactivation assay using rice protoplasts, we demonstrated that OsNAC5 is a transcriptional activator, and in subcellular localization studies using OsNAC5-sGFP fusion proteins, we showed its nuclear localization. Pull-down assays revealed that OsNAC5 interacts with OsNAC5, OsNAC6 and SNAC1. To analyze the function of OsNAC5 in rice plants, we generated transgenic rice plants that overexpressed *OsNAC5*. The growth of the *OsNAC5*-overexpressing plants was similar to that of control plants whereas that of the *OsNAC6*-overexpressing transgenic rice plants was retarded. The *OsNAC5*-overexpressing plants improved their tolerance to high-salinity stress. By microarray analysis, we found that many stress-inducible genes, including the late embryogenesis abundant (LEA) protein gene *OsLEA3*, were upregulated in rice plants that overexpressed *OsNAC5*. By gel mobility shift assay, OsNAC5 and OsNAC6 were shown to bind in the *OsLEA3* promoter. Collectively, our results indicate that the stress-responsive OsNAC5 and OsNAC6 are transcriptional activators that enhance stress tolerance by upregulating the expression of stress-inducible genes, such as *OsLEA3*, in rice although the effects of these proteins for growth are different. OsNAC5 might be a useful gene that can improve the stress tolerance of rice without affecting growth.

03020

AUXINS REVERSE MALE STERILITY CAUSED BY HIGH TEMPERATURES

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With global warming, plant high-temperature injury is becoming an increasingly serious problem. In wheat, barley and various other commercially-important crops, the early phase of anther development is especially susceptible to high temperatures. Here, we found that under high temperature conditions, endogenous auxin levels decreased in the developing anthers of barley and *Arabidopsis*. In addition, expression of the YUCCA auxin biosynthesis genes was repressed by increasing temperatures. Application of auxin completely reversed male sterility in both plant species. These findings suggest that tissue-specific auxin reduction is the primary cause of high temperature injury, which leads to the abortion of pollen development. Thus, the application of auxin may help sustain steady yields of crops despite future climate change.

03021

TOWARD THE RECONSTRUCTION OF HORMONE-MEDIATED TRANSCRIPTIONAL REGULATORY PATHWAYS AND SYSTEMATIC SIGNALS UNDER FLOODING IN ARABIDOPSIS

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Flooding triggers multiple regulatory pathways that are mainly associated with hypoxia responses in plants. We have shown previously that ethylene is necessary, but not sufficient, for hypoxia induction of *ADH* gene in *Arabidopsis*. Ethylene and gibberellic acid (GA) had been shown to mediate metabolic and developmental responses to submergence in rice. In *Arabidopsis*, we have identified groups of ethylene- or GA-responsive genes from microarray analysis in response to flooding. We have filtered lists of putative transcription factors (TFs) and transcription elements in regulatory pathways under flooding. We have performed clustering analyses to group genes that are co-ordinately regulated during hypoxia. Based on these groupings, we will attempt to identify target genes for some of these hypoxia inducible TFs.

Moreover, we have established an open system in which only root is subjected to hypoxia treatment. Through microarray studies, we identified three different classes of genes that are induced during hypoxia: (1) genes that are induced only in roots; (2) genes that are induced in both roots and shoots; and (3) genes that are induced only in shoots. The existence of Class 2 and 3 genes suggests that hypoxia triggers systematic signals that travel from roots to shoots to trigger responses. To investigate these systematic signals, we firstly hypothesize that ACC and/or ethylene might be a systematic signal and are performing experiments to test this hypothesis.

03022**DISSECTION OF DIVERSE LIGHT INPUTS AND THEIR EFFECTS ON THE CIRCADIAN CLOCK**

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The circadian clock is an endogenous oscillatory mechanism common to most eukaryotes and responsible for driving 24 hour rhythms. Indeed, circadian rhythms are important for controlling many metabolic, physiological and behavioural processes. A defining trait of the circadian clock is the ability to become entrained in order to exactly match the local environment. Light is the most important signal involved in entrainment.

In *Arabidopsis*, several of the clock components are regulated by light, which can reset the clock relative to the current photoperiod, ensuring it is in sync with the local environment and this in turn allows for adaptation to seasonal changes in day length.

The *Arabidopsis* circadian gene network responds diversely to different light inputs. Indeed different light pathways are likely to prevail at particular times of the day, whilst their effects are gated at others.

Our new data shows that variations in timing, duration, wavelength and intensity of light produce distinct responses, and allows the separation of the light input pathways. Additionally, dissecting the complexity of the light input signals and their corresponding responses in the clock gene regulatory pathways provides vital information for the parameters required to further refine mathematical models of the *Arabidopsis* clock.

The combination of these approaches leads towards an understanding of responses to natural light conditions where intensity and wavelength vary both within and between days.

03023**RNA MASKING SYSTEM DURING COLD DEACCLIMATION OF *ARABIDOPSIS* PLANTS**

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Low temperature is a major environmental limitation on plant geographical distribution and productivity. Overwintering plants are capable of exhibiting high levels of cold tolerance, which is acquired through the process of cold acclimation (CA). For cold adaptation *Arabidopsis* needs around 1 week to obtain a high level of tolerance during CA. In contrast, the acquired freezing tolerance is rapidly reduced in deacclimation (DA). It takes only 1 day and plants resume growth after sensing warm temperature. It is important to clarify the mechanism of DA in understanding plant growth and development. However the detailed mechanism of DA is not fully understood.

In order to understand the molecular mechanism of DA, we focused on the RNA masking system, which is an RNA regulation mechanism in translational step. RNA masking regulates protein expression by repressing translations and these translations are resumed when environmental changes or developmental signals occur. To identify the target mRNAs of RNA masking, we performed comparative analyses between transcriptome and proteome. According to these analyses, we identified several candidates of target mRNAs. These mRNAs were expressed during CA but corresponding proteins were specifically expressed during DA without increment of mRNA levels. This shows plants have a translational regulation system where transcripts, which are prepared during CA, are translated in DA. In addition, these target mRNAs encoded enzymes involved in primary metabolism or cell divisions. This data suggested that plants have the system to resume plant growth by providing energy and starting cell divisions to respond to environmental change rapidly. The translational profiling of the RNA masking targets and initial response system in DA of plants through RNA regulation will be discussed.

03024**INVOLVEMENT OF MIR169 IN THE NITROGEN-STARVATION RESPONSE IN ARABIDOPSIS**

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miR169 has been reported to play important roles in symbiotic nodule development and drought resistance. We report here that the *Arabidopsis* miR169 was strongly down-regulated, whereas its target NFYA family members were highly induced by nitrogen (N) starvation. Analysis of the expression of miR169 precursors showed that miR169a was substantially down-regulated in both roots and shoots by N starvation. Accumulation of the NFYA family members was suppressed in transgenic *Arabidopsis* with constitutive expression of miR169a. Furthermore, transgenic *Arabidopsis* plants overexpressing miR169a accumulated less N and were more sensitive to N stress than the wild type. N sensitivity of 35S::MIR169a might be due to impaired uptake systems. Using the split-root approach, we found that the expression of miR169 was mainly controlled by the local N status of the roots. These results suggest that miR169 is important for plant responses to N starvation.

03025**EXPRESSION OF *ARABIDOPSIS* CALRETICULIN 1, 2 AND 3 ARE INVOLVED IN WOUND AND HEAT STRESS RESPONSES**

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Calreticulin (CRT) is ubiquitously expressed, an abundant Ca²⁺-binding protein that is multifunctional protein involved in intracellular Ca²⁺ homeostasis and molecular chaperoning resides in the lumen of endoplasmic reticulum. To investigate the regulation of *CRT* gene expression, the promoters of *Arabidopsis* *CRT* genes, *AtCRT1*, *AtCRT2*, and *AtCRT3*, were fused to a *GUS* reporter gene. Histochemical study of these transgenic plants revealed those promoters of *AtCRT1*, *AtCRT2*, and *AtCRT3* are expression nearly constitutively in *Arabidopsis* and expression was high in younger tissue and typically diminished with age. Strong expression of both *AtCRT1* and *AtCRT3* genes was observed in radical during germination on 1 day but expression was absent from hypocotyls 4 days later. In addition, GUS activity observed in elongation tissues, such as the root elongation zone, and in flower parts including anther filament, sepal, petal, and abscisic zone of developing siliques. Interestingly, the *AtCRT1* and *AtCRT3* promoter are responsive to wounding and heat stress; and the *AtCRT2* promoter only to wounding. The transcript of *AtCRT* genes was also rapidly stimulated under wound stress and *AtCRT1* and *AtCRT3* are slightly induced under heat stress. Together with the results of histochemical and transcriptional analysis in transgenic *Arabidopsis* plant, our data suggest that *AtCRT* promoters are developmentally regulated in elongation zone under wound and heat stresses.

03026

COMPARATIVE EXPRESSION ANALYSIS OF TWO ARABIDOPSIS CYSTATIN GENES, ATCYS1 AND ATCYS2, DURING DEVELOPMENT AND ABIOTIC STRESS CONDITIONS

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The phytocystatins of plants are members of the cystatin superfamily of proteins, which are potent inhibitors of cysteine proteases. The *Arabidopsis* genome encodes seven phytocystatin isoforms (AtCYSs) in two distantly-related *AtCYS* gene clusters. We selected *AtCYS1* and *AtCYS2* as representatives for each cluster and then generated transgenic plants expressing the *GUS* reporter gene under the control of each gene promoter. These plants were used to examine *AtCYS* expression at various stages of plant development and in response to abiotic stresses. Histochemical analysis of *AtCYS1* and *AtCYS2* transgenic plants revealed that these genes have similar but distinct spatial and temporal patterns during normal development. In particular, *AtCYS1* was preferentially expressed in the vascular tissue of all organs, including leaves, roots and flowers, whereas *AtCYS2* was expressed in trichomes and guard cells in young leaves, root caps, and in regions connecting the immature anthers and filaments and the style and stigma in flowers. In addition, each *AtCYS* gene has a unique expression profile during abiotic stresses. High temperature and wounding stress enhanced the expression of both *AtCYS1* and *AtCYS2*, but the temporal and spatial patterns of induction differed. From these data, we propose that these two *AtCYS* genes play important, but distinct, roles in plant development and stress responses.

03027

FUNCTIONAL ANALYSIS OF THE *OSPIF1* GENE DOWN-REGULATED BY DROUGHT STRESS IN RICE

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Regulatory mechanisms of stress responses in rice largely remain unclear. Using rice microarray, we identified many abiotic stress-responsive genes. Among them, a gene for a bHLH transcription factor down-regulated by drought stress has been studied. The bHLH protein showed a high sequence homology with *Arabidopsis* PIF, driving us to name the transcription factor OsPIF1. The level of *OsPIF1* mRNA in rice seedlings grown under non-stressed condition with light/dark cycles oscillated in a circadian manner with peaks at the middle of the light period. Under drought stress condition, the expression of *OsPIF1* was not elevated during the light period. We found that *OsPIF1* was highly expressed in the node portions of the stem using promoter-GUS analysis. In a transient experiment, OsPIF1-sGFP fusion proteins were shown to be localized in nucleus, and the transcription of a *GUS* reporter gene driven by the promoter containing E-box elements was activated by OsPIF1, suggesting that OsPIF1 acts as a transcriptional activator. Transgenic *Arabidopsis* plants overexpressing OsPIF1 displayed an increase in hypocotyl length. Conversely, dominant loss-of-function *Arabidopsis* mutants with a chimeric repressor showed a reduced hypocotyle length. Overexpression of OsPIF1 in transgenic rice plants promoted internode elongation. In contrast, dominant loss-of-function rice mutants resulted in short length of the internode sections. These data suggest that OsPIF1 functions as an important regulatory factor of plant height in response to drought stress.

Recently, we analyzed down-stream genes of OsPIF1 by oligoarray system using node sections of OsPIF1 transgenic rice plants. Those putative down-stream genes of OsPIF1 were down-regulated by drought stress.

03028

SPINDLY, A NEGATIVE REGULATOR OF GA SIGNALING, IS INVOLVED IN PLANT ABIOTIC STRESS RESPONSE

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Plant cells respond to the environmental stress through reversible posttranslational modifications of important signal molecules. The dynamic glycosylation of serine or threonine by *O*-linked β -N-acetylglucosamine (*O*-GlcNAc) is critical to many cellular responses and reciprocal to *O*-phosphorylation. In *Arabidopsis*, *SPINDLY* (*SPY*) gene encodes an *O*-GlcNAc transferase (OGT), which was originally identified as a negative regulator in GA signaling. In this study, we found that *spy-1* and *spy-3* mutants are more tolerant to osmotic stress, such as high salinity and dehydration. *SPY* gene expression is found to be drought-stress inducible. Although *spy* mutants phenocopy the plant treated by Gibberellins (GAs), application GA_3 was not able to enhance plant drought stress tolerance, indicating that *SPY* involved in plant abiotic stress response is independent of GA signaling. Transcriptome analysis of the *spy-3* mutant discovered that the expression of some drought-inducible genes was upregulated, in addition to many GA inducible genes. More evidently, in 2h-dehydrated *spy-3* plants, the expression of many drought-responsive genes, especially fifteen LEA protein genes, is higher than that in wild-type plants. In addition, a time course analysis revealed that some DREB2A specific downstream gene expressions were enhanced in the *spy-3* mutant, in early dehydration stress. Transgenic plants overexpressing *SPY*, and the coding sequence of tetratricopeptide repeat (TPR) domain, which is supposed to be a dominant negative of the *SPY* gene will be analyzed to further verify its function in plant abiotic stress response.

03029

RSS1, A NOVEL RICE FACTOR ESSENTIAL FOR THE MAINTENANCE OF CELL DIVISION ACTIVITY UNDER SALT STRESS CONDITIONS

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Post-embryonic growth of plants depends on a continuous supply of newly-divided cells from the meristems and organ primordia. It is critical that the balance between cell proliferation and differentiation in the meristems or organ primordia is maintained under stressful conditions. If the rate of differentiation were to exceed that of division, the number of proliferative cells could not be maintained. However, how the proliferative activity is ensured under stress conditions has not been understood. Here we report that a novel rice gene, *RSS1*, is required for maintenance of cell division activity under salinity conditions, antagonizing the organism's cellular checkpoints in response

to stress. *RSS1* was identified as a genetic factor important for adaptation to environmental changes, since the loss-of-function mutant *rss1* is sensitive to various stresses such as salinity, cold and heat. *RSS1* encodes a protein containing the destruction box, which is required for 26S proteasome-dependent proteolysis, mediated by APC. When expressed by a constitutive gene promoter, *RSS1* fused to GFP accumulated in a cell cycle phase-dependent manner, probably reflecting destabilization of *RSS1* during M phase and subsequent G1 phase. Moreover, *RSS1* was expressed preferentially in dividing cells, especially at G1 and S phases. Under salinity conditions, the number of cells in the 4C phase was decreased in the shoot proliferative tissues of *rss1*, concomitant with down-regulation of cell cycle-related genes. Furthermore, in the root of *rss1*, the size and cell number of meristematic zone were decreased depending on salinity, eventually leading to the loss of the meristematic zone. These results suggest that *RSS1* contributes for the balance between the cell division and differentiation under stress conditions, which is required for the maintenance of proliferative cells, possibly by ensuring G1-S transition and/or S phase progression.

03030

JUB1, A H2O2-REGULATED NAC TRANSCRIPTION FACTOR, NEGATIVELY CONTROLS SENESCENCE AND CONSTITUTES A CENTRAL ELEMENT IN H2O2 SIGNALING

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Leaf senescence is genetically programmed process that occurs during late stages of plant development or can be induced by various abiotic stresses including salinity. A candidate signal mediating the regulation of age-dependent/abiotic stress-induced senescence is hydrogen peroxide (H₂O₂). We have recently performed global expression profiling using quantitative RT-PCR and Affymetrix micro-array-based hybridization to discover transcription factors (TFs) undergoing expression changes during natural and salt-induced senescence. Among the responding TF genes, the NAC family was overrepresented. We have begun to analyze the gene regulatory networks administered by individual NACs. Our previous studies indicated that salt-triggered expression of ANAC092 (a positive regulator of senescence, also called ORE1) and its downstream gene regulon may at least in part be mediated through an elevation of cellular H₂O₂ level upon salt stress. We now discovered another NAC TF, dubbed *JUB1*, which functions as a negative regulator of senescence. Its overexpression dampens the level of cellular H₂O₂ and increases life span accompanied by an increased resistance to oxidative stress. In contrast, precocious senescence and a lowered tolerance against abiotic stresses were observed in a *jub1-1* knock-down line. *JUB1* expression is strongly and rapidly induced by external H₂O₂, indicating that it plays a role in H₂O₂-mediated signaling. To explore the *JUB1* gene regulatory network, we determined its preferred binding sites by BSSA (binding site selection assay) and performed microarray-based expression profiling using estradiol-inducible *JUB1* overexpression lines. We hypothesize that *JUB1* constitutes a central regulator of a finely tuned control system modulating cellular level of H₂O₂, regulating stress adaptation and the entry into senescence.

03031

SUN SMART PLANTS: A STUDY OF SYSTEMIC ACQUIRED ACCLIMATION IN RESPONSE TO HIGH LIGHT IN ARABIDOPSIS

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During the day as a sun moves across the sky and the light intensity varies across leaves of a canopy plants are forced to acclimate or suffer photo-inhibition and potentially photo-damage. Leaves under high light stress both acclimate and also propagate a signal to the rest of the plant. This signal pre-acclimates shaded leaves to high light stress. The communication between high light stress and distal tissues is known as systemic acquired acclimation (SAA). To study SAA spot treatments have been developed which use light emitting diode (LEDs) to apply high light (HL) to a small area of an *Arabidopsis* leaf. Utilizing this experimental set-up, we have monitored changes in the expression of key stress regulatory genes and other light-responsive genes with the goal of investigating the development of the SAA signal and the acclimatory response in distal tissues. Employing a set of the key SAA regulated genes an extensive study of SAA signal was carried out comparing different light intensities, wavelengths and treatment times. These experiments show SAA as distinct rapid signaling system employed by the plant to induce a quick acclimation response in the distal tissues in response to high light.

03032

INSIGHT INTO A CHLOROPLAST-NUCLEAR SIGNAL THAT REGULATES GENE EXPRESSION AND STOMATAL CLOSURE DURING ABIOTIC STRESS

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Compartmentation of the cell has been critical to the success of Eukaryotes, but it requires a complex interplay of signals to ensure optimal operation. One such compartment, the chloroplast, can regulate nuclear gene expression in response to environmental stress by a process known as retrograde signalling^{1,2}. Although signals such as chlorophyll precursors and ROS have been proposed, their nature and mode of action are not resolved and they are the subject of rigorous debate³. Here we show that the metabolite targeted by SAL⁴ functions as a high light and drought stress retrograde signal in that its accumulation is regulated in the chloroplast, yet its primary site of action is the nucleus. The enzyme, SAL1⁴, negatively regulates PAP levels in chloroplasts and accumulation of the metabolite alters the expression of nuclear stress responsive genes. We show that stress gene expression is highly similar in SAL1 and XRN mutants. Furthermore, SAL1 mutants restore ABA responsiveness in stomata of abscisic acid (ABA) insensitive mutants, open stomata 1 (*ost1*) and ABA insensitive 1 (*abi1*). Thus, it activates an ABA signalling pathway that is distinct to the well characterised OST/ABI1 pathway resulting in stomatal closure and enhanced tolerance in drought sensitive mutants. The nature of the metabolite and its action will be discussed.

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2. Nott et al *Ann. Rev. Plant Physiol.* 57, 739-759 (2006).

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03033

FUNCTIONAL ANALYSES OF CYS2/HIS2-TYPE ZINC-FINGER PROTEIN AZF2 IN ARABIDOPSIS

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Transcriptional repressors were recently proposed to play a key role in modulating the plant response to abiotic stresses. A subset of these transcriptional repressors belongs to the C₂H₂-type zinc-finger gene family and contains an EAR repressor domain. We analyzed the function of *AZF2* (*Arabidopsis* zinc-finger protein 2), a C₂H₂-type zinc finger transcription factor that is strongly induced by dehydration, high-salinity and abscisic acid treatments. We generated transgenic *Arabidopsis* plants harboring an *AZF2-GFP* construct driven by the *AZF2* promoter. The *AZF2-GFP* protein was localized to the nuclei in roots under normal growth conditions and in leaves under NaCl stress. The transgenic plants overexpressing *AZF2* under its own promoter were more sensitive to salinity stress than wild-type plants. We also generated transgenic lines that express *AZF2* under the control of a glucocorticoid-inducible promoter. DEX-treated transgenic plants showed growth retardation and some lines which had relatively-high expression of *AZF2* appeared to be lethal even without stress. These results indicate that *AZF2* is involved in regulation of NaCl stress responses in plants. Currently, we are trying to identify downstream target genes of *AZF2*.

03034

FUNCTIONAL ANALYSIS OF TWO ABIOTIC STRESS INDUCIBLE CCCH-TYPE ZINC FINGER PROTEIN GENES IN RICE

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The molecular mechanism governing the response of plants to abiotic stresses is not yet fully understood. We describe two *Oryza sativa* stress-related CCCH-type Zinc Finger proteins genes (*OsSCZF1* and *OsSCZF2*) involved in drought, salt and cold stress responses in rice. The expression of *OsSCZF1* was induced by drought and NaCl treatments, whereas *OsSCZF2* was induced by drought and cold. GFP localization analyses showed that *OsSCZF1* and *OsSCZF2* move between the nucleus and cytoplasm. Transgenic plants over-expressing *OsSCZF1* gene showed a lesion mimic phenotype upon maturity, had an increased expression of pathogenesis related genes and showed tolerance to NaCl induced senescence. Transgenic plants over-expressing *OsSCZF2* were short in height, exhibited reduced seed setting and improved tolerance to drought and cold stress. A number of biotic and abiotic stress related genes were up-regulated in *OsSCZF2*-over-expressing rice plants. These results demonstrate that *OsSCZF1* and *OsSCZF2* encode functional proteins involved in modulating stress tolerance in rice.

03035

ROLE OF THE PHYTOCHROME AND CRYPTOCHROME SIGNALING PATHWAYS IN HYPOCOTYL PHOTOTROPISM

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Unilateral blue-light (BL) irradiation activates phototropin (phot) photoreceptors, resulting in the asymmetric distribution of the auxin phytohormone and induction of a phototropic response in higher plants. Other photoreceptors, including phytochrome (phy) and cryptochrome (cry), have been proposed as modulators of the phototropic responses. We here show that either phy or cry is required for hypocotyl phototropism in *Arabidopsis thaliana* under high fluence rate BL and that the constitutive expression of *ROOT PHOTOTROPISM 2* (*RPT2*) and treatment with the phytohormone gibberellin (GA) biosynthesis inhibitor paclobutrazol (PAC) partially and independently complements the non-phototropic hypocotyl phenotype of the *phyA cry1 cry2* mutant under high fluence rate BL. Our results thus indicate that the induction of *RPT2* and the reduction of the GA are crucial for hypocotyl phototropic regulation by phy and cry. We also reveal that GA suppresses hypocotyl bending via destabilization of DELLA transcriptional regulators under darkness but does not suppress the phototropic response in the presence of either phyA or crys, suggesting that these photoreceptors control not only the GA content but also the GA sensing and/or signaling that affects hypocotyl phototropism. The metabolic and signaling regulations of not only auxin but also GA by photoreceptors therefore appear to determine the hypocotyl growth pattern, including the phototropic and gravitropic responses and the inhibition of hypocotyl elongation, to adapt to light environments.

03036

ANALYSIS OF A STRESS-RESPONSIVE STABILIZATION MECHANISM OF ARABIDOPSIS DREB2A

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DREB2A is an important transcription factor that is involved in heat or water stress-inducible gene expression of *Arabidopsis thaliana*. It has been shown that DREB2A is selectively degraded under non-stressful conditions, whereas it becomes stable under stressful conditions. Deleting the negative regulatory domain (NRD) from DREB2A turns the protein into a constitutively active form (DREB2A CA), which is more stable than the wild type protein. However, mechanisms stabilizing DREB2A in response to environmental stresses have been uncovered.

We applied heat or drought stress to *A. thaliana* seedlings and analyzed the protein levels of DREB2A using an antibody specific to DREB2A. We found that the accumulation level of DREB2A was much higher in heat-treated seedlings than in drought-treated seedlings. We also applied heat stress to *A. thaliana* mesophyll protoplasts that were transiently expressing *DREB2A* or *DREB2A CA* under the control of the CaMV 35S promoter. Immunoblot analysis showed that the accumulation level of endogenous DREB2A was increased in response to heat stress as well as DREB2A CA. This result suggests the existence of a mechanism that helps DREB2A stabilization in response to heat stress independent of the NRD function in leaves. Taking advantage of the protoplast system, we are now testing effects of various stress treatments or inhibitors on the stability of the DREB2A proteins.

03037

COMPREHENSIVE ANALYSIS OF RICE DREB2-TYPE GENES THAT ENCODE TRANSCRIPTION FACTORS INVOLVED IN THE EXPRESSION OF ABIOTIC STRESS-RESPONSIVE GENES

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DREB2s (dehydration-responsive element-binding protein 2s) are transcription factors that interact with a *cis*-acting DRE (dehydration-responsive element)/CRT (C-repeat) sequence and activate the expression of downstream genes involved in water- and heat-shock stress responses and tolerance in *Arabidopsis thaliana*. In this study, we performed a comprehensive analysis of all five DREB2-type genes in rice (*OsDREB2s*: *OsDREB2A*, *OsDREB2B*, *OsDREB2C*, *OsDREB2E* and *OsABI4*) to determine which of them contribute to plant stress responses. We analyzed the expression patterns of these genes under abiotic stress conditions, and we examined the subcellular localization and transcriptional activation activity of their translational products in protoplasts. Only *OsDREB2A* and *OsDREB2B* showed abiotic stress-inducible gene expression. *OsDREB2B* has functional and non-functional forms of its transcript similar to its orthologs in the grass family. The non-functional transcript was more abundant than the functional transcript during non-stress conditions, but the functional transcript was markedly increased during stress conditions. Thus, stress-inducible alternative splicing of pre-mRNA is an important mechanism for the regulation of *OsDREB2B*. *OsDREB2B* showed nuclear specific localization and the highest transactivation activity among OsDREB2s. In addition, transgenic *A. thaliana* plants overexpressing the functional transcript of *OsDREB2B* showed enhanced expression of DREB2A target genes and improved drought and heat-shock stress tolerance. These results suggest that *OsDREB2B* is a key gene that encodes a stress-inducible DREB2-type transcription factor that functions in stress-responsive gene expression in rice.

03038

EARLY BIRD INTERACTS WITH ZEITLUPE TO MODULATE THE CLOCK'S SPEED

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The daily transitions between light and dark that most organisms are exposed to on the planet Earth have led to the evolution of a molecular timing mechanism that is called the circadian clock. It coordinates inner metabolic events with external conditions and is believed to be comprised of several transcription/translation feedback loops.

One mechanism of regulation of the clock's speed occurs post-transcriptionally by ZEITLUPE (ZTL), which recruits clock proteins for degradation in a timely manner.

We have recently identified a novel clock transcription factor *EARLY BIRD*, by mutations in *EBI* which shorten clock period of the clock controlled *CHLOROPHYLL A/B BINDING PROTEIN 2* fused to *LUCIFERASE* across all tested light intensities and wavelengths. These defects remain following light and temperature entrainment, suggesting EBI acts near the central loop to control the clock's speed. We have cloned and carried out studies on EBI's interactions with characterized clock components in protoplasts, and found that EBI interacts with ZTL. EBI is however not degraded by ZTL. Instead, EBI is a regulator of clock gene transcription, and its action modulated through its association with ZTL.

03039

TRANSCRIPTOME ANALYSIS OF FORMALDEHYDE RESPONSE IN WILD-TYPE AND FORMALDEHYDE-TOLERANT TRANSFORMANT OF ARABIDOPSIS

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Formaldehyde (HCHO) is one of the air pollutants causing sick building syndrome. Our ultimate goal is to produce ornamental plants whose capacity of absorbing and detoxifying HCHO is enhanced by genetic engineering. Although HCHO is toxic also to plants, its toxicity mechanism has not yet been elucidated. To obtain a clue to this mechanism, we investigated the genes responding to HCHO exposure for a short period of time using a newly constructed chamber in which HCHO concentration and humidity can be held constant. 7 weeks old *Arabidopsis thaliana* (Columbia) wild-type and HCHO-tolerant transformant plants (Chen et al. 2010) were used for the experiments. The transformant had previously been obtained by introducing the enzymes of ribulose monophosphate pathway of a methylotroph. The plants were exposed to gaseous HCHO at 1-2 ppm (low), 14-16 ppm (high), or less than 0.04 ppm (air control) at 24°C under light for 150 min. Total RNA was then isolated from rosette leaves and was subjected to microarray analysis using Affymetrix ATH-1. At low HCHO, the number of genes whose expression level changed more than 2-fold or less than 0.5-fold were 1938 (wild) and 1245 (transformant). The difference may be due to the mitigation of the stress in the transformant. At high HCHO, the respective numbers were 4490 (wild) and 4670 (transformant). No significant change was observed for the genes involved C1 metabolism. Prominent induction in both plants was observed with the genes for small heat shock proteins, GSH S-transferase and glucosyl transferase. Especially at low HCHO, there are differences in the response to HCHO in some gene clusters based on gene ontology such as plasma membrane, response to stress, and structural molecule activity. The differences between both plants may be due to difference in sensitivity to HCHO stress. In contrast, no clear difference could be seen at high HCHO, because the stress might have been too strong to be mitigated in the transformant.

03040

A SUBSET OF CYTOKININ TWO-COMPONENT SIGNALING SYSTEM PLAYS A ROLE IN COLD TEMPERATURE STRESS RESPONSE IN ARABIDOPSIS

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Multi-step two-component signaling system (TCS) is established as a key element of cytokinin signaling in *Arabidopsis*. Here we provide evidence for a function of TCS in cold stress response in *Arabidopsis*. Cold significantly induced the expression of a subset of A-type ARR genes and of GUS in ProARR7:GUS transgenic *Arabidopsis*. Analyses of A-type ARR expression in *ahk* mutants and in cytokinin-deficient or -overproducing transgenic *Arabidopsis* indicate that *AHK2* and *AHK3* might be involved in mediating cold to express A-type ARRs independent of the influence of endogenous cytokinin levels. Cold neither significantly induced *AHK2* and *AHK3* expression nor alter the cytokinin contents of wild type within the 4-h during which the A-type ARR genes exhibited peak expression in response to cold, indicating that cold might induce ARR expression via the *AHK2* and *AHK3* proteins without alterations in the cytokinin concentrations. The *ahk2*

ahk3 and ahk3 ahk4 mutants exhibited enhanced freezing-tolerance as compared with wild type. These ahk double mutants acclimated as efficiently to cold as did wild type. The overexpression of the cold-inducible ARR7 in *Arabidopsis* resulted in a hypersensitivity response to freezing temperatures without affecting the expression of CBF/DREB target genes under cold-acclimated conditions. By contrast, the arr5, arr6, and arr7 mutants showed increased freezing-tolerance, with or without cold acclimation. These results suggest that a subset of cytokinin two-component signaling system plays a negative regulatory role in cold stress signaling, occurring independently of the cold-acclimation pathway.

03041

QUADRUPLE SERAT MUTANTS DISPLAYED GENERAL SYMPTOMS OF MINERAL NUTRIENT DEFICIENCY

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Sulfur is an essential macronutrient required for plant growth. Plants have developed several strategies for coping with sulfur deficiency and other nutrient limitations. However, the coordinating network of adaptations to these nutrient deficiencies remains incompletely understood. Serine acetyltransferase (SERAT), which catalyzes O-acetylservine (OAS) formation, plays a key role in cysteine biosynthesis. OAS is a marker metabolite of sulfate deficiency and has been speculated to be a positive regulator for the expression of sulfur deficiency-inducible genes. In *Arabidopsis*, SERAT gene family comprises five members. We investigated quadruple knockout mutants of SERAT where only one functional isoform remained active. Some of them showed growth retardation and chlorosis with accumulation of amino acids. These phenotypes are also observed in plants during nutrient-depletion induced senescence. Thus, we compared metabolite and transcriptome data from quadruple *serat* mutants with S-, N-, P- and K- depleted plants in order to investigate what happens in the quadruple mutants. This revealed similarities to developmental programs associated with senescence or stress responses. Knockouts of SERAT genes result in decreased SERAT activity and OAS production, and consequently, flux into cysteine. Thus, the quadruple mutants feature of mimic sulfur deficiency. The constitutive mutation shifts the plant metabolism to a new homeostasis. Quadruple *serat* mutants provide a suitable experimental system for senescence induced by nutrient deficiency.

03042

TOWARDS UNDERSTANDING THE ROLE OF OAS IN SULPHUR STARVATION RESPONSE

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Plants rely on a sufficient supply of macro and micro nutrients to guarantee healthy growth. During evolution plants have developed mechanisms to optimize nutrient homeostasis while nutrient supply becomes suboptimal. To overcome limitations of sulphur plants induce sulphate uptake and assimilation but also several specific but still uncharacterised mechanisms (induction of -S marker genes). As O-acetylservine (OAS) accumulates upon sulphur starvation it has been discussed as positive regulator of the sulphur starvation response in plants. We investigated the role of OAS in coordinating the -S response in two independent experimental setups. One system allowed to induce OAS up to 2-3 fold within minutes and enabled to analyse the kinetic of the induction of -S marker genes within a timerange of 5-10 min. Remarkably, sulphur marker genes such as *APR1-3* (At4g21990, At4g04610, At1g62180), *MS5* (At5g48850, AT1G04770), *LSU* (At3g49580), *ChaC like* (At5g26220) and *SHM7* (At1g36370) increased up to 18 fold with a time lag of 10-20 min to the increase of OAS. Transgenic plants over-expressing a SERAT gene under control of an inducible promoter were used as a system to increase the OAS content in a controlled manner to experimentally evaluate the previous findings. The results of this approach reveal new insights into the signals and the kinetics of the sulphur starvation response and will be discussed in the context of the current knowledge of the regulation of sulphur homeostasis.

03043

IDENTIFICATION AND ANALYSIS OF EVOLUTIONARILY CONSERVED AND GLUCOSE RESPONSIVE NUCLEAR PROTEINS

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Sugars are the prime carbon and energy source, and also an important signal for growth and development in plants. To elucidate molecular mechanisms of sugar response in plants, we identified evolutionarily conserved and glucose responsive nuclear proteins, based on the results of proteome analysis with rice nuclear proteins and investigation of the sugar responsiveness of the corresponding genes. Identified proteins included two WD40 proteins (referred to as NuGWD1 and 2). Because we could confirm that these transcripts were induced by glucose and sucrose in both rice and *Arabidopsis*, we performed phenotypic analysis of *Arabidopsis* T-DNA insertion lines for *AtNuGWD1*. Although we isolated T-DNA insertion lines for *AtNuGWD1*, we could not obtain *atnugwd1* homozygous *Arabidopsis*. In the siliques of *atnugwd1* heterozygous plant, aborted ovules were observed. Reciprocal crosses between the *atnugwd1* heterozygous plant and the wild type revealed that the mutant allele could be transmitted through both male and female gametophytes and that *atnugwd1* homozygous embryo was lethal. The result of GST pull down assay using OsNuGWD1 protein revealed that OsNuGWD1 bound a complex containing ribosomal proteins. AtNuGWD2 was previously reported as SLOW WALKER1, which is involved in 18S ribosomal RNA biogenesis. Homologs of NuGWD1 and 2 are evolutionarily conserved from yeast to human. Taken together with the results in previous reports on function of yeast homologs, these results imply that glucose elevates levels of NuGWD1 and 2, which promote 18S ribosomal RNA biogenesis and assembly of 40S small ribosomal subunit in plants.

03044

EFFECT OF ALTERED EXPRESSION LEVEL OF APX1 GENE ON CELLULAR REDOX STATUS IN ARABIDOPSIS LEAVES EXPOSED TO HIGH-LIGHT STRESS

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Arabidopsis contains 7 genes encoding ascorbate peroxidase (APX), whose products widely located in cellular compartments including chloroplasts, microbodies, and cytosol. Among them, cytosolic APX (APX1) plays an important role in controlling cellular redox status in response to photooxidative stress. In this study, we evaluated the effect of overexpression (APX1-OE) or suppression (APX1-KO) of

APX1 on cellular H₂O₂ accumulation and gene expression levels under high-light stress. We obtained 7 independent *Arabidopsis* plants transformed with pBI/APX1 driven under 35S promoter and one APX1-KO line from TAIR. The APX activities in APX1-OE and APX1-KO plants were approximately 300-600 % and 30%, respectively, compared with that in wild-type plants. When exposed to high-light stress at 1,000 μmol m⁻² s⁻¹, the H₂O₂ levels in both wild-type and APX1-KO transiently increased within 1 h, but the level remained constant in APX1-OE. A q-PCR analysis revealed that the known H₂O₂-responsive genes like *HsfA2* and *HSP18.1* were significantly suppressed in APX1-OE even under high-light stress. Comprehensive analysis of gene expression by microarray comparing APX1-OE with wild-type plants indicated that over 200 genes are differentially regulated in response to cellular redox changes under high-light stress. The present study clearly shows that expression level of *APX1* gene has significant effect on cellular redox change followed by gene regulation under high-light stress.

03045

AREB/ABF-SnRK2 PATHWAY INVOLVED IN ABA SIGNALING IN RESPONSE TO WATER STRESS

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Under water stress conditions such as drought and high salinity, ABA levels increase in plants, and then the ABA regulates the expression of many genes that function not only in the stress response but also in the tolerance. In the presence of ABA, PYR/PYL/RCAR family START proteins that function as ABA receptors recognize and bind to group-A protein phosphatases 2C (PP2Cs). Then SNF1-related protein kinases (SnRK2s) are released from the PP2C-dependent negative regulation, which allows the subclass III of SnRK2s (SRK2D/SnRK2.2, SRK2E/SnRK2.6, and SRK2I/SnRK2.3) to regulate downstream transcription factors such as AREB/ABFs (AREB1/ABF2, AREB2/ABF4, and ABF3) and activate ABA-responsive gene expression or other ABA-related responses. In both *srk2d srk2e srk2i* and *areb1 areb2 abf3* triple knockout mutants, the expression of the ABA-responsive genes such as LEA protein genes and group-A PP2Cs was drastically and globally impaired in comparison to the WT plants under water stress conditions. Among the PP2Cs, three PP2C genes that were strongly induced in the vegetative tissues after exogenous ABA treatment were named *HAI1*, *HAI2* and *HAI3* (for *highly ABA-induced PP2C genes*). In this study, based on the molecular characterization of AREB/ABF transcription factors, SnRK2 protein kinases, and the downstream genes, we will discuss the role of the AREB/ABF-SnRK2 pathway in ABA signaling in response to water stress in *Arabidopsis* and rice.

03046

FUNCTIONAL ANALYSIS OF NOVEL SMALL RNA IN ARABIDOPSIS THALIANA

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Recently, a large number of non (protein) coding RNAs (ncRNAs) have been found in higher organism. It is reported that such as miRNAs and siRNAs are closely related with inhibition of genes expression, but a large number of ncRNAs functions are still unknown.

To find the novel gene regulation mechanisms by ncRNAs, we performed a genome wide *in silico* screening from *Arabidopsis thaliana* genome sequence, according to USE (Upstream Sequence Element) sequence, which is a specific *cis*-regulatory sequence motif of the plant U-snRNA genes, TATA-like sequence at downstream of USE element, and poly T stretch over 4 base pairs which is pol III-dependent transcription termination signal. Consequently a novel ncRNA (*Arabidopsis thaliana* Small RNA: AtsR8) was identified. The AtsR8 RNA was transcribed by Pol III with tobacco *in vitro* transcription system, and about 260-nucleotide RNA was yielded. Northern blot analyses revealed that AtsR8 RNA was exist within *Brassicaceae*, and showed root-specific expression. The RNA accumulated in *Arabidopsis* MM2d cultured cell except for nucleolus. Furthermore this small RNA expression was induced by low oxygen stress. To study AtsR8 RNA function, we are trying to identify AtsR8 RNA-binding proteins using biochemical technique from *Arabidopsis* MM2d cultured cells.

03047

MOLECULAR CHARACTERIZATION OF A CHLOROPLAST ANTIOXIDANT 2-CYS PRX ISOLATED FROM MUNGBEAN

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We isolated several low temperature inducible genes using suppression subtractive hybridization (SSH) method and were able to obtain to clone a gene encoding 2-cys peroxiredoxin. The full-length cDNA of *VrPrx* (*Vigna radiata* 2-Cys Prx) is 1,049 bp with an open reading frame (ORF) consisting of 261 amino acid (aa). Genomic southern blot confirmed that mungbean genome has one copy of *VrPrx* gene. Northern blot analysis was carried out for the gene expression during low temperature, ABA, NaCl, drought, wounding and hydrogen peroxide stresses. The RNA expression of *VrPrx* gene was significantly decreased by ABA, NaCl and drought stress, but wounding, low temperature and H2O2 stresses significantly induced *VrPrx* RNA expression. It was shown that *VrPrx*-GFP was targeted to chloroplast and the N-terminal chloroplast transit peptide is required for its targeting to the chloroplast in tobacco protoplasts. For the functional analysis of *VrPrx*, the *VrPrx* recombinant protein was heterologously expressed in *E. coli*. The *VrPrx* recombinant protein showed moderate antioxidant activity compared to other antioxidant enzymes. The role of *VrPrx* was investigated using *VrPrx* overexpressing *Arabidopsis* during environmental stresses. A mungbean gene homologous to *Arabidopsis CDSP* was shown to interact with *VrPrx* in yeast two hybrid system.

03048

A MUNGBEAN UBIQUITIN CONJUGATING ENZYME E2 CONFERS TOLERANCE ON ARABIDOPSIS TO SALT STRESS

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A low temperature-inducible cDNA designated as *VrUBC1* from mungbean (*Vigna radiata*) was isolated by suppression subtractive

hybridization method. By rapid amplification of cDNA end technique, the full-length cDNA of *VrUBC1* was obtained. The full-length cDNA of *VrUBC1* contains an open reading frame of 444 nucleotides in length and capable of specifying a 16.5-kDa protein of 148 amino acids (aa) with an isoelectric point of 7.72. *VrUBC1* mRNA was induced by NaCl and ABA, but not by wounding and low temperature stress. It was shown that *VrUBC1*-GFP was localized to the cytoplasm in tobacco cell. To examine the function of *VrUBC1*, *VrUBC1* was expressed in *Escherichia coli* as His-fusion protein. Purified *VrUBC1*-His recombinant protein was shown to have ubiquitination activity in vitro. For the in vivo functional analysis of *VrUBC1*, *VrUBC1* was expressed in yeast *ubc4/5* double mutant. Stress tolerance was tested in the *VrUBC1* overexpressing *Arabidopsis* transgenic plants. We propose that *VrUBC1* play an important role in protein degradation processes during abiotic stress in plants.

03049

FUNCTIONAL ANALYSIS OF A NOVEL HEAT SHOCK PROTEIN ISOLATED FROM SOYBEAN AND ARABIDOPSIS

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We have isolated wound-inducible genes from soybean using suppression subtractive hybridization (SSH) method and were able to obtain the full-length clone of *GmDlp1* gene encoding DnaJ-like protein. The full-length cDNA of *GmDlp1* is 689 bp with an open reading frame (ORF) consisting of 163 amino acid (aa). Genomic southern blot confirmed that soybean genome has two copies of *GmDlp1* gene. Northern blot analysis showed that the RNA expression of *GmDlp1* gene is specifically induced by heat, NaCl, wounding and drought stresses. It was demonstrated that *GmDlp1*-GFP was targeted to the nucleus in tobacco cell. Overexpressing *GmDlp1* showed more susceptible to salt and heat stress compared to WT. This indicates that *GmDlp1* may play a negative regulator to stress responses in plants. *Arabidopsis AtDlp1* gene DnaJ-like protein homologous to *GmDlp1* was further characterized for the functional analysis of DnaJ-like protein. It was shown that *AtDlp1* RNA expression is induced by heat shock stress and *AtDlp1*-GFP was targeted to the nucleus of protoplasts. The *AtDlp1* promoter (1 kb) was isolated and fused to the GUS reporter gene to investigate gene regulation of *AtDlp1* specific to heat shock stress or to developmental organ in the transgenic lines. RNAi construct was employed to generate *AtDnaJ* knock-out plants for the study of the function of *AtDlp1*. Molecular function of *AtDlp1* is discussed in relation to heat shock and also developmental stages in *Arabidopsis*.

03050

MOLECULAR CHARACTERIZATION OF SOYBEAN *SLT1629* PROMOTER INVOLVED IN OSMOTIC STRESS

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SLT1629 encodes KS-type dehydrin isolated from soybean (*Glycine max*). We were able to obtain about 1.5 kb upstream DNA sequences of *SLT1629* induced by osmotic stress in soybean. A series of promoter deletion constructs were made fused to GUS reporter gene. The promoter-GUS transgenic *Arabidopsis* and soybean plants were generated via *Agrobacterium*-mediated transformation. Tissue- and stress-specificity regulation by *SLT1629* promoter was analyzed using the transgenic plants. It will be discussed how the *SLT1629* promoter is specifically used for gene regulation further.

03051

ANALYSIS OF STO NUCLEAR LOCALIZATION SIGNAL AND COP1 INTERACTION DOMAIN

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Arabidopsis thaliana SALT TOLERANT (STO/BBX24), initially characterized as a protein conferring salt tolerance to yeast (Lippuner et al., 1996), was recently identified as a negative regulator of the light signal transduction (Indorf et al., 2007). BBX24 localizes in nucleus (Indorf et al., 2007) and interacts with CONSTITUTIVE PHOTOMORPHOREGENESIS1 (COP1) in the yeast 2-hybrid system (Holm et al., 2002). The BBX24 protein contains two B-box Zinc fingers at the N-terminus thought to mediate protein-protein interaction, and several amino acid residues at the C-terminal part necessary for interaction with COP1. BBX24 accumulates in the nucleus of cells during de-etiolation of young seedlings. Accumulation of BBX24 occurs during the first hours of exposition to white light and decreases after prolonged light exposition (Indorf et al., 2007). In this work we have identified the Nuclear Localization Signal (NLS) of the protein and by creating mutant molecules, analysed the effect of the different mutations by expressing them in the *bbx24-1* mutant background and analysing the phenotypes. The results indicate that accumulation of BBX24 in the nucleus is necessary to fulfil its function, and also that degradation of the protein occurs exclusively in the nucleus. In addition, the interaction with COP1 is required for degradation of BBX24 in light and for its function in photomorphogenesis.

03052

OVEREXPRESSION OF A NAC TRANSCRIPTION FACTOR, *AHNAC2* FROM PEANUT (*ARACHIS HYPOGAEA* L.), ENHANCES ABA SENSITIVITY AND DROUGHT TOLERANCE IN TRANSGENIC ARABIDOPSIS

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The NAC (NAM, ATAF and CUC) proteins constitute a large family of plant-specific transcription factors, displaying a variety of functions in higher plants. Previously, we reported isolation and transcriptional characterization of a peanut NAC gene, *Ahnac2* (*Arachis hypogaea NAC2*), from dehydrated leaves of peanut, and predicted to encode an AtNAC3 subfamily protein. In this study, it is indicated that AhNAC2 localized in nucleus and its C terminal has transcriptional activity. Moreover, the AhNAC2 protein could bind to specific NACRE (NAC response elements) *in vitro*. In peanut seedlings, the expression of the *Ahnac2* gene was induced by different treatments such as dehydration, high salt, cold, abscisic acid (ABA) and gibberellin (GA). When *Ahnac2* was overexpressed in *Arabidopsis* wild type (*Col-0*) driven by a 35S promoter, the transgenic plants exhibited a stronger tolerance to drought stress than control plants. As compared to wild

type, transgenic plants overexpressing *AhNAC2* were highly sensitive to ABA in root growth, seed germination, and stomatal close, but the *AhNAC2* subfamily homology gene knockout mutants (*rd26-1*) were insensitive to ABA in root growth. The complementation test of *AhNAC2* gene in *rd26-1* plants could enhance drought resistance and ABA sensitivity. In addition, quantitative RT-PCR analyses showed upregulated expression of some stress-related genes in the *AhNAC2*-overexpressing transgenic Arabidopsis. The results suggest that *AhNAC2* may play an important role in the regulation of ABA response in Arabidopsis during water stress. Drought tolerance of transgenic plants can be improved by high expression of the *AhNAC2* gene in plants.

Key words: NAC transcription factor, overexpression, ABA sensitivity, drought tolerance, peanut.

Xu Liu and Lan Hong contributed equally to this work.

03053

IDENTIFICATION OF ALTERNATIVE SPLICING EVENTS OF GENES REGULATED BY ARABIDOPSIS SERINE/ARGININE PROTEINS, ATSR45A AND ATSR30, IN RESPONSE TO HIGH-LIGHT STRESS USING A TILING MICROARRAY

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Alternative splicing is a widespread process that expands both transcriptome and proteome diversity in higher eukaryotes. Notably, many genes involved in the defense responses to various environmental stresses in plants have been reported to produce alternative splicing variants. Families of serine/arginine-rich (SR) protein are known to be major factors involved in regulation of alternative splicing efficiencies. We have demonstrated that expression of *Arabidopsis* SR proteins, *atSR45a* (plant-specific type SR protein) and *atSR30* (the homologue of mammal ASF/SF2) is markedly induced by high-light stress (Tanabe et al., 2009, PMB 70, 241). In addition, both *atSR45a* and *atSR30* interacted with U1 and U2 small nuclear ribonucleoproteins and the other SR proteins. These facts suggest that *atSR45a* and *atSR30* participate in the regulation of high-light stress responsive-alternative splicing events.

Here, to identify alternative splicing events of genes regulated by *atSR45a* and *atSR30*, we compared gene expression profiles in the wild-type with the knockout *atSR45a* (*KO-sr45a*) or *atSR30* (*KO-sr30*) plants under high-light stress using the Affymetrix GeneChip *Arabidopsis* tiling 1.0R array. The expression levels of 227 and 230 genome regions in the *KO-sr45a* and *KO-sr30* plants, respectively, were altered significantly ($P < 0.01$) compared with those in the wild-type plants. There were genes encoding stress-responsive proteins and transcription and splicing factors in the vicinity of the genome regions. By semi-quantitative RT-PCR analysis, we confirmed changes in the transcript levels or alternative splicing patterns in those genes, suggesting that *atSR45a* and *atSR30* affect directly or indirectly not only alternative splicing efficiency but also transcription of the genes in response to high-light stress.

03054

ASCORBATE RESPONSIVE GENES IN ARABIDOPSIS THALIANA

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The D-mannose/L-galactose pathway for ascorbate (AsA) biosynthesis has been identified in *Arabidopsis* recently. However, the regulation mechanism of AsA biosynthesis is still largely unknown. In this work, AsA deficient *Arabidopsis* mutant *vtc2-1* was incubated with AsA precursor L-galactone-1,4-lactone (L-GaL) under light. The transcripts abundance of the *vtc2-1* treated with it for 16h was analyzed by whole genome DNA microarray. A dozen genes were up-regulated by the supplementation of L-GaL. Analysis of expression levels of the up-regulated genes by real-time PCR showed that genes encoding an aspartyl protease (ASP) and a C3HC4-type ring zinc finger (RZF) were synchronously expressed in response to L-GaL supplementation, but not to the addition of D-glucose. In addition, the expression levels of two genes were well synchronized with light/dark rhythm. Transgenic *Arabidopsis* plants containing the promoter::reporter system were developed and verified the response of *ASP* gene to altered AsA levels. The present work provides the first insight into two genes whose expression is responsive to L-GaL and AsA content in *Arabidopsis* wild type and *vtc2-1* mutant.

03055

TRANSCRIPTIONAL NETWORKS OF DEHYDRATION-RESPONSIVE GENES IN ARABIDOPSIS

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Drought is the major environmental threat to agricultural production and distribution worldwide. Plant dehydration-stress adaptation is a complex biological process that involves global changes in gene expression and metabolite composition. In the dehydration responsive pathway, there are two major cis-acting elements and transcription factors, ABRE-AREB and DRE-DREB2. AREB and DREB2 function in ABA-dependent and ABA-independent gene expression, respectively, in response to dehydration stress.

In this study, to search for novel master genes that regulate dehydration responsive pathway, we analyzed co-expressions of *Arabidopsis* genes using our dehydration-transcriptome data. We found 120 correlated-genes modules by co-expression analysis. We focused on some modules in which stress-inducible genes were co-expressed. Co-expression analysis showed that the global gene-to-gene correlations occurred among ABA-regulated genes. In ABA-regulated genes module, two cis-acting elements, ABRE and G-box, were significantly observed in their promoter regions. We also showed gene-to-gene correlations of dehydration-repressed genes. These genes function in protein synthesis and several motifs were frequency existed in their promoter regions. We will discuss regulatory networks of dehydration-increased and repressed genes in *Arabidopsis*.

03056

REGULATION OF RNA METABOLISM BY RNA CHAPERON IS IMPORTANT FOR PLANT RESPONSE TO A VARIETY OF ENVIRONMENTAL STIMULI

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In recent years, posttranscriptional regulation of gene expression is recognized as a key regulatory process in the response of plants to diverse environmental stimuli, and this cellular process is regulated largely by RNA-binding proteins (RBPs). To understand the functional role of RBPs harboring RNA-recognition motif at the N-terminal half and a glycine-rich region at the C-terminal half (glycine-rich RNA-

binding proteins, GRPs) in posttranscriptional regulation of plant response to various environmental stimuli, the roles of GRPs were characterized in *Arabidopsis thaliana*, rice (*Oryza sativa*), and *Brassica napus*.

All GRPs investigated were highly up regulated by cold stress, and were differently regulated by drought or high salinity stress. Analyses of the loss-of-function mutants and overexpression transgenic plants revealed that GRPs in different plant species play fundamental roles in cold, salt, or drought stress adaptation processes. GRPs contribute to cold and freezing tolerance in *Arabidopsis* and rice, and the ability of GRPs to enhance cold tolerance is closely correlated with their RNA chaperone activities. In addition, analysis of a specific family of RBPs in *A. thaliana* demonstrated that RBPs affect ABA-regulated seed germination and seedling growth of *Arabidopsis*. Taken together, these results demonstrate that the regulation of mRNA metabolism is important for plant response to diverse environmental stresses and ABA. [Supported by grants from APSRC (R11-2001-092-04002-0), NRF of Korea (R01-2007-000-10665-0), CFG (CG2112-1), and WCU (R32-2008-000-20047-0)]

03057

ISOLATION AND CHARACTERIZATION OF A NOVEL CHLOROPLAST GENE *CEST* THAT CONFERS SALT-STRESS TOLERANCE IN TRANSGENIC ARABIDOPSIS

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Salinity stress is one of the major factors limiting plant growth and crop production. To identify rice genes that improve salt tolerance, we have constructed a large population of transgenic *Arabidopsis* expressing about 13,000 kinds of rice full-length cDNAs randomly, and screened the library under salinity stress. From more than 20,000 transgenic lines, 208 lines were selected as candidates for salt-tolerant lines. Among them R07303 was a line with the most remarkable phenotype. R07303 contains an uncharacterized rice gene named *chloroplast protein-enhancing stress tolerance* (*OsCEST*). Newly constructed transgenic *Arabidopsis* overexpressing *OsCEST* or its *Arabidopsis* homolog, *AtCEST*, showed improved tolerance to salinity stress without obvious morphological change or growth retardation. They also showed enhanced tolerance to drought, high temperature, and paraquat that causes photooxidative stress. Under salt conditions, overexpression of *CESTs* modulated salinity-induced impairment of photosynthetic activity and lipid peroxidation. Expressions of *OsCEST* and *AtCEST* did not respond to salt stress. They were mainly transcribed in photosynthetic tissues. GFP-fused OsCEST and AtCEST proteins were localized to the chloroplast in the *Arabidopsis* leaf protoplast. Reduced expression of *AtCEST* by double-stranded RNA interference resulted in impairing of photosynthetic activity, reduction of green pigment, and growth retardation under light conditions. Microscopic examination of these RNAi plants revealed poorly developed chloroplasts in the mesophyll cells. These findings together may indicate that *CEST* contributes to protection from photooxidative damage.

03058

INTERRELATIONSHIP BETWEEN MAMP-INDUCED DEFENSE RESPONSES AND CELL CYCLE PROGRESSION USING SYNCHRONOUS-CULTURED ARABIDOPSIS CELLS

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Inhibition of plant growth and cell division are often accompanied by induction of various biotic and abiotic defense responses, suggesting some link between defense responses and cell cycle regulation. We showed that cryptogein, a proteinaceous elicitor from an oomycete, induce cell cycle arrest prior to the programmed cell death in the synchronous-cultured tobacco BY-2 cells (Kadota et al. *Plant J.* 2004; *Plant Cell Physiol.* 2005, 2006). However, molecular links between the cell cycle regulation and defense signal transduction are mostly unknown.

In this study, we established an alternative model system to characterize cell cycle progression and defense responses triggered by a microbe-associated molecular pattern (MAMP) using *Arabidopsis* MM2d suspension-cultured cells. Cell cycle was effectively synchronized, and flg22, a peptide MAMP originated from bacterial flagellin, was applied in various cell cycle phases. Flg22 induced various defense responses such as production of reactive oxygen species and expression of defense-related genes, but not cell death. Inhibition of cell proliferation and cell cycle arrest at G2/M phase were also observed. At the same time, expression of cell-cycle related genes and proteins was repressed, while that of defense-related genes was upregulated. Relationship between the cell cycle progression and expression of defense responses will be reported.

03059

EXPRESSION OF ARABIDOPSIS PLASMA MEMBRANE AQUAPORIN PIP2;3 IS ENHANCED IN RESPONSE TO HIGH TEMPERATURES

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Aquaporins facilitate the membrane transport of water and specific small molecules. Plants have aquaporins more than 30 isoforms and the number is extremely large compared to that of other eukaryotes. This abundance may be efficient for fast adaptation to new conditions.

Arabidopsis thaliana contains 13 members of the plasma membrane intrinsic protein (PIP) in addition to TIP (10), NIP (9) and SIP (3). The PIP members are divided into the PIP1 and PIP2 groups, and members of the latter group, but not the PIP1 group, exhibit high water channel activity. Previous studies revealed that PIP1 members are expressed constitutively in most tissues and that the expression of PIP2s is altered in response to growth stage and abiotic stresses, such as salt, drought and cold. Recently, information of physiological role of individual PIP2 members in the tissues has been accumulated. In the present study, we focused our attention to physiological function of PIPs under high temperature conditions.

Expression of 13 PIP genes was quantified by qRT-PCR. Among them, *PIP2;3* transcript level was dramatically increased in shoots and roots when the growth temperature rose from 21 to 36 degree. Then, the level was decreased within a few hours despite at high temperatures. This phenomenon was observed even under the constant light conditions, indicating that this transient increment mainly depends on the high temperatures. Immunochemical quantification revealed the increase in total protein level of *PIP2;3*, *PIP2;1* and *PIP2;2* at high temperatures. The increased level was decreased after a few hours as well as the mRNA level. We estimate that this transient increment of *PIP2;3* is tightly related to physiological adaptation of plants to high temperatures. We will report physiological properties of the *PIP2;3* knockout mutant grown at high temperatures.

03060

ARABIDOPSIS ANAC078 TRANSCRIPTION FACTOR REGULATES FLAVONOID BIOSYNTHESIS UNDER HIGH-LIGHT

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NAC (NAM, ATAF and CUC2) is one of the largest families of transcription factors in the plant genome, with 106 and 149 members predicted in Arabidopsis and rice, respectively. A small number of the Arabidopsis NAC members have been studied, but the function and regulation of most NAC genes are still largely unknown.

Recently, we have isolated a combination of high-light and heat-shock (HL+HS) stress-inducible genes, including a NAC transcription factor designated ANAC078 (Plant J, 2006, 48: 535). Here we explored the physiological function of ANAC078 under HL stress. Yeast transcription activity assays showed that ANAC078 functions as a transcriptional activator. A fusion protein composed of green fluorescent protein (GFP) and the full-length ANAC078 was detected in the nucleus and cytoplasm, while fusion proteins comprising GFP and ANAC078 deleted of a putative transmembrane motif were found only in the nucleus. The cyclic amplification and selection of targets (CASTing) technique showed that the ANAC078 recognition sequence contains T[A/T/C][A/T/G/C]C[T/G]TG[T/G]G as a DNA-binding site (Plant Signal Behav, in press).

In ANAC078-overexpressing Arabidopsis plants (Ox-ANAC078-43), the transcription of 166 genes was up-regulated compared with the levels in wild-type plants under HL (1,200 micro mol m⁻²/ s⁻¹, 30°C). These genes included some for transcription factors regulating the expression of genes related to the biosynthesis of flavonoids. Interestingly, the transcript levels of some genes related to flavonoid biosynthesis and the levels of anthocyanins were significantly increased in the Ox-ANAC078-43 plants and reduced in knock-out ANAC078 plants compared with the wild-type plants under HL stress (Plant Cell Physiol, 2010, 51: 486).

The present findings suggest that ANAC078 protein is associated with the induction of genes related to flavonoid biosynthesis, leading to the accumulation of anthocyanins, in response to HL stress.

03061

ISOLATION AND MOLECULAR ANALYSIS OF ARABIDOPSIS F-BOX CONTAINING PROTEINS INTERACT WITH 14-3-3 PROTEINS IN ARABIDOPSIS.

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14-3-3 proteins regulate many cellular processes by binding phosphorylated sites in various proteins. Previous experiments demonstrate that the SnRK2.8 kinase phosphorylates the 14-3-3 proteins in *Arabidopsis* (PNAS 104; 6460-6465) and suggest a connection between nutrient deprivation signaling and key metabolic pathways. Although the interaction of 14-3-3s with other proteins is well known, the regulations of 14-3-3 proteins themselves have barely been studied. To understand the protein regulation mechanism of 14-3-3s and their target proteins under nutrient deficient conditions, we have screened and characterized 14-3-3 interacting F-box containing proteins that may have E3 ligase activities using yeast mating assay. As the results of the screening, several putative F-box containing protein candidates that interact with 14-3-3 kappa, chi, and psi were isolated. The interactions between these candidates and 14-3-3s were confirmed using X-gal assays and yeast auxotrophic growth assay (SD-LTHA). According to sequence analysis, all candidates have F-box domain at N-terminus and contain various domains at C-terminus. The gene expression analyses of candidates were performed under nutrient deprived conditions to determine the connection with nutrient deficient signalings. To determine whether these candidates have E3 ligase activity or not, candidate proteins fused with either the GST tag or the MBP tag were produced in *Escherichia coli* and purified. *In vitro* ubiquitination assays using the *E. coli* purified F-box containing proteins were performed. These results suggest that 14-3-3s interacting F-box containing proteins that have E3 Ub ligase enzyme activity may regulate 14-3-3 proteins as well as their targets.

03062

CONSTITUTIVE ACTIVATION OF STRESS-INDUCIBLE GENES IN A BRI1 (BRASSINOSTEROID-INSENSITIVE 1) MUTANT RESULTS IN HIGHER TOLERANCE TO COLD

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Many plant hormones are involved in coordinating the growth responses of plants under stress. However, not many mechanistic studies have explored how plants maintain the balance between growth and stress responses. Brassinosteroids (BRs), plant-specific steroid hormones, affect many aspects of plant growth and development over a plant's lifetime. Here we determined that exogenous treatment of BR helped the plant overcome the cold condition only when pretreated with less than 1 nM, and the bri1 mutation, which results in defective BR signaling and subsequent dwarfism, generates an increased tolerance to cold. In contrast, BRI1-overexpressing plants were more sensitive to the same stress than wild type. We found that the bri1 mutant and BRI1-overexpressing transgenic plants contain higher basal level of expression of CBFs/DREB1s than wild type. However, representative cold stress-related genes were regulated with the same pattern to cold in wild type, bri1-9 and BRI1 overexpressing plants. To examine the global gene expression and compare the

genes that show differential expression pattern in bri1-9 and BRI1-GFP plants other than CBFs/DREB1s, we analyzed differential mRNA expression using the cDNA microarray analysis in the absence of stress. Endogenous expression of both stress-inducible genes as well as genes encoding transcription factors that drive the expression of stress-inducible genes were maintained at higher levels in bri1-9 than either in wild type or in BRI1 overexpressing plants. This suggests that the bri1-9 mutant could always be alert to stresses that might be exerted at any times by constitutive activation of subsets of defense.

03063

OXIDATIVE SIGNALING DERIVED FROM CHLOROPLASTS REGULATES STRESS AND HORMONAL RESPONSES

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Chloroplasts are one of the major sources of reactive oxygen species (ROS), and thus oxidative signaling derived from the organelles is thought to be a determinant for plant response to environmental stress. Two types of ascorbate peroxidase are localized in thylakoid membrane (tAPX) and stroma (sAPX) of chloroplasts. Recently, we demonstrated that both chloroplastic APXs, especially tAPX, play a dual role in ROS-detoxification and gene regulation under photooxidative stress (Plant Cell Physiol., 2010, 51: 190-200). To clarify the physiological role of oxidative signaling derived from chloroplasts, we generated the system for transient suppression of tAPX in *Arabidopsis*

plants using a estrogen-inducible RNAi method. Microarray analysis revealed that ROS derived from chloroplasts affect the expression of approx. 800 genes. The induction of the genes in response to tAPX suppression was eliminated by shading or treatment with ascorbate. The genes involved in the plant hormones such as salicylate (SA) and jasmonate (JA), heat shock response, and disease resistance were induced by suppression of tAPX, suggesting that oxidative signaling derived from chloroplasts regulates abiotic and biotic stress response by a crosstalk with plant hormones. Next, we isolated and characterized paraquat-induced photooxidative stress sensitive and insensitive mutants (*pss* and *psi*, respectively) from knockout lines, which are disrupted genes responsive to ROS derived from chloroplasts. Among 109 lines, three *psi* (*psi1~3*) and one *pss* (*pss1*) mutants were isolated. The transcript levels of *PSS1* and *PSI*s were affected by light and redox state of photosynthesis. Interestingly, *psi1* mutants showed a high sensitivity and insensitivity to the treatment with SA and methyl JA, respectively, suggesting that PSI1 is associated with a crosstalk between ROS derived from chloroplasts and plant hormones.

03064

AHK CYTOKININ RECEPTOR HISTIDINE KINASES REGULATES LOW POTASSIUM SIGNALING IN ARABIDOPSIS

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Plant hormones are one of main factors that regulate plants growth and development. Most plant hormones have been known to interact with the other hormones and signaling components. One of the plant hormones, cytokinin, regulates shoot initiation, cell and leaf differentiation, lateral root formation, drought and osmotic stress, iron uptake, sulfate and phosphate assimilation, and nitrate sensing and assimilation. There are 3 cytokinin receptor histidine kinases, AHK2, AHK3, and AHK4/CRE1, which play important roles in cytokinin signaling, ABA signaling and osmotic stress in *Arabidopsis*. Potassium is an important macronutrient to for growth increase. Plant hormones, such as ethylene, jasmonic acid, ABA, and auxin have been known to regulate potassium deficiency signal pathways. Although it is possible that cytokinin regulates low potassium signaling like other macronutrient signaling, the relationship between cytokinin and potassium are poorly understood. To investigate how cytokinin receptor histidine kinases is involved in low potassium signaling pathways, *Arabidopsis* root growth under potassium deficient condition were examined. The *ahk2ahk3* double mutants exhibited more sensitivity to low potassium than the col-0 wild type. The *ahk2ahk4* and *ahk3ahk4* double mutants without additional cytokinin were less sensitive to low potassium than the single mutants, *ahk2* and *ahk3*. Different from other AHK family members, AHK4 functions as a kinase only when there is additional cytokinin. Otherwise, it functions as a phosphate. Cytokinin deficient mutants were also tested under low potassium condition. Our data suggest that cytokinin as well as the kinase activity of AHK family members play an important role in low potassium signaling.

03065

IDENTIFICATION OF CIS-ELEMENT AND TRANS-ACTING FACTOR INVOLVED IN THE INDUCTION OF *HSFA2* EXPRESSION IN RESPONSE TO OXIDATIVE STRESS

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Heat shock transcription factor A2 (*HsfA2*) is induced under environmental stress and regulates transcription of various defense-related genes (Nishizawa et al., 2006, Plant J.). Thus *HsfA2* plays an important role in regulating induction of defenses against different types of environmental stress, but its mode of regulation remains unknown. Here, we identified a cis-element and a trans-acting factor involved in the induction of *HsfA2* expression in response to oxidative stress. The transient reporter assay using luciferase reporter constructs with different fragments of *HsfA2* promoter showed that the deletion of the sequence from -191 bp to -108 bp markedly reduced the luciferase activity under high light. This promoter region contained HSE elements, suggesting that some Hsfs mediate the induction of *HsfA2* expression. Next, to identify an Hsf regulating the expression of *HsfA2*, we took advantage of the chimeric repressor silencing technology (CRES-T). The ectopic expression of the chimeric *HsfA1d* or *HsfA1e* repressor significantly suppressed the induction of *HsfA2* expression in response to high light. Furthermore, the double knockout of *HsfA1d* and *HsfA1e* (*KO-HsfA1d/HsfA1e*) in *Arabidopsis* plants significantly suppressed the expression of *HsfA2*. Transient reporter assay showed that the HSE elements in the 5'-flanking region of *HsfA2* are essential for the transcriptional induction of *HsfA2* under the control of *HsfA1d* and *HsfA1e*. These results suggest that *HsfA1d* and *HsfA1e* directly regulate the induction of *HsfA2* expression via the HSE in the *HsfA2* promoter. We are currently investigating other *HsfA1d/HsfA1e* target genes using a transcriptome analysis of the *KO-HsfA1d/HsfA1e* and wild-type plants, and the effect of loss-of-function and overexpression of *HsfA1d* and/or *HsfA1e* on the phenotype in *Arabidopsis* plants under oxidative stress.

03066

F-BOX PROTEINS ZTL, FKF1 AND LKP2 TOGETHER CONTROL THE CIRCADIAN CLOCK BY REGULATING PRR5 AND TOC1 STABILITY IN ARABIDOPSIS

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Endogenous time keepers known as circadian clocks underlie daily and seasonal changes in the physiology and behaviors of many organisms including plants. The plant circadian clock is composed of several partially redundant negative feedback loops. Regulation of the core clock protein turnover mediated by ZEITLUPE (ZTL) constitutes an important layer in the circadian clock in *Arabidopsis thaliana*. We found that ZTL homologs, FLAVIN BINDING, KELCH REPEAT, F-BOX1 (FKF1) and LOV KELCH PROTEIN2 (LKP2), play similar roles in the circadian clock when ZTL is absent. In contrast to subtle circadian clock defects in *fkf1*, the clock in *ztl fkf1* has a considerably longer period than in *ztl*. In *ztl fkf1 lkp2*, several clock parameters were even more severely affected than in *ztl fkf1*. Although *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) expression levels are lower in *ztl* than in wild type, introducing both *fkf1* and *lkp2* mutations into the *ztl* mutant dramatically diminished *LHY* expression without further affecting *CCA1* expression. This demonstrates different contributions of ZTL, FKF1, and LKP2 in the regulation of *LHY* and *CCA1* expression. In addition, FKF1 and LKP2 also interacted with TIMING OF CAB EXPRESSION1 (TOC1) and PSEUDO-RESPONSE REGULATOR5 (PRR5) and both proteins were further stabilized in *ztl fkf1* and *ztl fkf1 lkp2* compared to in *ztl*. Our results indicate that ZTL, FKF1, and LKP2 together regulate TOC1 and PRR5 degradation and are major contributors to determining the period of circadian oscillation and enhancing robustness.

03067

A TOMATO BZIP TRANSCRIPTION FACTOR, SLAREB, IS INVOLVED IN WATER DEFICIT AND SALT STRESS RESPONSE

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Abiotic stresses such as cold, water deficit, and salt stresses severely reduce crop productivity. Tomato (*Solanum lycopersicum*) is an important economic crop; however, not much is known about its stress responses. To gain insight into stress-responsive gene regulation in tomato plants, we identified transcription factors from a tomato cDNA microarray. An ABA-responsive element binding protein (AREB) was identified and named SIAREB. In tomato protoplasts, SIAREB transiently transactivated luciferase reporter gene expression driven by *AtRD29A* (responsive to dehydration) and *SILAP* (leucine aminopeptidase) promoters with exogenous ABA application, which was suppressed by the kinase inhibitor staurosporine, indicating that an ABA-dependent post-translational modification is required for the transactivation ability of SIAREB protein. Electrophoretic mobility shift assays showed that the recombinant DNA-binding domain of SIAREB protein is able to bind *AtRD29A* and *SILAP* promoter regions. Constitutively expressed *SIAREB* increased tolerance to water deficit and high salinity stresses in both Arabidopsis and tomato plants, which maintained PSII and membrane integrities as well as water content in plant bodies. Overproduction of SIAREB in *Arabidopsis thaliana* and tomato plants regulated stress-related genes *AtRD29A*, *AtCOR47*, and *SIC17-likedehydrin* under ABA and abiotic stress treatments. Taken together, these results show that SIAREB functions to regulate some stress-responsive genes and that its overproduction improves plant tolerance to water deficit and salt stress.

03068

ECTOPIC EXPRESSION OF EAR-DELETED *SIERF* ENHANCES TOLERANCE TO SALT STRESS AND *R. SOLANACEARUM* IN TOMATO

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The ethylene responsive factor (ERF) transcription factors play an important role in the regulation of disease defense- and stress-related genes via specifically binding to GCC box cis-acting element in plants. In contrast to other ERFs acting as activators, class II ERFs contain an ERF-associated amphiphilic repression (EAR) domain and act as GCC-mediated transcriptional repressors. In this study, a class II ERF, *SIERF*, was isolated from tomato and characterized. To demonstrate whether the EAR-motif of class II ERF proteins participates in ERF-proteins-mediated functions in plants, the EAR repressor domain was deleted to generate *SIERFΔRD*. SIERFΔRD protein retained the character of a transcription factor and became a GCC-mediated transcriptional activator. Transgenic Arabidopsis and tomatoes constitutively expressing *SIERFΔRD* exhibited reduced levels of membrane lipid peroxidation and enhanced tolerance to salt stress. Further, overexpression of *SIERFΔRD* in transgenic tomato induced significantly higher expression of *PR1*, *PR2*, and *PR5* and enhanced tolerance to *Ralstonia solanacearum*. This is the first illustration of a crop gene enhancing tolerance to both biotic and abiotic stresses in transgenic plants with the deletion of a repressor domain. Findings of the present endeavor revealed the significance of class II ERF proteins in crop improvement through the operation of the EAR repressor domain.

03069

ATCPL5 ENCODES A NOVEL SER-2-SPECIFIC RNA POLYMERASE II C-TERMINAL DOMAIN PHOSPHATASE THAT IS A POSITIVE REGULATOR FOR ABA RESPONSES IN ARABIDOPSIS

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Arabidopsis RNA polymerase II (RNAP II) C-terminal domain (CTD) phosphatases regulate stress-responsive gene expression and plant development via the dephosphorylation of serine residues of the RNAP II CTD. Certain of these phosphatases (CPL1, CPL2, and CPL3) are known as negative regulators of ABA and stress responses. In this study, we isolated *AtCPL5* (*Arabidopsis thaliana* CTD phosphatase-like protein 5), a cDNA that encodes a novel RNAP II CTD phosphatase. The encoded protein was unique, possessing two highly conserved CTD phosphatase domains (CPD1 and CPD2) located downstream of each DXDX(T/V) acylphosphatase motif. *AtCPL5* was induced by ABA, drought, high salt, and cold treatments in Arabidopsis. Histochemical staining using *PAtCPL5:GUS* transgenic plants confirmed the induction of *AtCPL5* by ABA in leaves. The *AtCPL5*-overexpressing plants exhibited ABA hypersensitivity for seed germination and seedling growth inhibition as well as significantly higher stomatal closure upon ABA treatment, lower transpiration rates upon dehydration, and enhanced drought tolerance. In addition, overexpression of *AtCPL5* in the transgenic plants changed the expression of a number of genes, including the ones encoding DREB-type AP2/ERF transcription factors, such as RAP2.4, RAP2, and QRAP2 that are known to be involved in ABA-mediated plant responses. In contrast to the Ser-5-specific phosphatase activity of the negative stress response regulators (CPL1 and CPL2), purified recombinant AtCPL5 and each half of the CPD-containing protein specifically dephosphorylated Ser-2 in the Arabidopsis RNAP II CTD. In summary, AtCPL5 is a unique CPL family protein that functions as a positive regulator of the ABA-mediated developments and drought responses in Arabidopsis.

03070

REGULATORY ROLE OF ENDOGENOUS CYTOKININS IN DROUGHT AND SALT STRESS RESPONSES

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Cytokinins (CKs) play crucial roles not only for plant growth but also for stress signaling as indicated by numerous reports. Recent data have showed that CKs are signals traveling from roots to shoots, and ABA:CK ratios in xylem sap are important for stress signaling. Additionally, CK has been reported to be directly involved in mediating stress responses as it was required for the CK receptor AHK4/CRE1 histidine kinase to function as a negative regulator of salt stress through His-to-Asp phosphorelay. Although evidence has indicated that there is cross-talk among CK signaling, stress signaling and CK metabolisms, but strong conclusions about the relationship between CK metabolism/CK regulatory role and stress response are still premature because the complexity of the CK profiles under stress conditions has not been fully explored. To unravel the relationship between CK metabolism and stress regulation in *Arabidopsis*, we have investigated the function of adenosine phosphate-isopentenyltransferases (*AtIPTs*) and cytokinin oxidases (*AtCKXs*) genes, which encode key enzymes involved in CK metabolisms, in stress response and the influence of stresses on their expression. We examined the expression of the *AtIPTs* and *AtCKXs* genes under various stress conditions. Subsequently, we performed functional analyses of CK-deficient plants, *atipt* mutants and *AtCKX*-overexpressors, in drought and salt stress responses. The *atipt* mutants and *AtCKX*-OX plants with decreased CK contents showed increased drought and salt tolerance, demonstrating that endogenous CKs act as negative regulators in these stress responses. Furthermore, the CK-deficient *atipt* mutants displayed delayed germination even without ABA. Different molecular and genetic

approaches have been used to elucidate the mechanism of CK-dependent stress responses. From these data, we will discuss the crosstalk between CK, ABA and stress signaling.

03071

THIOREDOXIN-REGULATED PFA-DSPTPASES IN HIGH LIGHT RESPONSE OF ARABIDOPSIS THALIANA

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PFA-DsPTPase is a subfamily of atypical dual protein tyrosine phosphatases (DsPTPases) that only present in plant, fungi, and kinetoplastid. In Arabidopsis, five PFA-DsPTPases have been identified which we designated as Yu1, Yu2, Yu3, Yu4 and Yu5. To date, the physiological roles of PFA-DsPTPases in plant have been poorly understood.

Here we showed that the mutants of *Yu1* and *Yu4* (*yu1-1* and *yu4-1*, respectively) are more vulnerable than wild type when grown under high light condition. When detached leaves were exposed to high light, *yu1-1* and *yu4-1* were more severely damaged than wild type. The maximum quantum yields (Fv/Fm) of *yu1-1* and *yu4-1* became negligible, while the Fv/Fm of wild type and over-expression lines reduced to 0.1 and 0.3, respectively. By transmission electron microscopy, we found that thylakoids were severely impaired in *yu4-1* but not in *yu1-1*, while chloroplasts of *yu1-1* were dissociated from cell wall and move towards the cell center, reminiscent of programmed cell death. In addition, the nonphotochemical quenching of chlorophyll fluorescence (NPQ) of *yu4-1* was lower than that of wild type, while the NPQ of *yu1-1* was the same as that of wild type, implying that *Yu1* and *Yu4* are involved in photoprotection through a NPQ-independent or NPQ-dependent mechanism, respectively. Furthermore, we found that *Yu1* and *Yu4* had strong interaction with ferredoxin 2 (Fd2), ferredoxin-thioredoxin reductase variable subunit 1 (FTRV1), and three m type thioredoxins (i.e., THM1, THM2 and THM4) in yeast two hybrid system. *In vitro*, addition of reduced GST-THM1, GST-THM2, and GST-THM4 could significantly increase the phosphatase activities of GST-Yu1 and GST-Yu2.

Our study provides evidence that the phosphatase activities of PFA-DsPTPases *Yu1* and *Yu4* are involved in high light response in Arabidopsis which may be regulated through the interaction with thioredoxins THM1/2/4.

03072

MOLECULAR AND GENETIC FACTORS UNDERLYING DIVERGENT METABOLISM AND LOW WATER POTENTIAL RESPONSE IN ARABIDOPSIS ECOTYPES LANDSBERG ERECTA AND SHAKDARA

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Arabidopsis ecotypes Landsberg *erecta* (Ler) and Shakdara (Sha) were found to have divergent strategies of responding to low water potential as well as profound differences in metabolism and gene expression. Sha exhibited four- to five-fold less proline accumulation than Ler after transfer of seedlings to low water potential or ABA treatment. Further observations established that the reduced proline was part of a more broadly differing metabolic signature encompassing altered levels of other amino acids, organic acids and sugars in Sha compared to Ler. Sha was less sensitive to ABA in some aspects, including proline accumulation, but not in others. Sha also had lower chlorophyll content. Ler and Sha had similar expression of proline metabolism enzymes, indicating that transcriptional regulation of these enzymes was not likely to cause the variation in proline accumulation. Mapping of quantitative trait loci (QTL) for proline accumulation using a LerxSha recombinant inbred line population found different QTL for ABA and low water potential suggesting that these two treatments affect proline by different mechanisms. Near isogenic lines were generated to confirm the location of the low water potential-proline QTL of largest effect. Large scale mRNA sequencing of Ler and Sha found that Sha had increased expression of many stress-associated genes including DREB family transcription factors. Sha had reduced expression of reactive oxygen metabolism and photosynthetic genes, consistent with its reduced chlorophyll content. The combined data indicated that Sha was not unresponsive to low water potential or ABA but rather employs a different metabolic regulation and stress response strategy than Ler.

03073

FUNCTIONAL CHARACTERIZATION OF TWO AP2/ERF GENES INVOLVED IN ABIOTIC STRESS SIGNAL TRANSDUCTION IN ARABIDOPSIS

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To adjust to environmental stress conditions such as drought, salinity, cold and hypoxia, plants trigger rapid defense responses via a number of signal transduction pathways. In this study, we have studied roles of two stress-responsive genes, *AtAP2/ERF1* and *AtAP2/ERF2*, members of AP2/ERF transcription factor family in abiotic stress signal transduction. *AtAP2/ERF1* transcription was highly up-regulated under various abiotic stress conditions such as low oxygen, high salt, MV and ABA whereas *AtAP2/ERF2* transcription was down-regulated under those conditions except low oxygen condition where its expression was up-regulated. Interestingly, analysis of *AtAP2/ERF2 promoter*:*GUS* transgenic plants showed that promoter activity of *AtAP2/ERF2* increased under high salt, MV and ABA treatments, suggesting that *AtAP2/ERF2* expression might be posttranscriptionally regulated. *AtAP2/ERF1* and *AtAP2/ERF2* proteins were localized in the nucleus, suggesting that they function as transcriptional regulators. Overexpression transgenic plants of *AtAP2/ERF1* and *AtAP2/ERF2* genes showed more tolerance to abiotic stresses such as high salt, mannitol, MV and flooding than wild-type plants, whereas *AtAP2/ERF1-RNAi* suppression transgenic plant and T-DNA insertional mutants of *AtAP2/ERF2* were more sensitive to those stresses. Results of promoter analysis of *AtAP2/ERF1* using *AtAP2/ERF1 promoter deletions*:*GUS* transgenic plants indicated that both -350 to -287 and -172 to -118 regions are responsible for the response to low oxygen and high salt stresses. Our results suggest that both *AtAP2/ERF1* and *AtAP2/ERF2* might play important roles in abiotic stress signal transduction. (*: these authors equally contributed to this work.)

03074

ARABIDOPSIS CHLOROPHYLL DROUGHT-INDUCED STRESS PROTEIN (ATCDSP32) FUNCTIONS AS A MOLECULAR CHAPERONE

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Arabidopsis Chloroplastic Drought-induced Stress Protein of 32 kDa (AtCDSP32) was cloned from a cDNA library. AtCDSP32 consisted

of two thioredoxin folds, an N-terminal thioredoxin fold without active site and a C-terminal thioredoxin fold with conserved active site of CGPC, respectively. To investigate biochemical property, mature form which is removed chloroplast transit peptide, N-terminal and C-terminal thioredoxin folds of AtCDSP32 were expressed in E. coli and the proteins were purified by GST affinity method. In an assay to measure thioredoxin activity, only the C-terminal thioredoxin fold containing the conserved active site showed DTT-dependent insulin reduction activity. When the structural characteristic of the recombinant AtCDSP32 proteins were analyzed by size exclusion chromatography and native-PAGE, mature form and N-terminal domain existed as various size oligomers while C-terminal domain existed as only small size oligomers. In an experiment to measure chaperone activity, mature form and N-terminal domain displayed holoase chaperone activity in a concentration dependent manner but C-terminal domain with small oligomer did not show activity. [Supported by EB-NCRC & BK21 program]

03075

A CHLOROPLAST ARABIDOPSIS NADPH DEPENDENT THIOREDOXIN REDUCTASE FUNCTIONS AS AN ELECTRON DONOR OF 2-CYS PEROXIREDOXIN

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2-Cys peroxiredoxins (Prxs) play important roles in the antioxidative defense systems of plant chloroplasts. In order to determine the interaction partner for these proteins in Arabidopsis, we used a yeast two-hybrid screening procedure with a C175S-mutant of Arabidopsis 2-Cys Prx-A as bait. A cDNA encoding an NADPH-dependent thioredoxin reductase (NTR) isotype C was identified and designated ANTR-C. We demonstrated that this protein effected efficient transfer of electrons from NADPH to the 2-Cys Prxs of chloroplasts. Interaction between 2-Cys Prx-A and ANTR-C was confirmed by a pull-down experiment. ANTR-C contained N-terminal TR and C-terminal Trx domains. It exhibited both TR and Trx activities and co-localized with 2-Cys Prx-A in chloroplasts. These results suggest that ANTR-C functions as an electron donor for plastidial 2-Cys Prxs and represents the NADPH-dependent TR/Trx system in chloroplasts. [Supported by EB-NCRC & BK21 program]

03076

PLANT SPECIFIC CALCIUM SENSOR SCS NEGATIVELY REGULATES ACTIVITY OF SNRK2 PROTEIN KINASES

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SnRK2 (SNF1-related protein kinases 2) are a family of protein kinases containing 10 members (SnRK2.1-2.10) in *Arabidopsis thaliana*. They play an important role in the regulation of plant development, osmotic stress and ABA signaling. So far, the information concerning mechanism(s) of regulation of their activity is still limited. Using yeast two-hybrid system we have identified a putative calcium binding protein, which interacts with the SnRK2 family members. The isolated cDNA encodes a plant-specific 375 amino acid protein containing two predicted EF-hand motifs. The calcium binding constant of the protein is $K=2.5 +/- 0.9 \times 10^5 \text{ M}^{-1}$. The CD spectrum indicated that the secondary structure of the protein changes significantly in presence of calcium, suggesting its possible function as a calcium sensor in plant cells. We studied interaction of this protein with selected SnRK2 kinases using pull-down assay and shown that SnRK2.4, SnRK2.6 and SnRK2.8 interacted with the protein in a calcium independent manner. Using bimolecular fluorescence complementation assay (BiFC) we confirmed that the binding occurs also *in planta*, exclusively in the cytoplasm. Therefore the protein was named SCS (SnRK2 interacting calcium sensor). We have analyzed phosphorylation of SCS catalyzed by SnRK2.4 kinase. *In vitro* studies revealed that the kinase phosphorylates Ser-250 of SCS in the conserved region R-L-L-S. We also tested *in vitro* the effects of SCS on the activity of recombinant SnRK2s. The studies show that activity of analyzed SnRK2 kinases, both ABA-dependent and independent ones is negatively regulated in a calcium-dependent manner by the characterized SCS protein. Our data suggest that SCS is a potential regulator of signal transduction pathway in which SnRK2 kinases are involved.

03077

REGULATION OF EXPRESSION AND ACTIVITY OF ZmCPK11 BY WOUNDING, TOUCH AND COMPONENTS OF JA-DEPENDENT SIGNAL TRANSDUCTION PATHWAY

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Expression of *ZmCPK11*, a member of the Calcium Dependent Protein Kinases family, is induced by mechanical wounding both locally at the injury site and systemically in unwounded *Zea mays* L. leaves. Moreover, the activity of 56 kDa CDPK of molecular mass similar to *ZmCPK11* was rapidly (< 5 min) stimulated. Touch, a weaker stress not leading to injure plants, also increased expression of *ZmCPK11* and enzymatic activity of 56 kDa CDPK. Trials to identify activated by the stress protein kinase as *ZmCPK11*, using antibodies produced against peptides from N-terminal and C-terminal regions of *ZmCPK11*, failed. Therefore, we generate transgenic *Arabidopsis thaliana* plants in which *ZmCPK11* with a c-Myc tag was expressed under the control of CaMV 35S promoter. Analysis of the transgenic plants showed that *ZmCPK11* is activated upon wounding and touching. Above results indicate an involvement of this protein kinase in early events of local and systemic response to wounding.

In the wound response jasmonic acid (JA) and its methyl ester (MeJA) are known as endogenous signal molecules produced in the octadecanoid pathway, in which linolenic acid (18:3, LA) is a substrate for JA biosynthesis. The involvement of *ZmCPK11* in wound induced JA-dependent responses was investigated in maize and transgenic *Arabidopsis* plants overexpressing *ZmCPK11-c-Myc*. Expression of *ZmCPK11* and selected wound-responsive genes was analyzed by quantitative real-time PCR (Q-PCR) in maize leaves. MeJA and 18:3 induced the expression of *ZmCPK11* and previously known wound responsive genes. Also, *ZmCPK11* was activated by MeJA and 18:3 in the transgenic *Arabidopsis* plants overexpressing *ZmCPK11-c-Myc*. Regulation of *ZmCPK11* on two levels (expression and enzymatic activity) by components of the octadecanoid pathway suggests that *ZmCPK11* is an important component of the wound induced JA-dependent response in plants.

03078**NITRATE RESPONSIVE MIR393/AFB3 MODULE CONTROLS ROOT SYSTEM ARCHITECTURE IN ARABIDOPSIS THALIANA**

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One of the most striking examples of plant developmental plasticity to changing environmental conditions is the modulation of root system architecture (RSA) in response to nitrate supply. Despite the fundamental and applied significance of understanding this process, the molecular mechanisms behind nitrate-regulated changes in developmental programs are still largely unknown. Small RNAs (sRNAs) have emerged as master regulators of gene expression in plants and other organisms. To evaluate the role of sRNAs in the nitrate response, we sequenced sRNAs from control and nitrate-treated Arabidopsis seedlings using the 454 sequencing technology. We found that microRNA miR393 was induced by nitrate in these experiments. miR393 targets transcripts that code for a basic helix-loop-helix (bHLH) transcription factor and for the auxin receptors TIR1, AFB1, AFB2, and AFB3. However, only *AFB3* was regulated by nitrate in roots under our experimental conditions. Analysis of the expression of this miR393/*AFB3* module, revealed an incoherent feed-forward mechanism that is induced by nitrate and repressed by N metabolites generated by nitrate reduction and assimilation. To understand the functional role of this N-regulatory module for plant development, we analyzed the RSA response to nitrate in *AFB3* insertional mutant plants and in miR393 overexpressors. RSA analysis in these plants revealed that both primary and lateral root growth responses to nitrate were altered. Interestingly, regulation of RSA by nitrate was specifically mediated by *AFB3*, indicating that miR393/*AFB3* is a unique N responsive module that controls root system architecture in response to external and internal N availability in Arabidopsis.

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03079**INVESTIGATING THE ROLE OF ER-LOCALIZED HSP90 ISOFORM ATHSP90.7 IN ARABIDOPSIS GROWTH AND DEVELOPMENT BY PROTEOMICS APPROACHES**

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Hsp90 is a highly conserved molecular chaperone that is involved in modulating a multitude of cellular processes under both physiological and stress conditions. In Arabidopsis, there are seven HSP90 isoforms (AtHSP90.1 to AtHSP90.7) that are localized in either cytoplasm/nucleus or organelles. The role of the cytosolic HSP90s in Arabidopsis has been implicated in resistance of abiotic and biotic stresses as well as in regulating normal plant growth and development. However, the function of organellar HSP90s in regulating plant growth and development under both normal and stress conditions is not well studied. We are interested in the function and specific role of Arabidopsis endoplasmic reticulum (ER) located AtHSP90.7. AtHSP90.7 was previously shown to be important for tissue proliferation and maturation of AtHSP90.7 was recognized in a shepherd mutant. We previously showed that overexpression of AtHSP90.7 altered the plant sensitivity to salt and drought. In this study, we use a set of proteomics approaches to investigate the physical interactors of Arabidopsis AtHSP90.7 in order to identify the potential client proteins of AtHSP90.7 in the endoplasmic reticulum. In addition, the biochemical and biophysical property of AtHSP90.7 is analyzed to elucidate the possible mechanism of its chaperone function.

03080**EXPLORING GENES INVOLVED IN THE REGULATION OF PHOTOSYNTHESIS EFFICIENCY BY MEASUREMENTS OF CHLOROPHYLL FLUORESCENCE**

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Plants need light energy to drive photosynthesis. However, too much light can cause photo-oxidative damage to the photosynthetic machinery. To deal with light stress, plants have evolved several defense mechanisms. In plants grown under high light intensity the photosystem (PS) II-antenna size and the ratio PSII/PSI are smaller than in those grown in shade. In addition, excess light energy can be immediately quenched and dissipated as heat. All these are necessary for plants in order to optimize photosynthesis efficiency under changeable environmental conditions. However, various aspects especially of the signal reception pathway are still not understood.

Recently, 3246 *Ds/Spm* transposon- or T-DNA-tagged *Arabidopsis* lines for genes encoding 1369 chloroplast proteins were collected and their seedling phenotypes were observed (Myouga et al., 2010, <http://large.psc.riken.jp/chloroplast/>). We not only isolated 111 mutants showing abnormal seedling and 122 mutants of which homozygous lines were not obtained, but also collected 1290 homozygous lines without visible phenotypes. Here, using those homozygous lines, we aimed to identify and characterize genes involved in the regulations of photosynthesis.

Plants grown under white light ($70\text{-}230\text{ ME m}^{-2}\text{s}^{-1}$) for 10 days were used for 2-D analysis of chlorophyll fluorescence with pulse amplitude modulation. We focused on two photosynthetic parameters, the effective quantum yield of PSII (Φ_{II}) and non-photochemical quenching (NPQ), and identified about 620 mutant candidates at the 68.3% significance level. Taking into consideration genomic information, about 400 lines were extracted. For the second screening, both extracted mutants and wild-type controls were grown under high light intensity (230 ME) for 24 hours after 10 days of growth under lower irradiation (30 ME). So far, the second screening has been done for about 300 lines, and the detail will be presented.

03081**DEVELOPMENT OF LEAF PALISADE CELL IN RESPONSE TO BLUE LIGHT**

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Plants develop sun or shade leaves to optimize absorption of light energy. Sun leaves are thicker than shade leaves and have developed palisade tissue consisting of elongated columnar mesophyll cells. Development of palisade tissue was reported to be regulated by long-distance signaling from mature leaves in *Chenopodium album* and was reported to be promoted by blue light in *Arabidopsis thaliana*. To gain insight into the light sensing mechanisms, we investigated palisade development in various intensity of blue or red light using

Arabidopsis and analyzed palisade development of new leaves in plants which is partially illuminated with strong light or blue light using Nicotiana tabacum. In Arabidopsis, elongation of palisade cell was not observed in any intensity (50-300 μ mol photons m⁻² s⁻¹) of red light while it was observed in blue light above 200 μ mol photons m⁻² s⁻¹ and also in 10% blue light with red light (300 μ mol photons m⁻² s⁻¹). These results indicate that blue light is essential for elongation of palisade cell but light intensity is also required for palisade development in Arabidopsis. In Nicotiana tabacum, although elongation of palisade cell was a little observed in strong red light illumination, it was promoted by 10% blue with strong red light illumination. The result shows that blue light involve in palisade development also in Nicotiana tabacum. In Nicotiana tabacum, palisade development of new leaf was observed in plants whose mature leaf was partially illuminated with strong light; however, it was not observed in case of partial blue light illumination (50 μ mol photons m⁻² s⁻¹) under strong red light background (300 μ mol photons m⁻² s⁻¹). The results imply that blue light signal is not transduced to new leaves but act in a cell-autonomous manner on palisade development.

03082

THE ARABIDOPSIS RING-H2 TYPE E3 LIGASE AIRP1 IS A POSITIVE REGULATOR OF DROUGHT TOLERANCE BY MODULATING ABA SIGNALING.

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Ubiquitination, one of the post-translational modifications in eukaryotic cells, is mediated by the cascade of E1 ubiquitin (Ub) activating enzyme, E2 Ub conjugating enzymes, and E3 Ub ligases. The 26S proteasome degrades most of poly-Ub tagged target proteins in the cytoplasm, nucleus, and ER. We screened the *Arabidopsis* T-DNA insertion mutants of E3 Ub ligases and isolated ABA-hyposensitive mutants. One of those mutants was named *atairp1* (*Arabidopsis thaliana* ABA Insensitive Ring Protein 1). *AtAIRP1* encodes a protein containing a single C3H2C3-type Ring finger domain, and its transcript level was induced by ABA and abiotic stresses, including drought, low temperature, and high salinity. Recombinant AtAIRP1 protein showed E3 ubiquitin ligase activity *in vitro*. *atairp1* mutant plants exhibited ABA insensitive phenotypes, such as decreased stomatal closure and increased germination ratio, relative to wild type plants. In addition, this mutant was more sensitive to drought stress than wild type. In contrast, 35S:*AtAIRP1-GFP* transgenic *Arabidopsis* plants were significantly tolerant to drought stress and showed hypersensitive phenotypes to exogenous ABA. Histochemical detection of H₂O₂ with DAB staining in wild type, *atairp1*, and 35S:*AIRP1-GFP* plants exposed to ABA showed that the 35S:*AIRP1-GFP* plants were much darker than wild type and *atairp1* mutant plants. It suggests that higher amount of ROS was accumulated in 35S:*AIRP1-GFP* transgenic plants than wild type and *atairp1* mutants by exogenous ABA. Also, transcript levels of several ABA signaling-related genes were higher in 35S:*AIRP1-GFP* transgenic plants than wild type and *atairp1*. In conclusion, our results suggest that AtAIRP1, Ring-H2 type E3 Ub ligase, plays a role as a positive regulator in the drought response by modulating ABA sensitivity.

03083

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF TWO HOMOLOGOUS *ATRDUF1* AND *ATRDUF2*, WHICH ENCODE THE RING-H2 TYPE E3 UBIQUITIN LIGASES IN ARABIDOPSIS

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Ubiquitination is mediated by the cascade of ubiquitin (Ub) activating enzyme (E1), Ub conjugating enzyme (E2), and Ub ligase (E3). E3 Ub ligases are categorized based on the presence of specific domains, such as RING, HECT, F-box, and U-box. RING E3 Ub ligases exist over approximately ~500 genes in *Arabidopsis*. Based on the *in silico* data (<https://www.genevestigator.com>), we selected two homologous genes (*At3g46620* and *At5g59550*), whose transcriptional levels were up-regulated by a broad spectrum of abiotic stresses (salt, cold, hydrogen peroxide, and drought) in *Arabidopsis*. *At3g46620* and *At5g59550* encoded proteins that contained a single RING domain and also C-terminal DUF1117 (Domain of Unknown Function 1117), and thus they were named *AtRDUF1* and *AtRDUF2*. Northern blot analysis showed that *AtRDUF1* and *AtRDUF2* mRNA were up-regulated by drought, cold temperature (4°C), high salinity (300 mM), hydrogen peroxide (20 mM), and ABA (100 μ M) treatment in *Arabidopsis* seedlings. Bacterially expressed MBP-*AtRDUF1* and MBP-*AtRDUF2* recombinant proteins exhibited E3 Ub ligase activity *in vitro*. To examine the cellular roles of *AtRDUF1* and *AtRDUF2*, loss-of-function T-DNA knock-out *atrduf1* and *atrduf2* mutant plants were obtained. In germination assay, *atrduf1atrduf2* double knock-out mutants showed insensitive phenotype to the herbicide paraquat (0.1–0.3 μ M methyl viologen), which generates reactive oxygen species, in comparison with wild type plants. More detailed cellular roles of *AtRDUF1* and *AtRDUF2* will be presented.

03084

DISSECTION OF POTASSIUM SIGNALING IN ARABIDOPSIS THALIANA

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Potassium (K⁺), along with nitrogen and phosphorus, is one of essential macronutrients used by plants for growth. Due to its positive impact in plant development, high quantities of K⁺ is present in fertilizers and applied to soil to be absorbed through the roots system of crop. The importance of K⁺ availability has been demonstrated to enhance maturity in corn, to prevent premature senescence in cotton and to improve soybean production. To ensure an adequate supply of K⁺, plants have developed multiple mechanisms for K⁺ uptake and translocation. In *Arabidopsis thaliana*, a family of 13 genes named AtKT/KUP (*Arabidopsis thaliana* K⁺ uptake transporter) has been identified as a potential potassium transporter. Among them, only HAK5 shows variation in expression under K⁺ starvation and high affinity K⁺ uptake activity. Considering its importance in K⁺ deprivation signaling, HAK5 promoter was used as a tool to perform a genetic screen for identifying new partners in the low K⁺ signal pathway. A collection of FOX library containing full-length *Arabidopsis* cDNAs expressed independently under the CaMV 35S promoter was transformed with HAK5 promoter fused with a firefly luciferase reporter gene (HAK5 promoter::LUC). After harvesting T1 progeny, transgenic seedlings were screened on selective medium and luciferase activities were measured under full nutrient condition. Since the expression of HAK5 promoter::LUC was only detected under low K⁺ condition, the transformants that have higher LUC activities under full nutrient condition were selected for further studies. Pattern of LUC activity was confirmed in T2 generation of selected lines and induction of endogenous HAK5 gene expression was analyzed by real time PCR. So far, 20 lines displaying strong luciferase activities in roots and/or leaves have been selected from the screening and sequence analyses have been performed. Further characterization of the candidate genes are in progress.

03085

STOMATAL RESPONSE TO GREEN LIGHT IN INTACT LEAVES

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Green light is a major constituent of sunlight and is enriched in the leaf canopy. Green light could have some effects on photosynthesis and transpiration of herbaceous plants that adapt to the canopy light environment. Compared with the stomatal responses to red light or blue light, virtually nothing is known for the stomatal responses to green light. Using intact sunflower leaves, we demonstrated that green light induces stomatal opening (Wang *et al.*, 2008, PCE 31: 1307). We also obtained the data suggesting the existence of a green light receptor. In this study, we used intact leaves of *Arabidopsis thaliana* to examine whether cryptochromes and phototropins are the green light receptors.

Single leaves of ca. 2 cm² from ten-week-old plants of *A. thaliana* (wild-type and mutants) were used for gas exchange measurements with a laboratory-constructed system. Stomatal conductance (Gs) of the whole leaf of wild-type (Columbia) was markedly higher in green light than that in the dark, indicating the stomata of *A. thaliana* respond to green light. Gs of a cryptochrome double mutant (*cry1cry2*) was lower than that of the wild-type, whereas Gs of a phototropin double mutant (*phot1phot2*) was comparable to its background, indicating that cryptochromes rather than phototropins are involved in stomatal green light response.

03086

IDENTIFICATION AND CLASSIFICATION OF ABIOTIC STRESS-RELATED 100 RING E3 UBIQUITIN LIGASES BASED ON THE IN SILICO DATA IN ARABIDOPSIS

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Understanding the cellular roles of environmental stress-induced actions of E3 ubiquitin (Ub) ligases in relation with plant growth and development is an important issue. About 1300 E3 Ub ligases exist in *Arabidopsis thaliana* and each has their specific target substrates. We decided to investigate RING E3 Ub ligases because, among ~ 500 genes, few RING E3 genes have yet been identified to be related with abiotic stresses. Based on the in silico data (<http://www.genevestigator.com>), we selected 100 RING E3 genes, of which expression was changed in response to abiotic stresses, including drought, high salinity, and cold as well as phytohormone ABA. Forty-four genes are strongly regulated by drought stress. Among the 44 genes, 13 are regulated by drought and salt stresses, while 12 genes are regulated by drought and ABA. To examine the gene expression profiles of those RING E3 genes in *Arabidopsis*, mRNA levels of At1g20823, At1g76410, At5g01880, At3g10910, and At2g17450, which belong to the same group of a clade and contain a single membrane anchoring domain, were monitored in response to abiotic stresses in *Arabidopsis*. The expression of At1g20823 was elevated by drought, salt, and cold stress, while At3g10910 was induced by drought and ABA. The At2g17450 gene showed an induction to ABA and At5g01880 showed a little induction to salt stress. At1g76410 was constitutively expressed. These results suggest that RING E3 Ub ligases, which belong to the same group of a clade, are differentially regulated by different abiotic stresses.

03087

CHLOROPLASTIC DEHYDROASCORBATE REDUCTASE 5 IS ESSENTIAL FOR RESPONSE TO PHOTOOXIDATIVE STRESS

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Ascorbate (AsA) is one of the most abundant antioxidants and acts as a redox buffer in higher plants. The AsA recycling systems consisting of monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) are thought to be essential for AsA functions. The genes encoding MDAR (MDAR1-5) and DHAR (DHAR1-5) existed in the *Arabidopsis* genome. To clarify the physiological role of the AsA recycling system in response to photooxidative stress, we characterized single knockout and knockdown *Arabidopsis* mutants lacking MDARs (MDAR2, 3, 4) or DHARs (DHAR3, 5). Among them, the knockout and knockdown mutants lacking DHAR5 (*dhar5-1* and *dhar5-2*, respectively) showed the highest sensitivity to high light (1000 μmol/m²/s) or treatment with methylviologen (50 μM). The redox state of AsA (reduced AsA/total AsA) was significantly lower in the mutant plants than the wild-type plants under photooxidative stress. Interestingly, the redox state of glutathione (GSH) (reduced GSH/total GSH) decreased in the wild-type plants but not in the mutant plants under photooxidative stress, suggesting that DHAR5 is involved in the oxidation of GSH. Under the same conditions, the accumulation of oxidized proteins and the decrease in the activity of photosynthetic electron transport were larger in the mutant plants than the wild-type plants. Moreover, the absence of DHAR5 accelerated drastically the expression of H₂O₂-responsive genes under photooxidative stress. A GFP-fusion of DHAR5 was localized in the chloroplasts of *Nicotiana benthamiana*. The present findings suggest that DHAR5 is essential for response to photooxidative stress via the redox regulation in chloroplasts.

03088

FUNCTIONAL ANALYSIS OF AN AP2 DOMAIN TRANSCRIPTION FACTOR, ATDS1 OF ARABIDOPSIS THALIANA

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ATDS1 containing one AP2 domain, encodes a member of the DREB subfamily A-5 of ERF/AP2 transcription factor family of *Arabidopsis thaliana*. *AtDS1* overexpression (OE) transgenic plant showed small plant size, late flowering, short internode as well as sensitive to drought than wild type plants. Seeds germination and stomatal closure test showed *AtDS1OE* plant was ABA insensitive. *AtDS1* mRNA was mainly induced by dehydration but not induced by ABA. We also found that ABA and proline level of *AtDS1OE* plants were lower than wild type after dehydration treatment. Microarray dataset of *AtDS1OE* plants revealed the possible mechanism of late flowering time, ABA insensitive, and dehydration sensitive phenotype. *FLC*, *CYP707a1* (the ABA 8'-hydroxylase), *ATAF1* and *ABR1* were up-regulated in 35S::*AtDS1* plants, while *SAD2* and *PLDalpha1* (an ABA signal component) was down-regulated making *AtDS1OE* plants exhibited ABA insensitive and not tolerate to dehydration. *ABI2* and *ATPP2CA* were up-regulated causing ABA insensitive in stomatal closure test. *GLN1.1* and *GLN1.3*, two glutamine synthase genes were down-regulated in *AtDS1OE* relating to lower proline level than WT. *AtDS1* protein was located in the nucleus and the transcriptional activation domain was located at C-terminal. These results suggest that *AtDS1* are involved in dehydration stress response and ABA signal transduction pathway. Further investigation will lead to a better understanding of the negative role played by *AtDS1* in the drought response pathway.

03089

RCAR3/PYL8 IS POSITIVE REGULATOR OF ABSCISIC ACID SIGNALING DURING GERMINATION AND ROOT GROWTH

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The phytohormone abscisic acid (ABA) regulates physiologically important developmental processes and stress responses. The functional protein phosphatase type 2C (PP2C) were a negative regulator of ABA signaling in seeds. Recent identification of soluble ABA receptors, regulatory compound of ABA receptors (RCARs)/pyrabactin resistances (PYRs), have provided a major breakthrough in understanding the signaling mechanisms of ABA and revealed the importance of PP2Cs. By transient expression of RCAR3/PYL8 fused with GFP in onion (*Allium cepa*) epidermal cells, we obtained evidence supporting the subcellular localization of this protein mainly in both the cytosol and the nucleus, respectively. Constitutive overexpression of RACR3/PYL8 confers ABA hypersensitivity in *Arabidopsis* seeds. *Arabidopsis* 3S: PCAR3/PYL8 transgenic plants showed a strong inhibition of early root growth as compared with the wild type. On the contrary, constitutive RNAi of RCAR3/PYL8 showed a resistance of germination and root growth in several ABA concentration media. Both overexpression and RNAi transgenic plants confirm that RCAR3/PYL8 positively regulates ABA signaling during germination and root growth.

03090

TOXICITY OF THALLIUM(I) ACETATE IN *ARABIDOPSIS THALIANA*, L.

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Thallium is naturally occurring trace element widely distributed in earth's crust but at very low concentration. Due to its high toxicity to all living organisms, thallium pollution and its impact on food chain quality have become increasing environmental concerns. Increasing use in emerging new technologies and demanding high-tech industry constantly raise the concern about exposure risk to all living organisms. However, it has not been studied extensively and published toxicity studies are scarce.

In order to study the toxicity effect of thallium(I) acetate, seeds of *Arabidopsis thaliana* were allowed to germinate on agar plates containing different thallium(I) acetate concentration. Following a three week dose-response assay, the inhibition of seedlings growth was proportional to the increase of thallium concentration. A random amplified polymorphic DNA (RAPD) assay was employed to detect DNA damage in response to thallium toxicity. For the DNA polymorphism analysis, sixty 10-base pair random primers with 60-70% GC content were screened. Among them, ten RAPD primers, that gave uniformly reproducible bands were chosen for further evaluation of thallium genotoxicity. Since the major drawback of the RAPD technique as a PCR-based method, is the reproducibility of the fingerprints banding, different concentration of DNA template were tested. In conclusion, after suitable optimization of PCR procedure as well as primer choice, the RAPD assay could detect DNA alteration in response to thallium exposure. These results show changes in intensity, gain and loss of bands due to thallium exposure.

03091

ARABIDOPSIS ZAT6 AFFECTS POTASSIUM/SODIUM HOMEOSTASIS TO ENHANCE TOLERANCE TO SALT-STRESS

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Salinity is one of the plant abiotic stresses that would obviously affect not only the growth, morphology but the yield. Among the salt stress, various genes would regulate for this physiological changes. In our laboratory, we found one of the C2H2 type zinc finger protein, Zat6, might be early-response to various abiotic stresses and was chosen for further study.

Among ZAT6, ZAT7 and ZAT10 proteins, there are one conserved L/FDLNL/F(x)P motif (also known as EAR-motif) as repressor domain in their C-terminal. These motif were first identified in class II AP2/ERFs (Apetala2/Ethylene Response Factor) and C2H2-type zinc-finger proteins (Ohta et al., 2001). The mechanism of repressor domain is not yet well known. Deletion or mutation in the EAR-motif of Zat7 abolishes salinity tolerance without affecting growth suppression which demonstrated that the EAR-motif of Zat7 is directly involved in enhancing the tolerance of transgenic plants to salinity stress (Ciftci-Yilmaz et al., 2007). Overexpression of ZAT6 in *Arabidopsis* was reported to regulate root development and phosphate homeostasis, while RNAi knockdown of ZAT6 was lethal (Ballachanda et al., 2007). The root abundant phenotype was observed in serial deleted promoter-derived GUS transgenic lines. Because ZAT6ox confer salt stress but not oxidative stress, we proposed that ZAT6 functions primarily in root by affecting ion homeostasis. The results positively show that ZAT6 reduces the accumulation of sodium ion after treatment. Further, we performed microarray and proteomic analysis to compare wild-type with ZAT6ox transgenic line. We group the identified genes by GO term and several genes were up- or down-regulated in response to stress between WT and ZAT6ox line under normal growth condition. Extra efforts are needed to verify how ZAT6 affects these genes to accomplish tolerance to salt-stress.

03092

ATPUB18 AND *ATPUB19* ARE INVOLVED IN ABA SIGNALING PATHWAY AS A NEGATIVE REGULATOR IN *ARABIDOPSIS*.

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As sessile organisms, plants have developed elaborate regulation mechanisms to adapt to various abiotic stresses. For fast and effective regulation, higher plants make use of the phytohormone ABA, a well-known mediator in responses to stresses. Plants have a large number of U-box E3 ubiquitin (Ub) ligases compared to humans and yeasts. This implies that U-box E3 Ub ligases may have plant-specific functions. We identified two ABA-induced genes, *AtPUB18* and *AtPUB19*, which encode U-box E3 Ub ligases in *Arabidopsis*. Both genes were upregulated not only by ABA treatment but also by broad spectrum of abiotic stresses. To explore gene expression patterns, each promoter of *AtPUB18* and *AtPUB19* was fused to a β -glucuronidase gene and introduced into *Arabidopsis*. The results show that these two genes were expressed in leaves, hydathodes, and roots under drought stress and ABA treatment. Notably, expression of these two genes was detected in stomatal guard cells. For loss-of-function assay, we obtained *atpub18* and *atpub19* single knock-out mutant lines and subsequently generated *atpub18atpub19* double knock-out mutant plant. These mutant plants displayed ABA hypersensitive phenotypes, such as increased stomatal closure and decreased germination ratio, relative to wild type plant. In addition, mutant plants were significantly tolerant to severe drought stress. For gain-of-function assay, we generated *AtPUB18* and *AtPUB19* over-expressing transgenic plants. These transgenic plants had reduced ABA sensitivity. Stomatal closure was decreased and germination ratio was higher compared to wild type plant under ABA treatment. Dehydration tolerance was also reduced in *AtPUB18*- and *AtPUB19*-overexpressing plants. These results indicate that *AtPUB18* and *AtPUB19* are involved as a negative regulator in ABA signaling pathway in *Arabidopsis*.

03093

IDENTIFICATION OF GENES INVOLVED IN OSMOTIC STRESS RESISTANCE USING ACTIVATION TAGGING SYSTEM IN ARABIDOPSIS

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In spite of many studies, there still remain unknown genes related to plant stress response. In this study, *Arabidopsis* genes involved in osmotic stress resistance were identified using activation tagging system. To do this, an activation tagging vector, pFGL942, containing four copies of CaMV35S enhancer and basta-resistant gene as a selectable marker was constructed and transformed into wild-type *Arabidopsis*. Approximately 6,000 T₁ activation tagging lines have been generated and among them 2,705 lines were screened to select salt-resistant lines. So far, 19 lines showed salt resistance at seed germination and/or at seedling stages in both T₂ and T₃ generations. Interestingly, 9 lines among the 19 lines showed significant salt resistance only at germination, not at seedling stage whereas other 10 lines were salt-resistant at both developmental stages, suggesting that a different set of genes might be involved in osmotic stress resistance at each of germination and seedling stages. The flanking sequences of T-DNA inserts of 11 lines among the 19 lines were recovered by TAIL-PCR and the genes activated by the CaMV35S enhancers were determined using RT-PCR. Until now, 5 activated genes encoding a DEAD box RNA helicase, a RNA-binding protein and so on were identified and their expression characteristics were analyzed.

03094

THE ROLE OF CYCLIC ADENOSINE MONOPHOSPHATE (cAMP) IN ARABIDOPSIS THALIANA

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Adenosine 3',5'-cyclic monophosphate (cAMP) is a ubiquitous signalling intermediate that regulates many intracellular processes in plant, particularly in response to environmental stress. This second messenger is synthesized from adenosine 5'-triphosphate (ATP) by adenylyl cyclase (AC). The *Arabidopsis* genome encoded two genes, At3g14460 (*AtAC1*) and At3g14470 (*AtAC2*) with high homology to maize AC and contain N-terminal nucleotide binding domain characteristic of adenylyl cyclases. Expression of *AtAC1* and *AtAC2* complemented *E.coli* cAMP mutant, *cya*, suggesting that both proteins produce functional cAMP in vivo. Gene expression studies using GUS staining and RT-PCR showed that *AtAC1* is expressed in root tips, inflorescence and anther while *AtAC2* is expressed in caulin, senescent, roots and inflorescence. T-DNA insertion mutants of *AtAC1* and *AtAC2* showed their phenotypes during germination under salt stress suggesting the role of cAMP as a signalling intermediate in salinity tolerance.

03095

TGA1 AND TGA4 TRANSCRIPTION FACTORS CONTROL NITRATE RESPONSES IN ARABIDOPSIS THALIANA ROOT ORGANS

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Nitrate is one of the most important sources of nitrogen (N) in agricultural soils. Nitrate regulates plant root morphology and acts as a potent signal to control global gene expression in *Arabidopsis*. However, the mechanisms involved in regulating gene expression in response to nitrate in plants are still mostly unknown. We used a bioinformatics approach to identify regulatory factors that mediate nitrate responses in *Arabidopsis*. Our bioinformatics strategy produced a ranking of genes based on their "nitrate regulatory potential". The top candidate of our ranking was TGA1, a bZIP transcription factor. TGA4, is a closely related member of the bZIP family that was also found in the ranking. Both TGA1 and TGA4 mRNAs accumulated strongly and quickly after nitrate treatments. To evaluate the function of these transcription factors, we analyzed tga1/tga4 double mutant phenotypes under different N-nutrient conditions. The tga1/tga4 double mutant showed a shorter primary root than wild-type plants grown in a medium containing a sufficient amount of nitrate. To understand the molecular basis of this phenotype, we performed transcriptomic analysis to evaluate the effect of nitrate in the wild type and tga1/tga4 double mutant plants using the Affymetrix ATH1 chip. Interestingly, 97% of the genes that depend on TGA1/TGA4 for normal expression are regulated by nitrate treatments. Among the nitrate-responsive genes that depend on TGA1/TGA4, we found the nitrate transporters NRT2.1, NRT2.2 and the nitrite reductase genes. These genes are key for nitrate uptake and reduction. Using chromatin immunoprecipitation assays we discovered that TGA1 is bound to NRT2.1 promoter in a nitrate-dependent manner. These results indicate that TGA1 and TGA4 are important regulatory factors mediating the nitrate response of *Arabidopsis* root organs.

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03096

FUNCTIONAL ANALYSIS OF OSMOTIC STRESS-ACTIVATED SUBCLASS II SNRK2 PROTEIN KINASES IN ARABIDOPSIS

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SNF1-related protein kinases 2 (SnRK2s) are activated by ABA- and/or osmotic stress and are involved in stress signaling pathways in plants. Ten members of *Arabidopsis* SnRK2 are classified into three subclasses. Recently, three subclass III SnRK2s including SRK2E/OST1/SnRK2.6 were shown to play crucial roles in ABA signal transduction pathways. However, the functions of other two subclasses of SnRK2s are still unclear. In this study, we studied in planta functions of the subclass II SnRK2s (SRK2C/SnRK2.8 and SRK2F/SnRK2.9) in *Arabidopsis*. To analyze their functions, we established the *srk2cf* double mutant. Microarray analysis showed that the many ABA-inducible genes were downregulated in *srk2cf* under drought stress conditions. However, those genes showed normal response to ABA even in *srk2cf*, suggesting that the subclass II SnRK2s mediate drought stress signaling significantly, but that their roles are only supportive in ABA-dependent pathway. We could not observe significant phenotypes of *srk2cf*. We will discuss the role of the subclass II SnRK2s in *Arabidopsis*.

03097

OSGAP1 FACILITATES VACUOLAR TRAFFICKING OF V-ATPASE SUBUNIT VHA-A UNDER SALT STRESS CONDITION

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Vacuolar H⁺-ATPases are significantly involved in vacuolar pH regulation, and endocytic trafficking. The molecular mechanism of the regulation is almost unknown in plant until now. To understand this mechanism, we attempted to screen some regulatory proteins as an interaction partner of OsVHA. Here, we show that the N-terminus of rice VHA-a subunit interacts with OsGAP1, a GTPase-activating protein for the Ypt/Rab family of small G-proteins, and they colocalizes into the TGN and/or prevacuolar compartment. Moreover, it was also demonstrated that the GAP domain as well as a C-terminal region of OsGAP1 contributed to efficient binding with OsVHA. In case of the OsGAP1 deletion mutant defective of two binding domains, green fluorescent protein tagged OsVHAs(OsVHA-GFP) were not delivered to the central vacuole, but diffused into the cytoplasm. From this, it was confirmed that GAP1 is essential in the trafficking of OsVHA from the prevacuolar compartment to the central vacuole. From the previous report on the major role of vacuolar H⁺-ATPases in ion and/or nutrient transport and in salt tolerance mechanism, our result supported that OsVHA could play some important roles in maintaining cytosolic Na⁺ homeostasis in rice. RT-PCR analysis showed that amounts of OsGAP1 and OsVHA transcripts were increased immediately in roots under salt stress. Furthermore, in the immunoprecipitation assay, it was assumed that the enhanced interaction between OsGAP1 and OsVHA under the same condition may facilitate vacuolar trafficking of OsVHA. In conclusion, it was indicated that VHA could adjust ion imbalance by interacting with OsGAP1 during salt stress through sequestration of it to vacuole.

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03098

IDENTIFICATION OF TRANSCRIPTION FACTORS INVOLVED IN OZONE TOLERANCE IN *ARABIDOPSIS THALIANA*.

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The tropospheric ozone, which is the major component of photochemical oxidants, is considered as the most phytotoxic air pollutant. A lot of studies have provided important information on understanding ozone stress response and mechanism. High concentration of ozone causes significant damage in plant growth and induces oxidative stress that activates programmed cell death. It has been proven that ozone triggers the hormone-signaling network, such as ethylene, salicylic acid and jasmonic acid, and gene expression associated with pathogen infection. Therefore, analysis of the responsive pathway of ozone would be related to biotic and abiotic stress response in plants. However, detailed molecular mechanisms of this subject remained to be clarified, and there were no reports regarding ozone-tolerant mutants. In this study, we applied our CRES-T system, in which a transcription factor is converted into a strong repressor, to identify transcription factors involved in ozone stress response. CRES-T is gene silencing technology, which could efficiently suppress functions of both target and functionally-redundant transcription factors. We identified three candidate genes, of which CRES-T transgenic plants exhibit tolerance to ozone stress. Among them, HR0294 plants that exhibited a higher tolerance to ozone also had tolerances to paraquat (methyl viologen) and SO₂, which also cause oxidative stresses. In HR0294 plants, O₂⁻ accumulation was reduced and steady-state mRNA levels of superoxide dismutases (SODs) in the chloroplasts were higher than in wild-type (Col-0). Our results indicate that the tolerance of HR0294 to oxidative stress is in part due to enhanced activities of the chloroplastic SODs.

03099

IDENTIFICATION OF WRKY TRANSCRIPTION FACTORS INVOLVED IN SULFUR DEFICIENCY INDUCIBLE SULFATE UPTAKE ACTIVITY

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SULTR1;2 high-affinity sulfate transporter facilitates sulfate uptake from the environment. In *Arabidopsis*, expression of *SULTR1;2* was induced upon sulfur deficiency (-S), which enables the plants to survive under -S condition. The -S response of *SULTR1;2* is controlled by SLIM1 transcription factor. Previously, we have identified *cis*-acting element involved in the -S-inducible expression of *SULTR1;2*, which contains WRKY binding sequence. In this study, we have tried to identify the WRKY transcription factors that control -S-inducible expression of *SULTR1;2*. Among the 72 members of WRKY transcription factor in *Arabidopsis*, 3 members exhibited the up-regulated transcript levels by -S in the wild type plants but not in *slim1* mutant. Then we isolated T-DNA insertion mutants of 3 WRKY members, and analyzed *SULTR1;2* expression and sulfate uptake activity. Both were significantly down-regulated under -S condition in these mutants comparing with wild-type plants, suggesting the involvement of these WRKY transcription factors in -S-inducible expression of *SULTR1;2* and sulfate uptake.

03100

FUNCTIONAL ANALYSIS OF A NITROGEN SUPPLY-INDUCIBLE TRANSCRIPTION FACTOR, OSMYB-NR1, IN RICE

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Supply of nitrogen, one of macronutrients, affects expression levels of numerous genes in plants. Previously, we performed transcriptome analysis using rice seedlings treated with ammonium nitrate to identify nitrogen supply-inducible genes in rice, and then identified two genes encoding putative nitrogen-inducible transcription factors (TFs), OsMYB-NR1 and OsMYB-NR2. Here we show characterization of OsMYB-NR1. RT-PCR analysis revealed that the expression of *OsMYB-NR1* was specifically induced by nitrate but not ammonia and that rapidly induced expression of *OsMYB-NR1* gradually reduced even in the presence of nitrate. We also detected the expression of *OsMYB-NR1* in various tissues, including seed, leaf sheath and blade, root, and panicle, suggesting that OsMYB-NR1 may be a ubiquitous TF. To investigate whether OsMYB-NR1 really functions as a TF, we investigated its subcellular localization using green fluorescence protein. DNA-binding activity was also investigated by the DNA-binding site selection experiment and electrophoresis mobility shift assays. Because the results of these analyses suggested that OsMYB-NR1 bind DNA in a sequence-specific manner in nuclei, we investigated its activity as a TF using a reporter plasmid that contains the identified sequence fused to the 35S minimal promoter. By protoplast transient assay using the reporter plasmid and two expression vectors for native OsMYB-NR1 and OsMYB-NR1 fused to the VP16 transcriptional

activation domain, it was shown that the expression of OsMYB-NR1 reduced expression of the reporter gene whereas OsMYB-NR1-VP16 fusion protein activated transcription from the synthetic promoter, suggesting that OsMYB-NR1 functions as a repressor. Furthermore, we found putative OsMYB-NR1 binding sites in the *OsMYB-NR1* promoter, and indicated that OsMYB-NR1 represses its own promoter activity. Based on these results, we will discuss a possible feedback regulation mediated by OsMYB-NR1.

03101

COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF METABOLIC ALTERATIONS BY P STARVATION IN PLANT ROOTS.

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Plants have developed many strategies to grow under low P conditions. To clarify the differences among plant species in strategies to grow under low P conditions, comparative transcriptomic analysis for roots of *Arabidopsis thaliana*, rice (*Oryza sativa*), *Lotus japonicus* and soybean (*Glycine max*) was performed. Plants were hydroponically cultured with or without P as +P and -P treatments, respectively. The roots were harvested to use for transcriptomic analysis. Oligonucleotide arrays provided by Agilent or Filgen were used for the transcriptomic analysis.

Many genes involved in known strategies to uptake P such as acid phosphatases and Pi transporters were commonly up-regulated by -P in all plants. It is considered that these genes contribute to efficient P uptake. It was also found that the lipid metabolism, such as sulfolipids and glycolipids synthesis, was commonly altered. The increase of non-phospholipids in -P conditions could contribute to internal P recycling.

The difference of metabolic alteration in carbon metabolisms was found between legumes and non-legume plants. Whereas the stimulation of glycolysis in -P roots of *Arabidopsis* and rice to maintain the supplement of carbon skeletons were suggested, the alteration of the genes involved in glycolysis and TCA cycles were not indicated in -P roots of legume plants. This fact suggests that the strategy of legume plants in carbon metabolism is different from non-legume plants.

The alteration of secondary metabolisms was specific in each plant species. The alteration of genes involved in flavonoid synthesis was specific in legume plants. Whereas the synthesis of isoflavones was repressed in -P *Lotus* roots, it was stimulated in -P soybean roots. These results may imply the importance of the secondary metabolites in the rhizosphere control by the legume-specific root exudates.

03102

PHYSIOLOGICAL AND MOLECULAR-GENETIC STUDY OF PLAGIOGRAVITROPISM IN LATERAL ROOTS OF ARABIDOPSIS

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Growth directions of lateral organs are major determinant of architecture of a plant body. Lateral roots of *Arabidopsis* temporary grow obliquely relative to the gravity vector after the initiation and then grow downward, whereas the transition from oblique to downward growth direction of mutant *hy5* is retarded. We study molecular mechanism controlling the growth directions of lateral roots by analyzing *hy5*. In order to quantitatively describe plagiogravitropic reorientation, we built a mathematical model by expressing bending rate as a linear combination of sine and cosine of deviation of growth direction from gravity vector. The model fitted well, implying that a mechanism sensing the radial outer- and apical component of gravity independently controls the bending rate. We further estimated gravitropic set-point angle (GSA) based on the model and found GSA declined with root elongation, and the decline was retarded in *hy5*. Because many auxin-responsive genes are up-regulated in *hy5*, we asked whether auxin is involved in growth direction of lateral roots. An application of anti-auxin BH-IAA caused lateral roots of *hy5* to grow more downward direction, suggesting auxin sustains oblique growth direction. However, IAA concentration at root tips was not significantly different between *hy5* and wild-type or among roots with various growth directions. Because part of ABA signaling is decreased in *hy5*, we also asked whether ABA is involved growth direction of lateral roots. ABA application caused lateral roots of *hy5* to grow more downward direction, and expression level of ABA-responsive gene *RD29A* at root tips was lower in *hy5* than wild-type. ABA signal at root tips of lateral roots may promote transition to downward growth direction. We also screened for revertants of *hy5* with earlier transition from oblique to downward growth in lateral roots and found a candidate line for the revertants.

03103

DISTINCT FUNCTIONS OF *BOR1* FAMILY IN BORON TRANSPORT IN *A. THALIANA*

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Boron (B) is an essential trace element for plants. Borate crosslinking of rhamnogalacturonan-II (RG-II) in cell walls is a primary physiological role of B in plants. Both B deficiency and toxicity have negative effects on plant growth and development. *Arabidopsis thaliana* *BOR1* is an efflux transporter of B that is required for the xylem loading under B limitation. Six *BOR1* homologous genes (*BOR2-7*) are present in the *A. thaliana* genome. Here we present their distinct functions for B nutrition.

T-DNA insertion in *BOR2*, the most similar gene to *BOR1*, impaired root cell elongation rather than shoot growth under the limited supply of B. Severer growth retardation both in roots and shoots was observed in the double T-DNA mutant of *BOR1* and *BOR2*. All of these lines grew normally under B sufficiency. These results suggest that *BOR2* has at least in part distinct function from *BOR1*. Total B concentrations in roots in *BOR2* mutant were not different from that of the wild type plants, however, dimerization of RG-II-B was reduced in these mutant plants under low B supply. It is likely that borate transport by *BOR2* from symplasts to apoplasts or into vesicles is required for effective dimerization of RG-II-B and promotes root cell elongation under B limitation.

BOR3 mutant plants did not show an apparent phenotype, but a triple T-DNA insertion mutant line of *BOR1*, *BOR2* and *BOR3*, exhibited more severe root growth defects under low B compared to the double mutant of *BOR1* and *BOR2*, suggesting its supportive role.

It is previously reported that *BOR4* is free from B-dependent protein degradation which down-regulates *BOR1* under high B and enhanced expression of *BOR4* improved high B tolerance. Slight growth reduction was observed under toxic level of B supply in T-DNA insertion mutants of *BOR4*, further supporting the role of *BOR4* for high B tolerance.

03104

FUNCTIONAL CHARACTERIZATION OF ARABIDOPSIS DJ-1A

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DJ-1 superfamily includes a broad spectrum of proteins characterized by having a highly conserved DJ-1 domain. DJ-1 was first identified as an oncogene in mouse and has recently been identified as the causative gene of familial Parkinson's disease. DJ-1 has multiple functions in transcriptional regulation, apoptosis and molecular chaperone. Although several plant genes containing DJ-1 like sequence have been identified, no other information on these proteins is available to date. In this study, we report the functional characterization of a cDNA encoding Arabidopsis DJ-1A (AtDJ-1A). AtDJ-1A is a 1167 bp cDNA with an open reading frame to encode a 389 amino acid protein with molecular weight of 41 kDa. AtDJ-1A contains two tandem arrays of DJ-1 like sequences, which is different from the other known DJ-1 superfamily members. Transcripts of AtDJ-1A increased during heat stress. AtDJ-1A protein exhibits a chaperone activity preventing thermal aggregation of malic dehydrogenase and a refolding activity renaturating the unfolded glucose-6-phosphate dehydrogenase. Furthermore, H₂O₂ treated AtDJ-1A protein showed higher chaperone activity than the native protein.

03105

NITRATE GROWTH RESPONSE OF HYDROPONICALLY GROWN ARABIDOPSIS THALIANA

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Improving nitrogen (N) use efficiency in crop plants is a way of reducing both the costs and detrimental environmental effects associated with nitrogen fertilisation. Generally, cereal crops are not efficient at acquiring N from the soil or utilising N once in the plant. To improve these processes we need to better understand how nitrogen is taken up and utilised by plants.

We know relatively little of how the nitrogen uptake processes in plants are regulated or how they respond to N limitation. The most well known system is that in *Arabidopsis thaliana*. However, much of the work with nitrogen in Arabidopsis has been carried out growing plants on solutions derived from tissue culture work and some with very high nitrate concentrations (up to 20 mM). To be able develop an assay to study the response of the Arabidopsis nitrogen transport system to N limitation we characterised the response of a number of Arabidopsis ecotypes to a range of nitrate concentrations.

Arabidopsis plants (Col_0, C24 and WS) were grown hydroponically in an 'ebb and flow' nutrient delivery system where plants can be grown to maturity at regulated concentrations of nitrate as low as 0.1 mM. Nitrate concentrations in the hydroponic solutions were maintained at 0.1, 0.2, 0.4 and 2.0 mM and plants grown for 5 weeks before harvesting and biomass measurement.

Our results were surprising in that shoot growth had reached its maximum at the relatively low nitrate concentration of 1 mM. The response to nitrate was also seen in the root:shoot ratio where below 0.4 mM nitrate the root as a proportion of shoot biomass was consistently higher.

These results suggest that studies investigating nitrogen uptake characteristics in Arabidopsis should use much lower nitrogen concentrations than are currently used.

03106

ATHAK5 AND AKT1 ARE REQUIRED FOR SEEDLING ESTABLISHMENT AND POST-GERMINATION GROWTH UNDER LOW K⁺ CONDITIONS

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Potassium (K⁺) is a major plant nutrient required for plant growth and development. Plant roots absorb K⁺ through uptake systems operating at low concentrations (high-affinity transport) and high external concentrations (low-affinity transport). To understand the molecular basis of high-affinity K⁺ uptake in *Arabidopsis*, we analyzed loss of function mutants in AtHAK5 and AKT1, two transmembrane proteins active in roots. Compared to wild-type under NH₄⁺-free growth condition, *athak5* mutant plants exhibited growth defects at 10 μM K⁺ but at K⁺ concentrations of 20 μM and above *athak5* mutants were visibly indistinguishable from the wild-type. While germination was only slightly decreased in *athak5 akt1* double mutants on low K⁺ media, double mutants failed to grow on media containing up to 100 μM K⁺ and growth was impaired at concentrations up to 450 μM K⁺. Interestingly, *athak5 akt1* double mutants are more sensitive to NH₄⁺ and Na⁺ than wild-type at intermediate K⁺ concentrations. Determination of Rb⁺(K⁺) uptake kinetics in wild-type and mutant roots using ⁸⁶Rb⁺ as a tracer for potassium revealed that high-affinity Rb⁺(K⁺) uptake into roots is almost completely abolished in double mutants and impaired in single mutants. These results strongly indicate that ATHAK5 and AKT1 are the two major, physiologically relevant molecular entities mediating high-affinity potassium uptake into roots during seedling establishment and post-germination growth. [This work was supported by the Crop Functional Genomics Center funded by the Ministry of Science and Technology of the Korean government (grant no. CG2152) and the Second Phase of the BK21 program (YBRI) of Korea]

03107

DISSECTING THE GENETIC CONTROL OF NATURAL VARIATION IN SALT TOLERANCE OF ARABIDOPSIS THALIANA ACCESSIONS

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Many accessions (ecotypes) of *Arabidopsis* have been collected. Although few differences exist among their nucleotide sequences, these subtle differences induce large genetic variation in phenotypic traits such as stress tolerance and flowering time. To understand the natural variability in salt tolerance, we performed large-scale soil pot experiments to evaluate salt tolerance among 350 *Arabidopsis thaliana* accessions. The evaluation revealed a wide variation in the salt tolerance among accessions. Several accessions, including Bu-5, Bur-0, LI-1, WI-0, and Zu-0, exhibited marked stress tolerance compared with a salt-sensitive experimental accession, Col-0. The salt-tolerant accessions were also evaluated by agar-plate assays. The data obtained by the large-scale assay correlated well with the results of a salt-acclimation (SA) assay, in which plants were transferred to high-salinity medium following placement on moderate-salinity medium for 7 days. Genetic analyses indicated that the salt tolerance without SA is a quantitative trait under polygenic control, whereas salt tolerance with SA is regulated by a single gene located on chromosome 5 that is common among the markedly salt-tolerant accessions. These results provide important information for understanding the mechanisms underlying natural variation of salt tolerance in *Arabidopsis*.

03108

ARABIDOPSIS LTP3 IS INVOLVED IN WOUND-INDUCED JASMONATE SIGNAL TRANSMISSION ALONG STEM

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Plant non-specific Lipid transfer proteins are a group of small and basic proteins having four disulfide bonds with eight conserved cysteine residues and a hydrophobic cave inside with a lipid binding ability. They are regarded to exert lipid binding function on flower development, cell wall extension, pathogen resistance, and systemic signal delivering. LTP3 is one of the non-specific lipid transfer proteins and it was considered to be a defense related protein with ABA-responsive gene expression. LTP3 promoter GUS fusion protein was detected in guard cell, inflorescence, cauline leaf, mid vein, stem and developing lateral root. Subcellular analysis using green fluorescent protein fused with LTP3 showed that it co-localized with endosome marker FM4-64. We found that wound-responsive jasmonate-related gene expression in systemic cauline leaves was impaired in *ltp3* knockout mutant, although no obvious phenotype was observed in rosette leaves. These evidences suggest that LTP3 is involved in wound-responsive systemic signal transmission particularly in stem region.

03109

GLUTATHIONE S-TRANSFERASE, AT1G10370, A NEW COMPONENT OF ABA SIGNALING PLAYS POSITIVE AND NEGATIVE ROLE IN ABIOTIC STRESS

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Glutathione S-transferase family has 63 members and plays major role in oxidative stress metabolism whereas no observation so far was provided to support a role on the regulation in droughted plants. A Glutathione S-transferase gene (At1g10370) was found in this study when mutated making the plant tolerant to drought and salt stresses but impaired in oxidative stress tolerance. The knock-out plant, *At1g10370* exhibited higher ABA accumulation, smaller stomata aperture, lower transpiration rate, higher glutathione content, better development of primary and lateral root system as well as longer vegetative growth in normal growth condition. *At1g10370* is mainly expressed in the guard cell, primary and lateral root primordia in addition to the vascular tissue, especially enhanced in the stressed condition contributing to open stomata and limited root growth in over-expressed plants. Under ABA treatment, the knockout plants are hyposensitive in seed germination, and have much profound root system than wild type. These stress adaptations contribute to an advantage to Arabidopsis in the later stage of water-limited environments. *At1g10370* may involve in different signaling pathways, including one in the ABA signaling upstream to the AREB1 pathway. We propose a negative regulatory role of *At1g10370* in the adaptive responses to drought and salt stresses, functioning as a modulator of the ABA-mediated signal transduction pathways.

03110

FUNCTIONAL CHARACTERIZATION OF CALRETICULIN GENE IN ARABIDOPSIS THALIANA

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The endoplasmic reticulum (ER) serves a platform for the folding and modification of membrane-associated and secreted proteins in eukaryotic cells. Since misfolded proteins may threaten the cell viability, those proteins are degraded in 26S proteasome of the cytoplasm that is called ER-associated degradation (ERAD). The unfolded protein response (UPR) is elicited by dysfunction of the ER which is a cellular organelle equipped with well-orchestrated protein folding machinery composed of a variety of proteins. Sensor proteins detecting the occurrence of misfolded proteins and protein chaperones helping the re-folding of those proteins participate in the surveillance mechanism called "Quality control" to prevent the accumulation of misfolded proteins that are not functioning properly. In order to understand the function of calreticulin (CRT) in plant growth and development, we isolated a triple knockout mutant, designated t123, lacking CRT1, CRT2 and CRT3 genes and present its characterization with respect to abiotic stress tolerance.

03111

MULTIPLE FUNCTION OF ARABIDOPSIS THALIANA TPR-CONTAINING THIOREDOXIN, ATTDX IN A STRUCTURE-DEPENDENT.

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We found that Arabidopsis AtTDX, a heat-stable and plant-specific thioredoxin (Trx)-like protein, exhibits multiple functions, acting as a disulfide reductase, foldase chaperone, and holdase chaperone. The activity of AtTDX, which contains 3 tetratricopeptide repeat (TPR) domains and a Trx motif, depends on its oligomeric status. The disulfide reductase and foldase chaperone functions predominate when AtTDX occurs in the low molecular weight (LMW) form, whereas the holdase chaperone function predominates in the high molecular weight (HMW) complexes. Because deletion of the TPR domains results in a significant enhancement of AtTDX disulfide reductase activity and complete loss of the holdase chaperone function, our data suggest that the TPR domains of AtTDX block the active site of Trx and play a critical role in promoting the holdase chaperone function. The oligomerization status of AtTDX is reversibly regulated by heat shock, which causes a transition from LMW to HMW complexes with concomitant functional switching from a disulfide reductase and foldase chaperone to a holdase chaperone. Overexpression of AtTDX in Arabidopsis conferred enhanced heat shock resistance to plants, primarily via its holdase chaperone activity.

03112

COMPARATIVE ANALYSIS OF TWO CYTOSOLIC HEAT SHOCK PROTEIN 90S IN ARABIDOPSIS

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The 90-kDa heat shock protein (Hsp90) is an abundant and highly conserved molecular chaperone that is essential for cell survival. A search for Hsp90 sequences in the *Arabidopsis thaliana* genome revealed that this family includes 7 members localized differentially. Among them, AtHsp90.1 to AtHsp90.4 is localized in cytoplasm. Despite the abundant information on animal Hsp90, there is minimal data on functional activity of *Arabidopsis* cytosolic Hsp90 proteins. Here, we carried the comparative analysis of two *Arabidopsis* cytosolic Hsp90, AtHsp90.1 and AtHsp90.3. AtHsp90.3 showed higher holdase and foldase chaperone activity than AtHsp90.1, although both of AtHsp90 proteins exhibited an effective chaperone activity. Using size-exclusion chromatography, we found that two proteins showed different oligomeric states, even though they had a high sequence similarity (86%). While AtHsp90.1 existed as various oligomeric forms such as monomer, dimer and high oligomer, AtHsp90.3 exists mainly as high oligomeric form. To determine the functional differences between each oligomeric forms of AtHsp90.1, chaperone activity was examined. High oligomeric forms of AtHsp90.1 showed higher activity than monomer or dimer forms. In addition, ATPase activity of AtHsp90.3 was also higher than that of AtHsp90.1. Thus, we concluded that high oligomeric Hsp90 may increase the capacity of substrate binding, and furthermore, increase the chaperone activity.

03113

ARABIDOPSIS MYB60 PLAYS AN IMPORTANT ROLE IN ABIOTIC STRESS TOLERANCE VIA ITS INVOLVEMENT IN STOMATAL REGULATION AND ROOT GROWTH

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In response to environmental challenges, several signaling pathways that trigger expression of transcription factors are activated in plant cells. *Arabidopsis* MYB60 was reported to be involved in stomatal regulation under drought conditions. Here, we show that two splice variants are generated from the MYB60 gene and that both play a crucial role in stomatal closure and subsequent drought tolerance. This role was demonstrated by over-expressing each variant, which conferred enhanced sensitivity to water deficit stress. The second splice variant of MYB60, which lacks the first exon and whose gene product localizes to the nucleus, also promoted guard cell deflation in response to water deficit. Moreover, MYB60 is expressed in root tissues when plants are exposed to auxin but not other plant hormones, which appears to contribute to altered root growth. Analysis of microarray data sets revealed that a diverse array of genes exhibits altered expression under drought stress in MYB60.2-ox plants that overexpress the second splice variant. MYB60.1-ox and MYB60.2-ox plants exhibited enhanced root growth in response to mannitol as compared to the wild type plants. Taken together, these data indicate that MYB60 plays a dual role in abiotic stress tolerance in *Arabidopsis*, through its involvement in stomatal regulation and root growth.

03114

HEAT SHOCK-DEPENDENT STRUCTURAL AND FUNCTIONAL SWITCHING OF THIOREDOXIN H-TYPE 3 IN ARABIDOPSIS THALIANA

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A large number of thioredoxins (Trxs), small redox proteins, have been identified from all living organisms. However, many of the physiological roles played by these proteins remain to be elucidated. We isolated a high molecular weight (HMW) form of h-type Trx from the heat-treated cytosolic extracts of *Arabidopsis* suspension cells and designated it as AtTrx-h3. Using bacterially expressed recombinant AtTrx-h3, we find that it forms various protein structures ranging from low and oligomeric protein species to HMW complexes. And the AtTrx-h3 performs dual functions, acting as a disulfide reductase and as a molecular chaperone, which are closely associated with its molecular structures. The disulfide reductase function is observed predominantly in the LMW forms, whereas the chaperone function predominates in the HMW complexes. The multimeric structures of AtTrx-h3 are regulated not only by heat-shock but also by redox status. Two active Cys residues in AtTrx-h3 are required for disulfide reductase activity, but not for chaperone function. AtTrx-h3 confers enhanced heat-shock tolerance in *Arabidopsis*, primarily through its chaperone function.

03115

A NOVEL TYPE OF ARABIDOPSIS MYB TRANSCRIPTION FACTOR MYB-H POSITIVELY REGULATES HYPOCOTYL ELONGATION IN THE LIGHT

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Complicated endogenous and exogenous signals exhibit coordinated regulation of plant growth and development. We were interested in MYB genes that are involved in the regulation of abiotic stress tolerance. Searching publicly available microarray analysis allowed us to identify several MYB genes showing altered expression in response to abiotic stresses. To examine their function, these MYB genes were constitutively expressed in *Arabidopsis*. Among them, one myb gene designated MYB-H (hypocotyls) exhibited significantly enhanced hypocotyls under light condition. The MYB-H seems to act as a repressor because of the presence of the R/KLFGV conserved motif which is found in the transcription repression. Fused MYB-H and GFP protein revealed that MYB-H was localized exclusively in the nuclear compartment. Quantitative RT-PCR analysis indicated that MYB-H expression responded to dark at the transcript level. Interestingly, enhanced elongation of the MYB-H overexpressor hypocotyls were completely blocked by the presence of GA biosynthesis inhibitor, paclobutrazol. Taken together, our results suggested that MYB-H is capable of positively regulating the elongation of *Arabidopsis* hypocotyls in GA-dependent manner.

03116

CYTOSOLIC ASCORBATE PEROXIDASE 1 REGULATES PHYSIOLOGICAL ACTIONS OF REACTIVE OXYGEN SPECIES

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In higher plants, reactive oxygen species (ROS) act not only as toxic molecules but also signaling molecules, controlling a diverse range of physiological functions. The action of ROS is strictly regulated by the cellular redox state consisting of a delicate balance between ROS-production and ROS-scavenging. Cytosolic ascorbate peroxidase 1 (APX1) is a key enzyme for cellular redox regulation in *Arabidopsis* plants. To clarify the involvement of APX1 in the regulation of ROS action, we investigated the response of knockout mutants lacking APX1 (KO-APX1) to wounding or treatment with methyl jasmonate (MeJA). It is well known that at early times after wounding, plants transiently produce ROS by NADPH oxidase, which is activated by jasmonic (JA) acid and its methyl ester, methyl JA (MeJA), to induce defense genes.

When the wild-type and KO-APX1 plants were wounded on two leaves, the growth of the KO-APX1 plants, but not the wild-type plants, was inhibited. In leaves of the wild-type, H₂O₂ accumulated in the vicinity of the site of wounding, while in leaves of the KO-APX1 plants H₂O₂ accumulated extensively from the site of wounding to unwounded region. After wounding, oxidized proteins largely accumulated in the KO-APX1 plants than the wild-type plants. Similar results were obtained in the KO-APX1 plants under the treatment with MeJA. These findings suggest enhancement of ROS toxicity due to the lack of APX1.

Next, we investigated the expression of MeJA-responsive and ROS-responsive genes under the treatment with MeJA in the wild-type and KO-APX1 plants. The transcript levels of MeJA-responsive genes were increased in response to MeJA treatment in both plants. Interestingly, the transcript levels of ROS-responsive genes were induced by MeJA treatment in the KO-APX1 plants, but not in the wild-type plants, suggesting that APX1 is essential for maintaining the specificity of oxidative signaling in plant cells.

03117

VARIATION IN OXIDATIVE STRESS TOLERANCE IN ARABIDOPSIS ACCESSIONS

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Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. Because so far the molecular mechanism involved in each stress has been revealed comparatively independently, our understanding of convergence points between biotic and abiotic stress signaling pathways remain rudimentary. However, recent studies including microarray analysis and target gene analysis of stress-responsive transcription factors support the existence of several molecules, including reactive oxygen species (ROS) as promising candidates for common players involved in crosstalk between stress signaling pathways. Some ROS are toxic byproducts of aerobic metabolism, whereas ROS also function as signaling molecules.

To understand the crosstalk in stress signaling, we focused on analysis of oxidative stress response and identified an ecotype that shows tolerance to oxidative stress-related chemicals. Analysis of hormone- or stress response of the identified ecotype and screening of another oxidative stress tolerant ecotypes are in progress. In this meeting, we will report the phenotypic and genetic analysis of the isolated stress tolerant ecotype.

03118

ALTERATION OF MORPHOLOGY AND GENE EXPRESSION UNDER COOL TEMPERATURE CONDITIONS ON ANTER DEVELOPMENT OF *ORYZA SATIVA* L.

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Cool temperature conditions, about under 20°C, are known to lead to pollen sterility and reduce grain yield in rice. Thus, understanding the genetic mechanism of cool temperature tolerance in rice at the microspore/pollen developmental stage is important. In this study, we compared the morphological features and gene expression profiles of anther tissues in cultivars, Sasanishiki and Hitomebore, at the microspore/pollen development stages, under cool temperature conditions.

Under cool temperature conditions, the fertility of Sasanishiki was only 57% while Hitomebore as more than 80% fertility. Under normal temperature conditions, tapetum become thinner at the uninucleate microspore stage, and then degenerated at the binucleate pollen stage. In the case of the cultivar, Hitomebore, such morphological features were observed even under cool temperature conditions. However, in the case of the cultivar, Sasanishiki under cool temperature conditions, the tapetum did not degrade at the uninucleate microspore stage but instead expanded, and pollen grains at the binucleate stage were collapsed. These findings suggest that tapetum degeneration affects a degree of cool temperature tolerance between two cultivars. Gene expression analysis using the Agilent 44K system showed that 74 genes were up-regulated in Hitomebore, and 36 genes were down regulated in Sasanishiki under cool temperature conditions. These results suggest that the up-regulated genes for the cool temperature tolerance in Hitomebore and the down regulated genes were affected by cool temperature conditions in Sasanishiki.

Taken these findings together, it is suggested that these genes have important roles in microspore/pollen development and tapetum degeneration, and integration/disintegration of such genes' expression affects a degree of cool temperature tolerance in rice cultivars.

03119

IDENTIFICATION OF STRESS TOLERANCE GENES VIA FOX HUNTING OF *THELLUNGIELLA HALOPHILA* FULL-LENGTH CDNA

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Thellungiella halophila has been identified as a model system for understanding abiotic stress tolerance, and it shows extreme salt tolerance. *Thellungiella* is closely related to *Arabidopsis*, and its genes show 90% identity to those of *Arabidopsis*. Recently, full-length cDNA library with a total of 9,569 unique genes were constructed from *Thellungiella* plants treated with salinity, cold, freezing stresses or ABA treatment (Taji et al., 2008). Using ectopic expression of full-length cDNAs, a novel gain-of-function system, termed the FOX hunting system (Full-length cDNA Over-expressing gene hunting system) was developed. To identify the genes conferring salt tolerance to *Arabidopsis* plants, we developed two strategies of FOX hunting, a whole genome FOX hunting and several mini-scale FOX hunting. In the mini-scale FOX hunting, we extracted genes by their functions such as transporters, transcription factors and abiotic stress inducible genes from the cDNA library. We have generated 3,500 T1 and 2,000 T2 lines. As a result of FOX huntings, we identified 9 salt-, 2 osmo- and 4 heat-tolerance related genes.

03120

THE METABOLIC BREAKDOWN OF SPHINGOID LONG-CHAIN BASE 1-PHOSPHATES: INVOLVED IN THE DEHYDRATION STRESS RESPONSE

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Sphingolipid metabolites, sphingoid long-chain base (LCB) 1-phosphates (LCB-1Ps), are involved in ABA signaling pathways. The LCB-1Ps synthesized by LCB kinase are dephosphorylated by LCB-1P phosphatase or degraded by LCB-1P lyase. Two independent *Arabidopsis* mutants (*dpl1-1* and *dpl1-2*) with T-DNA insertions in the LCB-1P lyase gene (*AtDPL1*, *At1g27980*) were characterized. The rates of fresh weight decreases of *dpl1-1* and *dpl1-2* mutants were significantly slower than those of the wild-type plants. This ability to limit their transpiration reflected the leaf temperature of the mutant plants higher than that of wild-type plants, suggesting that *AtDPL1* plays a role in dehydration stress. In the present study, we also show that the *At3g58490* gene encodes *AtSPP1*, a functional LCB-1P phosphatase involved in ABA-mediated stomatal responses. We isolated and characterized *Arabidopsis spp1* mutants, in which the *AtSPP1* gene is knocked out by T-DNA insertion. After drought stress, the rate of decrease in fresh weight was significantly slower in *spp1* mutants than in the wild-type plants. A stomatal aperture bioassay showed that stomatal closure occurs in responses to ABA in *spp1* mutants, suggesting that *AtSPP1* is involved in guard-cell signaling.

03121

INVOLVEMENT OF PHYTOCHROME A IN REGULATION OF WATER DYNAMICS AND AQUAPORIN EXPRESSION IN ARABIDOPSIS ROOTS

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Aquaporins are water channel proteins which are located in biological membranes and involved in water transport across them. Previously, we reported that the transcripts of several aquaporin genes in the root of *Arabidopsis* increase during dark adaptation, while decrease under far-red light (FR) illumination (Sato-Nara et al. 2004). One of the aquaporin genes, *TIP2;2* is tightly regulated by light and phytochrome A (phyA) seems to be involved in this FR repression. To demonstrate phyA regulation of water transport in the root and an involvement of aquaporin gene expression, we visualized the distribution of water in the root of the wild type and the *phyA* mutant and analyzed the amounts of aquaporin proteins (PIP1s, PIP2s, TIP1s). ¹H-NMR imaging revealed that water content in the *Arabidopsis* root gradually increases and keeps a high level during dark adaptation. The water content in the wild type root was reduced by following FR illumination. However, the increase of water content during dark adaptation and the decrease under FR illumination were not observed in the *phyA* mutant. Immunoblotting indicated that the amounts of PIP1s, PIP2s, and TIP1s in the root of the dark-grown seedling were larger than in the light-grown seedling. The amounts of PIP1s and PIP2s in the wild-type plant were reduced by 1 h exposure of FR but this reduction was not observed in the *phyA* mutant. In *Arabidopsis* transgenic plants expressing a *TIP2;2*-GFP fusion protein driven by its own promoter, *TIP2;2*-GFP was localized in the developed region of the root. The *TIP2;2*-GFP signals in the dark-grown plants were extremely brighter than in the light-grown plants, suggesting that the amount of a *TIP2;2* protein increases during dark adaptation. The similarities among the amounts of aquaporins and water content in the root imply the existence of phyA-regulation of water transport in the root.

03122

NITROGEN REGULATORY NETWORKS IN THE CONTROL OF FLOWERING TIME.

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Nitrogen (N) availability is one of the most limiting factors for plant growth and agricultural productivity. Plant growth and developmental aspects such as root architecture, leaf development, seed dormancy and flowering time can be dramatically affected by N supplied to plants. To investigate how plants sense and respond to N at the molecular level to coordinate flowering time, we integrated known floral gene networks with N-networks obtained from publicly databases. Our bioinformatics approach identified important floral genes regulated by N. Among them, several repressors of flowering time belonging to the AP2-type transcription factors are regulated by nitrate including, two *TARGET OF EAT 1 and 2* (*TOE1* and *TOE2* respectively), *SCHLAFMUTZE* (*SMZ*) and *SCHNARCHZAPFEN* (*SNZ*). These genes are targets of microRNA172 (miR172), a regulatory factor that controls flowering time by the photoperiod pathway. To analyze the possible role of these transcription factors in the N-response, we first evaluated the effect of nitrate treatments on the expression of these genes. *TOE1*, *TOE2*, *SMZ* and *SNZ* mRNA accumulated quickly after KNO₃ treatments but not after KCl treatments (as control) indicating they are nitrate responsive genes. We also found that miR172 was down regulated by nitrate treatment. Interestingly, analysis of *toe1 toe2 smz snz* quadruple mutant does not exhibit late flowering in N-response as observed in wild-type plants. These results prompt a model for nitrate control of flowering time that involves miR172 and their targets, the repressors of flowering time.

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03123

THE ARABIDOPSIS COLD SHOCK DOMAIN PROTEIN 3 (ATCSP3) INTERACTS WITH NUCLEAR POLY (A) BINDING PROTEINS (PABNS) IN THE NUCLEUS.

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Arabidopsis COLD SHOCK DOMAIN PROTEIN 3 (AtCSP3) shares a domain with bacterial cold shock proteins (CSPs) and is involved in acquisition of freezing tolerance. AtCSP3 functions as an RNA chaperon and is able to complement the cold-sensitive phenotype of an E.coli CSP quadruple mutant. AtCSP3 is involved in regulation of a CBF-independent pathway during cold acclimation. However, it is not well-understood how AtCSP3 controls freezing tolerance in Arabidopsis. To extend our understanding of the molecular functions of AtCSP3, we conducted a screening of AtCSP3-interacting proteins using the yeast two-hybrid system. Our screening identified several candidate interactors including the three isoforms of the nuclear poly (A) binding protein (PABN1-PABN3). The interaction between AtCSP3 and PABNs was further analyzed with Bi-molecular Fluorescence Complementation (BiFC). The whole AtCSP3 protein was fused with N-terminal portion of YFP (AtCSP3-nYFP) and similarly whole PABN1 was fused with the C-terminal portion of YFP (PABN1-cYFP). The fused constructs were transiently expressed in onion cells and fluorescent images were observed under microscope. The YFP signal was observed in the nucleus of the transformed onion cells. The data indicated that AtCSP3 and PABN1 interact in vivo and the complex is localized to the nucleus. Similar approach demonstrated in vivo interaction between AtCSP3 and PABN2 or PABN3, respectively. However, the protein complexes were localized to the nuclear speckles. In contrast to the cytosolic polyA-binding proteins (PABCs), functions of PABNs are not well documented. It has been suggested that PABNs are involved in the synthesis of poly (A) tails by interacting with poly (A) polymerase. Together, our data suggested a possibility that AtCSP3 is involved in mRNA processing that occurs within the nucleus.

03124

A POSSIBLE FUNCTION OF ARABIDOPSIS THALIANA 26S PROTEASOME SUBUNITS RPT2A AND RPT5A RESPONDING TO ZN DEFICIENT STRESS

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26S proteasome is a large multi-protein complex, which catalyzes active proteolysis, and is required for many process of plant development. Among >40 subunits of plant 26S proteasome, most of them are encoded by duplicated genes. It is suggested that plants can form different combinations of 26S proteasome corresponding to developmental stages and environmental conditions. Here we report the possible function of *A. thaliana* 26S proteasome responding to Zn deficiency. We found that the mutants of 26S proteasome subunits RPT2a and RPT5a were hyper sensitive to Zn deficiency. The shoot growth of *rpt2a-1, 2* and *rpt5a-5, 6* were about a half of that of Col-0 on medium without Zn supplementation. In addition, *rpt2a-1, 2* and *rpt5a-5, 6* showed increased lipid peroxidation in leaves under Zn deficiency, whereas Col-0 did not show any changes of lipid peroxidation. This result suggested that *rpt2a-1, 2* and *rpt5a-5, 6* suffers from oxidative stress in Zn deficiency. On the other hand, Zn concentration of shoot was similar between *rpt5a-5, 6* and Col-0 in both normal and Zn deficient conditions. Expression patterns of Cu/Zn superoxide dismutase (Cu/ZnSOD) in *rpt5a-5, 6* were similar to those in Col-0. Taken together, our results suggested that RPT2a and RPT5a have a crucial role in preventing the oxidative stress induced by Zn deficiency, and they are not seemed to be involved in regulation of Zn uptake and distribution in shoot.

03125

REGULATORY ROLE OF N-TERMINAL REGION OF MEKK1 ON ITS PROTEIN KINASE ACTIVITY

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Mitogen activated protein kinase (MAPK) cascades act as important signaling modules composed of three classes of enzymes, MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). MEKK1 is one of the best characterized *Arabidopsis* MAPKKK. It has been reported that MEKK1 has multiple downstream targets and activates particular MAPKKs depending on extracellular biotic and abiotic stimuli such as wound, cold, salinity stress and pathogen infection. How MEKK1 is activated has not yet been clarified. In this study, we investigated a regulation of MEKK1 activity in relation to the domain structure of MEKK1.

MEKK1 has kinase catalytic domain at C-terminal region. We prepared recombinant MEKK1 protein consisting of kinase domain (MEKK1 KD) and examined its activity toward 10 *Arabidopsis* MAPKKs by *in vitro* kinase assay. The results showed that MEKK1 KD phosphorylated MKK1, MKK2 and MKK6. We also prepared a series of recombinant MEKK1 whose N-terminal noncatalytic region was partially deleted and they were allowed to react toward MKK1, MKK2 and MKK6. The kinase activity of each truncated MEKK1 was suppressed depending on the length of remaining N-terminal region. These results suggest that the N-terminal region of MEKK1 is involved in a regulation of its kinase activity.

To check the effect of the N-terminal region more, we measured the kinase activity of MEKK1 KD under the presence of the various lengths of N-terminal fragments. The result showed that the kinase activity of MEKK1 KD was not affected by addition of any N-terminal fragments. Furthermore these N-terminal fragments did not interact to the kinase domain on yeast two-hybrid assay. These results suggest that kinase activity of MEKK1 could not be inhibited by a specific sequence in N-terminal region but could be regulated by conformational state of its N-terminal region.

03126

INVOLVEMENT OF LOV2 ARG427 IN LIGHT RECEPTION OF ARABIDOPSIS PHOTOTROPIN 2

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Phototropin (phot), a blue light (BL) receptor in plant, has two LOV (LOV1, LOV2) and Ser/Thr kinase (KD) domains in the N- and the C-terminal half, respectively. In the dark, the kinase activity is inhibited by mainly LOV2 which binds an FMN non-covalently (D450 state). Upon BL activation, a transient covalent bond is formed between FMN and a conserved Cys residue (S390 state), and then the inhibition is canceled through structural changes of LOV2. S390 is returned to D450 thermodynamically with a half decay time from seconds to minutes. Activated kinase induces autophosphorylation that is necessary for full expression of the photoresponses. Arabidopsis has two isoforms of phot (phot1, phot2). Phot1 senses BL in a wide range of light intensity, whereas phot2 acts as a high BL sensor. Both phot1 and 2 regulate phototropism, chloroplast movement, stomatal opening, etc.

In this study, we focused on Arg427 in phot2 which interacts with the phosphate tail of FMN and prepared mutated LOV2-KD peptide

(R/K) whose Arg427 was replaced to Lys. By UV-Vis spectroscopy, R/K peptide showed a photocycle like wild type, however its recovery time was about 15 times as fast as that of wild type. R/K peptide showed light-dependent kinase activity under high BL irradiation. We also produced Arabidopsis *phot1/phot2* double mutant rescued with a R/K mutated *phot2* and measured chloroplast movement. Accumulation and avoidance responses in R/K mutant were observed by 200 and 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiation, respectively. The results can be interpreted by the large reduction of the photosensitivity in the R/K mutant shifting the fluence-response curve to the higher light intensity. These *in vitro* and *in vivo* data suggested that the decay time of S390 in *phot2* LOV2 is one of important factors that regulate sensitivity for light intensity in plant.

03127

NOVEL TRANSCRIPTION FACTORS REGULATING THE EXPRESSION OF *OSDREB1B*

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Plants have evolved several mechanisms in order to withstand adverse environmental conditions, which are major constraints for their development and productivity. Understanding these mechanisms is of paramount importance in order to uncover strategies to cope with abiotic stress.

Plant responses to abiotic stress involve changes in gene expression, which are regulated by transcription factors (TFs). A single TF can regulate the expression of many genes, making them appealing targets for plant improvement. Among previously identified stress-responsive TFs, the Arabidopsis CBF/DREB1 family is known to play a prominent role in cold stress tolerance. Nevertheless, it is not well understood how these TFs are regulated, since not much is known about the control of their gene expression. Our work is focused on the rice gene *OsDREB1B*, homolog of the Arabidopsis gene *CBF1/DREB1B*, chosen due to its high and specific induction by cold, and we aimed to identify and characterize novel TFs that regulate its expression.

Using the Yeast One-Hybrid system to screen a rice cold-induced cDNA expression library, we have identified eight novel TFs that bind to the promoter of *OsDREB1B*. Their gene expression pattern was analyzed under different treatments (cold, salt, drought, and ABA) and the most interesting genes, based on stress responsiveness and putative function, were selected for further work. We are now focusing on the biochemical and molecular analysis of these genes and on their functional characterization in Arabidopsis. Moreover, a collection of Arabidopsis T-DNA insertion mutants (mutations in genes homologous to the rice genes identified) is also under study in order to understand the similarities and differences between signaling pathways in both species.

With this work we aim to provide new insights into the genetic control of stress responses in plants by identifying and characterizing new players in the signaling pathways.

03128

A MODEL SYSTEM TO STUDY PLANT-PATHOGEN INTERACTION UNDER SNOW

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Snow mold diseases are quite severe problem for winter wheat production in boreal regions with heavy and persistent snow. Snow molds include several different types of fungi that prefer low temperature and humid environments. Snow molds develop symptoms slowly during deep snow coverage. Therefore, the study of plant-snow mold interaction is limited to pathological and ecological aspects. During cold acclimation, overwintering plants develop resistance against snow molds. However, the mechanism of the resistance against pathogens is less understood than that of cold-induced freezing tolerance. Here we report potential use of *Arabidopsis thaliana* as a model system to study snow mold and host plant interactions at molecular levels. Overwintering capability was tested for a set of ecotypes of *A. thaliana* in Sapporo. While most of the ecotypes including Columbia appeared healthy after snow melting in the spring, the Eniwa ecotype, which is collected in a city near Sapporo, developed lesions on rosette leaves. We have successfully isolated three species of snow molds, *Typhula ishikariensis*, *Typhula incarnata*, and *Sclerotinia trifoliorum* from lesions formed in Eniwa leaves. Inoculation experiments revealed that *T. ishikariensis* and *T. incarnata* infected on leaves of Eniwa and Columbia ecotypes and formed sclerotia on them. Several different methods were tested for evaluation of *Arabidopsis* resistance against *Typhula*. Finally a simple method was devised, which utilizes mycelium with agar pieces placed on rosette leaves to evaluate lesion size area formed during a week exposure to cold and humid environments. The developed *Typhula-Arabidopsis* system was utilized to determine resistance of *Arabidopsis* plants overexpressing TaMDC1, a cold-induced cystatin of wheat, against snow molds. The assay system clearly demonstrated that 35S:TaMDC1 plants are more resistant against *T. ishikariensis* under a condition mimicking snow cover.

03129

INVOLVEMENT OF ARABIDOPSIS THALIANA PHOSPHOLIPASE DZETA2 IN ROOT HYDROTROPISM THROUGH THE SUPPRESSION OF ROOT GRAVITROPISM

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Root hydrotropism is the phenomenon of directional root growth toward moisture under water-deficient conditions. Although physiological and genetic studies have revealed the involvement of the root cap in the sensing of moisture gradients, and those of auxin and abscisic acid (ABA) in the signal transduction for asymmetric root elongation, the overall mechanism of root hydrotropism is still unclear. We found that the promoter activity of the *Arabidopsis* phospholipase Dzeta2 gene (PLDzeta2) was localized to epidermal cells in the distal root elongation zone and lateral root cap cells adjacent to them, and that exogenous ABA enhanced the activity and extended its area to the entire root cap. Although pldzeta2 mutant root caps did not exhibit a morphological phenotype in either the absence or presence of exogenous ABA, the inhibitory effect of ABA on gravitropism, which was significant in wild-type roots, was not observed in pldzeta2 mutant roots. In root hydrotropism experiments, pldzeta2 mutations significantly retarded or disturbed root hydrotropic responses. A

drought condition similar to that used in a hydrotropism experiment enhanced the PLDzeta2 promoter activity in the root cap, as did exogenous ABA. These results suggest that PLDzeta2 responds to drought through ABA signaling in the root cap and accelerates root hydrotropism through the suppression of root gravitropism.

03130

LOCAL AND SYSTEMIC REGULATION OF IRON STARVATION RESPONSES IN ARABIDOPSIS

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Iron (Fe) is an important component of biological redox systems. Although abundant in the lithosphere, under aerobic conditions it is highly insoluble. Therefore, plants have evolved morphological and physiological adaptation responses to acquire iron from the soil and for internal Fe remobilization. These responses are controlled by a local signaling pathway sensing the Fe availability in the soil solution and by a systemic signaling pathway monitoring the Fe status of the plant. To investigate the interplay of the local and systemic pathways and the responses that depend on them, we conducted split-root experiments with plants that had either an Fe-sufficient or -deficient shoot. This approach allowed all possible combinations of local and systemic sufficiency and deficiency signals. We found four regulatory modules that control the morphological and molecular physiological responses to Fe starvation. Three of these modules were controlled by systemic signals including all molecular responses as the expression of *IRT1*, *FRO2*, *BHLH* genes, *FRO3*, *OPT3*, and *NRAMP4* as well as root hair branching. Only the root growth response was solely regulated by local Fe signals. Hence, systemic signals play a predominant role in the regulation of adaptation responses to Fe deficiency presumably to avoid Fe toxicity. The local regulation is rather subordinate; in some cases it has an effect only in concurrence with the systemic deficiency signal. The only exception was the root growth control, where the local pathway controls the deficiency response regardless of the shoot Fe status.

04001

DIVERSITY OF EPIGENETIC CONTROL IN THE FWA GENE WITHIN THE GENUS ARABIDOPSIS

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There is natural variation in epigenetic state both in mammals and plants, but the significance of this variation remains unclear. The *FWA* gene was identified as a late flowering epi-mutant in *Arabidopsis thaliana*. *FWA* is silenced by DNA methylation in vegetative tissue, but it is specifically demethylated in the central cell of the female ovule and continues to be expressed in the endosperm from the maternal copy. A sequence similar to the SINE retroelement found near the transcription start site is conserved in the genus Arabidopsis and is the target of DNA methylation in *A. thaliana* (Lippman et al. 2004, Fujimoto et al. 2008). In *A. thaliana*, *FWA* is stably silenced in vegetative tissue, but in the related species, *FWA* expression and DNA methylation levels vary in vegetative tissue. In this study, we examined vegetative *FWA* expression in these species. Variation of vegetative *FWA* expression correlated with differences of methylation at non-CG sites in the region upstream of the transcription start site in the SINE-like region, and we suggest that this region is the critical methylated region for *FWA* silencing. In *A. thaliana*, the *FWA* expression is affected by methylation in regions both upstream and downstream from the transcription start site. The critical methylated region may have spread during evolution by the acquisition of *thaliana*-specific large tandem repeats. Ectopic *thaliana* *FWA* expression causes a late flowering phenotype, but over-expression of *lyrata* *FWA* does not. Stable *FWA* silencing might result from the selection of the large tandem repeats during evolution to inhibit late flowering in *A. thaliana*.

04002

GENOME-WIDE EPIGENETIC SILENCING BY HDA6 IN ARABIDOPSIS

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Histone deacetylase affects gene silencing and heterochromatin formation in eukaryotes. In *Arabidopsis*, histone deacetylase 6 (HDA6) is involved in epigenetic gene silencing. To understand the gene silencing mechanism by HDA6 on genome-wide, we studied the correlation between histone modifications and gene expression on the target genes of HDA6 in *Arabidopsis*.

Approximately 2000 AGI-assigned regions were identified as the direct targets of HDA6 by ChIP-on-chip analysis using anti-HDA6 specific antibody. Enrichment of histone H4 hyperacetylation was significantly elevated on about twenty percent of these HDA6 target genes and transposable elements in *hda6* mutant. Moreover, we determined the target sites of histone modification by HDA6 deacetylation activity using ChIP-qPCR on the several HDA6 targets in vivo. The correlation between HDA6 binding and elevation of histone H4 hyperacetylation was stronger on the regions of transposable element than protein coding regions. A large portion of transposable elements affected by HDA6 was located near centromeres and heterochromatin regions on all chromosomes in *Arabidopsis*. It suggests that HDA6 is required for maintenance of chromosome via repression of transposable elements on heterochromatic regions. Surprisingly, there are no changes in histone H4 hyperacetylation and also gene expression on about eighty percent of HDA6 target regions. To elucidate the HDA6 function on genome-wide in more detail, profiles of gene expression and some repressive marks of histone modifications by expression and ChIP-on-chip analyses using tiling array are in progress.

We would like to discuss the histone modification status and genome-wide epigenetic silencing mechanism by HDA6 in *Arabidopsis*.

04003

HPR1, A NOVEL COMPONENT OF RNA SILENCING IN PLANTS

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Transgene-mediated post-transcriptional gene silencing (PTGS) involves the propagation of a systemic silencing signal and serves as a model for the study of siRNA-based antiviral defense. Sense transgene-mediated PTGS (S-PTGS), inverted repeat transgene-mediated PTGS (IR-PTGS) and virus-mediated PTGS (V-PTGS) pathways converge at the level of DCL4, which processes double-stranded RNA (dsRNA) into short interfering RNA (siRNA). DCL4-derived siRNA are methylated by HEN1 and incorporated intoAGO1 to guide mRNA cleavage. Phloem-specific expression of the dsRNA trigger and grafting experiments implicated RNA-DEPENDANT RNA POLYMERASE 2 (RDR2), a component of the endogenous 24-nt siRNA pathway, in both short and long distance signaling of IR-PTGS. Here, we show that *rdr2* does not impair or delay the establishment of S-PTGS, which instead requires the action of RNA-DEPENDANT RNA POLYMERASE 6,

a component of the endogenous 21-nt tasiRNA pathway, suggesting independent modes of signaling for S-PTGS and IR-PTGS. However, through a forward genetic screen, we identified a mutation in HPR1 (At5g09860), a component of the THO/TREX RNA export complex, which delays the establishment of both S-PTGS and IR-PTGS. Like other mutations in the S-PTGS pathway, *hpr1* also impacts siRNA-based plant antiviral defense and tasiRNA accumulation. Because *hpr1* compromises the spreading of S-PTGS and phloem-triggered IR-PTGS, our results establish a first link between S-PTGS and IR-PTGS signaling pathways.

04004

CHARACTERIZATION OF THE NON-CELL AUTONOMOUS EFFECT OF MIRNAS AND TASIRNAS IN ARABIDOPSIS THALIANA

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Small RNAs (sRNAs) comprise an important class of molecules involved in the regulation of gene expression and also part of a response mechanism against biotic and abiotic stresses. sRNAs provide specificity to RISC complexes that act at both transcriptional and post-transcriptional levels to downregulate target genes. In plants, an important feature of gene silencing triggered by sRNAs is that some classes of sRNAs, including small interfering RNAs (siRNAs), can trigger silencing beyond distant from their production site. siRNA-triggered silencing has been shown to move from one cell to the next and also systemically via the phloem, which has important consequences for plant physiology. While the mobility of siRNA-triggered silencing is well accepted, the extent of non-autonomous silencing triggered by different classes of sRNAs is still controversial. Trans-acting small interfering RNAs (tasiRNAs) form a plant specific class of sRNAs. tasiRNA movement has been suggested as being important part in plant development, but the details of this movement are only partially characterized. The picture seems to be more complex regarding a third class of sRNA called microRNAs (miRNAs). Both scenarios, cell-autonomy and non-cell autonomy, have been suggested for miRNAs, without a clear conclusion about it. To answer these questions and to characterize the putative movement of tasi- and miRNA-triggered silencing we have used artificial miRNAs and tasiRNAs (amiRNAs and atasiRNAs, respectively) to track the spreading of silencing from phloem companion cells. Our data suggest that both miRNAs and tasiRNAs can generate a signal that spreads beyond the production site, with the later having a longer range. In addition we have investigated some of the features that could influence movement of the sRNA-triggered silencing signal.

04005

CHROMATIN AND DNA DYNAMICS ON THE TRANSCRIPTIONAL REPRESSION THROUGH THE EAR-MOTIF REPRESSION DOMAIN IN PLANTS

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The ERF-associated amphiphilic repression (EAR) motif is a plant specific repression domain (RD). The minimum core sequence is 6 amino acids and it acts as a repressor when fused with heterologous DNA binding domain. However, the mechanism of repression via EAR-motif (RD) is not known and it should be clarified whether a plant specific mechanism of transcriptional repression may exist. We attempted iTRAQ proteome analyses to identify factor(s) that interact with RD, and found that plant specific histone deacetylases, a histone demethylase and several nuclear proteins of unknown function were isolated. We are analyzing protein-protein interactions between these factors and RD, respectively, both in yeast and in vitro system. A sub-nuclear localization of representative proteins is examined by confocal microscopy. We present genetic evidences, that the nuclear protein involves in the RD related repression, using transgenic Arabidopsis and mutant lines. We will discuss a possible mechanism of transcriptional repression caused by the RD, including changes of histone codes, such as acetylation and methylation.

04006

TILING ARRAY ANALYSIS UNDER DROUGHT, COLD, HIGH-SALINITY AND ABA TREATMENTS REVEALED THE SENSE-ANTISENSE TRANSCRIPTS OF POSSIBLE REGULATORY ROLES IN ARABIDOPSIS

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Plants respond and adapt to drought, cold and high-salinity stresses in order to survive. We thought that non-coding RNAs have functions in plant abiotic stress responses and have applied *Arabidopsis* Affymetrix tiling arrays to study the whole genome transcriptome under drought, cold, high-salinity stress and ABA treatment conditions. The tiling array experiments showed that 7,719 novel transcription units (TUs) exist in the *Arabidopsis* genome. Most of the novel TUs are hypothetical non-coding RNAs and about 90% of them are mapped on the antisense strand of the AGI code genes in the sense-antisense transcripts (SATs). Significant linear correlation between the expression ratios (treated/untreated) of the sense TUs and the ratios of the antisense TUs was observed in the SATs of AGI code/non-AGI TU. We confirmed the presence of several stress- or ABA-inducible TUs on the antisense strand by real-time RT-PCR and Northern analyses. Analysis of the biogenesis mechanism and the function of the novel stress-responsive non-coding antisense RNAs are in progress.

04007

RNA-DIRECTED RNA POLYMERASES RDR5 IS ESSENTIAL FOR TRANSCRIPT LEVEL-MEDIATED POST-TRANSCRIPTIONAL GENE SILENCING

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The presence of multiple copies of an expression cassette driven by a strong promoter can result in transcript level-dependent post-transcriptional gene silencing (PTGS). We are testing the potential roles of RNA-directed RNA polymerases (RDRs) in this pathway in a reverse-genetic approach. Of six gene family members present in the *Arabidopsis thaliana* genome, *RDR1*, *RDR2*, *RDR5* and *RDR6* are considered to be functional, while *RDR3* and *RDR4* are most likely pseudogenes. *RDR1* has a role in the defence against viruses. *RDR2*

is essential for cell-to-cell and systemic signalling in PTGS and for RNA-directed DNA methylation (RdDM) of particular sequences. *RDR6* is a key factor in PTGS induced by amplicon- or inverted repeat-constructs and in the formation of endogenous tasiRNAs. No function has been assigned to *RDR5* so far. In contrast to the constitutively expressed *RDR1*, *RDR2* and *RDR6*, expression of *RDR5* shows organ-specific differences with highest levels found in seeds. Introgression of mutant *rdr6* and *rdr5* alleles into a line containing four copies of a *pro35S-GUS* reporter construct released the silencing of *GUS* expression. *GUS* 21nt siRNAs and RdDM of the *GUS* coding region were also reduced. A mutant *rdr2* allele reduced *GUS* silencing to a much lesser extent and had only a minor effect on *GUS* siRNA formation or DNA methylation. Thus, *RDR5* and *RDR6* are pivotal to transcript-level dependent PTGS and correlated RdDM and should have a central roles in the initiation or maintenance of PTGS. In contrast, *RDR2* is more marginally involved, most likely via a role in generating a mobile silencing signal.

04008

THE ARABIDOPSIS NMD PATHWAY - THE FATE OF UORF CONTAINING TRANSCRIPTS AND FEEDBACK REGULATION BY THE NMD FACTOR UPF3

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Approximately 20% of plant genes possess upstream open-reading frames (uORFs). The effect of uORFs on gene expression was mainly studied at the translational level. Very little is known about the impact of plant uORFs on transcript content. The nonsense-mediated mRNA decay (NMD) pathway degrades transcripts bearing premature termination-codons (PTCs). The termination codon of an uORF may be recognized as premature and lead to NMD. However, it is not known to which extent uORF-containing plant genes are exposed to NMD. We investigated the impact of natural uORFs on transcript exposure to NMD in Arabidopsis. Several uORFs whose upstream AUG (uAUG) codons had a strong Kozak context (the strength of this context determines AUG recognition and hence the likelihood of PTC formation) were found not to cause NMD, possibly due to their short length. In contrast, the transcript content of the AtMHX gene was reduced by a short uORF with a weak-context uAUG. We showed that this weak-context uAUG was efficiently recognized due to the secondary structure of its 5'UTR. It is possible that this secondary structure was responsible for the strong reduction of AtMHX transcript content by the short (13 codon) uORF. Several lines of evidence suggested that this reduction in transcript content, which was not dependent on the uORF-peptide, was due to NMD. Yet, AtMHX transcript levels were almost unaltered in mutants of the NMD factors UPF3 and UPF1. We discuss several possible reasons, including the existence of NMD-compensatory mechanisms. We also describe a new NMD compensatory mechanism that links weak function of the NMD machinery to increased expression of the NMD factor UPF3. Attempts to determine the mechanistic cause of this feedback regulation are illustrated. Our findings highlight that uORFs can not only inhibit translation but also strongly affect transcript accumulation. Since uORFs are abundant in plants, our findings may be relevant to a large number of plant genes.

04009

GERMLINE TRANSPOSITION OF THE CHROMOVIRUS *LORE1* IN THE ENDOGENOUS HOST *LOTUS JAPONICUS*

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Long Terminal Repeat (LTR) retrotransposons are very abundant and constitute the predominant family of transposable elements in plant genomes. They have been classified into two superfamilies, *Gypsy* and *Copia*, according to their structural features. Recent studies have identified chromoviruses to be a widely-distributed lineage of *Gypsy* elements. Chromoviruses contain chromodomains in their integrases, which suggests a preference for insertion into heterochromatin. In turn, this preference might have contributed to the patterning of heterochromatin observed in host plant genomes. Here, we report a detailed analysis of the spatio-temporal activity of a plant chromovirus in the endogenous host. We examined *LORE1a*, a member of the endogenous chromovirus *LORE1* family from the model legume *Lotus japonicus*. We found that *LORE1a* is stochastically de-repressed in plant populations regenerated from de-differentiated cells and that *LORE1a* transposes mainly in male germline. Bisulfite sequencing of the 5' LTR and its surrounding region suggests that tissue culture induces a loss of epigenetic silencing of *LORE1a*. Since histochemical GUS assay of transgenic plants containing an LTR::GUS fusion revealed that the *LORE1* LTR promoter is pollen specific, we conclude that male germline-specific *LORE1a* transposition is controlled transcriptionally by its own *cis*-elements. These distinctive features of *LORE1* indicate that this chromovirus has considerable potential for generating genetic and epigenetic diversity in the host plant population. Our results also define conditions for the use of *LORE1a* as a genetic tool.

04010

TRANSCRIPTION-DEPENDENT H3K27ME3 LEVELS AT THE *FLC* LOCUS

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During plant development, epigenetic regulation is involved in controlling expression of many genes. The polycomb group (PcG) system is one of the epigenetic mechanisms for gene repression. Analogous to animal systems, PcG gene silencing is associated with the trimethylation of Lys27 on histone 3 (H3K27me3) which is carried out by the PRC2 complex. Although the H3K27me3 is associated with genes with repressed transcriptional activity, little is known about the mechanisms for adding the H3K27me3 mark. Because of the absence of other polycomb components including PRC1 and the distinct features of Arabidopsis H3K27me3 distribution patterns, it has been suggested that plants may use different mechanisms to establish and spread the trimethylation mark.

We are investigating how the H3K27me3 mark is placed and removed from target sites using the Arabidopsis *FLC* gene, a gene that represses the transition to flowering. There is an inverse correlation between H3K27me3 levels and *FLC* transcription. In winter annual ecotypes, *FLC* transcription is decreased upon vernalisation with an increase in H3K27me3 levels. Arabidopsis genotypes and mutants with low *FLC* transcription also show high trimethylation levels. We used transgenes containing sequences from the *FLC* locus to directly test the relationship between transcription and H3K27me3 levels. In the absence of the transcription we observed high H3K27me3 levels at an *FLC* fragment, that were decreased by transcription from a 35S promoter. By manipulating transcription using an inducible

promoter, H3K27me3 levels rapidly decreased upon activation of transcription. These results support our model of co-transcriptional removal of H3K27me3 mark at the *FLC* locus.

04011

THE ARABIDOPSIS SUVR1, SUVR2 AND SUVR4 SET DOMAIN PROTEINS ARE HISTONE METHYLTRANSFERASES ASSOCIATED WITH THE NUCLEOLUS

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Histones are subject to a variety of post-translational modifications, among them methylation. These post-translational modifications contribute to the chromatin organization in the nucleus, where the dynamic DNA-protein structure can exist as either transcriptionally repressive heterochromatin or permissive euchromatin. The lysines of the histones can be mono-, di-, or trimethylated. The different levels of methylation status determine downstream chromatin organization and have great implications for regulation of eukaryotic gene expression. The enzymes responsible for catalyzing transfer of methyl groups to histone lysines contain the evolutionary conserved SET domain. Proteins most similar to the *Drosophila* SU(VAR)3-9 are generally associated with gene repression and heterochromatinization.

In *Arabidopsis*, there are 10 SUVH and 5 SUVR genes that can be classified as SU(VAR)3-9 proteins. The SUVR1, SUVR2 and SUVR4 proteins are closely related and define a SU(VR)3-9 subgroup. The SUVR transcripts are alternatively spliced, and the different splice variants encode proteins that localize to euchromatin, the nucleolus or subnuclear domains. This localization gives us a hint to involvement in regulation of rDNA chromatin and transposon sequences.

The SUVR proteins all contain a novel N-terminal domain named the WIYLD domain, which we show is a protein-protein interaction domain. We demonstrate that the WIYLD domain is important for the *in vitro* histone dimethylase activity of SUVR4, and NMR analysis has identified amino acids important for the protein-protein interaction activity of the domain. To better understand the *in vivo* function of SUVR1, SUVR2 and SUVR4, we are currently analyzing single and double knock-out mutants in addition to different overexpression lines for molecular and morphological phenotypes.

04012

PLANT ELONGATOR REGULATES AUXIN-RELATED GENES DURING RNA POLYMERASE II TRANSCRIPTION ELONGATION

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In eukaryotes, transcription of protein-encoding genes is strongly regulated by posttranslational modifications of histones that affect the accessibility of the DNA by RNA polymerase II (RNAPII). The Elongator complex was originally identified in yeast as a histone acetyltransferase (HAT) complex that activates RNAPII-mediated transcription. In *Arabidopsis thaliana*, the elongator mutants elo1, elo2, and elo3 with decreased lateral leaf and primary root growth due to reduced cell proliferation, identified homologs of components of the yeast Elongator complex, Elp4, Elp1, and Elp3, respectively. Here we show that the Elongator complex purified from plant cell cultures as a six-component complex. The role of plant Elongator in transcription elongation was supported by colocalization of the HAT enzyme, ELO3, with euchromatin and the phosphorylated form of RNAPII, and reduced histone H3 lysine 14 acetylation at the coding region of the SHORT HYPOCOTYL 2 auxin repressor and the LAX2 auxin influx carrier gene with reduced expression levels in the elo3 mutant. Additional auxin-related genes were downregulated in the transcriptome of elo mutants but not targeted by the Elongator HAT activity showing specificity in target gene selection. Biological relevance was apparent by auxin-related phenotypes and marker gene analysis. Ethylene and jasmonic acid signaling and abiotic stress responses were upregulated in the elo transcriptome and might contribute to the pleiotropic elo phenotype. Thus, although the structure of Elongator and its substrate are conserved, divergence in target gene selection has occurred and is specific in plants and shows that auxin signaling and influx are under chromatin control.

04013

FIXING THE SWITCH: ROLE OF POLYCOMB-GROUP GENES IN COMMITMENT TO FLOWERING

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Although the control of cell fate in plants is thought to be relatively variable, the switch to flowering in response to photoperiodic induction is extremely stable. Thus, plants typically show persistent flowering even if the induction is only transient. The Polycomb-Group (Pc-G) genes are obvious candidates, as they can stably repress gene expression in response to transient environmental signals, as occurs in the vernalization response where the floral repressor *FLC* is stably repressed in response to transient periods of cold. To test whether the Pc-G also play a more general role in floral commitment, we are characterising floral development in the *emf2-10 vrn2-1* mutant, which has severely depleted Pc-G activity.

EMF2 and *VRN2* encode components of the conserved POLYCOMB REPRESSIVE COMPLEX2 (PRC2) which controls gene expression negatively by its histone methyltransferase activity. We showed recently that the targets of Pc-G proteins are characterised by trimethylation of lysine 27 on the histone H3 tail (H3K27me3) in *Arabidopsis*. *emf2-10 vrn2-1* plants fail to maintain the inflorescence meristem (IM) identity and produce leaves afresh if the plants are shifted to short-day after transient floral photoperiodic induction by long-day. This reversion of the IM to vegetative state is partially suppressed in *emf2-10 vrn2-1 flc-5* triple mutants demonstrating once more the importance of the epigenetic control of *FLC*.

Furthermore, the flower phenotype of *emf2-10 vrn2-1* mutants show that Pc-G function is also essential in the establishment of boundaries, e.g. of the ABC-functions and in the limitation of floral stem cell proliferation. The latter phenotype is dependent of the stem cell factor *WUSCHEL* which is a target of H3K27me3 likely direct by PRC2.

Finally, we are taking whole genome profiling approaches to determine which genes are a target of PRC2 during the floral transition.

04014

SIGNIFICANCE OF PRE-MRNA SPLICING CAPACITY CONTROL IN DEDIFFERENTIATION AND ORGANOGENESIS OF ARABIDOPSIS

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We have been investigating molecular mechanisms of organogenesis in vitro with temperature-sensitive mutants of *Arabidopsis thaliana*. Of these mutants, *srd2* and *rid1* were characterized by the temperature sensitivity of hypocotyl dedifferentiation and meristem neo-formation. Studies with the *srd2* mutant at the molecular level have revealed that *SRD2* regulates snRNA level by activating snRNA transcription. Under high temperature conditions, the *srd2* mutation has been shown to cause snRNA deficiency, leading to particular defects in several aspects of development. For example, lateral root primordia of the *srd2* mutant failed in generating auxin gradient patterns due to the loss of expression of PINs at the restrictive temperature, and possibly as a result of this, developed into unorganized hemispherical structures.

Almost all phenotypic characters of the *srd2* mutant were observed also in the *rid1* mutant. Hence the *SRD2* and *RID1* genes were expected to be involved in closely related molecular events. The *RID1* gene was identified as encoding a putative DEAH-box RNA helicase. Sequence analysis showed a high similarity of *RID1* to budding yeast *Prp22*, which is known to participate in pre-mRNA splicing. This implies that the major physiological significance of *SRD2*-mediated activation of snRNA transcription in organogenesis lies in the increase of pre-mRNA splicing capacity. In favor of this hypothesis, by RT-PCR analysis, we found that the *srd2* mutation affects splicing patterns of several genes during hypocotyl dedifferentiation. More comprehensive analysis of splicing patterns in *srd2* and *rid1* is in progress with the whole genome tiling array.

04015

ARABIDOPSIS ARGININE METHYLTRANSFERASES ATPRMT5 AND ATPRMT10 REGULATE FLOWERING TIME IN DIFFERENT MANNER

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Protein arginine methylation plays an essential role in regulating transcription, RNA processing, nuclear transport, DNA-damage repair and signal transduction. Arginine methylation is catalyzed by a small group of protein arginine methyltransferases (PRMTs). Type I (asymmetric di-methylation) and type II (symmetric di-methylation) arginine methyltransferases represent two most important types in animals and plants. Our previous studies showed that both *Arabidopsis* type II arginine methyltransferase AtPRMT5 and type I methyltransferase AtPRMT10 are involved in regulating floral transition, but *atprmt5* *atprmt10* double mutants display an additive effect on flowering time and FLC expression, indicating that the mechanisms of regulating flowering by these two PRMTs are different. In this study, we found that in addition flowering time, AtPRMT5 also affects pre-mRNA splicing. RNA-sequence analyses revealed that several hundreds of pre-mRNA splice alterations in *atprmt5*, including several RNA processing factors which have flowering time phenotype, which may contribute partially to the late-flowering phenotype of *atprmt5*. Furthermore, through characterizing biochemical feature of AtPRMT10, we found that several sites play key roles in its methyltransferase activity. Transgenic analyses reveal that the methyltransferase activity of AtPRMT10 is essential for its role in flowering time regulation.

04016

ROLES OF SIRNAS AND MIRNAS IN GENOME STABILITY AND NONADDITIVE GENE EXPRESSION IN ARABIDOPSIS INTERSPECIFIC HYBRIDS AND ALLOPOLYPLOIDS

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Small RNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and trans-acting siRNAs (tasiRNAs), control gene expression and epigenetic regulation. Although the roles of miRNAs and siRNAs have been extensively studied, their expression diversity and evolution in closely related species and interspecific hybrids are poorly understood. Here, we show comprehensive analyses of miRNA expression and siRNA distributions in two closely related species *Arabidopsis thaliana* and *Arabidopsis arenosa*, a natural allotetraploid *Arabidopsis suecica*, and two resynthesized allotetraploid lines (F1 and F7) derived from *A. thaliana* and *A. arenosa*. We found that repeat- and transposon-associated siRNAs were highly divergent between *A. thaliana* and *A. arenosa*. *A. thaliana* siRNA populations underwent rapid changes in F1 but were stably maintained in F7 and *A. suecica*. The correlation between siRNAs and nonadditive gene expression in allopolyploids is insignificant. In contrast, miRNA and tasiRNA sequences were conserved between species, but their expression patterns were highly variable between the allotetraploids and their progenitors. Many miRNAs tested were nonadditively expressed (deviating from the midparent value, MPV) in the allotetraploids and triggered unequal degradation of *A. thaliana* or *A. arenosa* targets. The data suggest that small RNAs produced during interspecific hybridization or polyploidization serve as a buffer against the genomic shock in interspecific hybrids and allopolyploids: Stable inheritance of repeat-associated siRNAs maintains chromatin and genome stability, whereas expression variation of miRNAs leads to changes in gene expression, growth vigor, and adaptation.

04017

CALMODULIN SIGNALING AND mRNA ALTERNATIVE POLYADENYLATION: THE DEVELOPMENTAL CONNECTION

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Increasing evidence suggests that mRNA polyadenylation plays an important role in gene expression regulation. It has been demonstrated that mutation of polyadenylation factors can alter developmental processes and the responses of plants to environmental stimuli. Previously, we have shown that RNA binding activity of *Arabidopsis Cleavage and Polyadenylation Specificity Factor 30* (AtCPSF30) is regulated by calcium through its calmodulin-binding domain (Delaney et al. 2006. Plant Physiol 140: 1507). A T-DNA insertion in the CPSF30 gene, called *oxt6*, was found to cause the use of alternative polyadenylation sites (Zhang et al 2008, PLoS One, 3:e2410). We now have data to support the biological significant of this calcium connection between polyadenylation and plant development / environmental responses. The *oxt6* mutant possesses a number of developmental defects, including reduced lateral root formation at the initiation stage, shorter stamen filaments resulting in reduced fertility. The mutant also responses to a number of environmental stimuli differently, including resistance to oxidative stresses, and increased sensitivity to exogenously added 6-BA, IAA, and GA3. All of these defects can be complemented by the wild-type CPSF30 transgene. However, a transgene encoding a mutant CPSF30 protein with a mutated calmodulin binding domain can restore some wild-type phenotypes (short filament and oxidative resistance), but not others (lateral root defect and hormonal responses). These results are indicative of differential responses of CPSF30 to calmodulin, and by extension, to calcium

signaling. Our data support the novel notion that at least some of the regulatory roles of mRNA alternative polyadenylation in gene expression can be modulated through calcium signaling pathways. A working model for such regulatory pathways will be presented.

04018

ROLE OF THE ARABIDOPSIS EXOSOME COMPLEX IN REGULATION OF TRANSCRIPTION FROM HETEROCHROMATIC LOCI

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Exosome is a large, evolutionarily conserved and complex 3'-5' exoribonucleolytic macromolecular machine that is present in both nuclear and cytoplasmic compartments. It is highly versatile, and can carry out very distinct types of RNA transactions: (i) processing (3'-end trimming) of precursor RNA species into their respective mature products, (ii) total degradation of certain RNAs, and (iii) quality control/surveillance of yet another RNAs, which results in selective elimination of those species that are not properly processed or packaged into RNP particles.

In addition to a number of known substrates our genome-wide survey of exosome targets in Arabidopsis revealed many novel substrates including stable structural RNAs, select subset of mRNAs, byproducts of miRNA biogenesis as well as hundreds of non-coding RNAs, the vast majority of which have not been previously described. Furthermore, we have found that the plant exosome complex has a general role in regulating the transcripts emanating from heterochromatic loci, repetitive elements, transposons and processed pseudogenes which are targeted by RNAi, chromatin modification and DNA methylation pathways. This large collection of RNAs belongs to a "hidden" layer of the transcriptome that is tightly repressed and can only be visualized through transient inhibition of exosome activity.

Our most recent data in this area indicate that the exosome complex downregulates unwanted transcription from heterochromatic loci in a manner that is synergistic with the RNA-dependent DNA methylation (RdDM) pathway that is dependent on the RNA Polymerase IV and/or V. Results of further examination of the division of labor between exosome and other gene silencing pathways (using genetic analysis and Illumina-based deep sequencing) will be presented.

04019

DEEP SEQUENCING OF BRACHYPODIUM SMALL RNAs AT THE GLOBAL GENOME LEVEL IDENTIFIES MICRORNAs INVOLVED IN COLD STRESS RESPONSE

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MicrRNAs (miRNAs) are endogenous small RNAs having large-scale regulatory effects on plant development and stress responses. Extensive studies of miRNAs have only been performed in a few model plants. Although miRNAs are proved to be involved in plant cold stress responses, little is known for winter-habit monocots. *Brachypodium distachyon*, with close evolutionary relationship to cool-season cereals, has recently emerged as a novel model plant. There are few reports of *Brachypodium* miRNAs.

High-throughput sequencing and whole-genome-wide data mining led to the identification of 27 conserved miRNAs, as well as 129 predicted miRNAs in *Brachypodium*. For multiple-member conserved miRNA families, their sizes in *Brachypodium* were much smaller than those in rice and *Populus*. The genome organization of miR395 family in *Brachypodium* was quite different from that in rice. The expression of 3 conserved miRNAs and 25 predicted miRNAs showed significant changes in response to cold stress. Among these miRNAs, some were cold-induced and some were cold-suppressed, but all the conserved miRNAs were up-regulated under cold stress condition.

Our results suggest that *Brachypodium* miRNAs are composed of a set of conserved miRNAs and a large proportion of non-conserved miRNAs with low expression levels. Both kinds of miRNAs were involved in cold stress response, but all the conserved miRNAs were upregulated, implying an important role for cold-induced miRNAs. The different size and genome organization of miRNA families in *Brachypodium* and rice suggest that the frequency of duplication events or the selection pressure on duplicated miRNAs are different between these two closely related plant species.

04020

IDENTIFICATION OF MIRNA BIOGENESIS MODULATORS BY FORWARD AND CHEMICAL GENETICS

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The production of mature miRNAs requires multiple and coordinated steps that lead to a target mRNA cleavage or the inhibition of its translation. Although a variety of plant proteins involved in miRNA processing and function has been identified by mutant screens, their mutant phenotypes are not always the same. How much of this reflects redundancy between closely related proteins, differential requirements in several RNA silencing pathways, or differential activity during development is still unknown. An alternative to traditional forward genetic screens is chemical genetics, a powerful approach for studying signaling mechanisms in a variety of organisms. This approach utilizes small molecules to perturb a signaling pathway, permitting the identification of relevant factors at any stage of development without a continuous perturbation in a gene product, which may be essential for organismal or cellular survival. Additionally the identification of specific inhibitors of a pathway, such as miRNA biogenesis, could provide new powerful tools in further studies. Herein we report the development of a high-throughput assay for miRNA-mediated gene regulation in whole *Arabidopsis* seedlings and the identification of several miRNA inhibitors. Our strategy used transgenic lines carrying the luciferase gene as a reporter of the activity of an artificial miRNA (amiRNA) that targets it. We screened a commercial library of 10,000 structurally diverse small molecules, and identified a group of compounds with different levels and apparent modes of inhibition. Measurement of endogenous miRNAs together with the expression level of its precursors and targets genes confirmed the inhibitory effect of these compounds. Additionally, the compounds have been tested for their ability to affect other related pathways such as siRNA and tasiRNA. Most affect the tasiRNA production, only a few affect all the three pathways.

04021

RNA DEGRADATION IN THE PLANT NUCLEUS.

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RNA surveillance of stable RNAs in the nucleus involves polyadenylation-dependent degradation of aberrant, incorrectly processed or

superfluous transcripts by nuclear ribonucleases, the 3'-5' complex Rrp6/exosome or 5'-3' Rat1. In a model plant, *Arabidopsis thaliana*, this process is poorly described but plant homologues of nuclear exosome components have been shown to participate in RNA quality control. Here we show that also *Arabidopsis* homologues of Rat1, AtXRN2 and AtXRN3, contribute to polyadenylation-mediated nuclear RNA surveillance. In the absence of these nucleases there is a marked accumulation of polyadenylated rRNA precursors and excised spacer fragments. Interestingly, they differ from those observed in the plant *rrp6* mutant, indicating that two decay pathways may eliminate at least partly distinct set of substrates. In addition, although both AtXRN nucleases are highly homologous (65% identity and 90% similarity within conserved XRN family domains), only *xrn3* mutants showed a significant upregulation of several mRNAs when analysed by Affymetrix gene arrays. Some of these transcripts were also elevated in plants lacking the major component of the NMD pathway, UPF1, but not UPF3. This observation suggests an intriguing possibility that plant UPF1 may possess an additional function, separate from its role in NMD, which is related either to mRNA decay in the nucleus or, considering that XRN3 was found to act as an endogenous silencing suppressor, in one of the RNAi pathways. Our preliminary data also indicates that non-coding RNAs constitute an important class of AtXRN3 substrates.

04022

GLOBAL MAPPING OF ARABIDOPSIS HETEROCHROMATIN

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Heterochromatin, as opposed to euchromatin, was first identified as tightly condensed regions at cytological level, and it often proved to be resistant to nuclease treatment. Heterochromatin of the *Arabidopsis* is thought to be of low complexity and to contain mainly centromeric and pericentromeric repeat regions and nucleolar organizer. However, these conclusions were often based on fluorescence in situ hybridization (FISH) experiments, which show only a local snapshot but cannot easily reveal genome-wide information.

Here, we present a genome-wide map of candidate heterochromatin sequences in *Arabidopsis* using relative resistance to DNaseI as an operational criterion for the heterochromatic state. This is achieved at genomic level and high resolution using DNase-array, a technique to quantify chromatin sensitivity to DNaseI as a function of genome position, using AGRONOMICS1 *Arabidopsis* Affymetrix tiling arrays.

After limited digestion of permeabilized *Arabidopsis* leaf nuclei by DNaseI, DNA fragments larger than 17 kb were hybridized to AGRONOMIC1 arrays. DNaseI-resistant heterochromatin was strongly enriched at centromeric and pericentromeric regions and was also found in dispersed hypersensitive islands along chromosome arms. While the centromeric and pericentromeric DNaseI-resistant regions were strongly enriched in H3K9me2, which is considered as a consensus heterochromatic histone modification in *Arabidopsis*, the dispersed DNaseI-resistant regions where only partially enriched in H3K9me2. Such differential histone modifications in DNaseI-resistant chromatin suggest different molecular mechanisms mediating chromatin compaction.

We will present a detailed characterization of DNaseI-resistant heterochromatic regions of the *Arabidopsis* genome in relation to chromosomal localization, transcriptional activity and chromatin modifications; and we will discuss how these results help to illuminate heterochromatin - a poorly understood dark matter of the genome.

04023

S6, A DOWNSTREAM TARGET OF TOR SIGNALING IS INVOLVED IN AN EPIGENETIC REGULATION OF rRNA SYNTHESIS

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The TOR (target of rapamycin) pathway regulates various biological processes including the synthesis of ribosomal proteins, and transcription and processing of rRNA. A ribosomal protein S6 (RPS6) is one of the well-known downstream components of TOR pathway. Recently, ribosomal proteins have been known to have diverse functions in regulating cellular status as well as protein synthesis. However, so far, little is known about the roles of RPS6 in plants except in serving as a component of the 40S ribosomal subunit. Here, we report that RPS6 has a novel function via the interaction with HD2B (histone deacetylase 2B) which belongs to the plant-specific histone deacetylase HD2 family. RPS6 was localized to the nucleolus and the nucleoplasm where HD2B are present. Interestingly the co-expression of RPS6 and HD2B caused a change in the location of both RPS6 and HD2B to one or several nucleolar spots. TOR and HD2A (Histone deacetylase 2A), another member of the HD2 family, have been reported to regulate transcription of rDNA genes. We propose that the interaction between RPS6 and HD2B may play an important role in linking TOR signaling to rDNA transcription in plants. In addition, we will investigate the effect of S6K, a key downstream effector of the TOR signal, on the cell cycle regulation.

04024

DE-REPRESSEION OF RNA-DIRECTED DNA METHYLATION UPON DE-CONDENSATION OF CONSTITUTIVE HETEROCHROMATIN

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Centromeric constitutive heterochromatin is marked by DNA cytosine methylation (5mC) and histone H3 Lys9 methylation (H3K9me) in animals and plants. In *Arabidopsis*, 5-methylcytosine is detected in CG, CHG and CHH contexts. MET1, a homologue of the mammalian Dnmt1, is responsible for the maintenance of CG methylation. The majority of CHG and a small fraction of CHH methylation are achieved by CMT3. DRM2, a Dnmt3 homologue, and two functionally diversified plant-specific RNA polymerases, Pol IV and Pol V, play specific roles in RNA-directed DNA methylation (RdDM) in all sequence contexts. RdDM uses 24-nt siRNAs to guide *de novo* methylation of homologous DNA sequences. Unlike mutations in *MET1*, *CMT3* and the chromatin remodeling factor gene *DDM1*, mutations that affect Pol IV and Pol V do not appreciably influence methylation of centromeric repeats, despite the presence of its corresponding 24-nt siRNAs. This suggests that RdDM does not work efficiently at constitutive heterochromatin. Here we report that the vegetative cell nuclei (VN) of the male gametophyte (pollen) invariably undergo extensive de-condensation of centromeric heterochromatin and lose centromere identity. VN show greatly reduced H3K9me2, phenocopying nuclei carrying a mutation in *DDM1*. However, unlike the situation in *ddm1* nuclei, the de-condensed heterochromatin in VN retains dense CG methylation and transcriptional silencing and, unexpectedly, is subjected to hypermethylation in non-CG contexts. *drm2*, *polIV* and *polV*, but not *cmt3*, mutants displayed a substantial decrease in the centromeric non-CG hypermethylation in VN, indicating the involvement of RdDM in this process. The findings implicate the condensed form of constitutive heterochromatin in blocking the RdDM machinery.

04025

ANALYSIS OF RID2, A TEMPERATURE-SENSITIVE MUTANT THAT IS DEFECTIVE IN PRE-RRNA PROCESSING AND IN SEVERAL ASPECTS OF CELL PROLIFERATION, AND ITS SUPPRESSOR MUTANT, SR1/W1.

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rid2 (*root initiation defective 2*) is a temperature-sensitive mutant of Arabidopsis that is defective in the initial process of adventitious rooting and callus formation, and therefore is expected to serve as a useful tool for studying dedifferentiation and reactivation of cell division. *rid2* is also characterized by enlargement of the nucleolus at the restrictive temperature. Our analyses revealed that *RID2* encodes a methyltransferase-like protein, which was shown to localize mainly in the nucleolus, and that its function is required for pre-rRNA processing.

For a genetic approach to the action mechanism of *RID2*, we isolated a suppressor mutant of *rid2* and designated it as *sriw1* (*suppressor of rid two 1*). The *sriw1 rid2* double mutant could form adventitious roots and callus at the restrictive temperature, although the impairment of pre-rRNA processing was not recovered. Chromosomal mapping and genome sequencing detected a base substitution in At5g09330 encoding a NAC transcription factor (ANAC082), which was responsible for the suppression of the *rid2* defects in *sriw1*. Several lines of data suggest that this NAC transcription factor functions as a transcriptional activator and is expressed in positive correlation with cell proliferation.

We then examined the effect of *sriw1* on two other temperature-sensitive mutants, *rid1* and *srd2* (*shoot regeneration defective 2*), which share phenotypes with *rid2* in some aspects. *RID1* and *SRD2* encode a putative RNA helicase and an snRNA transcription activator, respectively, and both are likely involved in pre-mRNA splicing. Interestingly, *sriw1* also suppressed in part various defects of *rid1* and *srd2*. It is supposed that SRIW1 plays an important and extensive role in the RNA-processing-dependent regulation of cell proliferation.

04026

ROLE OF A JUMONJI DOMAIN PROTEIN IBM1 IN DIFFERENTIAL DNA METHYLATION AND HISTONE MODIFICATION BETWEEN GENES AND TRANSPOSONS IN *ARABIDOPSIS THALIANA*

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Differential cytosine methylation of genes and transposons is important for maintaining integrity of plant genomes. In Arabidopsis genome, transposons are heavily methylated at both CG and non-CG sites, while the non-CG methylation is rarely found in active genes. We previously reported that a jmjC-domain-containing putative histone demethylase IBM1 (increase in *BONSAI* methylation 1) prevents ectopic deposition of non-CG methylation, and this process is necessary for normal Arabidopsis development. To directly determine the genomic targets of IBM1, we conducted high-resolution genome-wide analysis of DNA methylation using genomic tiling array. The *ibm1* mutation induced extensive hyper-methylation in thousands of genes. On the other hand, methylation in transposons was unaffected. Bisulfite sequencing of some affected genes showed that hyper-methylation in *ibm1* mutant was almost exclusively in CHG sites, whereas methylation of genes was limited to CG sites in wild type. Notably, long and transcribed genes were most severely affected. The *ibm1*-induced hyper-methylation did not depend on previously characterized components of the RNAi-based DNA methylation machinery. Our results suggest novel transcription-coupled mechanisms to direct genic methylation not only at CG but also at CHG sites and highlight the importance of negative regulation of non-CG methylation to maintain distinct pattern of DNA methylation in genome. IBM1 prevents the CHG methylation in genes, but not in transposons. Furthermore, using ChIP-chip, we found that *ibm1* also induced ectopic H3K9me2 in thousands of genes, which parallels the *ibm1*-induced DNA methylation. We will discuss the mechanisms that are involved in differential epigenetic modifications in genes and transposons.

04027

CONTROL OF SEED GERMINATION BY POSTTRANSLATIONAL HISTONE MODIFICATION

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Seed germination, which distinguishes post-embryonic development from embryonic development, is one of the important developmental phase transitions in seed plants. For optimal survival, various environmental and endogenous factors should be monitored properly to determine appropriate timing for seed germination. Among environmental factors, light is perceived by phytochromes and promotes seed germination. Light-dependent activation of phytochromes modulates ABA and GA levels by regulating both their metabolic and signaling pathways. Several negative regulators of seed germination that act when phytochromes are inactive have been reported. However, neither positive regulators of seed germination nor direct mechanisms for the regulation of the hormonal levels have been reported. Here we report that two functionally redundant histone modifiers act as positive regulators of seed germination. We show that loss of these factors leads to reduced germination efficiency when red light pulse is treated. Our study also shows the control of some key germination genes and modification of their chromatin by these novel factors as well as a regulatory pathway involving them.

04028

BUSRTS OF RETROTRANSPOSITION REPRODUCED IN ARABIDOPSIS

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Retrotransposons, which proliferate by reverse transcription of RNA intermediates, comprise a major portion of plant genomes. Plants often change the genome size and organization during evolution by rapid proliferation and deletion of LTR-retrotransposons. Precise transposon sequences throughout the Arabidopsis genome and the *trans*-acting mutations affecting epigenetic states make it an ideal model organism to study transposon dynamics. Here, we report mobilization of diverse families of endogenous Arabidopsis LTR-retrotransposons identified through genetic and genomic approaches using high-resolution genomic tiling-arrays and mutants in the chromatin-remodelling gene *DDM1* (decrease in DNA methylation 1). Using multiple lines of self-pollinated *ddm1* mutant, an increase in the copy number was detected and verified for diverse retrotransposons in a gypsy family (*ATGP3*) and copia families (*ATCOPIA13*, *ATCOPIA21*, *ATCOPIA93*), as well as DNA transposons of a Mutator family, *VANDAL21*. Notably, a burst of retrotransposition occurred stochastically and independently for each element, suggesting an additional autocatalytic process. Furthermore, comparison of the identified LTR-retrotransposons in related Arabidopsis species revealed that lineage-specific burst of retrotransposition of these elements indeed occurred in natural Arabidopsis populations. The recent burst of retrotransposition in natural population is targeted to centromere repeats, which presumably is less

harmful than insertion to genes. The *ddm1*-induced retrotransposon proliferations and genome rearrangements mimic the transposon-mediated genome dynamics during evolution and provide experimental systems to investigate the controlling molecular factors directly.

04029

H3K4 DEMETHYLATION BY JMJ14 IS REQUIRED FOR PROPER REGULATION OF FLORAL INTEGRATORS AND FLOWERING TIME

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Histone lysine methylation plays an essential role in regulating chromatin functions such as transcription and heterochromatin formation. Histone H3 lysine 4 (H3K4) methylation is linked to active transcription. Recent findings in mammals have demonstrated that histone methylation is reversible by a family of Jumonji C (JmjC) domain-containing proteins. KDM5/JARID1 family proteins have been shown to be able to demethylate H3K4me1,2,3 in mammals. Previously, we identified six proteins showing high sequence similarity to KDM5/JARID1 family proteins in Arabidopsis, including JMJ14, MEE27/JMJ15, JMJ16, JMJ17, JMJ18 and JMJ19. Here we demonstrate that JMJ14 is a histone H3K4 demethylase that can reverse mono-, di- and tri-methylated H3K4. Disruption of JMJ14 leads to early flowering under both long day and short day conditions. Analysis of the molecular basis of early flowering in *jmj14* mutants shows that the expression level of the central flowering repressor *FLOWERING LOCUS C (FLC)* is not altered, whereas the floral integrators, *FLOWERING LOCUS T (FT)*, *SUPPRESSOR OF OVEREXPRESSION 1 OF CONSTANS (SOC1)*, *APETALA1 (AP1)* and *LEAFY (LFY)* are de-repressed. However, chromatin immunoprecipitation coupled with qPCR or high-throughput sequencing revealed that H3K4me3 was not changed at these genes, suggesting additional layer of regulation. In summary, we demonstrate that JMJ14 is an active histone demethylase and is involved in flowering time regulation.

04030

THE EVOLUTION OF EPIGENETIC CONTROLS

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Epigenetics plays an important role in cellular differentiation allowing distinct cell types to have specific characteristics despite sharing the same genome sequence. However, there are confusing cause and effect relationships between epigenetics and gene expression. We propose that epigenetic controls have evolved in parallel with genetic controls. We predict that there will be correlations between a gene's epigenetic profile (or Epitype) and its evolutionary divergence from a common ancestral gene. To test this hypothesis, we examined multiple gene families in *Arabidopsis thaliana*, looking at different types of epigenetic structures (histone modifications, nucleosome position, and 5-methyl Cytosine DNA methylation). Data were generated using Chip-chip Affymetrix Tiling Arrays. By overlaying this data onto the gene family phylogenies, we will attempt to show that epigenetic controls and associated phenotypes evolve by gene duplication, divergence, and subfunctionalization. In future work we will determine if there is a hierarchy of evolutionary conservation among these different epigenetic structures. We will use the Teiresias algorithm to search for statistically significant sequence patterns among gene family members that might facilitate an epitype. Through comparing different gene family members, their evolutionary divergence, and the presence or absence of these controls/patterns, we hope to determine if certain types of epigenetic controls are more stable or heritable on an evolutionary time-scale.

04031

A GENETIC DISSECTION OF DNA DE-METHYLATION USING ARABIDOPSIS THALIANA ENDOSPERM

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Both DNA methylation and de-methylation has been associated with parent of origin specific gene expression of the *Arabidopsis FWA* gene in endosperm. FWA contains DNA methylation during male and female gametogenesis. Just prior to fertilization the maternal FWA copy losses DNA methylation, in an active process mediated by the DNA glycosylase DEMETER, and becomes transcriptionally active thought endosperm development. No other component involved in the DNA de-methylation process has been described so far.

We are using a transgenic FWA fused with GFP that mimics endogenous FWA imprinting in a mutagen screen for DNA de-methylation machinery components. Here we present the initial characterization of several selected mutants.

04032

GROWTH HABIT DETERMINATION BY THE BALANCE OF HISTONE METHYLATION ACTIVITIES IN ARABIDOPSIS

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In *Arabidopsis*, the rapid-flowering summer-annual versus the vernalization-requiring winter-annual growth habit is determined by natural variation in *FRIGIDA(FRI)* and *FLOWERING LOCUS C(FLC)*. However, the biochemical basis of how FRI confers a winter-annual habit remains elusive. Here we show that FRI elevates *FLC* expression by enhancement of histone methyltransferase (HMT) activity. *EARLY FLOWERING IN SHORT DAYS (EFS)*, which is essential for *FRI* function, is demonstrated to be a novel dual substrate (histone H3 lysine 4 (H3K4) and H3K36)-specific HMT. FRI is recruited into *FLC* chromatin by EFS and in turn enhances EFS activity and engages additional HMTs. At *FLC*, the HMT activity of EFS is balanced by the H3K4/H3K36- and H3K4-specific histone demethylase (HDM) activities of autonomous-pathway components, *RELATIVE OF EARLY FLOWERING 6* and *FLOWERING LOCUS D*, respectively. Loss of HDM activity in summer annuals results in dominant HMT activity, leading to conversion to a winter-annual habit in the absence of FRI. Thus, our study provides a model of how growth habit is determined through the balance of the H3K4/H3K36-specific HMT and HDM activities and the biochemical role of FRI in this balance.

04033

ARABIDOPSIS DICER-LIKE 4 REQUIRES THE DSRNA-BINDING PROTEIN DRB4 TO CLEAVE LONG-DSRNAs INTO 21 NT SMALL RNA *IN VITRO*.

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In RNA silencing pathway which is widely conserved among eukaryotes, specific interactions between a Dicer-family protein and a dsRNA binding protein plays an important role. *Arabidopsis thaliana* has four Dicer-like (DCL1-4) proteins and five dsRNA-binding (DRB1/HYL1 and DRB2-5) proteins. We have previously reported that DCL1 and DCL4 interact with DRB1/HYL1 and DRB4, respectively.

In this study, we characterized the dsRNA cleavage activity of DCL4 and the function of DRB4 *in vitro*. We prepared crude extracts from *Arabidopsis* seedlings, and incubated them with 500 bp dsRNA derived from actin gene. As a result, the extracts from wild-type plants produced 21 nt small RNA while the extracts from *dcl4-2* and *drb4-1* mutants did not. Then, we purified the DRB4 complexes from crude extracts by immunoprecipitation using anti-DRB4 antibody. The DRB4 complexes produced 21 nt small RNA from 500 bp dsRNA *in vitro*. The DCL4 complexes purified by anti-DCL4 antibody showed dsRNA cleavage activity similar to the DRB4 complexes. The DCL4 complexes from the *drb4-1* extracts (*drb4*-DCL4 complexes) could not produce any small RNAs, but addition of recombinant DRB4 to these complexes recovered the activity of 21 nt small RNA production. These results suggest that DRB4 plays an essential role in the DCL4-dependent dsRNA cleavage and production of 21 nt small RNA. Additionally, amino acid substitutions on dsRNA-binding domains of DRB4 impaired both its dsRNA-binding activity and the recovery of dsRNA cleavage by the *drb4*-DCL4 complexes. This result indicates that dsRNA-binding activity of DRB4 is critical for the dsRNA cleavage by DCL4. In our previous report, DCL4 was shown to have little dsRNA-binding activity *in vitro*, suggesting that DCL4 requires the dsRNA-binding protein DRB4 to access dsRNAs because DCL4 itself is incapable to bind them.

04034

FOLDING OF NASCENT PEPTIDE IS IMPORTANT FOR TRANSLATION ARRET IN ARABIDOPSIS CGS1 MRNA

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Cystathionine γ -synthase (CGS) catalyzes the key step of methionine biosynthesis in plants. CGS is encoded by *CGS1* gene in *Arabidopsis thaliana*. Expression of *CGS1* is feedback-regulated at the step of mRNA degradation in response to S-adenosyl-L-methionine(SAM) (1). A short amino acid sequence termed the MTO1 peptide that is encoded in the exon 1 of *CGS1* itself is important for this regulation as a cis-acting element (2). In vitro translation system of wheat germ extract reproduces this regulation (3). In vitro analyses revealed that *CGS1* mRNA degradation is induced by the translation arrest at the Ser-94 codon which is located immediately downstream of the MTO1 region (4). Translation arrest is induced at the translocation step when the A-site of the ribosome is occupied by the peptidyl-tRNA (Ser) (4).

Since the MTO1 peptide most likely exists inside the exit tunnel of the arrested ribosome, and its amino acid sequence is important, we hypothesized that the MTO1 peptide functions within the exit tunnel. We compared the folding status of MTO1 peptide inside the arrested ribosome or non-arrested one by pegylation assay using in vitro translation system. The result indicated that MTO1 nascent peptide takes a compact structure in the SAM-induced arrested ribosome. Therefore, folding of the MTO1 peptide is suggested to be important for this regulation. Folding status of the MTO1 peptide and the translation arrest will be discussed.

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 - (2) Ominato et al., J. Biol. Chem., 277: 36380-6, 2002
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04035

ATSF1, A SPLICING FACTOR-RELATED PROTEIN THAT FUNCTIONS TO CONTROL DEVELOPMENT IN ARABIDOPSIS.

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Pre-mRNA splicing is an essential step in the regulation of gene expression and it is associated with many component proteins of spliceosome complexes. AtSF1 gene encodes a splicing factor similar to the human (mBBP/SF1) and yeast SF1 (BBP/ScSF1) proteins and interact with U2AF, which are functional at an early stage of spliceosome assembly.

A T-DNA insertion line (atsf1-2) of AtSF1 showed a range of developmental defects such as dwarfism and ABA-hypersensitivity during seed germination. Microarray data revealed that various genes were found to be up- or down-regulated, including heat-shock transcription factors, heat-shock proteins, and CYP707A3 gene, in atsf1-2 mutant. Microarray data of some genes were confirmed using RT-PCR assay. The expression levels of the CYP707A family and heat-shock protein genes were confirmed again through real-time PCR. Some genes showed changes in their alternative splicing patterns. Promoter GUS reporter assay also showed that the coding sequence of the AtSF1 gene was necessary for tissue-specific reporter gene expression in transgenic plants.

In addition, the protein interaction between AtSF1 and AtU2AF65 was confirmed through GST-pull down assay. These results suggest that AtSF1 is the *Arabidopsis* ortholog of the yeast and metazoan SF1 protein, a pre-mRNA splicing factor required for the early stage of spliceosome assembly.

04036

BATTLES BETWEEN PLANT GENOME AND ITS PARASITE THROUGH THE ACTION OF A SMALL RNA

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RNA silencing is a defense system against genomic parasites such as transposons which are potentially harmful to host genome. The majority of transposons are epigenetically silenced by RNA silencing to stabilize the host genome. Despite the presence of this defense system, transposons constitute a major component of eukaryotic genome. This implies the existence of a pathway, which enables transposons to escape host defense. There is increasing knowledge about the mechanism of silencing, however, the strategies to avoid epigenetic silencing for transposons are poorly understood. Here we show that a class of transposons in rice utilizes a microRNA (miRNA) as a strategy to suppress silencing. We found that *miR820* is a miRNA, which is expressed from CACTA DNA transposon in rice and whose

target is a DNA methyltransferase gene, *OsDRM1a*, one of the components of epigenetic silencing. We confirmed that *miR820* regulates the expression of *OsDRM1a* by using small RNA deficient mutants. Although, this regulation is conserved at least among *Oryza* species, the nucleotide sequence of *miR820* and its recognition site of the target gene in some species co-evolved to keep their base pairing, suggesting the contribution of this regulation to adaptive evolution. Our results demonstrate that RNA silencing, intrinsically a host defense machinery, is utilized as a countermeasure of transposons in rice. Furthermore, our analysis of the regulation of *OsDRM1a* by *miR820* shed light on the action of small RNAs both in the battle and cooperation between plant genome and its parasite.

04037

QUANTITATIVE EPIGENETICS OF ROOT DEVELOPMENT IN ARABIDOPSIS

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Loss or gain of DNA methylation can affect gene expression and is sometimes transmitted across generations. Such epigenetic alterations could thus be a source of heritable phenotypic variation in the absence of DNA sequence change. To test this possibility, we are carrying out a series of quantitative genetic analyses on a population of near isogenic, epigenetic Recombinant Inbred Lines (epiRLs).

We have previously shown that the epiRLs exhibit stable inheritance of multiple parental DNA methylation variants (epialleles) over at least eight generations as well as a low amount of DNA sequence variation caused by transposable element mobilization. In addition, we could show that these lines exhibit continuous variation and high heritability (~30%) for flowering time and plant height (Johannes et al, PLoS Genet 2009). Analysis of a third complex trait in the epiRLs population, length of the primary root, indicates similarly high heritability (35 to 50%). This trait is not genetically correlated with flowering time or plant height, suggesting a largely distinct heritable basis in each case.

We will report our progress in epigenotyping selected lines using immunoprecipitation of methylated DNA coupled to hybridization on whole genome tiling arrays. This selective epigenotyping will serve to identify causative epigenetic quantitative trait loci by classical linkage mapping methods.

04038

CRITICAL ROLE OF ALAC1 CHROMATIN RELATED GENE IN ESTABLISHMENT OF GENOMIC IMPRINTING AND DNA DEMETHYLATION

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Genomic imprinting is the epigenetic phenomenon that results in mono-allelic gene expression according to their parent-of-origin. Flowering plants control seed development in part by genomic imprinting in the endosperm. The establishment of imprinting is known to require DNA demethylation by DNA glycosylase, but little is known how DNA demethylation is regulated.

To better understand the regulatory mechanism, we screened *alarm clock for FWA imprinting (alac)* mutants that are defective in genomic imprinting using GFP reporter constructs of a maternally expressed gene, *FWA*. We isolated *alac1* mutant that affected activation and DNA demethylation of maternally expressed imprinted genes. *alac1* also exhibits endosperm over-proliferation and autonomous endosperm development without fertilization, which mimics the mutants of imprinted genes. These results indicate *ALAC1* controls the establishment of genomic imprinting through DNA demethylation. *alac1* encodes chromatin related protein. The relationship of chromatin remodeling and DNA demethylation will be discussed.

04039

HISTONE DEACETYLASE 19 IS REQUIRED FOR THE REPRESSION OF SALICYLIC ACID BIOSYNTHESIS AND SALICYLIC ACID-MEDIATED DEFENSE RESPONSES IN ARABIDOPSIS

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Histone deacetylases remove acetyl groups from the N-terminal tails of histones, which leads to repressive chromatin structures or to the modification of transcription factor-binding surfaces. Therefore, the role of histone deacetylases is often closely associated with the repression of transcriptional activity. Histone deacetylase 19 (HDA19), an Arabidopsis RPD3/HDA1-class histone deacetylase has been implicated in multiple developmental processes and defense responses. Here, we show that HDA19 is involved in the repression of salicylic acid (SA)-mediated defense responses in Arabidopsis. Loss of HDA19 activity increased SA content and the expression of a group of genes required for SA accumulation and pathogenesis-related (PR) genes, resulting in enhanced resistance to *Pseudomonas syringae*. The acetylation levels of histone H3 within *EDS5*, *GDG1*, *PR1*, and *PR2* promoters are increased in the *hda19* mutants compared to in wild type. The increased histone acetylation in *PR1* promoter in *hda19* was not sufficient for the transcriptional activation of *PR1*. Furthermore, we found that HDA19 directly associates with *PR1* and *PR2* promoters. Taken together, these results indicate that HDA19 plays a key role in repressing defense responses prior to pathogen attack and modulating the activity of defense responses by preventing harmful overstimulation of defense responses through histone deacetylation-mediated repression of PR-gene transcription.

04040

TRANSCRIPTIONAL CONTROL OF JUVENILE-TO-ADULT PHASE TRANSITION IN ARABIDOPSIS

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Plants undergo a series of phase transitions from juvenile to adult of the vegetative phase and to the reproductive phase during their growth and development. Recent studies have revealed that the vegetative phase transition and flowering in *Arabidopsis* are regulated by SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) factors. SPL mRNAs are post-transcriptionally regulated by microRNA156 (miR156) in an age-dependent manner and this comprises the endogenous pathway of phase transition. However, transcriptional regulation of SPLs has not been known. In this study, we provide evidences that a histone modifier is required for the transcriptional activation of SPLs and thus regulates phase transitions in *Arabidopsis*. Mutations of the histone modifier resulted in delay in the production of abaxial trichomes(a characteristic of adult leaves) and flowering. Moreover, the levels of SPL mRNAs and a histone H3 modification within SPL chromatin were reduced by the mutations. Thus, the histone modifier acts as a transcriptional co-activator of SPLs.

04041

THEORETICAL ANALYSIS OF EPIGENETIC REGULATION OF FLOWERING LOCUS C

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A central gene in the vernalization pathway is *FLOWERING LOCUS C* (*FLC*), a MADS-domain-containing transcription factor that acts as a floral repressor. *FLC* prevents plants from flowering until winter has passed. The repression of *FLC* by prolonged cold involves chromatin remodeling by addition of repressive histone modification at *FLC* chromatin, such as histone H3 lysine 27 trimethylation (H3K27me3), as well as the loss of histone modifications associated with active transcription, such as histone H3 acetylation and histone H3 lysine 4 trimethylation. In *A. thaliana*, the repressive histone modifications persist in *FLC* even after a return to warm conditions, and *FLC* expression is stably repressed. In contrast to *A. thaliana*, in the perennial herb *A. alpina*, the level of H3K27me3 decreases in the ortholog of *A. thaliana* *FLC* and *PEP1* is reactivated after the plant returns to warm conditions. The difference between histone modifications at *FLC* and at *PEP1* may be one of the mechanisms that separates annuals and perennials, but a detailed mechanism is greatly unknown. We developed a mathematical model for epigenetic regulation of *FLC* and showed that bistability caused by a positive feedback is necessary to explain the robust repression of *FLC*. Addition and removal of active/repressive modifications per histone was modeled as a stochastic process. The fraction of histones with repressive modifications per cell changed according to temperature and converged to the equilibrium state. *FLC* expression level per cell population changed according to the state change of each cell. We found that *FLC* expression was completely and robustly repressed even under noisy temperature environment when each cell revealed bistability. We also showed that the speed of addition of repressive modifications was a critical factor that separates annuals and perennial.

04042

IDENTIFICATION AND CHARACTERIZATION OF THE COMPONENTS RESPONSIBLE FOR THE ENDOSPERM-SPECIFIC EXPRESSION OF FWA

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In *Arabidopsis* seed formation, one sperm fertilizes the egg cell, and the other fuses with the central cell derived from female gametophyte, producing the diploid embryo and the triploid endosperm, respectively. Endosperm tissue is essential for healthy embryo growth. It has been known that endosperm has a unique transcriptional feature that is parental-origin expression of imprinted genes. One of those imprinted genes is *FWA* gene that has the heavily methylated transcriptional start site and is transcriptionally silent in vegetative tissue. On the contrary, in endosperm tissue, the expression of *FWA* occurs with decreasing of the methylation level at the transcriptional start site. However, the mechanism underlying this transcription regulation remains unknown. To identify new components involved in this molecular mechanism, we screened a mutagenized population of *FWA* promoter-GFP transgenic plants for mutants defective in the *FWA* expression in endosperm. We isolated several mutants, called *alarm clock for FWA imprinting* (*alac*). One third of the total developing seed from *alac2* heterozygous mutant exhibited the weakened GFP expression pattern. Moreover, these endosperms also showed abnormal nuclear division or delayed nuclear division during early endosperm development, while this mutation has little effect on the expressions of maternally imprinted genes. In *alac3* mutant, the expression of other maternally imprinted genes, such as *MEDEA* and *FIS2*, decreased in the presumptive mutant ovules. Positional cloning of genes responsible for *alac* mutants are in progress.

04043

THE EPIGENETIC MECHANISM FOR RESETTING OF FLOWERING LOCUS C DURING REPRODUCTIVE STAGE

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Epigenetic regulation of *FLOWERING LOCUS C* (*FLC*), a major floral repressor, is one of the critical factors that determine flowering time in *Arabidopsis thaliana*. Epigenetic state of *FLC*, however, is reset during reproductive stage. In previous study, we showed that *FLC* expression disappeared in gametophytes and was reactivated after fertilization in the embryos, not in the endosperm regardless of the epigenetic state in adult plant. Although many *FLC* regulators and their effects on *FLC* chromatin have been extensively studied, the epigenetic resetting mechanism of *FLC* has not been thoroughly characterized yet.

In this study, we have been searching for key factors of the *FLC* resetting using *FLC::GUS* analysis and RNA expression analysis in many mutants of epigenetic regulators.

DNA methyltransferases as well as the small RNA interference pathway did not affect *FLC* resetting. Factors isolated as *FLC* activators in floral regulation had different effects on *FLC* resetting depending on their biochemical properties: some acted as positive components other showed no obvious roles. FRI and its associated components had roles in the maintenance of the reactivated *FLC* expression during late embryogenesis but not in initial reactivation. Thus, our results indicate that general epigenetic mechanisms do not regulate *FLC*.

resetting, rather specific classes of *FLC* regulators have potential to influence *FLC* resetting.

04044

MOLECULAR STUDY ON THE REGULATION SYSTEM OF THE WHEAT DOMESTICATION GENE *Q* IN POLYPLOID WHEAT AND ITS ANCESTRAL SPECIES

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Common wheat is a hexaploid species that has evolved from 3 diploid ancestral species after allopolyploidizations. Consequently, common wheat contains 3 genomes, namely A, B, and D. The wheat *Q* gene, which has pleiotropic effects on spike morphology, is *APETALA2*-like and was designated as *WAP2*. Although the *Q* is located on the chromosome 5A, 5B and 5D contain its homoeoloci. Bread and durum wheat harbor the *Q* (*WAP2A⁰*), whereas their ancestors and older species of cultivated wheat harbor the *q* (*WAP2A^q*). This implies that *Q* has evolved from *q*. The aim of this study is to determine the difference between the functions of *WAP2A⁰* and those of homoeogenes and *WAP2A^q* by analyzing their structure and the system for regulation of gene expression. We used bread wheat (AABBDD), its near-isogenic line that harbors *q* (AABBD), durum (AABB), and the ancestral diploid species (AA, BB, and DD). Polymorphisms (SNPs and indels) were found not only in the coding sequences but also in the promoter regions. *WAP2B* has a large deletion in its exons, leading to the translation of an unusual protein only in hexaploid wheat. The expression level of the *WAP2A* was highest in homoeogenes in both hexaploid and tetraploid wheats. Since the expression levels of the *WAP2* in diploid species were similar to those of *WAP2A* in hexaploid wheat, both *WAP2B* and *WAP2D* must have been suppressed after polyploidization. The expression level of the *WAP2A⁰* was almost twice that of *WAP2A^q*. Bisulfite sequencing revealed that *WAP2D* promoter was preferentially methylated in hexaploid wheat but to a lesser extent in diploid species. These results suggest that *WAP2* homoeogene expression is differentially regulated by genetic and epigenetic mechanisms, depending on the allopolyploidizations.

04045

CLASS 1 RNASE III IN *ARABIDOPSIS THALIANA* (AtRTL2) CLEAVES DOUBLE-STRANDED RNAs SPECIFICALLY

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Double-stranded RNA replicons (dsRNA replicons) have been founded in healthy plants. The concentrations of these plant dsRNA replicons (endornaviruses) are maintained in host cells. We hypothesized that double-stranded ribonucleases in host plants could be involved in the regulation of their concentrations. One of the candidates of host factor regulating endornaviruses is class 1 RNase III. Class 1 RNase IIIs founded in bacteria and yeasts cleave dsRNAs. However the activities of plant class 1 RNase IIIs are unknown. We investigated the *in vitro* activities of class 1 RNase III in *Arabidopsis thaliana* (AtRTL2) with its recombinant protein. The recombinant AtRTL2 cleaved dsRNAs specifically and produced about 25 nt products which were longer than the cleavage products by bacteria and yeasts class 1 RNase IIIs. The recombinant AtRTL2 *in vitro* cleaved purified dsRNAs of endornaviruses. These results suggest that plant class 1 RNase III could be involved in the regulation of endornaviruses.

04046

GENOME-WIDE ANALYSES OF ALLELE-SPECIFIC EXPRESSION AND DNA METHYLATION IN *ARABIDOPSIS THALIANA*

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A diploid organism has two copies of each gene in the genome, one inherited from each parent. The allelic variation of gene expression is determined by various genetic and nongenetic factors. To understand how DNA methylation is linked to allelic gene regulation, we have used whole genome tiling microarrays and next-generation DNA sequencing to generate DNA methylation profiles (methylome) and transcription profiles (transcriptome) of the whole seedling of *Arabidopsis thaliana* accessions Columbia (Col-0) and C24.

Combinational analyses of the methylome and the transcriptome have indicated that a large number of the genes differentially expressed between the two accessions were associated with differential DNA methylation.

Quantitative real-time PCR and bisulfite-mediated genomic DNA sequencing of selected genes derived from the two accessions and from methylation-defective mutant (*met1* mutant; Col-0 background) confirmed the validity of our data. Some of these genes are likely to be involved in naturally occurring genetic variation in *Arabidopsis thaliana* accessions.

04047

FUNCTIONAL STUDY OF ARABIDOPSIS EXOSOME PUTATIVE CATALYTIC SUBUNITS, ATRRP44A AND ATRRP44B

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Exosome complex is widely conserved, functionally versatile macromolecular machine that plays an important role in regulation of gene expression in eukaryotes. Exosome functions include RNA degradation, processing, and RNA quality control. Also, we have found that the plant exosome complex has a general role in downregulation of transcripts emanating from heterochromatic loci, repetitive elements, transposons and processed pseudogenes. The conserved core of the eukaryotic exosome contains nine subunits. Six of the subunits are organized into a hexameric ring, which is capped by a trimer of subunits. With the exception of plant exosome, which possesses a catalytically active Rrp41 subunit, active sites are contributed by Rrp44 (Dis3) as well as by nuclear-specific subunit Rrp6. Exonucleolytic activity of Rrp44, is considered to be the tenth subunit of the exosome core. It had been shown recently that yeast Rrp44 also possesses endonucleolytic activity. Using proteomics approach we previously identified nine proteins corresponding to *Arabidopsis* exosomal core, but Rrp44 and Rrp6 proteins were absent from our preparations despite the existence of highly homologous genes in *Arabidopsis*. Both ATRRP44a and ATRRP44b genes contain RNB domain known to be responsible for 3' to 5' exonuclease activity. To investigate the function of

both AtRRP44a and AtRRP44b genes, we characterized T-DNA mutants of both genes and found that AtRRP44b has no phenotype, atrrp44a alleles show embryo lethal phenotype. To address the functions of AtRRP44a and AtRRP44b during vegetative growth we constructed inducible RNAi (iRNAi) alleles and found that depletion of AtRRP44a leads to molecular phenotype indicative of exosome malfunction and subsequently results in seedlings death. We also have found that AtRRP44a protein possesses 3' to 5' exonuclease activity. Results of further examination of the division of labor between two *Arabidopsis* AtRRP44a and AtRRP44b proteins will be presented.

04048

CONVERGENT ROLE OF THE DNA METHYLTRANSFERASE MET1 AND THE PUTATIVE HISTONE DEMETHYLASE IBM1 IN THE NEGATIVE CONTROL OF GENIC H3K9 DIMETHYLATION

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Methylation of histone H3 lysine 9 (H3K9) is a hallmark of transcriptional silencing in many organisms. In *Arabidopsis thaliana*, dimethylation of H3K9 is involved in the control of DNA methylation and silencing at transposons, repetitive sequences and occasionally genes when they present a repetitive sequence in their promoter. However, bodies of transcriptionally active genes are depleted of that mark. We constructed a high-resolution genome-wide map of H3K9me2 methylation in the *met1* mutant background by using chromatin immunoprecipitation coupled with whole-genome Roche Nimblegen microarrays (ChIP-chip). We could observe H3K9me2 hypermethylation in hundreds of genes in the *met1* background; at first, this seems paradoxical with the role of MET1 as a CG DNA methyltransferase, for CG DNA methylation is an epigenetic mark implicated in the maintenance of H3K9me2 patterns at some heterochromatic loci. IBM1 is a JMJ protein with putative H3K9me2 histone demethylase activity; *ibm1* mutants also display hyper-accumulation of H3K9me2 in the bodies of genes. To better understand the genetic interactions between IBM1 and MET1, we analysed the extent of H3K9me2 hypermethylation in the single and double-mutants. Our observations collectively suggest that there are at least two mechanisms for dimethylation of H3K9, and that both IBM1 and MET1 contribute to its negative regulation at genes, although to different degrees. We will discuss our evidence for those mechanisms and their relevance within the context of establishment, maintenance, and control of heterochromatin.

05001

REGULATION AND MEMBRANE DYNAMICS OF THE DISTAL ROOT STEM CELL NICHE

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Plants possess at the tip of the shoot and the root meristems that harbor pluripotent stem cells from which all cells of the plant body derive. Intercellular signaling processes mediated by small peptide ligands and their respective receptors play important roles in the necessary dynamic but also tight regulation of the transition from stem cell fate to differentiation. Stem cells in the *Arabidopsis* shoot apical meristem express and secrete the CLAVATA3 (CLV3) peptide and this signal is transmitted by the membrane localized leucine rich repeat receptor kinase CLAVATA1 (CLV1) and CLV2/CRN (CORYNE) to the subjacent organizing cells, which express the homeodomain transcription factor WUSCHEL (WUS). This signaling eventually leads to a negative feedback loop adjusting stem cell homeostasis in the shoot. A direct binding of CLV3 to the ectodomain of CLV1 was shown using radio labeled ligand and CLV1 extracellular domain [1]. Recently we discovered, based on genetic evidence that a similar regulation, consisting of the CLV3-related peptide CLE40, the membrane localized receptor kinase ARABIDOPSIS CRINKLY 4 (ACR4) and the WUS-homolog WOX5, also exists in the distal root meristem. The major difference to the shoot meristem regulation is that the peptide signal (CLE40) originates from differentiated cells, not from stem cells [2]. We are currently studying ligand-receptor interactions and putative complex formations involved in plant stem cell regulation. We want to analyze the dynamics of these processes, especially upon binding of the peptidic ligands to their respective receptors. We are currently establishing experimental setups using fluorescently labeled receptors and ligands for these analyses using fluorescence correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP), fluorescence resonance energy transfer (FRET) and fluorescent lifetime imaging (FLIM) techniques.

[1] Ogawa et al, Science (2008).

[2] Stahl et al, Curr Biol (2009).

05002

NOD FACTOR/ NITRATE-INDUCED CLE GENES DRIVE SYSTEMIC AND HAR1-DEPENDENT REGULATION OF NODULATION

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Host legumes control root nodule numbers by sensing external and internal cues. A major external cue is soil nitrate, whereas a feedback regulatory system in which earlier formed nodules suppress further nodulation through shoot-root communication is an important internal cue. The latter is known as autoregulation of nodulation (AUT), and is believed to consist of two long-distance signals: a root-derived signal that is generated in infected roots and transmitted to the shoot; and a shoot-derived signal that systemically inhibits nodulation. In *Lotus japonicus*, the leucine-rich repeat receptor-like kinase, HYPERNODULATION ABERRANT ROOT FORMATION 1 (HAR1), mediates AUT and nitrate inhibition of nodulation, and is hypothesized to recognize the root-derived signal. Here we identify *L. japonicus* CLE-Root Signal 1 (*LjCLE-RS1*) and *LjCLE-RS2* as strong candidates for the root-derived signal. A hairy root transformation study shows that overexpressing *LjCLE-RS1* and -RS2 inhibits nodulation systemically and furthermore, that the systemic suppression depends on HAR1. Moreover, *LjCLE-RS2* expression is strongly up-regulated in roots by nitrate addition. Based on these findings, we propose a simple model for AUT and nitrate inhibition of nodulation mediated by *LjCLE-RS1*, -RS2 peptides and the HAR1 receptor-like kinase.

05003

ROLES OF CLE1-CLE7 IN ROOT DEVELOPMENT IN ARABIDOPSIS THALIANA

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The CLE (CLAVATA3 ENDOSPERM SURROUNDING REGION) peptides are signaling molecules thought to regulate the size of meristems

and their differentiation. Despite the essential function of *CLAVATA3* (*CLV3*) in maintenance of shoot apical meristem, previous findings suggested its closest homologues, *CLE1-CLE7*, are abundantly expressed in *Arabidopsis* roots, and their roles in root development have not been elucidated.

The transcript levels of *CLE1*, *3*, *4*, and *7* were increased by low NO_3^- , but repressed by high NO_3^- . *CLE1*, *2*, *3*, *4*, and *7* were expressed in pericycle cells. *CLE1* and *CLE5* were expressed in root tip, and *CLE2* and *CLE4* were expressed in basal position of lateral roots. We could barely find any expression of *CLE6* in roots. Overexpression of *CLE1-CLE7* inhibited the growths of lateral roots, although the lateral root densities were unaffected. Accordingly, *CLE1-CLE7* were suggested to function as repressors of lateral root elongation.

The *clv1* mutants having mutations in *CLV1*, the receptor of *CLV3*, had longer lateral roots than the wild-type plants. The longer lateral root phenotype of *clv1* mutants was due to enhanced elongation rates of lateral roots. Microscopic analysis of *CLV1-GFP* revealed that *CLV1* was expressed in the phloem companion cells in roots. Analysis of the root lengths of *CLE3* overexpressors in wild-type and *clv1* mutant backgrounds indicated the *clv1* mutant was less sensitive to the overexpression of *CLE3* gene having inhibitory effects on lateral root elongation. These results suggested that *CLV1* acts as a receptor of *CLE1-CLE7* peptides and that *CLE-CLV1* signaling cascade regulates elongation of lateral roots in *Arabidopsis*.

05004

A SIGNALING MODULE CONTROLLING THE STEM CELL NICHE IN ARABIDOPSIS ROOT MERISTEMS

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Many processes in plant development are controlled by small signaling peptides, one of these signaling molecules is *CLE40*. Within the *CLE*-family *CLE40* is most closely related to *CLV3*, which is essential for the stem cell regulating *CLAVATA*-pathway (*CLV*) in the shoot apical meristem. Because of the close relation to *CLV3* and the fact that *cle40* mutants show defects in regulating the root meristem, a similar mechanism to the *CLV*-pathway of the SAM is supposed to function in the root. *CLE40* can rescue a *clv3* mutant if expressed from the stem cell domain. In the root, *CLE40* is expressed in the differentiating descendants of distal root stem cells and the differentiation zone of the stele. Reducing *CLE40* levels delays differentiation and allows stem cell proliferation. Conversely, increased *CLE40* levels drastically alter the expression domain of *WOX5* and promote stem cell differentiation. In addition to that our data strongly suggests that the receptor kinase *ACR4*, previously shown to control cell proliferation, is an essential component, and also a target, of *CLE40* signaling. Our results reveal that in contrast to the shoot system, signals, originating from differentiated cells, but not the stem cells, determine the size and position of the root niche (1).

(1) Stahl et al, Curr Biol (2009)

05005

SCREENING OF THE *CLV2* ENHANCER MUTANTS

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The shoot meristem and root meristem are continuously forming many tissues during plant development. To understand the mechanism of plant development, it is important to examine the mechanism of shoot and root meristem. It has been known that the secreted peptide *CLAVATA* (*CLV3*) restricts the size of the shoot meristem by negatively regulating the *WUSCHEL* (*WUS*) gene. This peptide has been received by receptors such as *CLV1* and *CLV2/CORYNE* (*CRN*). However, compared with shoot meristem, it has not been known how *CLE* pathways regulate root meristem development. *Arabidopsis* genomes contain at least 31 members of the *CLV3/ENDOSPERM SURROUNDING REGION* (*CLE*) family genes including *CLV3*. Over-expression of the *CLE* genes and treatments with *CLE*s resulted in consumption of root meristem. Furthermore, the mutants of the *clv2* and *crn* showed resistance against over-expression *CLV3* and treatments with *CLE* peptide. Therefore, it has been suggested that development of root meristem is regulated by similar *CLV3-CLV2/CRN* pathway. However, the *clv2* and *crn* mutants did not show obvious phenotype in root meristem. Furthermore, it has not been known that the mutants of *CLE* show phenotypes of root meristem. Therefore, it has been suggested that more redundant *CLE* pathways are regulating root meristem development. To discover novel factors related to *CLE* pathways in root meristem, we have screened for mutants are more resistant against *CLE* treatments in the *clv2* mutant background. All enhancer mutants enhanced peptide resistance of the *clv2* mutant. Some of them enhanced shoot phenotypes of the *clv2* mutant as well. We are identifying candidate genes of these *clv2* enhancer mutants. Further analysis of these mutants will be presented.

05006

IDENTIFICATION OF THE NOVEL PEPTIDE LIGANDS INVOLVED IN PLANT ORGAN DEVELOPMENT

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It is well known that intercellular signaling systems in shoot apical meristem (SAM) have important roles of lateral organ development and positioning. Recent analyses showed that one of the signaling molecules are peptides working as ligands, such as *CLV3*. It is suggested many peptide molecules have different roles at SAM, but only a few number of peptides was obtained. We started to identify novel peptide ligands working at SAM secreted into apoplastic space, by using the cauliflower mutant of *Arabidopsis* (double mutant of *ap1 cal*) and cauliflower heads of *Brassica oleracea* var. *botrytis*. We peeled several cell layers of cauliflower heads, extracted proteins by dipping in CaCl_2 solution in vacuo, and identified 41 proteins between 3,000 Da and 18,000 Da by 2D gel electrophoresis and protein sequencing. We also identified 339 proteins of 500-4000 Da from *Arabidopsis ap1 cal* mutant by using column chromatography technique and amino acid sequence analysis with LC-MS/MS. We then chose 30 proteins and started their bioassay by adding the synthetic peptides to *Arabidopsis* seedlings. Some of the synthetic peptides showed biological activity changing morphology in roots and/or shoots, such as abnormal cell division planes, disruption of QC, and aberrant direction of root cell elongation. We are currently examining the biological roles of the candidate proteins.

06001

INVOLVEMENT OF BOR6 AND BOR7, BORON EFFLUX TRANSPORTERS EXPRESSED IN POLLEN TUBES, IN REPRODUCTION

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Boron is an essential micronutrient for the plants. The male sterility is one of the B deficiency symptoms. The first boron transporter, BOR1, is identified through molecular genetics of *Arabidopsis thaliana* and seven BOR1 or BOR1-like genes are present in the genomes of *Arabidopsis thaliana*. BOR6 and BOR7 are boron transporters specifically expressed in pollen and pollen tubes and it was possible that BOR6 and/or BOR7 is involved in reproduction. We investigated possible roles of BOR6 and BOR7 in reproduction. We obtained T-DNA tag lines for these genes from ABRC. When plants are grown under B deficient condition and pollen tubes in pistils were observed, pollen tubes of double mutant for the BOR6 and BOR7 genes were shorter than the wild type. WT pollen tubes reached to the lower part of the pistil, while those of the double mutant did not. Pollen tubes of the double mutant tend to extend toward ovules in the upper part of the pistil rather than reaching to the lower part of pistils. T-DNA insertions in BOR6, BOR7 did not segregate from the corresponding heterozygous plants in the 1:2:1 ratio and wild type plants appeared in a higher frequency among the selfed progenies. Our results suggest that BOR6 and BOR7 play an important role in the pollen tube elongation and reproduction.

06002

THE ARABIDOPSIS ACYL-COA-BINDING PROTEINS ACBP1 AND ACBP2 ARE ESSENTIAL FOR EARLY EMBRYO DEVELOPMENT

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In *Arabidopsis thaliana*, a family of six genes (ACBP1 to ACBP6) encodes acyl-CoA-binding proteins (ACBPs) which show conservation at the acyl-CoA-binding domain. N-terminal transmembrane domains and C-terminal ankyrin repeats are also present in ACBP1 and ACBP2. ACBP1 has been observed to accumulate in embryos of developing seeds and has been proposed to function in lipid transfer. Using ACBP2-specific antibodies in immunolocalization, we showed that ACBP2 is similarly expressed in *Arabidopsis* embryos during seed development. Since ACBP1 and ACBP2 are highly conserved (82% identity) and are both expressed in embryos, we investigated if they function redundantly in lipid metabolism during seed development. Our findings revealed that the *acbp1* and *acbp2* single mutants are similar to wild type in growth and development, but the *acbp1acbp2* double mutant is embryo lethal and embryos aborted at early embryogenesis. Lethality in the double mutant was overcome by introduction of an ACBP1 cDNA. Lipid and acyl-CoA analyses indicated that siliques (but not leaves) of the *acbp1* mutant accumulate galactolipid monogalactosyldiacylglycerol and 18:0-CoA. In contrast, the levels of most polyunsaturated species of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine had declined. Further observations demonstrated that recombinant ACBP1 and ACBP2 bind unsaturated phosphatidylcholine and acyl-CoA esters *in vitro*. Taken together, our results suggest that ACBP1 and ACBP2 are essential in lipid transfer during early embryogenesis in *Arabidopsis*.

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06003

PLASTIC CHLOROPLAST DIFFERENTIATION IN ARABIDOPSIS ROOTS BY LIGHT AND PHYTOHORMONES

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Elucidation of mechanism of plastic chloroplast differentiation is beneficial for understanding of the assembly of photosynthetic apparatus, as well as agricultural purposes. Here, we studied the chloroplast differentiation in *Arabidopsis* roots. In the presence of light, chloroplast differentiation in roots is positively and negatively regulated by cytokinin and auxin, respectively. Light and phytohormones control the coordinated expression of genes involved in Chl biosynthesis and photosynthesis. For light signaling, a transfactor HY5 was prerequisite. Consistent with *in silico* prediction, promoter-GUS analysis shows that binding of HY5 to a G-box is essential for the transcriptional activation in roots. Comparison of Chl and HY5-YFP fluorescence shows that the former is limited in cortex, while the later is observed in almost all cells. Furthermore, phytohormones do not affect the HY5 stability, suggesting HY5 is essential but not sufficient for chloroplast differentiation in roots, and phytohormones may affect distinct pathway for transcriptional regulation. Expression analysis reveal the phytohormone-dependent expression of GLK2 in roots, an isoform of recently identified transfactor that controls the coordinate expression of photosynthesis genes. In fact, Chl accumulation in *glk1 glk2* is significantly lower, while those in overexpressors GLK1_{OE} and GLK2_{OE} are approximately 6- and 3-fold higher than WT, respectively, suggesting the phytohormonal control is primarily mediated via GLK2 in roots. Surprisingly, excised roots of GLK overexpressors are photosynthetically competent, as they show light-dependent O₂ evolution and CO₂ fixation. Our results show that the chloroplast differentiation in roots is regulated by combination of phytohormones- and light-dependent transfactors that coordinate the expression of photosynthesis genes causing the assembly of functional photosynthetic apparatus.

06004

TACKLING THE REGULATION OF GLUCOSINOLATE BIOSYNTHESIS IN ARABIDOPSIS THALIANA

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About 120 glucosinolates (GSLs) are predominantly synthesized in the plant family of the Brassicaceae. GSLs are sulphur- and nitrogen-containing secondary metabolites and their breakdown products confer e.g. resistance to herbivores and pathogens or exhibit anticarcinogenic properties in humans.

Synthesis of GSLs is tightly connected with the synthesis of amino acids in the primary metabolism, mainly methionine, phenylalanine and tryptophane.

These amino acids form the skeleton of the GSLs. Side-chain elongation, core GSL biosynthesis and side-chain modification lead to the final products of aliphatic, aromatic and indolic GSLs.

The combination of coexpression analysis and metabolite profiling led to the identification of biosynthetic genes and key regulators in glucosinolate biosynthesis. Additionally, the integration of transcriptomic and metabolomic data of sulphur-deprived *Arabidopsis* plants revealed coordinate changes of genes involved in glucosinolate and amino acid metabolism.

However, several genes identified through the latter approach are most likely environmentally regulated. At moment, we are trying to elucidate the distinct regulation of these genes by various abiotic challenges.

Furthermore, we are aiming to unveil the impact of the primary amino acid metabolism in glucosinolate biosynthesis by investigating the metabolomes of mutant plants impaired in amino acid synthesis and transport.

06005

IDENTIFICATION OF REGIONS REQUIRED AND SUFFICIENT FOR NITRATE-RESPONSIVE EXPRESSION OF *NIR1* AND *NIA1*

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Nitrate acts as a signaling molecule that induces changes in growth and gene expression in plants. Genes encoding nitrate reductase (NR) and nitrite reductase (NIR) are typical nitrate-inducible genes. To identify *cis*-element(s) involved in nitrate-responsive gene expression, we analyzed 5' and 3' flanking sequences of an NR gene, *NIA1*, and an NIR gene, *NIR1*, of Arabidopsis. The 1.9-kb promoter of *NIA1* was not activated by nitrate. However, when both the 5' and 3' flanking sequences of *NIA1* were fused to GUS gene, the expression of this fusion gene was strongly induced by nitrate. When the 3' flanking sequence was fused to the downstream of GUS gene under the control of the 35S minimal promoter, this chimeric gene also showed nitrate response. These results indicated that the 3' flanking sequence of *NIA1* is sufficient to confer nitrate response. On the other hand, GUS reporter gene under the control of the 3.1-kb promoter sequence of *NIR1* was expressed in response to nitrate treatment. Subsequent analyses revealed that the region from positions -188 to -1 (relative to the translation start site) was found to contain at least one *cis*-element necessary for the nitrate-dependent activation of the promoter. To define the nitrate-responsive *cis*-element (NRE) in this region, we compared the sequences of several NIR gene promoters from various higher plants and identified a conserved pseudo-palindromic sequence motif as the putative NRE. A synthetic promoter in which the four copies of a 43-bp sequence containing the motif were fused to the 35S minimal promoter was found to direct nitrate-responsive transcription. Furthermore, mutations in this pseudo-palindromic motif diminished the nitrate-responsive activity of the promoter, indicating that the 43-bp sequence is an NRE that is both necessary and sufficient for nitrate-responsive transcription.

06006

IN VIVO MEASUREMENTS OF AMINO ACIDS USING FRET-BASED METABOLITE SENSORS

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Methods to analyze multiple layers of regulations (genomic modification, control of transcript and protein levels, post-transcriptional modification, metabolite and ion levels) *in situ* are especially relevant in systems biology. Compared to the transcriptional and translational regulations, changes of metabolite levels in living cells are often very fast and reversible; therefore it is very difficult to capture a status in its native context. Moreover, responses to stimuli differ between different cell types in the same tissue, which makes it challenging to isolate the change in metabolic status of a single cell type by conventional biochemical approaches.

In order to understand cellular metabolism at higher temporal and spatial resolution, we developed protein-based biosensors using Fluorescent Resonance Energy Transfer (FRET). By taking advantage of the bacterial periplasmic binding protein family, sensors for many biologically important molecules, including sugars and amino acids (reviewed in Okumoto 2010), have been created. These FRET sensors can be expressed in wide range of model systems including cell cultures, and importantly, whole organisms. Also, when externally added, it provides an extremely fast method for the detection of metabolites (Dulla et al, 2008). Our preliminary data suggest that the metabolite sensors can be used for the analyses of cellular export activity in higher temporal resolution, providing a new powerful tool for the discovery of molecular mechanisms for such processes.

Okumoto, S. (2009) Imaging approach for monitoring cellular metabolites and ions using genetically encoded biosensors. Curr. Opin. Biotech. *in press*

Dulla, C., Tani, H., Okumoto, S., Frommer, W.B., Reimer, R.J., and Huguenard, J.R. (2008). Imaging of glutamate in brain slices using FRET sensors. J Neurosci Methods 168, 306-319.

06007

THE TOMATO MUTANT *ZETA* ENCODES A NOVEL ENZYME ESSENTIAL FOR CAROTENOIDS BIOSYNTHESIS IN PLANTS

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Carotenoids play essential roles in plants. They are synthesized in plastids from the isoprenoid pathway. Three enzymes have been reported to convert phytoene to trans-lycopene: Phytoene desaturase (PDS), Zeta-carotene desaturase (ZDS) and Carotene isomerase (CRTISO). Here we describe the characterization of a forth enzyme that is essential to metabolize zeta-carotene.

We have isolated a novel mutation in tomato (*S. lycopersicum* cv M82), termed *ZETA*, which blocks carotenoid biosynthesis in flowers and fruit at 9,15,9'-tri-cis-zeta-carotene. Etiolated cotyledons of *ZETA* accumulate zeta-carotene, similarly to flowers and fruit. However, exposure to light reverses the phenotype to normal. Young leaves of *ZETA* are virescent but turn green as they develop. Photosynthetic activity is implicated in the correction of the lesions inflicted by *ZETA*.

The gene encoding *ZETA*, called *Ziso*, was cloned based on homology to the *Arabidopsis* gene At1G10830. Genomic and cDNA sequences of *Ziso* in wild type tomato and two *ZETA* alleles revealed that each allele carries a unique nonsense mutation, thus confirming the molecular basis of *ZETA*. *Ziso* in tomato encodes a polypeptide of 369 amino acids which, upon cleavage of a predicted transit peptide, generates a mature polypeptide of 32.4 kDa with potentially five trans-membrane helices. Bioinformatic analysis revealed that *Ziso* occurs in all cyanobacteria, algae and plants. Deletion of the gene *Synpcc7942_1979*, which is the ortholog of *Ziso* in *S. elongatus* PCC7942, resulted in accumulation of cis-zeta-carotene. Taken together, these results demonstrate that ZISO is required for cis-to-trans isomerization of the C15-C15' double bond in zeta-carotene in both cyanobacteria and plants. The mechanism of action of ZISO is yet unknown. We speculate that ZISO contributes a redox-related function necessary for the isomerization reaction.

06008

A PRACTICAL METHOD WITH CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY TO PROFILE ANIONIC METABOLITES WITH A FUSED SILICA CAPILLARY

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Metabolite profiling is a powerful approach to reveal the metabolic response networks associated with genetic modification or environmental alteration. For comprehensive analysis of anionic metabolites, a variety of attempts have been made in the past. Here we propose the CE-MS analysis using a fused-silica capillary as a convenient method to profile primary anionic metabolites in plants. This method is composed of analysis of anionic metabolites in two different analytical modes, where the electro-osmotic flows (EOF) are

appropriately controlled. High-speed mode aimed at comprehensive analysis of a number of anionic metabolites in short time to generate fast EOF by selection of running electrolyte prepared in appropriate pH condition and initialization and preconditioning procedures of fused silica capillaries. Under the condition where ammonium formate (pH 8.0) is used as electrolyte, a standard mixture including 38 compounds could be analyzed in less than 16 min. However, the peaks of several isomers overlapped in this mode. Therefore, we also developed the other analytical mode, high-resolution mode, for separation of these isomers. A mixture of ammonium acetate (pH 10.0) and methanol is used as electrolyte, because high alkaline condition and several organic solvents are useful to increase resolution by suppressing fast EOF. Under this condition, the isomers could be separately determined, demonstrating an advantage in analysis of five hexose isomers, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate, galactose 1-phosphate and mannose 6-phosphate, which would be difficult to separate with the existing CE-MS methods. We also show the results of analysis of extracts from *Arabidopsis* leaf and the moss by this method. Based on these results, we will discuss how the proposed method is useful in comprehensive and precise profiling of anionic metabolites.

06009

LARGE-SCALE SCREENING OF *ARABIDOPSIS* MUTANTS IN THE EXPRESSION OF A GENE FOR SEED OIL SYNTHESIS BY HIGH THROUGHPUT REAL-TIME BIOLUMINESCENCE MONITORING SYSTEM.

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During the maturation of *Arabidopsis* seeds, a large portion of sugar carbons imported from the source are converted into oil for storage. An AP2-type transcription factor ASML1/WRI1 directly activates genes for fatty acid synthesis in the plastid, while expression of genes for the synthesis of triacylglycerol (TAG) in the endoplasmic reticulum requires additional factor(s). To search for factors involved in the regulation of TAG assembly in the ER, we introduced a firefly luciferase (LUC) reporter gene with the promoter of a gene for diacylglycerol acyltransferase (*DGAT1*; At2g19450) into *Arabidopsis*. The *DGAT1::LUC* plants showed strong LUC expression in fruits that contain maturing seeds, and weaker expression in young leaves, which showed a circadian rhythm with a peak in the evening. These LUC expression patterns were similar to the expression pattern of *DGAT1* mRNA. To screen for mutants with altered expression of LUC, we used high throughput real-time bioluminescence monitoring system, which can automatically monitor bioluminescence of 1,920 or 19,200 individual plants for a week. Seeds of a *DGAT1::LUC* plant were treated with EMS, and young M2 seedlings which showed low bioluminescence were further grown to obtain seeds. We then monitored bioluminescence of 32 to 48 individual M3 offsprings from each candidate M2 line. After screening ca 20,000 M2 seedlings, we obtained dozens of lines that consistently exhibit low LUC expression in M3 generation. At least two of them showed reduced bioluminescence in developing seeds and reduced seed oil content. We also screened ca 10,000 T1 *DGAT1::LUC* plants further transformed with enhancer T-DNA, and obtained dozens of lines exhibiting high LUC expression. In one of them, enhancer was inserted near a seed-specific gene with unknown function, and over-expression of this gene under the 35 S promoter enhanced bioluminescence in developing seeds and caused increased oil content per seed.

06010

TRANSCRIPTOME COEXPRESSION ANALYSIS LED TO IDENTIFICATION OF A NOVEL ANTHOCYANIN GLYCOSYLTRANSFERASE GENE IN *ARABIDOPSIS*

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In *Arabidopsis*, the presence of 32 flavonols and 11 anthocyanins has been reported. These flavonoid structures suggest that at least 12 UDP-sugar dependent glycosyltransferases (UGTs) are involved in the flavonoid glycosylation. Among them, 6 UGTs have already been identified (1-4) but others still remain unknown.

To identify flavonoid UGTs comprehensively, we utilized transcriptome coexpression analysis using known flavonoid-related genes as queries. Twenty-four genes had more than two correlations with flavonoid-related genes. Among them, *UGT3* was found to be highly correlated with anthocyanin biosynthetic genes. *UGT3* belongs to the flavonoid-UGT subfamily which glycosylate a hydroxyl group of sugar moiety linked to the flavonoid aglycone. The anthocyanin profiles of *ugt3* Ds-transposon inserted mutants are different from that of wild type when grown in high sucrose condition. *UGT3* recombinant protein could catalyze the xylosylation of cyanidin 3-O-glucoside. *UGT3* recognized UDP-xylose but not other UDP-sugars. These data show that *UGT3* encodes a novel anthocyanin O-xylosyltransferase.

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 - (2) Tohge *et al.*, *Plant J.*, 42, 218-235 (2005)
 - (3) Yonekura-Sakakibara *et al.*, *J. Biol. Chem.*, 282, 14932-14941 (2007)
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06011

COORDINATED REGULATION OF THYLAKOID BIOGENESIS AND PHOTOSYNTHETIC GENE EXPRESSION EVIDENCED BY A GALACTOLIPID-DEFICIENT MUTANT

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In plants, the galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are the predominant lipids in thylakoid membranes and are indispensable for photosynthesis. Among the three isoforms that catalyze MGDG synthesis in *Arabidopsis*, MGD1 is responsible for most galactolipid synthesis in chloroplasts, whereas MGD2 and MGD3 are required for DGDG accumulation during phosphate (Pi) deficiency. A null mutant of *Arabidopsis* MGD1 (*mgd1-2*), which lacks both galactolipids and shows a severe defect in chloroplast biogenesis under nutrient-sufficient conditions, accumulated large amounts of DGDG with a strong induction of *MGD2/3* expression during Pi starvation. The mutant showed internal membrane biogenesis and chlorophyll accumulation in the plastid without functional photosynthesis. In the mutant, light-harvesting/photosystem core complexes were improperly formed, suggesting a requirement

for MGDG for the proper assembly of these complexes. During Pi starvation, distribution of plastid nucleoids changed concomitantly with internal membrane biogenesis in *mgd1-2*. Moreover, reduced expression of nuclear- and plastid-encoded photosynthetic genes observed in Pi-sufficient *mgd1-2* was restored after Pi starvation. Thus, galactolipid biosynthesis triggers plastid gene expression and plastid-to-nucleus signaling independently of photosynthesis. These results suggest that glycolipid synthesis and subsequent membrane biogenesis inside the plastid are involved in regulating photosynthetic genes encoded in the nucleus and plastids. This concerted regulation between galactolipid biosynthesis and photosynthetic gene expression may be important for synchronizing the formation of photosynthetic complexes with the development of thylakoid membranes during chloroplast biogenesis.

06012

PLANT METABOLOMICS: WIDELY TARGETED AND UN-TARGETED ANALYSIS

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Metabolome data obtained from large-scale plant bioresources are expected to accelerate unraveling diverse plant metabolism. For the genome wide metabolomics, we established widely targeted metabolomics and un-targeted metabolomics based on two different types of LC-MS/MS [1-4]. Using UPLC-tandem quadrupole MS (TQMS), the targeted 734 standard compounds, available primary and secondary metabolites, were detectable using selected reaction monitoring (SRM), which is high sensitivity/selectivity analysis based on the selected MS1 (precursor ion) and MS2 (product ion). This methodology was reasonable for analysis of thousands of samples because high throughput data processing represent a significant advance as compared to un-targeted analysis. On the other hand, the un-targeted MS/MS spectral tag (MS2T), which was obtained by the UPLC-quadrupole time of flight MS, was essential for the estimation of the unknown metabolites. In the samples of *Arabidopsis thaliana* (Col-0) at various development stages, thousands of MS2T data have been measured and the approx. 300 MS2Ts were successfully annotated using reference data of the standard compounds and reported fragmentation patterns of metabolites [3]. Thus, the predicted SRM of the detectable metabolites could be accomplished by using the annotated MS2T. In this study, the 4,986 of predicted SRM traditions (277 MS2Ts x 3 product ions x 6 steps of collision energy ranging from 10 to 60 eV) were measured by UPLC-TQMS. In the seeds of Col-0, approx. 70% of predicted SRM could be detected. These results suggested that the targeted and predicted SRM driven ultra widely targeted metabolomics has the potential of improving the number of detectable metabolites.

[1-3] Sawada et al., Plant and Cell Physiology (2009) 50:37-47; 50:1181-1190; 50:1579-1586.

[4] Matsuda et al., The Plant Journal (2009) 57:555-577

[5] Matsuda et al., Plant Physiology (2009) 152:566-578

06013

IMPROVEMENT OF A METHOD FOR PLANT HORMONE ANALYSIS BY LC-MS/MS

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Current studies revealed that the hormonal regulations were not always independent or parallel but rather often synergistic or antagonistic. In order to better understand their interactive networks, we have developed a method for plant hormone analysis by using a liquid chromatography-tandem mass spectrometry (LC-MS/MS). Our objective is to establish a method for quantitative analysis of as many hormones and related metabolites as possible. A difficulty in this method is mainly depending on their low concentrations in plants. Because co-migrating impurities suppress ionization of target compounds, it is necessary to establish a simple but effective purification method before analysis by LC-MS/MS. We will report our current status of hormone analysis. Auxin-related compounds we analyze are indole-3-acetic acid (IAA), indole-3-aldoxime, indole-3-acetaldehyde, indole-3-acetamide and IAA-amino acid conjugates. Cytokinins are *trans*-zeatin, N^6 -(Δ^2 -isopentenyl)adenine, dihydrozeatin and their 3 ribosides. Abscisic acid (ABA) and its metabolites are phaseic acid (PA), dihidro-PA, 7'-OH-ABA, neo-PA and ABA-glycosyl ester (internal standards were kindly provided by Dr. Suzanne Abrams). Gibberellins are GA₁, GA₄ and other 12 GAs involved in GA₁ and GA₄ biosynthesis and metabolism. Jasmonic acid (JA) and precursors are OPDA, OPC:8 and metabolites including JA-Ile and 12-OH-JA. Salicylic acid has been analyzed relatively easily. Brassinosteroids are still difficult to analyze for now, and we can only detect castasterone. We will report our current results of *Arabidopsis* dry seeds, seedlings, leaves, roots and flowers. Minimum amount of plant materials we need were depending on concentrations of hormones. Usually we need approximately 250 mg fresh weight of plant materials for general hormone analysis. We can also extend the method we established for *Arabidopsis* to other plants.

06014

A NEW ENZYME ENCODED BY THE ROD1 GENE IN ARABIDOPSIS REGULATES SEED TRIACYLGLYCEROL COMPOSITION AND PLANT DEVELOPMENT

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Triacylglycerols (TAG) from vegetable oils are a major source of human diet, as well as an important feedstock for non-food industrial products and biofuels. Fatty acid composition is a major factor that affects the uses of the oils. In oil-accumulating cells of plant seeds, monounsaturated oleic acid 18:1, synthesized from acetyl-CoA in the plastids, is desaturated to polyunsaturated fatty acids 18:2 and 18:3 by two desaturase enzymes of the endoplasmic reticulum, FAD2 and FAD3. Before desaturation, 18:1 must be incorporated into phosphatidylcholine (PC), which is the only substrate recognized by the FAD2 and FAD3 desaturases. Current models of TAG synthesis in oilseeds propose that 18:1 can enter the PC pool either by action of LPCAT or by the action of CPT on 18:1-diacylglycerol. Here, we show that a previously unrecognized enzyme, phosphatidylcholine: diacylglycerol cholinephosphotransferase (PDCT), encoded by the *Arabidopsis* ROD1 gene, is a major reaction for transfer of 18:1 into PC for desaturation, and for reverse transfer of 18:2 and 18:3 PUFAs into the TAG-synthesis pathway. The PDCT enzyme catalyzes transfer of the phosphocholine headgroup from PC to diacylglycerol (DAG), and mutation of rod1 reduces 18:2 and 18:3 accumulation in seed TAG by 40%. Our discovery of PDCT is important for understanding glycerolipid metabolism in plants and other organisms, and provides new tools to modify the fatty acid compositions of plant oils for improved nutrition, biofuels and other purposes. In addition, mutant characterization of rod1 indicated that PDCT may also affect plant development in *Arabidopsis*, such as seedling development and flowering.

06015

THE ARABIDOPSIS NRT2.4 IS A HIGH-AFFINITY NITRATE TRANSPORTER IMPLICATED IN ADAPTATION TO LOW-N CONDITIONS

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Nitrogen (N) is one of the most important nutrients for plants; its availability is often a limiting factor of plant growth and productivity both in natural and agricultural systems. Therefore, plants have evolved a set of responses to adapt to low N availability. These include biochemical and morphological changes to enhance N acquisition and allocation. However, little is known about the molecular basis of these responses. Here we characterized *AtNRT2.4*, which encodes a putative high-affinity nitrate transporter, as a component involved in plant adaptation to low N availability in *Arabidopsis*.

AtNRT2.4 expression was induced as nitrogen availability getting scarce and repressed by supplement with nitrogen, irrespective of nitrogen species. Transgenic plants harboring promoter-GFP fusion gene revealed that *AtNRT2.4* is preferentially expressed in epidermis of lateral roots, indicating that *NRT2.4* plays a role in acquisition of nitrate under N-limiting conditions. To further investigate the role of *AtNRT2.4*, we characterized a T-DNA insertional mutant of the gene. The mutant exhibited a significant reduction in the high-affinity nitrate uptake compared with WT and this was evident only in plants grown under low-N conditions. Furthermore, the mutant showed greater reduction in nitrate uptake in lower nitrate concentrations. Together, these results suggest that *AtNRT2.4* is a high affinity nitrate transporter involved in adaptation to low-N conditions.

06016

EFFECT OF ALTERATION OF PHOTOSYNTHETIC CAPACITY ON VARIOUS METABOLISMS

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We have demonstrated that transgenic tobacco plants expressing cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase (FBP/SBPase) in chloroplasts exhibit increased photosynthetic capacity and dry matter compared with those of the wild-type plants (Nature Biotechnol. 19, 965, 2001; Plant Cell Physiol. 47, 380, 2006; Plant Cell Physiol. 49, 375, 2008). In higher plants, the balance between carbon and nitrogen must be tightly coordinated, suggesting that various metabolic pathways including nitrate metabolism are affected in the photosynthesis-elevated plants. To clarify the effect of alteration of photosynthetic capacity on various metabolisms, we generated and evaluated the transgenic *Arabidopsis* (ApFS) expressing FBP/SBPase in chloroplasts. The photosynthetic CO₂ fixation rate in ApFS was approx. 1.3-fold higher than that in the wild type under saturating irradiance conditions. After 8 weeks, the total fresh weight of ApFS was approx. 1.2-fold higher than that of the wild type. Here we analyzed the transcript levels of various genes involved in carbon and nitrogen metabolisms in ApFS and wild-type plants. In 5-weeks-old plants, expressions of many genes involved in the Calvin cycle were reduced, while expressions of genes encoding starch synthase and sucrose synthase were increased in ApFS. Interestingly various genes involved in nitrogen metabolisms were induced in ApFS. The contents of RuBP, Ru5P, FBP, and sucrose in ApFS were higher than those in wild type, whereas the contents of various amino acids were lower than those in the wild type. These results suggest that the enhancement of photosynthetic capacity in ApFS induces increase in the contents of photosynthetic intermediates, leading to the feedback inhibition of expression of the genes involved in the Calvin cycle, the temporary imbalance of carbon and nitrogen ratio, and thus the induction of various genes involved in nitrogen metabolisms due to maintenance of C/N balance in ApFS.

06017

REGULATION OF HORMONAL RESPONSE THROUGH THE FLAVIN METABOLISM BY CHLOROPLASTIC FAD PYROPHOSPHOHYDROLASE (ATNUDX23) IN ARABIDOPSIS PLANTS

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Although flavins, such as riboflavin (RF), FMN, and FAD, are essential for not only a variety of primary and secondary metabolisms but also responses to environmental stimuli and hormones in plants, their metabolic pathway(s) are still largely unknown. Recently, we found that an *Arabidopsis* Nudix hydrolase, AtNUDX23, has the activity of pyrophosphohydrolase toward FAD and is localized in chloroplasts (Plant Physiol., 2008, 148: 1412-1424). Here we studied the physiological role of AtNUDX23 in flavin metabolism using AtNUDX23-overexpressed (Ox-NUDX23) or -suppressed (KD-nudx23) *Arabidopsis* plants. The activity of pyrophosphohydrolase toward FAD was enhanced and reduced in the Ox-NUDX23 and KD-nudx23 plants, respectively. Interestingly, the levels of RF, FMN, and FAD in both Ox-NUDX23 and KD-nudx23 plants were lower than those in the control plants. The transcript levels of genes (*COS1* and *AtRibF2*) involved in the RF biosynthesis were significantly decreased in both Ox-NUDX23 and KD-nudx23 plants. Similarly, the transcription of those genes was decreased by the exogenous applications of RF, FMN, or FAD. These findings suggest a negative feedback regulation of the flavin biosynthesis and the maintenance of levels of flavins via AtNUDX23.

Next, we investigated the involvement of AtNUDX23 in the plant hormonal responses. The transcript levels of methyl jasmonate (MeJA)-responsive genes in the Ox-NUDX23 and KD-nudx23 plants were markedly higher than those in the control plants under the treatment with or without MeJA. In contrast, the transcript levels of salicylic acid (SA)-responsive genes were lower in the Ox-NUDX23 and KD-nudx23 plants than the control plants under the treatment with or without SA. These findings suggest that the intracellular levels of flavins have the opposite effect on the MeJA- and SA-responses, and thus the flavin metabolism by AtNUDX23 is prerequisite for the accurate regulation of plant hormonal responses.

06018

PLASTIDIAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE CONTROLS ROOT AND POLLEN DEVELOPMENT BY REGULATING THE CARBOHYDRATE AND AMINO ACID BALANCE IN ARABIDOPSIS

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Plant metabolism is highly coordinated with development. However, an understanding of the whole picture of metabolism and its interactions with plant development is scarce. The glycolytic glyceraldehyde-3-phosphate (GAP) dehydrogenase (GAPDH) converts GAP to 1,3-bisphosphoglycerate with reduction of NAD⁺ to NADH. Both cytosolic and plastidial GAPDH activities have been described but the *in vivo* functions of the plastidial isoforms remain unresolved. We have identified two *Arabidopsis* plastid localized GAPDH isoforms (GAPCp1 and GAPCp2). *gapcp* double mutants (*gapcp1gapcp2*) display a drastic phenotype of arrested root development and sterility. Down-regulation of GAPCp leads to drastic changes in the sugar and amino acid balance. We demonstrate that GAPCps are important for the synthesis of serine in roots. Serine supplementation to the growth medium rescues root developmental arrest, and restores normal levels of carbohydrates and sugar biosynthetic activities in *gapcp1gapcp2*.

GAPCp deficiency also leads to male sterility. Pollen from *gapcp1gapcp2* have shrunk and collapsed shapes and are unable to germinate when cultured in vitro. The pollen alterations observed in *gapcp1gapcp2* were attributed to a disorganized tapetum layer. We demonstrated that both the expression and catalytic activity of GAPCp in anthers are necessary for mature pollen development. A metabolomic study in flower buds indicated that the most important difference between the sterile *gapcp1gapcp2* and the fertile wild type plants was the increase in the signaling molecule trehalose. Our results provide insights into the in vivo functions of the GAPCps and emphasize the importance of the plastidial glycolytic pathway, and specifically of GAPCps, in the connections between plant primary metabolism and development.

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06019

RECONFIGURATION OF MITOCHONDRIAL ENZYME COMPLEXES UNDER SHORT TERM OXIDATIVE STRESS

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The reconfiguration of metabolic enzyme complexes is probably a mechanism to alter the metabolic network under stress conditions especially for short term adaptation. In previous studies, we found rapid changes of mitochondrial metabolism such as tricarboxylic acid cycle and amino acid metabolism in *Arabidopsis* cell culture and roots. To elucidate the involvement of reconfiguration of enzyme complexes in these responses we conducted proteomic analysis in order to identify mitochondrial protein complexes that change their composition under oxidative stress.

Liquid culture seedlings of *Arabidopsis* were treated with 120 µM of menadione for 2 h to induce oxidative stress. The strong inhibition of pyruvate dehydrogenase and aconitase acted as markers for the onset of oxidative stress. Metabolite profiling revealed that the changes in metabolite abundance were similar in liquid culture seedling to those seen earlier in roots. Mitochondria were isolated from control and menadione treated seedlings and protein complexes were analysed by 2D-blue native /SDS-PAGE. The intensities of 18 spots increased and 13 spots decreased in menadione treated sample suggesting these proteins associate to or dissociate from protein complexes, respectively. Twenty four of these spots were identified successfully by LTQ Orbitrap MS. Some proteins are known to be involved in response to oxidative stress such as annexin1, peroxiredoxin IIF and glutathione S-transferases. We also identified metabolic enzymes related to central carbon metabolism including malic enzyme, fumarase and glyceraldehyde-3-phosphate dehydrogenase. Total enzyme activity and mRNA level of the enzyme was not directly correlated to the spot intensity in case of many proteins suggesting the metabolic regulation in post-translational level. The physiological relevance of some of these metabolic regulations will be discussed.

06020

TRANSCRIPTIONAL REGULATION OF GENES FOR SUBUNITS OF ACETYL-COA CARBOXYLASE IN ARABIDOPSIS

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Acetyl-CoA carboxylase (ACCase) catalyzes the formation of malonyl-CoA from acetyl-CoA, the first committed step of fatty acid synthesis. Plants, with an exception of Gramineae, have two forms of ACCase: the eukaryotic form, composed of a large multifunctional polypeptide in cytosol, and the prokaryotic form, composed of four different polypeptides in plastids. Plant plastidic ACCase is a multienzyme complex composed of biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC), and carboxyltransferase (CT) complex made up of two pairs of α and β polypeptides. Of these four polypeptides, β polypeptide is encoded by the chloroplast genome, and the other three polypeptides are encoded by the nuclear genome.

In *Arabidopsis*, BC and CT are encoded by single gene, and there are two genes for BCCP. The expression of all of these four genes is increased during the seed development, as well as other genes for enzymes involved in fatty acid synthesis. To understand the mechanisms that control the expression of these genes, the upstream sequences from these genes were fused to the β-glucuronidase (GUS) gene, and introduced into *Arabidopsis*. By analyzing the GUS activity in transgenic plants, we found that 5'-UTR sequences of these genes were necessary and sufficient for expression in developing seeds, and there were (WRINKLED1) WRI1 binding sequences in these sequences. Our data also suggested that WRI1 binding sequences upstream of transcriptional start site cannot induce the expression in seeds, indicating the importance of the position of WRI1 binding sequences.

06021

GENE EXPRESSION ANALYSIS TO UNDERSTAND FUNCTIONS OF LOXES IN ARABIDOPSIS.

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Recently many oxylipins which are metabolites of α-linolenic acid have been found as signal factors of plant growth regulation. It has been reported that oxylipins activate various signal compounds and enhance stress tolerances like jasmonic acid (JA) as well. The biosynthesis of oxylipins starts from releasing of α-linolenic acid from membrane lipids by DADs-like enzymatic activities, and followed by other enzymes such as LOX and AOS to convert precursors into a wide array of bioactive oxylipins. Six LOXs exist in *Arabidopsis thaliana* and they are classified into two types due to positional specificity of enzyme activity (9 or 13 oxidation).

It's common that 9- and 13-oxidation types of LOXs and/or AOS coexist in one plant species, however *Arabidopsis* involves only one AOS in spite of six LOXs. In JA biosynthesis and response, it is already known that three 13-LOXs (LOX2, LOX3, LOX4) are activated, but the function of LOX6, another 13-LOX is still unknown. Moreover there is almost no information on the 9-LOXs (LOX1, LOX5) activities at *in vivo* experiments. Nevertheless *in vitro* enzymatic assay demonstrated that a combination of 9-LOX with the *Arabidopsis* AOS produced a 9-oxidized oxylipin. And it was also suggested that the putative 9-LOX product may bind to AOS according to the analysis based on the crystal structure. Therefore it is reasonable that the 9-LOXs and 9-AOS pathway is actually activated in *Arabidopsis*.

Here we focused on the 9-LOXs and LOX6 enzymatic activities in *Arabidopsis* using a molecular genetic approach. In the case of *aos*, every LOXs induced regardless of the stress, namely before and after wounding treatment. On the other hand the wild type of *Arabidopsis* only induced LOX5 and LOX6 after 1.5h after the treatment while LOX1 were not influenced. Therefore these results suggest that LOX5 and LOX6 should supply substrates of AOS a sort of the wounding response.

06022

MUTAGENESIS IN RICE B-CYANOALANINE SYNTHASE (OSCAS) COULD SHIFT THE ACTIVITY RATIO FROM CYANIDE DETOXIFICATION TO CYSTEINE SYNTHESIS

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Cyanide detoxification and cysteine biosynthesis are crucial metabolic steps in plant hormone ethylene biosynthesis. To remove the co-product cyanide during the oxidation of 1-aminocyclopropane-1-carboxylic acid to ethylene (ACC), cysteine acts as the co-substrate for the β -cyanoalanine synthase (CAS) to form cyanoalanine and the sulfide released in this reaction must be used to replenish the cysteine pool by the action of *O*-acetylserine sulfhydrylase (OASS) to sustain further cyanide removal. Interestingly CAS and OASS are encoded by a gene family with overlapping structure and catalytic activity. To delineate activity favors of these proteins in the β -substituted alanine synthase family, rice CAS (OsCAS) mutants have been generated according to amino acid differences between CAS and OASS and expressed in budding yeast. Single amino acid change from valine to alanine at position 284 on OsCAS decreased the cyanoalanine synthase activity half but increased the cysteine synthesis almost five times compared to the wild type. The feasibility in serine acetyltransferase (SAT) interaction is one of factors distinguishing CAS/OASS members. A failure in complex formation between watermelon SAT and OsCAS mutant in which the SAT binding interface was re-constructed, indicating that more amino acid residues are involved in forming the SAT interacting domain. It is speculated that ethylene biosynthesis, and thereby the co-product cyanide, is the major driving force for the speciation of a mitochondrial OASS member into CAS during evolution in higher plants. Over-expression of OsCAS in Arabidopsis mitochondria allowed a better growth of seedlings, with 1.5 folds increase in fresh weight in 7 day old seedlings, when germinated under 0.01 - 1mM ACC. This result provides evidence on CAS functions as cyanide detoxification in relation to ethylene biosynthesis *in planta*.

06023

IDENTIFICATION OF *IN VIVO* TARGET GENES OF *ARABIDOPSIS THALIANA* FLAVONOID PATHWAY REGULATORS

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Flavonoids are a class of plant secondary metabolites playing important roles in various plant functions such as pigmentation, protection against UV light damage and phytopathogens, fertility and dormancy. The most visible function of flavonoids is the formation of red and purple anthocyanin pigments. Anthocyanins are synthesized by the coordinated, consecutive action of several biosynthetic enzymes. The fine-regulation of flavonoid biosynthesis is achieved by combinatorial action(s) of transcription factors, expressed in a spatially and temporally controlled manner. Expression of biosynthetic genes for anthocyanin formation is regulated by a protein complex composed of a more or less ubiquitously expressed WD40-repeat (WDR) protein, a PRODUCTION OF ANTHOCYANIN PIGMENT (PAP)-group R2R3-MYB transcription factor and a R-like basic helix-loop-helix (bHLH) protein, designated as MBW complex.

Although it is well accepted that the combinatorial interacting factors PAP1, ENHANCER OF GLABRA3 (EGL3) and TRANSPARENT TESTA8 (TT8) act to regulate target gene expression, binding of these factors to their target gene promoters in the nucleus of living cells has yet to be demonstrated. Here, we used a chromatin immunoprecipitation (ChIP) procedure to identify *in vivo* binding sites of MBW components with the help of transgenic GFP-fusion complemented loss-of-function lines.

06024

INACTIVATION OF STROMA ENZYMES BY OXYLIPIN ALDEHYDES GENERATED UNDER ENVIRONMENTAL STRESS

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Environmental stresses cause light excess in leaves and increase the production of reactive oxygen species in chloroplasts. Lipid peroxides produced via the oxidation of polyunsaturated fatty acids are degraded enzymatically or nonenzymatically to various aldehydes (oxylipin aldehydes). Among them, alpha,beta-unsaturated aldehydes (2-alkenals) have high electrophilicity and can form covalent adducts with proteins and nucleic acids, to induce cell damage. Under strong light, 2-alkenals were accumulated in leaves of wild-type tobacco. The transgenic tobaccos that overexpress 2-alkenal reductase from *Arabidopsis thaliana* accumulated lower levels of 2-alkenals and showed tolerance to strong light. This suggested that 2-alkenals are associated with photooxidative damage. In this study, we comprehensively analyzed the sensitivity of chloroplast components to oxylipin aldehydes in order to reveal their targets in the chloroplast. Addition of C1-C9 aldehydes to chloroplasts inactivated CO₂ photoreduction. Acrolein was the most toxic. Because the thylakoid electron transport chain was not inactivated, the Calvin cycle was the primary target. We added various oxylipin aldehydes to protein fraction extracted from chloroplasts and determined the inactivation of stroma enzymes. Phosphoribulokinase, glyceraldehyde-3-phosphate dehydrogenase and dehydroascorbate reductase showed high sensitivity to 2-alkenals. Acrolein had the strongest toxicity. Ribulose-1,5-bisphosphate carboxylase/oxygenase, ascorbate peroxidase and superoxide dismutase were unaffected by aldehydes. Thus each enzyme has different sensitivity to aldehydes. In plant cells, glutathione scavenges aldehydes, but under severe oxidative stress, it is expected that enzymes may be inactivated by oxylipin aldehydes according to the sensitivity order we revealed in this study. Inactivation of these sensitive enzymes as initial event will lead to further accumulation of reactive oxygen species and oxylipin aldehydes.

06025

IDENTIFICATION OF A CDNA CODING FOR A PUTATIVE AROMATIC SUBSTRATE PRENYLTRANSFERASE IN HOP (*HUMULUS LUPULUS*)

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Hop (*Humulus lupulus*) is a perennial and dioecious bine plant that belongs to the Cannabinaceae. The female hops are widely cultivated and the flowers (hop cones) are used as a raw material for beer brewing. The basal part of hop cone bracts is covered with many yellow glandular trichome called lupulin glands, where a variety of essential oils and aromatic compounds are accumulated. Lupulin glands are a rich source of prenylated aromatic compounds, in particular the major components are xanthohumol (prenylated chalcone), and humulone and lupulone (prenylated phloroglucinol) that give bitter taste to beer. Those prenylated phenols have received a large amount of attention since they have multiple biological activities beneficial for human health, where the prenyl residues attached to aromatic rings is suggested to play an important role in their activities. Recent progresses in plant molecular biology have enabled us to isolate prenyltransferases for aromatic compounds. For instance flavonoid prenyltransferases are reportedly membrane-bound enzymes and share moderate homology with homogentisate prenyltransferases involved in the biosynthesis of vitamin E and plastoquinone. In this study, we have cloned an aromatic substrate prenyltransferase-like cDNA *HIPT-1* from ca.10,000 ESTs of the lupulin-enriched cDNA library. *HIPT-1* was preferentially expressed in lupulin glands, while low expression was also detected in leaves by semi-quantitative RT-PCR analysis of different tissues of hop. The subcellular localization of *HIPT-1* was suggested to be plastids by GFP fusion protein

analysis. In the phylogenetic analysis, the hop HIPT-1 appears to be from a common origin with the homogentisate solanesyltransferase of plastoquinone biosynthesis but is clustered in a new single branch, and not in the other flavonoid prenyltransferase group.

06026

SIGE IS A GLOBAL REGULATOR CONTROLLING SUGAR CATABOLISM OF A UNICELLULAR CYANOBACTERIUM *SYNECHOCYSTIS* SP. PCC 6803.

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Metabolic engineering of photosynthetic organisms have received much attention because of its value for utilization of light energy and external carbon dioxide. Engineering of primary metabolism, however, is often difficult due to their robustness. We previously demonstrated that disruption of a group 2 sigma factor SigE of *Synechocystis* sp. PCC 6803 represses transcription of sugar catabolic genes such as glycolysis, the oxidative pentose phosphate pathway, and glycogen catabolism. In this study, the strain overexpressing SigE is generated using *psbAII* promoter. Microarray analysis showed that genes for the oxidative pentose phosphate pathway and glycogen catabolism increased in the SigE overexpression strain, and subsequent Northern analysis confirmed them. Immunoblotting revealed that protein levels of sugar catabolic enzymes such as glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glycogen phosphorylase, and isoamylase increased by *sigE* overexpression. The level of glycogen, which is carbon storage of cyanobacteria, reduced in the SigE overexpression strain under normal growth conditions. Metabolomic analysis with CE/MS showed that metabolites of the TCA cycle and acetyl-CoA are altered by SigE overexpression. We also found that SigE overexpression results in defective growth under mixotrophic or dark conditions. Combined with all data, we conclude that SigE overexpression globally enhances sugar catabolism, opening a sigma factor-based engineering for the modification of carbon metabolism in photosynthetic organisms.

06027

METABOLOMIC ANALYSIS OF PLANT INTACT VACUOLES ISOLATED FROM GENETICALLY ENGINEERED CELLS

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Vacuole plays an important role in maintaining homeostasis of plant cell via the development of turgor pressure, the accumulation of various inorganic ions and metabolites and the degradation of discarded proteins or organelles. These functions of the vacuole are dependent on the vacuolar containing substances. Until now, however, there are few studies about vacuolar substances analyzed directly and comprehensively. In the former study, we established the isolation method of intact vacuoles from suspension-cultured cells or whole plants and reported the proteomic analysis of the vacuolar membrane proteins. In this study, we have analyzed the contents of intact vacuoles directly and comprehensively by using CE-MS and FT-ICR-MS. In consequence, we have detected not only well-known vacuolar substances but also some unexpected substances in the vacuole. The proteomic analysis of vacuolar membrane and vacuolar sap was also conducted. We have also prepared the transgenic Arabidopsis suspension-cultured cells over-expressing unknown tonoplast proteins and conducted the comparative analysis of the vacuolar metabolites of the wild-type and transgenic cells in order to elucidate the possible functions of the unknown membrane proteins.

06028

REGULATION OF COA HOMEOSTASIS BY ARABIDOPSIS NUDIX HYDROLASES, ATNUDX11 AND 15

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CoA is an obligate cofactor in all living organisms, where it is involved in over 100 different reactions in intermediary metabolism. The biosynthesis of CoA from pantothenic acid is essential and universal in prokaryotes and eukaryotes, which has well been characterized. CoA is known to be hydrolyzed at the pyrophosphate unit; however little attention has been given to the reaction so far. We have demonstrated that, among 27 types of Arabidopsis Nudix hydrolases (AtNUDX1-27), cytosolic AtNUDX11 and mitochondrial AtNUDX15 hydrolyze CoA (*Plant Physiol.* 2008, 148: 1412-24). To gain insight into the importance of such reaction in plant cells, we studied here the physiological roles of AtNUDX11 and 15 in the CoA metabolism. The RT-PCR analysis showed that *AtNUDX11* and *15* had highest expression in roots and inflorescences, respectively. Similarly, the activities of CoA pyrophosphohydrolase were higher in roots and inflorescences than leaves, stems, and siliques. The activities of CoA pyrophosphohydrolase in the knockout *AtNUDX11* and *15* mutants (*KO-nudx11* and *KO-nudx15*) were reduced to 80.4 and 46.2%, respectively. However, there was no difference in the levels of free CoA and acetyl-CoA (AcCoA) between the wild-type plants and both mutants grown in the MS medium supplemented with sucrose. Importantly, the expression levels of CoA biosynthetic enzymes (*PANK*, *PPCS*, *PPCD*, *PPAT*, and *DPCK*) were suppressed in the *KO-nudx11* and *KO-nudx15*, suggesting a feedback inhibition of CoA biosynthesis by intracellular CoA levels. On the other hand, the levels of free CoA and AcCoA in both mutants grown in the MS medium in the absence of sucrose, by which fatty acid metabolisms are activated, were increased approx. 1.5- and 1.7-fold, respectively, compared with those in the wild-type plants. These results suggest that AtNUDX11 and 15 act as regulators in CoA homeostasis through its hydrolysis.

06029

OVEREXPRESSION OF NICOTINAMIDE ADENINE DINUCLEOTIDE SYNTHETASE CAUSES EARLY AGEING IN *ARABIDOPSIS THALIANA*

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Nicotinamide adenine dinucleotides (NAD(P)(H)) are well-known coenzymes that mediates hundreds of redox reactions and are basis of a large number of cellular metabolic processes regulating responses to various stressful environments and developmental events. NAD synthetase (*NADS*) is encoded by At1g55090 and acts as a key enzyme that mediates the final step of NAD biosynthesis, amidation of nicotinate adenine dinucleotide (NaAD) to NAD. In this study, we generated transgenic *Arabidopsis* plants overexpressing *NADS* gene (SOX lines). During vegetative growth, overexpression of *NADS* did not show any remarkable morphological changes. Moreover, the levels of NAD and NaAD were unchanged in SOX lines. However, we found that *NADS*-overexpression caused precocious ageing after bolting. The ageing was accelerated not only at leaves but also at developing inflorescences and stems. After bolting, NAD was decreased and nicotinamide, a product of NAD turnover, was increased in wild type and SOX lines. Noticeably, in SOX lines, the increase of nicotinamide was significantly enhanced and the level of NaAD was drastically increased. Thus, both turnover and biosynthesis of NAD seemed to be facilitated by bolting-inducing or -induced signal(s) in SOX lines. Interestingly, *NADS* overexpression resulted in increase of GSH level, suggesting that SOX lines were exposed to oxidative stress condition. These results indicate that the acceleration of NAD turnover results in oxidative stress and leads to runaway cell death and early ageing.

06030

FUNCTIONAL CHARACTERIZATION OF *A. THALIANA* ETHE1

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The glyoxalase system has been studied in a number of organisms. It has been proposed that detoxification of 2-oxoaldehydes is its primary function, although the exact role(s) of this system, which consists of two enzymes: glyoxalase I (lactoylglutathione lyase) and glyoxalase II (hydroxyacylglutathione hydrolase) is unknown. While glyoxalase I is well studied, much less is known about glyoxalase II enzymes, which exist as multiple isozymes in plants. In *Arabidopsis thaliana*, the protein product of the gene At1g53580 was originally called Glx 2-3 and considered one of the five Glx II isozymes. Recently it has been found that this gene possesses 54 % sequence identity to human Ethe1 (compared to 14% to other Glyoxalase II Isozymes of *A. thaliana*), which has been implicated in the rare disorder Ethymalonic encephalopathy. Since then this gene was renamed *A. thaliana* ETHE1. Studies from our lab have shown that the enzyme is required for plant survival (endosperm development). Furthermore over expression of the gene has a positive effect on plant growth that is affected by the cellular localization of the enzyme. In particular we found that targeting of ETHE1 to the cytoplasm conferred resistance to L-Valine stress. In an effort to better understand the functional role of this enzyme, we are performing non targeted metabolic profiling using GC-MS to compare the polar and non polar metabolite profiles in plants with altered ETHE1 levels. Extracts prepared from plants grown under various conditions are being compared to identify metabolic changes associated with altered ETHE1 expression and distribution. Results from these experiments will be discussed.

06031

METABOLIC CHANGES BY THE OVEREXPRESSION OF A PUTATIVE VACUOLAR MEMBRANE TRANSPORTER GENE IN ARABIDOPSIS LEAVES AND SUSPENSION CULTURED CELL

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Vacuole of plant cells is a multi-functional organelle that contributes to the storage of various types of metabolites. Proteomic analysis revealed that there are more than 30 unknown trans-membrane proteins that putatively act as transporters of metabolites across the tonoplast membrane. However, actual functions of these trans-membrane proteins have not been clarified yet. We are currently investigating functions of several unknown tonoplast trans-membrane proteins, and mechanisms with which they control cellular and vacuolar metabolisms. In this study, we report changes observed in metabolite profiles of transgenic lines of *Arabidopsis* suspension cultured cells T87 and *Arabidopsis* plants that overexpress a gene, At3g21690, encoding a putative MATE efflux family protein. Analysis of cell extract from transgenic T87 cells demonstrated that the accumulation levels of amino acids and sugars did not change. However, significant increase was observed in the accumulation of flavonols. This increase coincided with the upregulation of several transcriptional regulators including TTG1. These results suggest that, in suspension-cultured cells, overexpression of At3g21690 affects the vacuolar accumulation of flavonols or affects the biosynthesis of flavonols by unknown mechanisms. On the other hand, in rosette leaves of the transgenic plants, overexpression of At3g21690 did not alter the levels of flavonols. Instead, significant increase was observed in the accumulation of putative glucosinolates and sinapate derivatives. This result implies that a transporter encoded by At3g21690 may transport multiple substrates according to the cell-type dependent metabolic activities. Metabolite profiling of isolated vacuoles and production of At3g21690 gene-silencing lines are currently ongoing. This work was partly supported by CREST, JST.

06032

PROMOTER ANALYSIS OF SAPONIN BIOSYNTHETIC GENES IN LICORICE

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Roots and stolons of *Glycyrrhiza* plants (licorice) are among the most important crude drugs in oriental traditional medicine, and are also used as natural sweetener. Licorice contains two major triterpenoid saponins, glycyrrhizin and soyasaponins, both of these are derived from β -amyrin, one of the most commonly found triterpenes in plants. We have recently identified, from *G. uralensis*, the genes encoding bAS (β -amyrin synthase) and two cytochrome P450 monooxygenases, CYP88D6 (β -amyrin 11-oxidase) and CYP93E3 (β -amyrin 24-hydroxylase), participate in the pathway for glycyrrhizin and soyasaponins, respectively. To understand the molecular mechanisms for the regulation of triterpenoid saponin biosynthesis in licorice, 5'-upstream region of the each of bAS, CYP88D6 and CYP93E3 was isolated using PCR-based genome walking method. Obtained 1.8 - 2.0 kb region upstream of translation start site was fused to GUS reporter gene, subsequently introduced into the several different tissues of *G. uralensis* by particle bombardment. The GUS expression, under the control of bAS- or CYP88D6 promoter, was detected in the cultured stolons; however, not detected in the leaves and stems. Transgenic *Arabidopsis* plants containing the bAS- or CYP88D6 promoter-GUS fusion also showed GUS expression predominantly in roots. These observations were consistent with the expression patterns of corresponding genes as revealed by RT-PCR analysis, as well as the accumulation pattern of glycyrrhizin in *G. uralensis*. Several cis-motifs, including W-boxes and SURE (Sucrose Responsive Element), are

commonly found in *bAS* and *CYP88D6* gene promoters, suggesting that both genes may be regulated by the same or similar transcription factor(s). To narrow down the *cis*-regulatory regions, 5'-deletion analysis of *bAS* and *CYP88D6* promoters is in progress.

06033

IDENTIFICATION OF CYTOCHROME P450S INVOLVED IN TRITERPENOID SAPONIN BIOSYNTHESIS IN LICORICE

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Glycyrrhizin, a major bioactive compound derived from the underground parts of *Glycyrrhiza* (licorice) plants, is a triterpenoid saponin that possesses various pharmacological activities and is used as a natural sweetener. Due to its economic value, the biosynthesis of glycyrrhizin has received considerable attention. The biosynthesis of glycyrrhizin involves the initial cyclization of 2,3-oxidosqualene, a common precursor of both triterpenes and sterols, to a triterpene β -amyrin, one of the most commonly occurring triterpenes in plants. The subsequent steps involve a series of oxidative reactions at the C-11 and C-30 positions, followed by glycosyl transfers to the C-3 hydroxyl group; however, no genes encoding relevant oxidases or glycosyltransferases have been identified. As a resource for gene discovery in glycyrrhizin biosynthesis, we have generated an EST library from the stolons of *G. uralensis* plants, comprising approximately 56,000 cDNAs. Mining of EST data set for putative P450s and subsequent transcript profiling-based selection led to the identification of CYP88D6. CYP88D6 was characterized by *in vitro* enzyme assays and shown to catalyze the sequential two-step oxidation of β -amyrin at C-11 to produce 11-oxo- β -amyrin, a possible biosynthetic intermediate between β -amyrin and glycyrrhizin. The expression of CYP88D6 was predominantly detected in the roots and stolons, which is consistent with the accumulation pattern of glycyrrhizin *in planta*. These results suggest a role of CYP88D6 as a β -amyrin 11-oxidase in the glycyrrhizin pathway. We also isolated, by PCR-based method, CYP93E3 that participates in soyasaponin biosynthesis as a β -amyrin 24-hydroxylase. These observations clearly indicate that in *G. uralensis* and most probably in other legumes as well, the P450 enzymes involved in triterpenoid saponin biosynthesis are recruited in at least two very distant CYP families, CYP93 and CYP88.

06034

DISCOVERY OF P450 GENES IN TRITERPENOID SAPONIN BIOSYNTHESIS

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Triterpenoid saponins are a diverse group of secondary metabolites produced by many plant species. These compounds have been associated with a variety of biological activities. Legumes show a pool of different triterpenoid saponins, however, most of its biosynthesis pathways are yet unknown. Barrel medic (*Medicago truncatula*), a model legume with extensive EST data set, exhibits a pool of different triterpenoid saponins. Its aglycones are most likely derived from β -amyrin, the initial product of cyclization of 2,3 oxidosqualene. This first step catalyzed by β -amyrin synthase (bAS) was already reported, but subsequent steps in the modification of triterpenoid backbone are yet unknown. In a previous work, licorice (*Glycyrriza spp.*) CYP88D6, was reported as a β -amyrin 11-oxidase, catalyzing 2 sequential steps in the glycyrrhizin pathway. Similarly, licorice CYP93E3 was characterized as a β -amyrin 24-hydroxylase implicated in the soyasaponin pathway. In this work, using *M. truncatula* EST database, 2 homologs of CYP88D6 and a homolog of CYP93E3 were identified and analyzed for their potential β -amyrin oxidizing activity using a yeast expression system. The results showed that CYP93E2 was able to modify β -amyrin to β -amyrin-24-oic acid via 24-OH- β -amyrin. Furthermore, CYP93E2 expression was highly correlated with bAS expression, suggesting that it plays a key role in the modification of β -amyrin *in planta*. Although CYP88D2 and CYP88D3 were not able to oxidize β -amyrin; when their activity was tested in combination with CYP93E3, presumable β -amyrin derivatives were detected in the transgenic yeast co-expressing bAS, CYP88D3 and CYP93E3. These results suggest that CYP88D3 was able to oxidize C-24 oxidized derivatives of β -amyrin. Finally, the identified P450 enzymes collectively constitute a molecular toolbox that can be utilized toward the combinatorial biosynthesis of triterpenoids.

06035

MULTIPLE BIOSYNTHETIC PATHWAYS FOR BRASSINOSTEROIDS ARE FUNNELED INTO CASTASTERONE IN *A. THALIANA*

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A large-scale analysis for brassinosteroids (BRs) in *A. thaliana* revealed that dolichosterone (DS) and homodolichosterone (HDS) are contained in the plant. Together with the presence for 28-norcastasterone (28-norCS) and castasterone (CS), this suggests that multiple biosynthetic pathways to produce BRs are operating in *A. thaliana*. To investigate biosynthetic connections of the multiple BRs biosyntheses, crude enzyme solution was prepared from *A. thaliana*, and *in vitro* conversions of the end products of the multiple biosynthetic pathways were examined. When HDS was used as a substrate, DS and HDS were detected as the enzyme products. When DS was used, CS was identified as a product. Coupled with our previous result that 28-norCS is converted into CS, the results indicate that the multiple biosynthetic pathways are biosynthetically connected to produce, an active BR, CS in *A. thaliana*. To identify the gene catalyzing the conversion of DS to CS, DWF1 deficient mutant was selected, and metabolic study was carried out. Unfortunately, conversion of DS to CS was detected, indicating that DWF1 does not catalyze the reduction of DS to CS in *A. thaliana*. In the presentation, SMTs and cytochrome P450s involved in the biosynthetic connection will be also discussed.

06036

SIMULTANEOUS MEASUREMENTS OF METABOLITES AND PLANT HORMONES IN *ARABIDOPSIS THALIANA* USING CAPILLARY ELECTROPHORESIS ELECTROSPRAY QUADRUPOLE TIME-OF-FLIGHT (CE-ESI-QTOF)

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Metabolome analyses using mass spectrometry such as GC-MS or LC-MS have been developed to identify various substances in plants with an improved efficiency. Recently, CE-MS is reported to be a useful tool to identify plant metabolites, especially the charged species.

In this study, we performed a simultaneous quantitative analysis of metabolites such as various primary metabolites including amino acids, organic acids, and phosphate compounds in *A. thaliana* shoots and roots at different growth stages by using CE-ESI-QTOF. As plant hormones, indole-3-acetic acid (IAA), cytokinin, abscisic acid (ABA), and jasmonic acid (JA) were also detected with CE-ESI-QTOF.

We also investigated the changes in primary metabolites response to the externally applied auxin (IAA) during the growth.

We performed a simultaneous quantitative analysis of metabolites and plant hormones successfully under the same condition. In the case of non-target analysis in the positive mode, 548 and 603 peaks were found in shoots and roots, respectively. Of these, 25 and 23 compounds in shoots and roots respectively could be identified e.g. as amino acids. In the negative mode, 199 and 214 peaks in shoots and roots respectively were identified. Of these, some compounds in shoots and roots were identified; these included organic acids, phosphate compounds and others.

06037

A PLASTIDIC INVERTASE REGULATES PHOTOSYNTHESIS AND NITRATE ASSIMILATION DURING GREENING

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Since the photosynthetic apparatus contains a massive amount of nitrogen in plants, the regulation of its development by sugar signals is important to the maintenance of the carbon-nitrogen balance. Here, we isolated an *Arabidopsis* mutant (*sicy-192*) whose cotyledon greening was inhibited by treatments with sugars, such as sucrose, glucose, and fructose. In the mutant, the gene encoding plastidic alkaline/neutral (A/N) invertase (INV-E) was point-mutated at codon 294, with Tyr substituted for Cys (C294Y). Interestingly, the greening of cotyledons in the knockout-INV-E lines was not inhibited by treatment with the sugars. In addition, the knockout-INV-E lines expressing an INV-E:C294Y or INV-E:C294A gene had the same phenotype as *sicy-192* mutants, while the lines expressing a wild-type INV-E gene had the same phenotype as wild-type plants. A recombinant INV-E:C294Y protein had the same enzymatic activity and substrate specificity as a recombinant INV-E protein. Though the transcriptional level of INV-E was not altered, the protein and activity levels of INV-E were higher in the mutants than in the wild-type plants. These findings indicated that the mutant INV-E is more stable than the wild-type INV-E and Cys294 is necessary to regulate the stability of INV-E in vivo during greening. On treatment with sucrose, the expression of photosynthesis-related genes was weaker in seedlings of mutant plants than wild-type seedlings, while the activity of nitrate reductase was stronger in the mutant plants than wild-type plants. These findings suggest that Cys294 of INV-E is associated with the development of the photosynthetic apparatus and the assimilation of nitrogen in *Arabidopsis* seedlings to control the ratio of sucrose content to hexose content (*J. Biol. Chem.* in press).

06038

BIOCHEMICAL ANALYSIS OF AUXIN BIOSYNTHETIC PATHWAYS IN ARABIDOPSIS

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Indole-3-acetic acid (IAA) is a naturally occurring auxin that regulates most aspects of plant growth and development. Plants have several proposed pathways for IAA biosynthesis, but none of them are fully characterized at enzyme/intermediate levels. Identification of IAA intermediates in each pathway will accelerate genetic and enzymatic studies on IAA biosynthesis. In this study, we analyzed proposed IAA intermediates in *Arabidopsis* mutants in which IAA biosynthesis genes are genetically modified. LC-MS/MS analysis of indole-3-acetaldioxime (IAOx) in CYP79B-deficient *Arabidopsis* mutants and non-crucifer plants demonstrated that the CYP79B pathway mainly contributes to IAA biosynthesis via IAOx in *Arabidopsis*, and may not operate in non-crucifers. LC-MS/MS analysis of indole-3-acetaldehyde (IAAld) in *Arabidopsis* will also be discussed.

06039

METABOLIC PROFILING AND CYTOLOGICAL ANALYSIS OF PROANTHOCYANIDINS IN IMMATURE SEEDS OF FLAVONOID ACCUMULATION MUTANTS OF ARABIDOPSIS THALIANA

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Arabidopsis TRANSPARENT TESTA19 (TT19) encodes a glutathione S-transferase (GST)-like protein that is involved in the accumulation of proanthocyanidins (PAs) in the seed coat. PA accumulation sites in *tt19* immature seeds were observed as small vacuolar-like structures, whereas those in *tt12*, a mutant of the tonoplast-bound transporter of PAs, and *tt12 tt19* were observed at peripheral regions of small vacuoles. We found that *tt19* immature seeds had small spherical structures showing unique thick morphology by differential interference contrast microscopy. The distribution pattern of the thick structures overlapped the location of PA accumulation sites, and the thick structures were outlined with GFP-TT12 proteins in *tt19*. PA analysis showed higher levels of solvent-insoluble PAs in *tt19* immature seeds compared with wild-type. Metabolic profiling of the solvent-soluble fraction by LC-MS demonstrated that PA derivatives such as epicatechins and epicatechin oligomers were absent in *tt19*. We also revealed that *tt12* specifically accumulated glycosylated epicatechins, the putative transport substrates for TT12. Given the cytosolic localization of functional GFP-TT19 proteins, our results suggest that TT19, which acts prior to TT12, functions in cytosol for regular accumulation of PA precursors such as epicatechin and glycosylated epicatechin in the vacuole. The PA pathway in *Arabidopsis* seed coat is discussed in relation to the subcellular localization of PA metabolites.

06040

STRUCTURAL AND FUNCTIONAL ANALYSES OF VOLATILE COMPOUNDS- AND HEAVY METALS- INDUCED ARABIDOPSIDES IN ARABIDOPSIS THALIANA

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In recent research for bioactive substances of *Arabidopsis thaliana*, seven new oxylipins named arabidopsides A- G were isolated from the aerial parts of this plant. Arabidopsides A- G were monogalactosyl diacylglycerides or digalactosyl diacylglycerides containing 12-oxophytodienoic acid (OPDA) and/or dinor-oxophytodienoic acid (dn- OPDA) which were known as precursors of jasmonic acid (JA) and have received much attention because they play important roles in regulation of developmental and defense gene expression of plants as JA and methyl jasmonate. On the other hand, it is known that alpha-, beta- unsaturated carbonyl compounds and some heavy metals induce OPDA and JA in various plants like *Arabidopsis thaliana* and *Oryza sativa*. In this research, we investigated the effect of volatile compounds and heavy metals as potent inducers for triggering accumulations of arabidopsides in mature leaves of *A. thaliana*. In addition, volatiles- and heavy metals- induced arabidopsides weren't able to detected in the *coi 1-1* mutant. These results indicate that abiotic stress- induced arabidopsides are connected with the mechanism of stress response through COI 1 signaling in *A. thaliana*.

06041

PHYSIOLOGICAL CONTRIBUTION OF PURINE METABOLISM IN DROUGHT ACCLIMATIZATION OF ARABIDOPSIS

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In plants, catabolism of purine compounds constitutes one of the fundamentals of nitrogen metabolism, but its physiological role remains elusive in most species except legumes. Among several plant enzymes participating in purine catabolism, xanthine dehydrogenase (XDH) serves as the key enzyme responsible for the first and rate-limiting reaction in the oxidative degradation pathway. We have recently demonstrated that RNAi-mediated knockdown of Arabidopsis XDH genes (*AtXDH1* and *AtXDH2*) causes defects in normal growth and development as the plants grow to maturity [1]. Although widely regarded as a housekeeping enzyme, XDH is also implicated in plant responses and acclimatization to various biotic and abiotic stress including pathogen attack, high salinity and drought. In this study, we examined the physiological impact of XDH knockdown, hence impaired purine metabolism, on drought tolerance of Arabidopsis plants [2]. Compared with the wild-type strain, severely *XDH*-silenced RNAi plants showed significantly reduced biomass and chlorophyll content following drought-shock treatment. Conversely, the drought-hypersensitive growth of RNAi plants was restored to normal by pre-stress treatment of exogenously supplied uric acid, the product of XDH catalysis. These RNAi plants constitutively accumulated high levels of H₂O₂ and significantly enhanced cell death upon drought shock. Our results indicate that purine metabolism has a critical role in plant acclimatization to environmental stress such as drought. We suggest that the purine degradation pathway might contribute, by producing antioxidant metabolites, to defense mechanisms against reactive oxygen species (ROS) under stress conditions, since uric acid and other certain metabolites of purine degradation possess efficient ROS-scavenging activities.

[1] Nakagawa et al. (2007) Plant Cell Physiol. 48: 1484-1495.

[2] Watanabe et al. (2010) FEBS Lett. 584: 1181-1186.

06042

MOLECULAR ANALYSIS OF STEROL BIOSYNTHESIS-RELATED GENES IN BARLEY CHROMOSOME ADDITION LINES OF COMMON WHEAT

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Barley chromosome addition lines of common wheat have been developed through wide hybridization between the hexaploid (2n = 6x = 42) wheat cultivar Chinese Spring (CS) and the diploid (2n = 2x = 14) barley cultivar Betzes (ISLAM et al., 1975). It is expected that the barley chromosome with the genetic background of wheat will produce new or more bioactive substances. In this study, we focused on plant sterols that could reduce the plasma cholesterol levels of animals when administered orally. Stigmastanol, campesterol, and sitosterol are the main molecular species of plant sterols; of these, stigmastanol is reported to be the most effective in reducing plasma cholesterol levels. The level of stigmastanol in wheat is lower than that in barley.

We used a series of barley chromosome addition lines in the full complement of wheat chromosome sets. On determining the plant sterol levels in 2-week-old seedlings, we observed that the level of stigmastanol in the barley chromosome 3 addition (3H) line was 1.5-fold higher than that in CS and in other barley chromosome addition lines. To elucidate the reason for the higher level, we isolated full-length cDNAs of wheat and barley *CYP710A* genes; *CYP710A* is a cytochrome P450 subfamily that encodes sterol C-22 desaturase in plants. We mapped these *CYP710A* genes on chromosome 3 in barley and wheat (3A, 3B, and 3D). The expression levels of *CYP710A* genes in the 3H line were higher than those in the other barley chromosome addition lines. Overexpression of the *CYP710A* genes in *Arabidopsis* increased the stigmastanol level but did not alter the total plant sterol level. Finally, we determined the overall expression patterns of genes related to plant sterol biosynthesis in CS and in each addition line, using a 44k oligo-DNA microarray (Agilent) customized for wheat. Our results provide an approach for altering plant sterol profiles and for increasing the effective plant sterol levels in wheat.

06043

IDENTIFICATION OF METABOLIC QTL CANDIDATE GENES IN ARABIDOPSIS

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Recently we identified quantitative trait loci (QTL) of metabolites from primary metabolism in *Arabidopsis thaliana* using GC-MS and suggested candidate genes on the basis of functional annotations in TAIR (Liseck et al., 2008). We now test if these candidates are the cause of natural diversity in the populations used to detect the QTL. For this, we measured the metabolite content in *A. thaliana* Col-0 leaves with knocked out (KO) candidate genes to determine if the candidate has any effect on the respective metabolite. A first analysis of a subset of KO lines showed that several of them in fact had increased or decreased levels of the respective metabolite (e. g. fumarate, tyrosine, urea, nicotinate and phosphate). With one exception, this affected mainly the metabolite of interest (136 metabolites measured in total) and did not cause major alterations in growth.

Currently, we assess if the alleles of the two parental accessions used for mapping in the initial study have a different effect on the respective metabolite level. For this, we transform genomic sequences from Col-0 and C24 comprising the candidate gene into the KO background. Allelic effects will be displayed as differences in the accordant metabolite levels between the transformants.

In the rare cases where no KO lines were available, RNAi and TILLING lines are under investigation to explore the potential gene-metabolite interaction and the metabolic effects of synthetic alleles.

06044

MITOCHONDRIAL RESPIRATORY SYSTEM POTENTIALLY REGULATES ISOPRENOID BIOSYNTHESIS IN CYTOPLASM AND PLASTID
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Isoprenoids are a wide variety compounds that are important for all organisms and useful for human life. Unlike animals, plants synthesize isoprenoids via two pathways, the cytosolic mevalonate (MVA) pathway and the plastidial 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. Little is known about the mechanisms that regulate these biosynthetic networks over multiple organelles. To understand such mechanisms, we have isolated and characterized the *Arabidopsis* mutant, *lovastatin Insensitive 1* (*loi1*), which is resistant to lovastatin, a specific inhibitor of HMG-CoA reductase (HMGR), the key enzyme of the MVA pathway. *loi1* also showed resistant phenotype to a specific inhibitor of the MEP pathway. HMGR activity in *loi1* grown under lovastatin was higher than that in wild type (WT) grown in the same condition. We consider that higher HMGR activity gives rise to lovastatin resistant phenotype in *loi1*. *LOI1* encodes a pentatricopeptide repeat (PPR) protein localized in mitochondria that is thought to have RNA binding ability and function in post-transcriptional regulation. *LOI1* belongs to the DYW subclass of PPR proteins, which is hypothesized to be correlated with RNA editing. As a result of analysis of RNA editing of mitochondrial genes, a defect in RNA editing of three genes, *nad4*, *ccb203*, and *cox3*, was found in *loi1*. These genes are related to the respiratory chain. WT treated with some respiration inhibitors mimicked the *loi1* phenotype. HMGR activity of WT treated with lovastatin combined with antimycin A, an inhibitor of complex III in the respiratory chain, was higher than that of WT treated with only lovastatin, despite the lack of alteration of transcript or protein levels of HMGR, suggesting that HMGR enzyme activity is regulated through the respiration. Our studies show the novel possibility that mitochondrial respiration plays potentially regulatory roles in isoprenoid biosynthesis.

06045

CPL1 IS INVOLVED IN SULFUR-DEFICIENCY RESPONSE AND TOLERANCE IN ARABIDOPSIS THALIANA

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Plants regulate gene expressions involved in sulfur uptake and assimilation under conditions of limited sulfur supply (refereed to as -S). Although some regulatory factors are already identified, the whole story of plant response to sulfur deficiency is not well understood. To identify novel regulatory factors for plant -S responses, we took a forward genetics approach using a transgenic *Arabidopsis thaliana* line expressing green fluorescent protein (GFP) under the control of a -S-inducible chimeric promoter. We screened for mutants with altered patterns of -S-responsive gene expressions. One of these mutants was further analyzed in this study. This mutant showed reduced -S-inducible reporter gene expression both under normal and -S conditions. In addition, transcript accumulations of other sulfur-responsive genes were affected in this mutant such as serine acetyltransferase (*SAT1*) and a branched-chain aminotransferase (*BCAT4*). The mutation was mapped to a 39.2 kb region in chromosome IV, where only one mutation was found at an exon-intron junction of *CPL1* gene. *CPL1* is an RNA polymerase II C-terminal domain phosphatase with two double stranded RNA binding domains. We obtained four *cpl1* alleles and all of them showed reduced relative growth under -S, indicating that *CPL1* is required for the maintenance of growth under -S condition. Increased accumulations of serine, O-acetyl-L-serine and agmatine in the shoots of *cpl1* mutants were consistent with the hypersensitivity of *cpl1* mutants to -S. Taken together, our study demonstrated the involvement of *CPL1* in the regulation of sulfur metabolism, gene expression and tolerance to -S stress.

06046

INTERACTION BETWEEN A SULFATE TRANSPORTER AND CYSTEINE SYNTHASE

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The sulfate ion (SO_4^{2-}) is transported into plant root cells by SO_4^{2-} transporters and then mostly reduced to sulfide. The S^{2-} is then bonded to O-acetylsulfide (OAS) through the activity of cysteine synthase (O-acetylsulfide (thiol)lyase or OASTL) to form cysteine, the first organic molecule of the SO_4^{2-} assimilation pathway. Here we show that a root plasmamembrane SO_4^{2-} transporter of *Arabidopsis*, SULTR1;2, physically interacts with OASTL. The interaction was initially demonstrated using a yeast two-hybrid system and corroborated by both *in vivo* interaction studies. The domain of SULTR1;2 shown to be important for association with OASTL is called the STAS domain. This domain is at the carboxyl terminus of the transporter and extends from the plasmamembrane into the cytoplasm of the cell. The functional relevance of OASTL-STAS interaction was investigated using yeast mutant cells devoid of endogenous SO_4^{2-} uptake activity, but co-expressing SULTR1;2 and OASTL. The analysis of SO_4^{2-} transport in these cells suggests that the binding of OASTL to the STAS domain negatively impacts transporter activity in this heterologous system. In contrast, the activity of purified OASTL measured *in vitro* was enhanced by co-incubation with the STAS domain of SULTR1;2, but not with the analogous domain of the SO_4^{2-} transporter isoform SULTR1;1, even though the SULTR1;1 STAS peptide also interacts with OASTL based on the yeast two hybrid system and *in vitro* binding assays. These observations suggest a regulatory model in which interactions between SULTR1;2 and OASTL coordinate internalization of SO_4^{2-} with the energetic/metabolic state of plant root cells.

06047

LIPID PROFILING BY LC-MS REVEALED A UNIQUE GLYCEROLIPID CLASS RELATED TO PHOSPHATE-LIMITING GROWTH OF ARABIDOPSIS THALIANA

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Phosphorus limitation leads to a drastic change in membrane lipid composition in plants. Lipidome analysis by liquid chromatography-ion trap mass spectrometry confirmed compositional changes of membrane lipid in *Arabidopsis thaliana* by phosphorus limitation, i.e., decreases of phospholipids (PC, PE, PG, and PI) and increases of two glycoglycerolipids (DGDG and SQDG). In addition to these well-known changes of lipid profiles, an unknown lipid group was found to accumulate in the leaves of *A. thaliana* by phosphorus limitation. Based on the MS/MS analyses, this inducible lipid group was elucidated as diacylglycerol bound to hexuronosyl moiety. During the course of the investigation, an *Arabidopsis* mutant, which does not accumulate this hexuronosyldiacylglycerol under phosphate-limiting condition, was isolated. Since a glycosyltransferase gene was disrupted in this mutant, this glycosyltransferase is postulated to be involved in the final condensation reaction of diacylglycerol and hexuronic acid. Under phosphate-depleted condition, the mutant showed an enhanced senescence compared with the wild-type plant, suggesting that the hexuronosyldiacylglycerol plays a role to mitigate the depletion stress of phosphorus in *A. thaliana*. This inducible lipid group was also found in leaves of rice, and the level of this lipid increased by phosphorus limitation, suggesting a general physiological significance of this lipid class across plant species.

07001

TARGETED INTERACTOMICS REVEALS A COMPLEX CORE CELL CYCLE MACHINERY IN ARABIDOPSIS THALIANA

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Cell proliferation is the main driving force of plant growth. Although genome sequence analysis revealed a high number of cell cycle genes in plants, little is known about the molecular complexes steering cell division. In a targeted proteomics approach, we mapped the core complex machinery at the heart of the *Arabidopsis* cell cycle control. Besides a central regulatory network of core complexes, we distinguished a peripheral network that links the core machinery to up- and downstream pathways. Over 100 new candidate cell cycle proteins are predicted and an in-depth biological interpretation demonstrates the hypothesis-generating power of the interaction data. Furthermore, we could demonstrate that plants have evolved a combinatorial toolkit comprising at least hundred different CDK/cyclin complex variants, which strongly underscores the functional diversification among the large family of cyclins and reflects the pivotal role of cell cycle regulation in the developmental plasticity of plants.

07002

STUDIES OF GENES INVOLVED IN UPTAKE AND METABOLISM OF ARSENICS IN PLANTS, FOR DEVELOPING NEW VARIETY OF CROPS TO PROTECT PEOPLE FROM ARSENIC CONTAMINATION

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Arsenic poisoning through consumption of cultivated crops is a severe health problem in many countries of South Asia especially in Bangladesh, Burma, India and Thailand. For instance, in Bangladesh more than 30 millions people are affected from rice-derived arsenic contamination leading to severe damage of kidney, liver, lungs, bladder etc and many other neurological and vascular disorders. To solve this severe problem we aim to generate genetically modified variety of crops either by inhibiting/activating native gene(s) responsible for arsenic uptake or by insertion of foreign genes responsible for arsenic metabolism "in planta". For identification and characterization of genes responsible for arsenic uptake or metabolism of arsenics "in planta" we have employed data mining, an *in silico* analysis based on searching of the existing databases. Data mining experiments resulted in identification of four candidate genes that are involved either in uptake, transport or cellular localization of arsenics in plants. For further study of these genes we have also employed *in vivo* analyses in which we have included *Arabidopsis thaliana* as a model plant. Interestingly, all four candidate genes we identified by *in silico* analyses are present in *A. thaliana* genome. We also show that by increasing or decreasing the level expression of the candidate genes in various combinations we can generate a "super plant" with new characteristics. When exposed to high arsenic concentrations, the amount of arsenics accumulated in the shoots of this "super plant" is 60% lower than that found in the wild-type control plants. Validation of these *in silico* results by *in vivo* experiments (generation of the transgenic "super plant") are in progress.

07003

MODULE NETWORK ANALYSIS OF A LARGE COMPENDIUM OF ARABIDOPSIS EXPRESSION DATA

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Biological functions are performed by sets of various molecules (non-coding RNA, proteins, metabolites, etc.) forming a functional module, cooperating tightly to achieve a specific goal. One of the main goals of systems biology approaches is the identification of those modules through the analysis of large sets of various high-throughput data, often referred to as "omics" data. In this study, we have analyzed a very large compendium of *Arabidopsis* expression data, collecting data from more than 1,200 microarray experiments mostly focused on cell cycle, stress and development. Using a robust probabilistic module network algorithm, we have built a regulatory module network composed of 233 modules representing more than 7,500 genes and 1,100 regulators associated to the modules. By crossing the results with independent microarray experiments, we can show that some modules are highly enriched in genes relevant for more specific biological responses like hydrogen peroxide stress response or lateral root development. Further integration of other data information like the cell cycle phase, proteomics data, miRNA and transcription factor targets, identifies modules specifically enriched for one or more of those data types. We are currently setting up a large scale experimental analysis using a novel high-throughput transcriptomics technology to further explore the properties and function of some modules, specialized in response to stress and lateral root development. We have also developed a user-friendly web interface to allow biologists to easily mine the module network for modules of interest. Users can identify modules enriched for a given set of genes of interest and have additional information displayed like GO enrichment, gene annotations or gene expression profiles. Our approach and the results we have compiled so far illustrate the power of top-down systems biology analysis to exploit efficiently the avalanche of high-throughput data.

07004

ARABIDOPSIS FERROMICS: PARALLEL INTERROGATION OF CHANGES IN THE QUANTITATIVE (PHOSPHO-) PROTEOME AND TRANSCRIPTOME OF ARABIDOPSIS ROOTS UPON FE DEFICIENCY USING iTRAQ AND MRNASEQ
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Iron (Fe) deficiency is counteracted by a suite of responses to ensure the maintenance of vital processes for which Fe is essential. Although significant progress has been made in elucidating the function of individual components in the Fe deficiency response at the transcriptional level, information on both post-transcriptional changes and the composite interactions among these components remain scarce. In the present study, we generated transcriptional profiles of Fe-deficient plants were generated by mRNA sequencing using the Solexa platform. By means of the iTRAQ technology combined with 2D-LC-MS/MS analysis on an LTQ-Orbitrap, we quantitatively identified ~2500 proteins and ~200 phosphoproteins in Arabidopsis roots. Proteins with functions in the response to heat, cold and oxidative stress as well as proteins involved in primary metabolism were found to be most abundant in the proteome. Fe deficiency dramatically induced proteins involved in methionine metabolism, in particular in the synthesis of S-adenosyl Met and nicotianamine, an important Fe chelator that plays a crucial role in Fe transport and homeostasis. In addition, enzymes mediating the flavonoid pathway were induced. Strong induction of a Phe ammonia lyase, two coumarate CoA ligases, and a caffeoyl-CoA 3-O-methyltransferase point to increased production of phenolics, a key response for the utilization of root apoplastic Fe. Marker of Fe deficiency such as FRO2 and the iron storage protein ferritin FER1 were among the most up or down-regulated proteins upon Fe deficiency treatment. Correlation analysis of transcript and protein abundance in Arabidopsis roots revealed a variable protein-to-transcript ratio and identified several proteins that are regulated exclusively at the post-transcriptional level. In summary, we here provide a quantitative (phospho) protein reference map for Arabidopsis roots and add a further layer of information with regard to the physiological responses to Fe deficiency.

07005

GENOME WIDE ANALYSIS OF TRANSCRIPTIONAL REGULATION BY THE CIRCADIAN TRANSCRIPTION FACTOR LHY

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The MYB transcription factor LHY functions redundantly with CCA1 as one of the core components of the *Arabidopsis* circadian clock, and regulates expression of many output genes. Genome-wide binding sites for LHY were identified by chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-Seq). Over 5000 sequences were identified, approximately 50% of which showed rhythmic expression. GO term analysis showed that LHY regulates many biological processes, including aspects of developmental regulation, electron transport and energy pathways, and responses to biotic and abiotic stimuli. However, there was no significant enrichment for genes implicated in transport or in cell organization and biogenesis. Most of the known clock-associated genes contained binding sites for LHY. Binding was also detected within the promoters of *LHY* and *CCA1*, providing novel evidence for direct, negative autoregulation.

Expression of the LHY protein peaks shortly after dawn, but that of its known target genes exhibits a wide range of phases ranging from early morning to late night. Our current work aims to investigate the mechanisms by which the effect of LHY can be modulated to give rise to such a broad range of phases and waveforms. We hypothesize that (i) the different affinity of LHY for different target promoters may result in different timing of transcriptional activation, or (ii) the effect of LHY on expression of individual target genes may be altered by interaction with other regulatory proteins. Analysis of motif enrichment within LHY-binding regions identified several sequences, including Evening Element-like sequences, G-box sequences and a CT-rich motif. Analysis is under way to determine whether different classes of binding sites for LHY or for cofactors are associated with expression at different times of the day.

07006

BLUE-LIGHT INPUT TO THE ARABIDOPSIS CIRCADIAN CLOCK-THE ROLE OF EARLY FLOWERING 3.

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The circadian clock mechanism provides plants with temporal information that enables the co-ordination of cellular and therefore physiological events. This temporal co-ordination, based on an approximate 24 hour pattern provides a framework into which environmental factors can be incorporated. Specifically, the circadian clock is entrained by light and temperature which facilitates seasonal regulation, including photoperiodic flowering. The resolution of the molecular mechanism by which the clock measures photoperiods has been aided by the work of Yu et al, 2008 where it was demonstrated that the clock proteins EARLY FLOWERING 3 (ELF3) and GIGANTEA (GI) interact with the E3 Ubiquitin Ligase CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1). We have taken this framework and mathematically modelled the protein interactions, including the regulation of the blue-light photoreceptors, CRYPTOCHROMES (CRY1 and CRY2) on COP1 and the stabilisation of another blue-light photoreceptor, ZEITLUPE (ZTL) on GI. This model addresses blue-light conditions and allows light-dark cycle regulation. As such the model has aided our understanding of the temporal profiles of protein complex formation as well as indicating the requirement for other specific functions of the proteins involved, furthering the molecular understanding of how photoperiodic time measurement can occur.

07007

AN INCOHERENT FEED FORWARD LOOP DEFINES DISCRETE EXPRESSION PATTERNS DURING EARLY ARABIDOPSIS THALIANA TRICHOME DEVELOPMENT

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The development of trichomes from pluripotent epidermal cells in *Arabidopsis thaliana* provides a powerful model for the study of gene regulatory networks involved in plant cell differentiation. We have previously shown that the R2R3-MYB protein GL1 and bHLH transcription factor GL3 together directly regulate approximately 20 genes involved in trichome initiation, including the WRKY transcription factor gene TTG2 [1, 2]. To better establish the trichome initiation network, we combined literature-based ChIP analyses with ChIP-chip experiments using TTG2-GFP transgenic plants and GFP antibodies. Among 372 genes identified as TTG2 direct targets, many were shared by GL3 (with or without GL1). In addition to the TTG2 genome-wide location, gene expression experiments indicated that several GL3/TTG2 common targets showed opposite regulation by GL3 and TTG2. Further expression analyses conducted on mutant strains suggest

that GL3, TTG2, and target genes participate in an Incoherent Feed Forward Loop (I-FFL) in which the target gene is up-regulated by GL3 and down-regulated by TTG2. We propose that it is this network architecture that is responsible for the narrow window of gene expression for several GL3 targets that occurs at early stages of trichome development. We are currently using a combination of experimental and mathematical modeling experiments to probe the functional relationships of factors in I-FFLs, and further delineate the mechanisms underlying epidermal cell fate determination.

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07008

SYSTEMS ANALYSIS OF SECONDARY THICKENING IN THE ANTER ENDOTHECIUM

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Male fertility requires both the generation of viable pollen, but also the subsequent release of this pollen. Anther dehiscence relies upon a specialised maternal cell layer in the anther, the endothecium, which undergoes specific secondary thickening during the post-meiosis stages of microspore maturation and pollen mitosis. Cellulose and lignified thickenings are deposited in bands within the endothecium and as the anther subsequently dehydrates these generate the forces required for dehiscence. Mutation of the MS35/MYB26 gene results in an absence of secondary thickening in the endothecium and failure of anther dehiscence with associated male sterility (Dawson et al., 1999). *Arabidopsis* MYB26 is a regulator of endothecium secondary thickening that when ectopically expressed can induce thickening in other tissues, indicating a conservation of the secondary thickening regulatory network between reproductive and vegetative tissues (Yang et al., 2007).

We have used transcriptomic analysis to construct a preliminary MYB26 regulatory network and interactions between selected components have been tested by ChIP-PCR, yeast-2-hybrid screens, mutant analysis and ectopic expression. DEX-inducible lines have been used to analyse the dynamics of expression changes by conducting time-series RT-qPCR analysis. These data have subsequently been analysed to determine the dynamics of the expression changes observed and used for inference studies to establish a defined regulatory network during anther dehiscence.

Key components in the regulatory network have been tested for their functional roles in anther dehiscence and secondary thickening. This and the process of network development will be discussed.

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07009

AUXIN BIOSYNTHESIS INHIBITORS ARE IDENTIFIED BY GENOMICS-BASED APPROACH USING ATGENEXPRESS GENE EXPRESSION DATA

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We established transcriptome data of hormone series in AtGenExpress project. Here, we conducted correlation analysis of DNA microarray data from AtGenExpress to estimate hormone status in microarray experiments by using hormone-inducible genes as markers. It was found that a chemical compound, aminoethoxyvinylglycine (AVG) had the strongest anti-auxin activity in *Arabidopsis*. We also identified other effective compounds such as L-aminoxyphenylpropionic acid (AOPP) through additional screening. These inhibitors shared characteristics in that they inhibited pyridoxal enzymes and/or aminotransferases. The compounds inhibited growth, auxin accumulation, and expression of *Aux/IAA* genes in *Arabidopsis* seedlings, independently from ethylene action. The seedlings were recovered from the inhibition after exogenous application of IAA, but not of ACC. Since the inhibitors have characteristics to inhibit pyridoxal-phosphate (PLP)-dependent enzymes, we analyzed all possible PLP-dependent steps of auxin biosynthesis in enzyme extracts from *Arabidopsis* and wheat. AVG and AOPP inhibited L-Trp aminotransferase, suggesting that this is the first auxin-biosynthesis inhibitor that functions both in monocot and dicots.

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07011

TWO NOVEL PROTEINS REGULATE RESPONSE TO IRON DEFICIENCY IN ARABIDOPSIS ROOTS.

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Iron deficiency induced anemia is the most prevalent nutritional disorder in the world. Plants are the primary source of iron therefore understanding how plant roots regulate the uptake, transport, and utilization of iron under low iron conditions is important for providing long-term solutions for iron deficiency. A systems biology approach was taken to elucidate the regulatory mechanisms involved in plant responses to iron deficiency. Using a combination of high-resolution transcriptional profiling, ChIP on chip analysis, and Yeast 2 Hybrid analysis we discovered two novel components of the iron deficiency gene regulatory network that appear to play distinct roles in mediating plant responses to iron deprivation. One of these components, POPEYE (PYE), is a transcription factor that binds to the promoter of key iron homeostasis genes and suppresses their expression. The other protein, BRUTUS (BTS), may act as a component of the ubiquitin conjugation pathway that is involved in the degradation of iron homeostasis regulatory proteins. Loss of PYE function leads to decreased tolerance of iron deprivation, while loss of BTS function enhances tolerance to low iron. Moreover, PYE and BTS are tightly co-regulated within the pericycle, and both bind to close PYE homologs. Our data suggests that PYE and BTS and their targets function individually or as part of a complex in response to iron deprivation.

07013

EPLANT: VISUALIZING AND INTEGRATING LARGE-SCALE ARABIDOPSIS DATA SETS FROM THE NANOMETER TO KILOMETER SCALES IN 3 DIMENSIONS FOR HYPOTHESIS GENERATION

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Visualization tools for biological data are typically two-dimensional and often limited in their ability to integrate data at multiple scales. To demonstrate the utility of three-dimensional data visualization in overcoming these limitations we have developed "ePlant", a suite of open-source world wide web-based tools for analyses of the model organism *Arabidopsis thaliana*, rendered as interactive three-dimensional models permitting integration and visualization of biological data from the nanometer to the kilometer scale. Currently, ePlant at bar.utoronto.ca/eplant consists of the following modules, in increasingly coarser scale: a protein structure model explorer, a molecular interaction network explorer, subcellular localization of gene products, expression patterns at the tissue level, and a sequence conservation and polymorphism explorer. We also report a high-throughput protein structure prediction and annotation collection with >70% coverage of the *Arabidopsis* proteome. The ePlant framework can be applied to any model organism. To facilitate the development of three-dimensional displays of biological data on the world wide web we have established the "3D Data Display Initiative" (3ddi.org).

07014

AGENT BASED MODELLING OF AUXIN TRANSPORT CANALISATION

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Auxin transport canalisation describes how auxin organises and promotes its own transport through cells. Auxin is able to enter cells passively or actively, but can only leave cells by being actively pumped out by proteins including PIN (a membrane bound auxin transporter). During vascular tissue formation a narrow transport path, or canal, forms from an area where auxin is accumulating, to a sink elsewhere in the tissue. A missing link in this process is the regulation of the positioning of the PIN proteins on the cell membrane. During canalisation, PIN proteins are observed to be polarised in the direction of auxin flux. This could be by targeted removal or targeted insertion of the PIN proteins. Causing them ultimately to become polarly localised on the cell membrane in the correct location to pump auxin in the direction of the sink. To investigate canalisation, in the hope that we can direct future wet laboratory work, we have developed a flexible agent based modelling framework to allow for testing of different hypotheses of how PIN localisation might be regulated. The models are built by putting agents representing auxin, and proteins including PIN, into a tissue consisting of a number of cells. We then look for the global behaviour of canalisation as an emergent property of the simple interactions between the agents and their environment.

07015

ARABIDOPSIS PROTEIN MICROARRAYS FOR PROTEIN FUNCTIONAL STUDIES

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Since the majority of proteins lack discernable enzymatic or biochemical functions, large-scale characterization of proteins will depend on the identification of molecules that associate with them. In this respect protein microarray platform is an exciting tool for large-scale protein functional studies. In the past five years under NSF-Arabidopsis 2010 funding, we have generated an *Arabidopsis thaliana* expression collection (ATEC) containing ~15,000 *Arabidopsis* genes. We have used these expression clones and produced >10,000 *Arabidopsis* proteins to-date using an optimized medium-throughput plant-based expression system.

We have successfully used purified proteins to produce version1 high-density *Arabidopsis* protein microarrays containing 1133 proteins and used them to perform protein-protein interaction studies in an unbiased, high-throughput manner (Popescu et al., 2009, PNAS 104: 4730-4735). We have version2 protein microarrays containing 2158 *Arabidopsis* proteins and used it to infer a MAPK phosphorylation network (Popescu et al., 2009, Genes Dev 23:80-92). We identified 570 putative MAPK phosphorylation targets, many of which were annotated with transcription factor functions, involved in responses to abiotic and biotic stimuli and development.

We have generated version3 protein microarrays containing 5160 *Arabidopsis* proteins. These arrays are available to the scientific community through ABRC. Our goal this year is to add 10,000 additional proteins to the microarrays.

During this meeting, we will discuss importance of our protein microarray platform and also new set of protocols to analyze protein-protein and protein-DNA interactions using these microarrays. We strongly believe that protein microarray resources will help in elucidating function of *Arabidopsis* proteins. Currently, we are using these protein microarrays to study programmed cell death and innate immune signaling.

07016

COMPARATIVE METABOLITE PROFILING CHARACTERIZES THE IMPACT OF GENOTYPE-DEPENDENT METHIONINE ACCUMULATION IN ARABIDOPSIS THALIANA

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Methionine (Met), an essential amino acid for all organisms, functions as a precursor of plant hormones, polyamines, and defense metabolites. The regulatory mechanism of Met biosynthesis is highly complex and, despite its great importance, remains unclear. To address the key question of how accumulation of Met influences metabolism as a whole in *Arabidopsis*, we analyzed three *methionine over-accumulation* (*mto*) mutants using a established gas chromatography (GC)-time-of flight (TOF)/mass spectrometry (MS) protocol for metabolite profiling. Multivariate statistical analyses showed distinct metabolomic phenotypes of the three *mto* mutants, *mto1*, *mto2*, and *mto3*. A more sophisticated approach based on orthogonal projection to latent structures—discriminant analysis highlighted genotype-related differences. Though Met accumulation in *mto1* had no dramatic effect on other metabolic pathways except for the aspartate family, metabolite profiles of *mto2* and *mto3* indicated that several extensive pathways were affected in addition to over-accumulation of Met. The marked changes in metabolic pathways in both *mto2* and *mto3* were associated with polyamines. Our results suggest that comparative metabolomics can not only reveal the impact of Met over-accumulation on metabolism, but also may provide clues to identify crucial pathways for regulation of metabolism in plants.

07017

INTEGRATED METABOLITE AND TRANSCRIPT PROFILING REVEALS COMPREHENSIVE METABOLIC REPROGRAMMING INVOLVED IN TWO INDEPENDENT PATHWAYS OF ARABIDOPSIS TO ULTRAVIOLET-B LIGHT

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Plants produce secondary metabolites following strong light exposure such as UV-B irradiation. Anthocyanins, flavonoids and phenolic compounds are regarded as protective pigments in *Arabidopsis* leaves. To understand the mechanism of production of these protective pigments in response to UV-B light, we compare the metabolic responses of wild type *Arabidopsis* to that of *Arabidopsis* mutants impaired in flavonoid (TRANSPARENT TESTA4 [tt4] and TRANSPARENT TESTA5 [tt5]), or sinapoyl-malate (sinapoylglucose accumulator 1 [sng1]) biosynthesis, to a short, 24h or a longer 96h exposure to UV-B imposed stress. In control experiments we subjected the same genotypes to long day conditions as well as to 24, and 96h treatments of continuous light. Following these treatments we evaluated the dynamic response of metabolites including flavonoids, sinapoyl-derivatives and ascorbate which are well established to play a role in cellular protection from UV-B stress, as well as a broader range of primary metabolites, in attempt to more fully comprehend the metabolic shift following cellular perception of this stress. Our data reveals that short-term responses occur only at the level of primary metabolites suggesting that these effectively prime the cell in order to facilitate the later production of UV-absorbing secondary metabolites. In addition, transcript profiling was conducted on samples to capture affected responses in transcript levels by irradiation of UV-B. The combined results of these studies are discussed in the context of current models concerning the metabolic response of plants to the stress imposed by excessive UV-B irradiation.

07018

KINEMATIC ANALYSIS OF PLOIDY EFFECTS ON ROOT APICAL GROWTH IN ARABIDOPSIS THALIANA

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Effects of polyploidization have been described macroscopically for many aspects of plant growth, but how an increase in the ploidy level influences cell proliferation and cell volume growth remains to be elucidated. We generated tetraploids from four strains of *Arabidopsis thaliana*, Columbia, Landsberg erecta, Wassilewskija, and C24 by treatment with colchicine. By comparison of kinematic data of the primary root growth between these tetraploids and their parental diploids, we quantitatively analyzed the ploidy effects on plant growth.

The first step of the kinematic analysis was the measurement and calculation of spatial profiles of volume growth rate (dV/dt), cell volume (dV/dN), cell production rate (dN/dt), and cell number (N) in the cortex as a function of cumulative volumes of the cortical cell file (V). We also determined relative elementary growth rate (REGR) and local cell production rate (LCPR) by differentiating dV/dt and dN/dt with respect to V. The spatial profiles of REGR and LCPR indicate the locations of volume growth and cell proliferation, respectively. The obtained data depicted growth characteristics of the tetraploids common to all strains. The final cell volumes were much larger in the tetraploids than in the diploids. The volume growth zones were enlarged in the tetraploids compared to the diploids, while no significant changes were found in the rate and location of cell proliferation.

These results suggest that the ploidy change from diploid to tetraploid generally raises volume growth without affecting cell proliferation, leading to the increase in the cell volume. We are now further analyzing the kinematic data by our original mathematical model to estimate efficiencies of three aspects of root growth; cell proliferation, volume growth, and organ maintenance. We will discuss cellular mechanisms underlying growth modification by the ploidy level based on the mathematical analysis.

07019

DENSITY GRADIENT OF FLORIGEN SIGNALS MEDIATES DIVERSITY OF INFLORESCENCE ARCHITECTURES:

PHLOEM TRANSPORT MODEL BASED ON SOURCE-SINK BALANCE

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Arabidopsis thaliana is an annual herb that flowers only once and completes their life cycle after reproduction in a year. However, it has been reported that double mutants of SUPPRESSOR OF OVEREXPRESSION OF CONSTANTS 1 (SOC1) and FRUITFULL (FUL) revealed an inflorescence meristem reversion and created aerial rosettes, establishing phenotypes common to perennial plants. We hypothesize that this transition in growth habit is caused by a density gradient of florigen signals. Some of florigen signals, like FT products in *A. thaliana*, are transported from leaves to shoot apex through phloem to initiate flowering.

Phloem transport of these florigen signals should be driven by pressure difference between source (mature leaves) and sink (meristems). We developed a phloem transport model of florigen signals based on a pressure flow hypothesis.

The model system of plant consists of an array of the meristem cells connected by sieve tubes. The end of branch is initially immature, and it grows to be a flower or a leave, depending on the flowering signals transported from leaves. Thus, the whole system is a dynamical network system with active nodes acts as source or sinks. By using numerical simulations, we have found a variety of inflorescence reversion patterns. In particular, it is shown that the threshold parameter for flowering and effective diameter of sieve tube are key parameters.

07020

ANALYSIS OF THE ARABIDOPSIS CYTOSOLIC PROTEOME HIGHLIGHTS ITS DIVERSE ROLES AND NOVEL FUNCTIONS WITHIN THE PLANT CELL

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The cytosol is the intracellular fluid that contains all eukaryotic cellular components and importantly is the conduit that allows interactions between partitioned metabolic processes. The cytosol is a crowded environment containing a highly complex mixture of dissolved molecules; ionic solutes, small molecule metabolites, and macromolecules, including proteins and nucleic acids. In plants, it is estimated that over 200,000 metabolites are present, reflecting the innumerable cellular reactions that occur within this matrix. The cytosol is also the location of many of the most important processes that occur with the plant cell, including glycolysis, protein biosynthesis, protein degradation and metabolite biosynthesis. This intracellular matrix thus represents an intriguing system that likely

contains many thousands of proteins. While there have been numerous proteomic studies that focused on the organelles and other subsystems within the plant cell, no such characterizations have been undertaken on the cytosol. The cytosol was isolated from cell suspensions of *Arabidopsis thaliana*, using a gentle homogenization method and differential centrifugation to remove cellular debris and intact organelles. Multidimensional protein identification technology was used to identify over 1300 proteins that comprise the cytosolic proteome. This included glycolytic enzymes, ribosome and proteasome subunits, ubiquitin, cytosolic heat shock proteins, 14-3-3 proteins, glutathione-s transferases, cell wall biosynthetic enzymes, protein kinases and phosphatases, proteins involved in vesicular trafficking and proteins with unknown function. The wide range of proteins identified in the *Arabidopsis* cytosolic proteome highlights its diverse functions in the plant cell and the identification of many proteins with unknown function could further build on our understanding of how reactions in the cytosol can influence plant function.

08001

PYROPHOSPHATE OVER-ACCUMULATION IN *FUGU5/AVP1* MUTANT INHIBITS CELL DIVISION EARLY AFTER GERMINATION AND INDUCES COMPENSATED CELL ENLARGEMENT

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Analyses of leaf development in various mutants of *Arabidopsis thaliana* revealed that excessive post-mitotic cell expansion is often triggered by decreased cell proliferation activity, a phenomenon that we named "compensation". Among five compensation-exhibiting mutants (*fugu1-fugu5*) that we have isolated and characterized (Ferjani et al., 2007), here we report about our recent findings on *fugu5* mutant. When *fugu5* is germinated on inorganic medium, cell division is almost completely lost in mesophyll cells of cotyledons, which are oblong in shape and exhibit strong compensation. However, we recently found that compensation in *fugu5* mutant is cancelled when sucrose is supplied in the media. However, the mechanism of sucrose action remained unclear. Cloning of *FUGU5* gene revealed that it is *AVP1*, which encodes for the vacuolar pyrophosphatase (PPase) that has two functions: hydrolysis of cytosolic pyrophosphate (PPi) and vacuolar acidification. So, what is the triggering factor of compensation in *fugu5*? As an approach to answer this question, we introduced the cytosolic inorganic pyrophosphatase (*IPP1*) gene of *Saccharomyces cerevisiae*, under the control of *FUGU5* promoter. *IPP1* was used because it only hydrolyzes the cytosolic PPi without contributing to vacuolar acidification, thus providing a good system to analyze *FUGU5* functions independently. Interestingly, our results clearly showed that *fugu5* mutant phenotypes were totally recovered after the introduction of *Pro_{FUGU5}::IPP1* transgene. This study provides evidence that PPi hydrolysis, rather than vacuolar acidification, is the major role of *FUGU5* in planta. Therefore, the removal of cytosolic PPi produced by active cellular metabolism early during germination is a prerequisite for sustaining cell division, proper developmental programs and to boost seedling growth in *Arabidopsis*.

08002

THE TRIHELIX TRANSCRIPTION FACTOR GTL1 REGULATES PLOIDY-DEPENDENT CELL GROWTH IN THE ARABIDOPSIS TRICHOME

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Leaf trichomes in *Arabidopsis* develop through several distinct cellular processes, such as patterning, differentiation and growth. Here we report a novel trichome mutation in *Arabidopsis*, which in contrast to previously identified mutants, increases trichome cell size without altering its overall patterning or branching. We show that the corresponding gene encodes a GT-2-LIKE1 (GTL1) protein, a member of the trihelix transcription factor family. GTL1 is present within the nucleus during the post-branching stages of trichome development and its loss of function leads to a ploidy increase in trichomes that have completed branching. Our data further demonstrate that the gtl1 mutation modifies the expression of several cell cycle genes and partially rescues the ploidy defects in the cyclin-dependent kinase (CDK) inhibitor mutant *siamese*. GTL1 functions downstream of genes involved in trichome patterning and early differentiation processes, such as TRANSPARENT TESTA GLABRA1 (TTG1) and GLABRA2 (GL2). Further genetic analysis between gtl1, glabra3 (gl3) and *tryptochon* (try) mutants illustrates that GTL1 acts independent from GL3- and TRY-related growth mechanisms.

Apart from the strong expression in trichomes, promoter-GUS and functional genomic GFP-fusions revealed expression of the GTL1 locus at lower levels in other vegetative tissues, although we could not detect any obvious developmental phenotypes. Our recent study demonstrates that at least two more GTL1 orthologues in *Arabidopsis* show a very similar spatial and temporal expression pattern, suggesting gene redundancy among these orthologues. Further double mutant analysis confirms this redundancy, and also illustrates that this group of transcription factors plays an important role for the transcriptional regulation of plant organ growth.

08003

A NOVEL TRANSCRIPTION FACTOR UPBEAT1 CONTROLS THE TRANSITION FROM PROLIFERATION TO DIFFERENTIATION IN THE ROOT VIA ROS SIGNALING.

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The balance between cellular proliferation and differentiation is a key aspect of development in multicellular organisms. However, due to the multitude of factors that maintain the balance between cellular proliferation and differentiation, the underlying regulatory network is poorly understood.

Using high-resolution expression data from the *Arabidopsis* root we identified a transcription factor, UPBEAT1 (UPB1) that plays a crucial role in this process. Comparisons of the expression patterns of transcriptional and translational reporter constructs suggested that UPB1 moves from its site of synthesis in the lateral root cap to the elongation zone where it becomes nuclear localized. This suggested that it acts both as a signal molecule and as a transcriptional regulator. Intriguingly the transition from cellular proliferation to differentiation is coincident with the last cells of the lateral root cap. Mutation of *UPB1* resulted in increased proliferation and delayed elongation, while ectopic expression of *UPB1* resulted in the opposite phenotype. Genome-wide expression profiling coupled with ChIP-chip analyses revealed that UPB1 directly regulates the expression of a set of enzymes that modulate the balance of reactive oxygen species (ROS) between the zones of cell proliferation and the zone of cell elongation where differentiation begins. Disruption of UPB1 activity alters this ROS balance, leading to a delay in the onset of differentiation. Modulation of either ROS balance or peroxidase activity through chemical reagents affects the onset of differentiation in a manner consistent with the postulated UPB1 function. This pathway may function independently of auxin and cytokinin plant hormonal signaling.

08004

REGULATION OF STOMATAL LINEAGE CELL PROLIFERATION BY THE ARABIDOPSIS MYB FOUR LIPS VIA DIRECT TARGETING OF CORE CELL CYCLE GENES

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Developmental programs coordinate cell proliferation with differentiation but the underlying mechanisms are incompletely understood. Stomata, which are epidermal pores surrounded by two guard cells, develop from a specialized stem cell lineage and function in shoot gas exchange. The *Arabidopsis* FOUR LIPS (FLP) and MYB88 genes encode closely related and atypical two-MYB-repeat proteins, which when mutated induce excess divisions and abnormal groups of stomata in contact. Consistent with a role in transcription, we show here that FLP and MYB88 are nuclear proteins with DNA-binding preferences distinct from other known MYBs. To identify possible FLP/MYB88 transcriptional targets, we used chromatin immunoprecipitation (ChIP) followed by hybridization to *Arabidopsis* whole genome tiling arrays. These ChIP-chip data indicate that FLP/MYB88 target the upstream regions especially of cell cycle genes including cyclins, cyclin dependent kinases (CDKs), and components of the pre-replication complex. In particular we show that FLP represses the expression of the mitosis-inducing factor CDKB1;1 which, along with CDKB1;2, we find is specifically required both for the last division in the stomatal pathway and for cell over-proliferation in fip mutants. FLP recognizes a distinct cis-regulatory element that overlaps with that of the cell cycle activator E2F-DP in the CDKB1;1 promoter suggesting that these MYBs may also modulate E2F-DP pathways. We propose that FLP and MYB88 together integrate patterning with the control of cell cycle progression and terminal differentiation through multiple and direct cell cycle targets.

08005

CYTOCHROME P450 FAMILY MEMBER CYP704B2 CATALYZES THE ω -HYDROXYLATION OF FATTY ACIDS AND IS REQUIRED FOR ANTER CUTIN BIOSYNTHESIS AND POLLEN EXINE FORMATION IN RICE
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The anther cuticle and microspore exine act as protective barriers for the male gametophyte and pollen grain, but relatively little is known about the mechanisms underlying the biosynthesis of the monomers of which they are composed. We report here the isolation and characterization of a rice (*Oryza sativa*) male sterile mutant, *cyp704B2*, which exhibits a swollen sporophytic tapetal layer, aborted pollen grains without detectable exine as well as undeveloped anther cuticle. In addition, chemical composition analysis indicated that cutin monomers were hardly detectable in the *cyp704B2* anthers. These defects are caused by a mutation in a cytochrome P450 family gene, *CYP704B2*. The *CYP704B2* transcript is specifically detected in the tapetum as well as the microspore from stage 8 of anther development to stage 10. Heterologous expression of *CYP704B2* in yeast demonstrated that *CYP704B2* catalyzes the production of ω -hydroxylated fatty acids with 16 and 18 carbon chains. Our results provide insights into the biosynthesis of the two biopolymers sporopollenin and cutin. Specifically, our study indicates that the ω -hydroxylation pathway of fatty acids relying on this ancient *CYP704B* family, conserved from moss to angiosperms, is essential for the formation of both cuticle and exine during plant male reproductive and spore development.

08006

THE ABORTED MICROSPORES (AMS) REGULATORY NETWORK IS REQUIRED FOR POSTMEIOTIC MALE REPRODUCTIVE DEVELOPMENT IN ARABIDOPSIS THALIANA
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The *Arabidopsis thaliana* *ABORTED MICROSPORES* (AMS) gene encodes a basic helix-loop-helix (bHLH) transcription factor that is required for tapetal cell development and post-meiotic microspore formation. However, the regulatory role of AMS in anther and pollen development has not been fully defined. Here we show by microarray analysis that the expression of 549 anther-expressed genes was altered in *ams* buds and that these genes are associated with tapetal function and pollen wall formation. We demonstrate that AMS has the ability to bind *in vitro* to DNA containing a 6-bp consensus motif, CANNTG. Moreover, 13 genes involved in transportation of lipids, oligopeptides and ions, fatty acid synthesis and metabolism, flavonol accumulation, substrate oxidation, methyl-modification and pectin dynamics were identified as direct targets of AMS by chromatin immunoprecipitation (ChIP). The functional importance of the AMS regulatory pathway was further demonstrated by analysis of an insertional mutant of one of these downstream AMS targets, an ABC transporter, White-Brown Complex homolog, WBC27, which fails to undergo pollen development and is male sterile. Yeast two hybrid screens and pull-down assays revealed that AMS has the ability to interact with two bHLH proteins (AtbHLH089 and AtbHLH091) and the ATA20 protein. These results provide insight into the regulatory role of the AMS network during anther development.

08007

CARBON STARVED ANTER (CSA) ENCODING A MYB DOMAIN PROTEIN REGULATES SUGAR PARTITIONING REQUIRED FOR RICE POLLEN DEVELOPMENT
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In flowering plants, sink tissues, such as those in developing flowers, rely on carbohydrates from leaf tissues (sources) for nutrition and energy. Photosynthetic carbohydrates (sugars) are synthesized in leaves, and transported to sink tissues. However, it is not clear how sugar partitioning in plants is regulated at the molecular level and how such regulatory mechanisms affect reproductive development. Here we describe the isolation and characterization of a rice (*Oryza sativa*) mutant, *csa* (*carbon starved anther*). Compared with wild-type plants, *csa* mutants had increased sugar contents in leaves and stems, and reduced levels of sugars and starch in floral organs including anthers. In particular, the *csa* mutant had reduced levels of carbohydrates in later anthers and was male sterile. Feeding assay using ¹⁴C-labeled sucrose indicated that the *csa* mutant had reduced accumulation of ¹⁴C-labeled sugars in the sink tissue of anther. CSA was isolated by map-based cloning and it encodes a transcription factor of the R2R3 MYB family; moreover, CSA is expressed preferentially in the anther tapetal cells surrounding developing pollen and in the sugar-transferring vascular tissues. In addition, the expression of

Os MST8, encoding a monosaccharide transporter crucial for the anther sugar unloading pathway, was greatly reduced in csa anthers. Furthermore, CSA was found to be associated in vivo and in vitro with the promoter of *Os MST8*. Our findings suggest that CSA is a key transcriptional regulator for sugar partitioning in rice during male reproductive development. This study also establishes a molecular model system for further elucidation of the genetic control of carbon partitioning in plants.

08008**DETERMINATION OF ADAXIAL/ABAXIAL ANTER POLARITY REQUIRES HYPONASTIC LEAVES 1**

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Lateral organ stamen produces pollen grains for pollination in higher plants. Coordinated development of stamen architecture is essential for normal fertility. A few of adaxial and abaxial identity genes, including members of HD-ZIP III and YABBY families, have been reported to regulate the stamen development. However, it remains unknown whether miRNA pathways are involved in polarity establishment of stamens and how HYPONASTIC LEAVES 1 (HYL1) that control miRNA biogenesis regulates stamen development. Here we report that hyl1 mutants have severe defects in stamen polarity, as two inner microsporangia in the anthers did not develop and the abaxial connectives was adaxialized. In situ hybridization demonstrates that expression of the adaxial/abaxial identity gene REVOLUTA (REV) extended to abaxial region of hyl1 anthers while abaxial marker gene FILAMENTOUS FLOWER (FIL) was repressed in abaxial side. The rev and jba-1D (dominant miR166g mutant) alleles partially rescued stamen polarity defects. These results suggest that HYL1 maintain stamen polarity by localization of REV and FIL transcripts in anthers through miR165/166, and thus plays an essential role in establishment of the stamen architecture and male fertility.

08009**K⁺ AS AN ENERGY CARRIER - A NEW ROLE OF AN UBIQUITOUS CATION IN PLANTS**

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Potassium is a key element in the life of plants. It is used as a major osmotically active solute to maintain turgor and to drive reversible changes in cell volume and irreversible cell expansion.

In plants a variety of transporter systems are involved in the uptake and redistribution of K⁺. An important role is played by voltage-gated K⁺ channels allowing either K⁺-influx or K⁺-efflux. A unique -so far not understood- role is played by a special class of voltage-gated K⁺ channels that can mediate both, K⁺-influx and K⁺-efflux. The exceptional representative in the model plant *Arabidopsis thaliana* is the K⁺ channel AKT2 that is mainly expressed in the phloem. Previous studies have shown that the features of AKT2 are regulated by post-translational modifications (Dreyer et al., 2001). Phosphorylation switches AKT2 from an inward-rectifying channel that mediates K⁺-uptake only into a non-rectifying channel that mediates also K⁺ release. By site-directed mutagenesis AKT2 could be locked in the inward-rectifying mode (i.e. dephosphorylated) or in the non-rectifying mode (i.e. phosphorylated) (Michard et al., 2005).

To evaluate the role of AKT2-regulation we generated transgenic plants expressing these (de)phosphorylation-mimicking mutants instead of the AKT2 wild-type. Physiological analyses of the diverse transgenic plants revealed that the post-translational regulation of AKT2 is an important process for enhancing plant growth under certain abiotic stress conditions. The physiological data could be complemented by computational models simulating the behavior of AKT2-expressing cells. These simulations reveal that phosphorylation of AKT2 switches on a 'K⁺-battery' that efficiently assists the plasma-membrane H⁺-ATPase in energizing transmembrane transport processes. Our results shed clear light on a new role of K⁺ in phloem transport. The K⁺ ions circulating in phloem provide an energy source that can be harvested by means of AKT2-regulation.

08010**BRASSINOSTERIOLS CONTROL MALE FERTILITY BY DIRECTLY REGULATING THE EXPRESSION OF KEY GENES INVOLVED IN ANTER AND POLLEN DEVELOPMENT OF ARABIDOPSIS**

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The development of anther and pollen is important for male reproduction, and this process is coordinately regulated by many external and internal cues, including many phytohormones. The first brassinosteroid (BR) was purified from pollen, one of the richest sources for the plant steroid hormone. Although it is well known that the mutants defective in BR-biosynthesis/signaling showed a significantly reduced male fertility, the underlying mechanism(s) of the reduced fertility is poorly understood. In this study, we systematically examined the male reproductive phenotypes of a series of brassinosteroid biosynthetic and signaling mutants, and found that, besides the expected cell expansion defects as indicated by the shortened filament, these mutants also showed reduced pollen number, viability, and release efficiency. These defects were related with abnormal tapetum and microspore development. Using both real-time quantitative RT-PCR and microarray experiments, we found that the expression of many key genes required for anther and pollen development was suppressed in these mutants and induced in the over-expression line of *BES1*, encoding an important transcription factor for BR signaling. Chromatin immunoprecipitation analysis demonstrated that BES1 could directly bind to the promoter regions of genes encoding transcription factors essential for anther and pollen development, *SPL/NZZ*, *TDF1*, *AMS*, *MS1*, and *MS2*. Taken together, we propose that brassinosteroids control male fertility at least in part via directly regulating key genes for anther and pollen development in *Arabidopsis*. Our work provides a novel mechanism to explain how a phytohormone regulates an essential genetic program for plant development.

08011**HYPOMORPHIC GENETICS ENLIGHTENS ELF3 FUNCTION IN THE CIRCADIAN CLOCK***Eva Herrero (Max Planck Institute for Plant Breeding Research, Germany), Nora Bujdoso (Max Planck Institute for Plant Breeding Research, Germany), Elsebeth Kolmos (University of California San Diego, USA), Andrew J Millar (University of Edinburgh, United Kingdom), Ferenc Nagy (Institute of Plant Biology, Hungary), Seth J Davis (Max Planck Institute for Plant Breeding Research, Germany) herrero@mpiz-koeln.mpg.de

EARLY FLOWERING 3 (ELF3) is an essential clock component for the generation of circadian rhythms. Moreover, ELF3 has been shown to gate light-resetting of the oscillator, and this has been proposed to be through the physical interaction with phytochrome B (phyB). Despite these pioneering genetic and physiological studies, the biochemical mechanisms of ELF3 action have remained elusive. Here, we report the isolation of elf3-12 as a new elf3 allele found in genetic screen for clock period mutants. Unlike elf3 loss of function alleles,

circadian oscillations persist in *elf3-12*, and this mutant displays wild-type flowering time and hypocotyl-growth inhibition. The *elf3-12* mutation encodes an amino-acid replacement in a conserved domain, and we therefore used this mutant to place ELF3 within the clock mechanism. For this, the expression-level phenotypes of clock genes in *elf3-12* revealed probable targets. From there, we could show light dependence for periodicity phenotypes in *elf3-12*; no clock phenotype was detected in darkness. This light-dependent *elf3-12* phenotype was shown to be enhanced by the overexpression of photoreceptors *phyB* and *phyA*. Moreover, *elf3-12* plants were found to have a reduced circadian ability to repress light-regulated gene expression. The *elf3-12* was perturbed in resetting the oscillator. Taken together, the circadian characterization of *elf3-12* has prompted us to propose a functional domain required to inhibit phytochrome action on ELF3. Furthermore, we inferred the targets of ELF3 transcriptional repression within the core oscillator. For the first time, we could separate the circadian function from the light-gating function of ELF3. This is consistent with ELF3 being a multifunctional protein that integrates light signals as a core oscillator component.

08012

PHOSPHOLIPID SIGNALING MODULATES INFLORESCENCE MERISTEM ACTIVITY IN ARABIDOPSIS

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Flowers are unique in unusually high level of certain lipids, such as phosphatidic acid (PA) and phosphoinositides, which play critical roles in signal transduction. In Arabidopsis, however, lipid metabolism in flowers is largely unknown mainly because flowers are too tiny to harvest in bulk according to the developmental stages. To explore the lipid signaling during flower development, we performed developmental stage-specific glycerolipid profiling using a system to synchronize flower development. The results revealed active changes in PA during flower development. The *Phosphatidic acid phosphohydrolase 1 / Phosphatidic acid phosphohydrolase 2 (pah1pah2)* double mutant is defective in two isoforms of PA phosphatase involved in PA metabolism, which shows high level of endogenous PA. Because the terminal flowers were produced occasionally in *pah1pah2*, an enhancer mutant, *Enhanced pah1pah2 1 (eph1pah1pah2)*, was screened, whose mutation site was mapped into *Terminal Flower 1 (TFL1)*. The *pah1pah2* mutation enhanced terminal flower phenotype of known *tfl1* mutant alleles, *tfl1-1* and *tfl1-14*. *TFL1* protein interacts with phosphatidylcholine (PC), whose level was increased in *pah1pah2* due to the increase in PA. The application of phosphocholine, a soluble substrate of PC biosynthesis, to the inflorescences of the *tfl1* mutants strongly enhanced terminal flower phenotype. Furthermore, inducible suppression of phosphatidylethanolamine biosynthetic pathway to increase relative PC level at the shoot apex reproduced the same effect. These results suggest that cellular PC level modulates inflorescence meristem activity in Arabidopsis flowers.

08013

RAP1 REGULATES ASYMMETRIC DIVISIONS IN THE ARABIDOPSIS ROOT

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Asymmetric divisions of stem cells to give rise to more differentiated progeny sustain growth in plants after embryogenesis. New cells are continuously generated in specialized proliferative tissues or meristems. The root meristem comprises an organizing center surrounded by stem cells that asymmetrically divide to produce the initial cells of the different root cell-type lineages. Asymmetric cell divisions of the cortex/endodermis stem cell and its daughter cell require the SHORT-ROOT (SHR) transcription factor (TF), which moves from the stele to the endodermis, as well as a related transcription factor, SCARECROW (SCR) that carries SHR into the nucleus. In addition, two zinc-finger proteins, MAGPIE (MGP) and JACKDAW (JKD) can differentially activate or restrain the SHR/SCR feedback loop.

By reverse genetics we have found a novel regulator of asymmetric cell division in the Arabidopsis root, Root Altered Pattern 1 (RAP1). RAP1 is specifically expressed in the endodermis and it is quickly activated prior to the first asymmetric division in a *shr* inducible system where SHR can be conditionally activated. By chromatin immuno-precipitation followed by real time PCR, we have found that RAP1 is a direct target of SHR and SCR. Furthermore, ectopic expression of RAP1 can rescue the lack of asymmetric cell divisions of the cortex/endodermis initial daughter cell of *shr* and *scr*, where these cells do not normally divide. *rap1* mutant shows defects in the root organizing center, and combination of this mutant with *scr*, *jkd* and *mgp* mutants greatly reduces formative divisions in the Arabidopsis root.

08014

PHYTOCHROME NUCLEAR BODIES AND PHOTOMORPHOGENESIS IN PLANTS

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Phytochromes are red and far-red photoreceptors regulating every facet of plant development and growth. Two early phytochrome signaling events have been described: (A) Light directly regulates the relocation of phytochrome A (*phyA*) and phytochrome B (*phyB*) from the cytoplasm to the nucleus, where they interact and colocalize with a group of bHLH transcription factors (PIFs) on discrete subnuclear foci called phytochrome nuclear bodies and regulate transcription; (B) Light triggers rapid degradation of *phyA* and some of the PIFs. The function of phytochrome nuclear bodies in relationship to phytochrome signaling events is unknown. We carried out a unique genetic screen looking for *phyB*:GFP mislocalization mutants. This screen identified a novel photomorphogenetic mutant, *hemera (hmr)*. Strikingly, besides defects in *phyB*:GFP nuclear body formation, the *hmr* mutant is impaired in all phytochrome responses examined, including chloroplast biogenesis and *phyA* degradation, suggesting that HMR is an essential regulator linking phytochrome nuclear body formation and light signaling. The tall and albino phenotypes of *hmr* make it the founding member of a new class of photomorphogenetic mutant. In addition, the *hmr* mutant is the first phytochrome signaling mutant defective in *phyA* proteolysis, which suggests a biochemical role of HMR and phytochrome nuclear bodies in protein degradation. Further characterization of HMR will likely to provide great insight into the mechanistic link between phytochrome nuclear bodies and early phytochrome signaling events.

08015

CHARACTERIZATION OF INTERCELLULAR SIGNALING THAT COORDINATES CELL PROLIFERATION WITH POST-MITOTIC CELL EXPANSION DURING LEAF DEVELOPMENT

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During leaf development, a genetic defect in cell proliferation often triggers enhanced post-mitotic cell expansion. This phenomenon is called 'compensation'. For example, in the leaves of *angustifolia3* mutant (*an3*), cell number is decreased by 70% and cell size is increased

by 50% compared with wild-type (WT). Cell proliferation and post-mitotic cell expansion occur in spatially distinct regions of the same leaf primordium. Thus, compensation suggests an interaction of cell proliferation with post-mitotic cell expansion. Previously, we established an experimental system to generate chimeric leaves for *AN3* expression by Cre/Lox-mediated recombination. Using this system, we showed that *an3*-dependent compensation is induced in a non-cell-autonomous manner.

Here, to deepen our understanding about the mechanism of compensation, we further analyzed the nature of intercellular signaling using *AN3* chimeric leaves. In our experimental system, expression of a translational fusion of *AN3* tagged with triple green fluorescence proteins is induced under the *an3-4* background upon heat-shock. We selected leaves that expressed *AN3* within a half side of the leaf under the *an3-4* genotype. In these leaves, *AN3* overexpressor (*AN3 o/x*) cells are separated from *an3-4* cells by midrib. Interestingly, in these chimeric leaves, *AN3 o/x* cells did not show compensation although *an3-4* cells did. Namely, size of *AN3 o/x* and *an3-4* cells in these leaves was similar to that of cells in WT and *an3-4*, respectively. This result suggested that midrib might be a structure to prevent spread of the intercellular signaling in *an3*-dependent compensation. We will discuss on the mechanism of compensation based on the nature of intercellular signaling including above-mentioned results.

08016

EXPLORING THE ROOT CLOCK AS A MOLECULAR MECHANISM FOR PERIODIC LATERAL ROOT FORMATION

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Formation of lateral roots by a plant's root system facilitates effective exploration of the soil environment. In *Arabidopsis*, lateral roots are initiated through specification of new stem cell populations (meristems) within the primary root. Formation of these meristems occurs in an apical-to-basal sequence and follows a temporal periodic distribution, however the molecular mechanisms underlying this pattern are unknown. Previous reports have shown that lateral root formation correlates with a peak of expression of the auxin response reporter DR5 in the basal meristem. Using real-time imaging to examine DR5 expression within the primary root tip, we found that DR5 reporter expression has a periodic oscillatory pattern. Furthermore, microarray analyses revealed that the expression of many genes oscillates within the root tip, with two dominant groups of oscillating genes. We hypothesize that these oscillating genes comprise a clock-like mechanism that we term the lateral root clock, which regulates periodic lateral root formation. Following each DR5 oscillation, we observe that a novel site is marked at which a lateral root primordium will ultimately form. Preliminary data suggest that competence to respond to the periodic clock signal is governed by two opposing signaling gradients and disruption of these gradients interferes with lateral root formation.

08017

PIZZA: A NEW PLAYER IN THE BRASSINOSTEROID METABOLISM?

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Brassinosteroids (BR) affect a range of developmental characteristics in plants, including organ growth, flowering and seed germination. Proteins involved in the biosynthesis as well as in signaling pathways of BR have been identified through loss-of-function mutant analysis. In contrast, screening of overexpression lines led to the identification of several enzymes involved in BR metabolism.

Searching the *Arabidopsis* FOX (Full-length cDNA Over-eXpressor gene) collection for genes that alter organ size, we isolated an overexpression line strongly resembling BR deficient plants. We confirmed that this phenotype is caused by overexpression of a single gene *PIZZA*. It could be rescued by supplying exogenous brassinolide and castasterone but not gibberellic acid. Based on the *PIZZA* overexpression phenotypes, we suggest a role for *PIZZA* in BR metabolism rather than biosynthesis. Analysis of promoter-GUS reporter lines shows a spatial and temporal control of *PIZZA* expression during development with strongest signals in roots, meristems and floral tissues. As *PIZZA* knock-out lines do not show strong developmental phenotypes, redundant enzymes are likely to exist. We are currently investigating the *in vitro* and *in vivo* function of the *PIZZA* protein, especially in comparison to the known P450 hydroxylases BAS1 and SOB7, which act redundantly in BR metabolism.

08018

ANALYSIS OF NEW ARABIDOPSIS MUTANTS WITH ALTERED ORGAN SIZE

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Final size of plant organs is determined by an increase in cell size and cell number, but how these processes are controlled by environmental cues is not well understood. The correlation between ploidy, the nuclear DNA content, and cell size has long been reported in many plant species, and we are interested in studying how an increase in ploidy impacts cell and organ size in response to environmental signals. Our previous study identified a novel trichome mutant in *Arabidopsis* and we have shown that the corresponding gene, encoding a plant-specific trihelix transcription factor *GTL1*, functions as a negative regulator of ploidy-dependent cell growth in trichomes. In this study we are analyzing organ size phenotypes of *gtl1* mutants under various light and photoperiodic conditions. We are also performing transcript analyses and cell biological analyses of *gtl1* mutants. Based on our progress, we will discuss how organ size is controlled in response to external cues.

08019

HD3A PROTEIN, THE MAJOR COMPONENT OF FLORIGEN, IS THE MOBILE BRANCHING SIGNAL IN RICE.

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Heading date 3a (Hd3a) protein in rice and its *Arabidopsis* ortholog FT are recently considered as the likely florigen, the crucial component of a long-distance flowering signal. Hd3a protein is generated at the leaf phloem tissue in flowering-promoting conditions, moves from leaves to apical meristem and causes flowering. Here we show overexpression of Hd3a in vasculature caused not only early flowering but also high branching, semi-dwarf, abnormal internode elongation and abnormal inflorescence architecture. Detailed observation revealed that high branching phenotype is caused by outgrowth of axillary meristems. Because Hd3a is expressed specifically in casuclature and not expressed in axillary meristem, we suspected that Hd3a protein moves from vasculature to axillary meristem to start meristem outgrowth. We successfully observed Hd3a-GFP fluorescence in the axillary meristem and we conclude that Hd3a protein moves from leaf to the axillary meristems and induces meristem outgrowth. These data suggest that Hd3a/FT-like proteins may define the

novel type of the mobile molecule that integrates the development between organs that sense and adapt the environment.

08020

CELLULAR MECHANICS CAN REGULATE THE AUXIN EFFLUX CARRIER PIN1 IN THE SHOOT APICAL MERISTEM

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In the shoot apical meristem, local maxima of the hormone auxin initiate leaf formation. Auxin accumulates at specific sites by directed, up-the-gradient transport, which is mediated by the efflux carrier PIN1. Plasma membrane localization of PIN1 is, therefore, critical for organogenesis. PIN1 intracellular localization is sensitive to osmotic changes: hypo-osmotic and hyper-osmotic treatments increase and decrease the PIN1 density on the membrane, respectively. The hyper- and hypo-osmotic effects were fully reversible. Application of external forces could bring more PIN1 to the plasma membrane and rescue the hyper-osmotic loss of PIN1 signal. In parallel, increasing the membrane-cell wall contact could enhance PIN1 plasma membrane localization. On the other hand, decreasing the membrane tension resulted in less PIN1 on the plasma membrane. Taken together, we propose that the cellular mechanics — particularly, membrane tension and turgor — can regulate PIN1 intracellular localization and thus function. This mechanism may be important for regulation of PIN1 abundance in different domains within the meristem, as well as meristem alteration in response to environmental stresses.

08021

RETINOBLASTOMA RELATED PROTEIN (RBR) CONTROLS ASYMMETRIC CELL DIVISION IN THE ROOT MERISTEM

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In *Arabidopsis* the Retinoblastoma Related protein (RBR) is the single pRB homolog. A canonical function for RBR in controlling cell cycle and delimiting the stem cell region by modulating cell transitions and differentiation has been previously reported. Local reduction of RBR in the primary root meristem expands the stem-cell pool without altering cell-cycle rates, indicating that RBR status defines stem-cell capacity. (Wildwater et al., 2005).

The A-B pocket domain of RBR protein displays a high conservation when compared to that of its homologs in humans, fruit fly and *C. elegans*. Residues which are crucial for protein interactions are highly conserved, not only those that define pRBs interactions with E2F transcription factors but also those indispensable for the binding to diverse proteins which contain the LxCxE motif (Dick., 2007). It has been shown that several of these physical interactions occur through the B-pocket of pRB and the LxCxE motif present in the interactor partner. Similarly, in *Arabidopsis* viral proteins bind RBR through the LxCxE motif.

By using the LxCxE motif as an in silico bait we have located this motif upstream of the GRAS domain of the SCARECROW protein, an important regulator of stem cell activity and asymmetric cell division. We demonstrate that RBR physically binds to SCR, via the LxCxE motif, and report on the consequences of this interaction for asymmetric cell division of the ground tissue. Our study describes a novel role for RBR in cell differentiation by directly binding to and modulating the activity of a plant-specific transcription factor.

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08022

DEVELOPMENT OF THE QUIESCENT CENTER AND DEFINITION OF THE AUXIN MAXIMUM IN ARABIDOPSIS ADVENTITIOUS ROOTS IN PLANTA AND IN THIN CELL LAYERS

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In *Arabidopsis* adventitious roots (ARs) are induced by darkness [Takahashi et al, J Plant Res (2003)]. Stem thin cell layers (TCLs) produce ARs *in vitro* under darkness and with auxin (IBA or NAA) and, mainly, auxin (IBA) plus cytokinin (Kin) [Falasca et al, Plant Cell Rep (2004)]. We investigated the definition of the quiescent center (QC) and the auxin maximum in the ARs *in planta* and in TCLs. We used QC marker lines previously used in lateral roots (LR) (i.e., QC25 and pAGL42:GFP) and a DR5:GUS line (harbouring uidA gene driven by the auxin-inducible DR5 promoter). The seedlings were grown under darkness either without hormones (HF) or with NAA, Kin, and IBA plus Kin. The definition of the QC and the auxin maximum were also investigated in the ARs from TCLs cultured with IBA plus Kin. AR formation *in planta* was enhanced by NAA and IBA plus Kin in comparison with the HF medium, and was sporadic with Kin alone. At day 11 after sowing ARs at various stages were similarly present in NAA- and IBA+Kin-grown seedlings, but AR anomalies increased with NAA. DR5:GUS activity was observed in the dividing hypocotyl cells involved in AR formation independently on the hormonal treatment. During AR development, a gradient of DR5 activity was established, and it was similar to the pattern of DR5 activity during lateral rooting [Benkova et al, Cell (2003)]. The QC was defined in the AR primordia *in planta* at the same stage in which it occurs in LR primordia [Malamy and Benfey Development (1997)], and the expression of the QC markers and DR5:GUS construct was concomitant. Anomalous expression of the QC markers and DR5:GUS construct occurred in the anomalous ARs of the NAA treatment. The ARs produced by the TCLs showed DR5:GUS activity at the tip, and QC markers expression as in ARs *in planta*. In conclusion, QC definition and auxin maximum are related events in the ARs *in planta* and *in vitro*.

08023

FINE-TUNING OF ADVENTITIOUS ROOT INITIATION IN ARABIDOPSIS HYPOCOTYL INVOLVES A SUBTLE BALANCE OF AUXIN RESPONSE FACTORS AND AUXIN INDUCIBLE GENES

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The root system is composed of the primary, lateral and adventitious roots. Lateral roots always develop from roots whereas adventitious roots develop from stem or leaf derived cells. Adventitious rooting is known to be a quantitative genetic trait with a high phenotypic plasticity due to multiple endogenous and environmental regulatory factors, including phytohormones, light, temperature and nutrients

but very little is known about the molecular mechanisms regulating adventitious rooting and in the past few years we have identified several candidate genes, some of which supposed to act at the crosstalk of auxin and light signaling pathways (Sorin et al. Plant Cell 2005, Plant Physiology 2006). We have shown that this highly plastic quantitative trait is controlled in Arabidopsis by a subtle balance between the negative regulator *AUXIN RESPONSE FACTOR17* (*ARF17*) and the positive regulators *ARF6* and *ARF8*. These genes display overlapping expression domains, interact genetically, and regulate each other's expression at both transcriptional and post-transcriptional levels by modulating the availability of their regulatory microRNAs *miR160* and *miR167* (Gutierrez et al. Plant Cell 2009). We show here that *ARF6* and *ARF8* are positive regulators of *GH3-3*, *GH3-5* and *GH3-6* whereas *ARF17* is a negative regulator. These *GH3* genes, but not the others, are required for fine-tuning of AR initiation in Arabidopsis hypocotyls and we propose that they act by degrading an inhibitor of AR formation upon auxin induction.

08024

AUXIN AND JASMONIC ACID INTERACTION IN THE CONTROL OF LATE STAMEN DEVELOPMENT IN ARABIDOPSIS

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Late stamen development starts at the end of meiosis and consists of three simultaneous processes: filament elongation, pollen maturation and anther dehiscence. We have previously demonstrated that auxin coordinates pollen maturation and anther dehiscence, and triggers filament elongation. Auxin is synthesized in the stamens by YUC biosynthetic proteins before the inception, and peaks at the initial stage, of late developmental processes; subsequently its concentration progressively declines before anthesis. The peak of auxin is responsible for promoting filament elongation whereas the subsequent decrease in auxin concentration allows triggering pollen maturation and anther dehiscence. When late stamen development begins, auxin is perceived by the TIR1 AFB receptors and the auxin- perception mutants *tir1afb* show early anther dehiscence, precocious pollen development and reduced filament elongation.

Late stamen development is also controlled by jasmonic acid (JA), which is involved in the final stages of pollen maturation and anther dehiscence. In particular, the *opr3* mutant defective in JA biosynthesis shows indehiscent anthers, reduced pollen viability and reduced filament length. To assess whether auxin controls the level of JA, the expression of genes involved in JA-biosynthesis and the JA content are being measured in *tir1afb* perception mutants at different stages of development. Preliminary data demonstrate an increase of JA biosynthesis in auxin perception mutants. Accordingly, genetic analysis shows that the *afb1opr3* double mutant has indehiscent anthers confirming that auxin acts on the level of JA. Auxin and JA interactions will be discussed in view of recent data.

08025

STRUCTURE AND DNA BINDING SPECIFICITY OF THE LEAFY TRANSCRIPTION FACTOR

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The LEAFY gene is present in all land plant and plays a central role in flower development of angiosperms (1). It encodes a plant specific transcription factor with no resemblance to other proteins and unknown origin. In Arabidopsis, LEAFY participates to triggering the floral transition and subsequently patterns the floral meristem by inducing the expression of several floral organ identity genes. Characterizing LEAFY's molecular action and evolution is central to understand the evolution of plant reproductive structures. By combining biochemical and structural analyses, we have shown that LFY is a novel type of Helix-Turn-Helix transcription factor, which binds DNA as a cooperative dimer (2). Combining SELEX (binding sites selection assay) experiments with quantitative affinity measurements, we have established a position specific weight matrix that allows accurate prediction of binding site affinity. The implications of these findings in Arabidopsis and other plants will be discussed.

(1) Moyroud et al. (2009) J. Plant Biology 52:177

(2) Hames et al. (2008) EMBOJ 27:2628.

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08026

FUNCTION OF A CALCIUM SENSOR / PROTEIN KINASE COMPLEX IN REGULATING BLUE AND RED LIGHT RESPONSES IN ARABIDOPSIS DEVELOPMENT

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Calcium serves as a critical messenger in many adaptation and developmental processes. Cellular calcium signals are detected and transmitted by sensor molecules such as calcium-binding proteins. In higher plants, calcineurin B-like (CBL) proteins and CBL-interacting protein kinases (CIPKs) represent important relays in calcium signaling. Although transient elevations in calcium concentration represent a long known phenomenon in light responses there exact contribution to deciphering light signals has remained poorly understood. We have identified an Arabidopsis *cbl1/cipk1* double mutant that displayed hypersensitive hypocotyl responses specifically to red and blue light. In addition, the double mutation attenuated the expression of marker genes, *CAB* and *CHS*, in response to red and blue light exposure. Moreover, we identified HYPERSENSITIVE TO RED AND BLUE1 (HRB1), which has been shown to function in cryptochrome and phytochrome signaling pathways, as a target of the CBL1/CIPK1 complex. Molecular interaction of CIPK1 with HRB1 was observed in yeast two hybrid and *in planta* BiFC assay, and an *in vitro* kinase assay revealed that CIPK1 phosphorylates HRB1. These results suggest that CBL1 and CIPK1 function in the light signalling pathway, and provide new evidence that light signal transduction involves calcium signals.

08027

ELUCIDATING THE ROLE OF THE F-BOX PROTEIN HAWAIIAN SKIRT IN MICRORNA REGULATION DURING FLORAL DEVELOPMENT

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HAWAIIAN SKIRT is an F-Box protein that has been characterised and the most conspicuous phenotype from the mutant *hws-1* is the

fusion of its sepals (Gonzalez-Carranza, et al. 2007). To identify potential substrates for HWS and to dissect the downstream events from this gene, several strategies have been pursued; including: 1) phenotypic analysis of double mutants, obtained from crossing the *hws-1* mutant to other floral mutants which share similar phenotypes with the over expressing lines of HWS; and 2) The isolation and characterization of suppressors from an EMS mutagenised *hws-1* population. Genetic crosses from our first approach revealed that HWS plays an important role in the micro RNA pathway and the accumulation of pri-MicroRNAs is affected in the *hws-1* mutant and over expressing lines of HWS. Screening of the *hws-1* EMS mutagenised population has resulted in the isolation of several suppressing lines that are currently being studied. One of these, *prb-1*, a dominant mutation, rescues the sepal fusion phenotype and increases the number of floral organs; it has been mapped and the nature of the mutation identified as a single nucleotide substitution in the miRNA binding site for miR164 in the *CUC1* gene. A novel allele from the *CUC2* gene (*cuc2-1D*) with a point mutation in the same regulatory module has been identified and characterised previously (Larue, et al. 2009); double and triple mutants with *prb1* and *hws1/prb1* have been generated; and analyses of these plants will be presented. Another suppressor line identified from the EMS screening; 23.1, has been identified and its mapping is ongoing. Further analysis of this line will be presented.

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08028

CLONAL DELETION ANALYSIS OF *RBR1* IN THE ROOT MERISTEM

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Rb mediates a verity of processes which determine cell fate in almost all lineages of life. It is a key component in the G1/S cell cycle transition as well as in apoptosis. *RBR1*, the *Arabidopsis Rb* homologue has been shown to regulate stem cell maintenance in the root meristem (1). We sought to determine *RBR1* role in specific cell types of the *Arabidopsis* root stem cell niche. However, mutations in this gene lead to gametophytic lethality, abolishing the possibility for clonal deletion analysis of F1 offspring by currently known systems. Using the BOB (Brother Of Brainbow) setting we could generate and track genotypically *rbr1* cells, marked by a double fluorescence signal (CyPet and TagRFP) in a fully complemented *RBR1* wt background. Although *RBR1* is expressed throughout the whole meristem, null homozygous clones (hereinafter NHCs) display different phenotypes followed by *RBR1* depletion. Columella stem cell and daughter NHCs undergo vast and aberrant cell proliferation with occasional cell death. QC NHCs proliferate but rarely exhibit cell death. Proximally, in the vascular tissue, cell death is the main outcome of *RBR1* null clones. Conversely, division zone and differentiated columella cells do not respond to *RBR1* deletion. Furthermore, stem cell death induced by the radiomimetic drug Zeocin is inhibited by *RBR1* overexpression while roots with decreased *RBR1* levels (*RCH1::RBR1-RNAi*) are hypersensitive and display earlier and broader stem cell death. These findings suggest a cell specific function of *RBR1* in controlling proliferation, orientation of division and cell death in *Arabidopsis* root.

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08029

TRANSCRIPTIONAL REGULATION OF FLORAL MERISTEM IDENTITY GENES: A NEW PATHWAY TO INITIATE FLOWER FORMATION

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The transition from vegetative to reproductive development in plants is controlled by environmental and endogenous cues to optimize reproductive fitness. These multiple cues need to be integrated temporally and spatially for the proper developmental regulation of the initiation of flower formation during the meristem identity (MI) transition. In *Arabidopsis*, the transcription factors LEAFY (LFY), APETALA1 (AP1) and FRUITFULL (FUL) play a key role in this process. How the relevant environmental and endogenous cues together trigger LFY, AP1 and FUL upregulation is still not fully understood. Inductive photoperiod activates expression of all three MI regulators, while gibberellin hormone signaling upregulates LFY expression. Recently, we and others identified a new pathway that activates LFY, AP1, and FUL expression during the MI transition (Wang et al., 2009; Yamaguchi et al., 2009). Overexpression of the micro RNA regulated SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3 (SPL3) transcription factor causes precocious developmental phase transitions. Genetic analysis revealed that LFY, AP1 and FUL are required for the precocious MI transition of SPL3 overexpressors. In addition, ful mutants suppress the early flowering phenotype of SPL3 overexpressors. SPL3 directly binds to the regulatory regions of LFY, AP1 and FUL and up-regulates their expression together with closely related SPL transcription factors. We show that the SPL pathway activates initiation of flower formation in parallel to the known photoperiod pathway. Using the FUL promoter as a model, we are further investigating the individual contributions of the relevant inductive pathways towards upregulation of an MI regulator and initiation of flower formation.

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08030

THE EPIDERMAL SPECIFICATION PATHWAY REGULATES THE CELL SIZE PATTERN IN THE ARABIDOPSIS SEPAL

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Cell division regulation contributes to complex developmental patterns, such as the diverse cell sizes present in the abaxial epidermis of the *Arabidopsis* sepal. The sepal cell length ranges from 360 μ m in an average giant cell to 10 μ m in the smallest cells. To first address if cells of different sizes represent extremes in a uniform cell type population or distinct cell identities, we analyzed transcriptional reporters. Two enhancer trap markers distinguish giant cells from small cells suggesting cells of different sizes also have different fates. Next we screened for EMS mutants that disrupt the formation of giant cells. Surprisingly, 4 of the 6 mutants identified from this random mutagenesis are alleles of genes previously linked to the epidermal specification pathway. Positional cloning showed that mutations in *ATML1*, a class IV homeodomain leucine zipper transcription factor involved in epidermal specification, block the formation of giant cells. Similarly a hypomorphic mutation in *DEK1*, a transmembrane calpain protease also involved in epidermal specification, blocks the formation of giant cells. Mutations in the crinkly repeat receptor kinase encoding gene *ACR4*, another member of the epidermal specification pathway, reduce the number of giant cells as do mutations in *HDG11*, a class IV homeodomain leucine zipper transcription factor in the same family as *ATML1*. All of these mutants have epidermal cells. We next asked whether these genes control the specification of giant cells as assayed by the expression of the giant cell enhancer trap marker. *DEK1* controls giant cell specification because the expression of the marker is strongly reduced in *dek1* mutants. In contrast, the giant cell marker is still expressed in multiple small cells in *atml1* mutants. We conclude that the epidermal specification pathway also controls the cell size patterning by promoting both the size

and cell fate of giant cells.

08031

SUMO E3 LIGASE HIGH PLOIDY2 REGULATES ENDOCYCLE ONSET AND MERISTEM MAINTENANCE IN ARABIDOPSIS

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Endoreduplication involves a doubling of chromosomal DNA without corresponding cell division. In plants, many cell types transit from the mitotic cycle to the endoreduplication cycle or endocycle, and this transition is often coupled with the initiation of cell expansion and differentiation. Although a number of cell cycle regulators implicated in endocycle onset have been identified, it is still largely unknown how this transition is developmentally regulated at the whole organ level. We have recently shown that the TIR1/AFB-AUX/IAA-ARF dependent auxin signal modulates the switch from the mitotic cycle to the endocycle in *Arabidopsis*; high levels of auxin signalling are required to repress the endocycle, thus maintaining cells in the mitotic cycle whereas lower levels of auxin signalling trigger exit from the mitotic cycle and entry into the endocycle. We have also reported that a nuclear-localized SUMO E3 ligase, HIGH PLOIDY2 (HPY2), functions as a repressor of endocycle onset in *Arabidopsis* meristems. Loss of HPY2 results in a premature transition from the mitotic cycle to the endocycle, leading to severe dwarfism with defective meristems. HPY2 possesses an SP-RING domain characteristic of MMS21-type SUMO E3 ligases, and we show that the conserved residues within this domain are required for the in vivo and in vitro function of HPY2. HPY2 is predominantly expressed in proliferating cells of root meristems and it functions downstream of meristem patterning transcription factors PLETHORA1 (PLT1) and PLT2. These results establish that HPY2-mediated sumoylation modulates the cell cycle progression and meristem development in the PLT-dependent signaling pathway.

08032

TOWARD UNDERSTANDING THE MECHANISM OF STORAGE ROOT FORMATION IN RADISH THROUGH ARABIDOPSIS AS A MODEL SYSTEM

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Root growth or thickening plays a key role in the final productivity and quality of storage roots in root crops. To clarify the mechanisms controlling the inheritance of different root shapes, we chose radish because of its large variations in roots.

Morphological and anatomical studies were carried out using 3 cultivars that varied in root shape; a long type (Lt) cv. Taibyousoubutori, a round type (Rt) cv. Fuyudorishougoi both show storage roots, and a skinny type (St) cv. Kosena shows non-storage root. We found that all cultivars began to diverge in root shape at 4 weeks after sowing. To identify the genes related to root shape, suppression subtractive hybridization was performed at the phase of root thickening. Two cDNA libraries were established; the first library was performed using the Lt and the St when the second library was prepared by doing subtraction between the Rt and the St. Over 1600 clones were obtained from both libraries. Of these, 695 ESTs were isolated and sequenced. BLAST search of the *Arabidopsis* information resource was done to find their putative functions. A total of 154 and 157 different genes were identified from the first and second library, respectively. Interestingly, 47 genes were overlapping in both libraries. Eleven subtracted cDNAs were selected and examined by real time RT-PCR to determine their tissue specificities and expression levels in roots at different developmental stages. All the genes were developmentally regulated and highly expressed in roots, however the relative expression level varied among root parts and/or cultivars. This study has led to the identification of candidate genes that may regulate root shape in radish. Meanwhile, *Arabidopsis* will be used to study the function of the candidate genes. We found the orthologs of the candidate genes in *Arabidopsis* and the T-DNA tagging knockout mutants. Twenty-eight lines having T-DNA are under investigation especially in root growth.

08033

A TWO-FOLD SWITCHER: MULTIPLE NUCLEAR AND PLASTID GENES ARE AFFECTED BY THE SENESCENCE-ASSOCIATED TRANSCRIPTION FACTOR WHIRLY1 IN ARABIDOPSIS

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Plant senescence is a highly regulated process requiring high activity of numerous transcription factors from a variety of families including NAC, WRKY and MYB (Guo et al. 2004). Here we suggested that an additional group of senescence-associated transcription factors is the plant specific Whirly protein family. In *Arabidopsis* three Whirly proteins, At-Why1, At-Why2 and At-Why3, can be found (Krause et al. 2005, 2009). Whirly1 is a protein with dual localization in the nucleus and in chloroplasts (Grabowski et al. 2008).

Quantitative RT-PCR analysis in rosette leaves and analysis of plants transformed with the *uidA* (*GUS*) gene under *At-Why1* promoter control indicated an increased expression of *At-Why1* before the onset of leaf senescence. *At-Why1* T-DNA-insertion-lines showed an early senescence phenotype, which could be recovered to wildtype by transformation with a construct of own-promoter fused with *At-Why1* coding sequence. Accordingly, transgenic lines overexpressing *At-Why1* showed a staygreen phenotype, indicating a senescence-suppressor function for *At-Why1*. Gene expression analysis in the different lines showed altered expression of senescence-associated nuclear genes (*wrky53*, *wrky33*, *why2*, *sag101*, *sag12*) and of plastid genes (*ndhf*, *atpf*, *rps12*, *rpl16*, *psaA*, *psaB*), pointing to a two-fold regulatory function of *At-Why1* on nuclear and plastid genes, according to its dual localization. To distinguish between the nuclear and the plastid form, transformants overexpressing isoforms of *At-Why1*, targeted to either the nucleus or the plastids, were analysed by quantitative RT-PCR. It was shown that nuclear gene expression is affected not only directly by the nuclear form of *At-Why1*, but also indirectly by the plastid locate form. Taken together this suggests a superior function for the Whirly1 protein in *Arabidopsis* leaf senescence coordinating chloroplasts and the nucleus by retrograde signaling.

08034

DOSAGE-SENSITIVE FUNCTION OF RETINOBLASTOMA RELATED AND CONVERGENT EPIGENETIC CONTROL ARE REQUIRED DURING THE ARABIDOPSIS LIFE CYCLE

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Understanding the convergent developmental mechanisms of core cell cycle genes is highly instructive in biology. When these genes

are essential in development, lethality precludes mutation analysis throughout the life cycle of an organism. We subjected a homozygous lethal mutation in *RETINOBLASTOMA RELATED* (*RBR*) of *Arabidopsis* for tetraploid genetic analysis to study the function of *RBR* during the plant life cycle. In diploids, while *RBR* deficient female gametophytes with features of aberrant cell fate and differentiation were analogous to what was previously reported for male gametophytes, we provide evidence that *RBR* controls gametic genome duplication, thus genome integrity in the gametophyte-derived progeny. Quantitative reduction of *RBR* in tetraploids led to identification of *rbr* heterozygous plants that displayed novel *RBR* dosage-dependent phenotypes in differentiation and development of the sporophyte albeit the absence of cell cycle defects. These phenotypes coincided with deregulation of conserved epigenetic factors such as Polycomb Repressive Complex 2 (PRC2) genes and *METHYLTRANSFERASE1* (*MET1*) in the sporophyte, as shown for the gametophytes as well. However, unlike the repression by the PRC2 in gametophytes, *RBR* is activated by the sporophytic PRC2 subunits, suggesting that distinct modules of the conserved *RBR-PRC2-MET1* loop control gametophyte and sporophyte generations in plants.

08035

DOWNSTREAM TARGETS OF THE *ARABIDOPSIS THALIANA* TRANSCRIPTIONAL ACTIVATOR STYLISH1

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Members of the plant-specific SHI/STY transcription-factor family have overlapping functions and regulate lateral organ development, partially by controlling auxin biosynthesis, e.g. via activation of *YUC4* (Sohlberg et al., 2006; Eklund et al., 2010). Using a *35Spro:STY1-GR* construct we have identified additional genes that are up-regulated by the SHI/STY-family members. To investigate if the identified downstream genes are direct targets of the SHI/STY-family members we are planning to do ChIP experiments and are using qRT-PCR to study if they are up-regulated by STY1 in the presence of the protein translation inhibitor cycloheximide. We are also studying the function of some of the potential direct downstream targets by characterizing insertion lines. Among the genes that we believe could be direct downstream targets are additional genes potentially involved in auxin biosynthesis, as well as genes encoding enzymes involved in cell wall metabolism or transcription factors, e.g. one regulating style development.

08036

DISSECTION OF THE ROOT GROWTH RESPONSE TO PHOSPHATE STARVATION IN *ARABIDOPSIS THALIANA*

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Phosphate (Pi) starvation triggers molecular, physiological and developmental responses aimed for recycling internal Pi, recovering external Pi, trapping metals and limiting growth (1). When grown on a low-Pi medium, the *Arabidopsis* root growth is inhibited (2). By using the *Arabidopsis* natural variation we have identified *LPR1* (At1g23010), and its parologue *LPR2* (At1g71040). They both encode for a multicopper oxidase, the activity of which reduces root growth when seedlings are on a Pi-deficient medium (3,4). The *LPR1* protein is located in the endoplasmic reticulum and genetically interacts with *PDR2*, a P5-type ATPase (At5g23630) (5). These results provide strong evidence for the involvement of the endoplasmic reticulum at the root tip in sensing and/or responding to Pi deficiency.

In order to get more insights into this pathway, we started new genetics and chemical genetics analyses. In a large scale EMS mutagenesis of *Arabidopsis*, we isolated 85 mutants with a long primary root on low-Pi medium, including 18 new *lpr1* alleles. Around 40% of these mutations are recessive. A physiological and molecular analysis of some of these mutants will be presented.

In a parallel approach, we screened a chemical library in order to find drugs altering the Pi signalling. Two of these drugs mimic the root low-Pi syndrome, whereas two other molecules have an opposite effect. Detailed analysis of the effect of these drugs will be presented.

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08037

THE AP2 DOMAIN TRANSCRIPTION FACTOR PLT2 REGULATES AUXIN ON MULTIPLE LEVELS.

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In the *Arabidopsis thaliana* root tip, a dynamic auxin maximum is required for establishment and maintenance of the quiescent centre (QC) and stem cells. The positioning of the QC is radially determined by the GRAS transcription factors SHORTROOT and SCARECROW, and overlaps longitudinally with expression of PLETHORA (PLT) double AP2 domain transcription factors. The *PLT* expression domain follows the auxin gradient. Additionally, PLTs are known to regulate expression of PIN proteins, auxin efflux carriers that provide the polar auxin transport underlying the auxin maximum.

Micro-array analysis of the transcriptome of seedlings overexpressing *PLT2* suggests that this transcription factor influences auxin in more ways than through auxin efflux: its targets include genes involved in auxin signaling, biosynthesis, influx, and conjugation. To investigate the complex feedback between *PLT2* and auxin we are doing a series of experiments: a) validate downstream targets for binding of *PLT2* to their promoter by ChIP, b) examine single and multiple target knockouts for phenotypes, c) inducibly overexpressing selected targets in a *plt1=plt2* double mutant background and test to what extent these targets complement the mutant phenotype, d) inducibly overexpressing *PLT2* in a mutant target background to test their requirement for the *PLT2* overexpression response, and e) locally inducing auxin biosynthesis in the *plt1=plt2* double mutant background and test whether an auxin increase is sufficient to rescue the meristem maintenance defect. Data on these ongoing experiments will be presented.

08038

ROLE OF THE TEMPRANILLO GENES IN ARABIDOPSIS FLOWER DEVELOPMENT

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In plants, the transition from the vegetative to the reproductive phase involves a change in the identity of the Shoot Apical Meristem (SAM) that gives rise to flowers rather than leaves. The timing of this transition is affected by both endogenous signals and environmental conditions. In *Arabidopsis*, genes that control flowering time were assigned to different genetic pathways. Under Long Days (LD) conditions, floral induction is mainly triggered via the activation of Flowering Locus T (FT) by CONSTANS (CO) in the vascular bundles of leaves.

In our group, two transcription factors of the RAV family, TEMPRANILLO 1 and 2 (TEM1, TEM2), have been recently described as novel negative regulators of FT (Castillejo and Pelaz, 2008). The fact that TEM1 was able to bind both in vitro and in vivo the 5' UTR of FT implies that these proteins act as direct repressors of FT transcription. In addition, TEM has been proposed to act in the photoperiod pathway in a quantitative balance with CO for the fine-tuning of FT expression and therefore floral transition under LD.

As the early flowering phenotype of the null mutant tem1-1 was more evident under Short Days (SD) than in LD, we are studying the role of TEM in the non inductive SD conditions by monitoring its expression in parallel with that of CO and FT and by investigating the TEM interaction with the gibberellins pathway, which is crucial for flowering induction in SD conditions.

As TEM genes are expressed not only in vascular tissue but also in the SAM we are additionally studying the function of TEM in different domains by down-regulating TEM expression either in the SAM, or in the leaf or in both domains.

Our latest results unravelling the roles of TEM in the different expression domains and its interaction with different genetic pathways will be presented.

08039

EVO-DEVO OF UNIFACIAL LEAVES IN MONOCOTS: MAKING OF FLATTENED LEAF BLADES WITHOUT ADAXIAL-ABAXIAL POLARITY.

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Angiosperm leaves generally develop as "bifacial" and flattened structures with adaxial and abaxial identities for efficient light capture and photosynthetic functions. By contrast, a number of monocot species, such as iris (Iridaceae) or leek (Alliaceae), develop "unifacial" leaves, in which leaf blades have only one face, the abaxial side. Despite longstanding interest in the mechanisms underlying unifacial leaf development and evolution, it remains unknown how unifacial leaves become abaxialized or how and why they have repeatedly evolved in monocots. Another interesting question is how unifacial leaves become flattened. Bifacial leaves require adaxial-abaxial polarity for leaf blade flattening, whereas many unifacial-leaved species can become flattened without adaxial-abaxial polarity. Thus, flattened leaves have independently evolved in bifacial and unifacial leaves. To answer these questions, we focused on the genus *Juncus* (Juncaceae) as a model, which has a wide variety of leaf forms and contains species suitable for molecular genetic studies (Yamaguchi and Tsukaya 2010, J. Plant Res. 123).

In this report, we present genetic mechanisms underlying flattened leaf blade formation in unifacial leaves. By molecular genetic studies using two *Juncus* species with flattened or radialized unifacial leaves, we identified a transcription factor responsible for laminar outgrowth in unifacial leaves. The locus from flattened unifacial-leaved species causes the leaves to flatten and expresses more transcripts. Differential gene-expression activities between two species are caused by tissue-specific *cis*-regulatory changes of the locus. Our studies also demonstrate that the leaf blade flattening is a key that triggers central-marginal leaf polarity differentiation. Based on these results, we propose a genetic model of laminar outgrowth in unifacial leaves, and discuss the mechanism of convergent evolution of flattened leaf blades.

08040

THE ROLE OF TCP TRANSCRIPTION FACTORS IN THE LEAF DIFFERENTIATION IN ARABIDOPSIS

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Higher plants continuously generate new organs from shoot apical meristem (SAM). Coordination of the maintenance of undifferentiated fate in SAM and the promotion of differentiation in an organ is essential for the development of plant shoots. CINCINNATA (CIN)-like TCP transcription factors are involved in this coordination via the negative regulation of CUP-SHAPED COTYLEDON (CUC) genes, which regulate the formation of SAM and the specification of organ boundaries. However, the molecular mechanism of action of CIN-like TCPs is poorly understood. In this study, we identified the direct target genes of TCP3, a CIN-like TCPs of *Arabidopsis*, by using microarray, chromatin immunoprecipitation, and analysis of activity of the promoters. The target genes of TCP3 were involved in the repression of expression of CUC genes, since gain of function of the target genes suppressed the formation of SAM and resulted in the fusion of cotyledons, while their loss of function induced ectopic expression of CUC genes in leaves. Our results suggest that TCP3 integrates activities of the specific set of genes for the repression of expression of CUC genes. Since CIN-like TCP genes were revealed to act dose-dependently in the leaf differentiation, we propose that CIN-like TCPs plays an important role in the signaling that results in the generation of different leaf forms without having any lethal effects on shoots.

08041

FLOWERING ON TIME IN THE MODEL LEGUME MEDICAGO TRUNCATULA

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The transition from vegetative to floral development is a crucial step in sexual reproduction and as such is under complex regulation by external and endogenous cues. Like *Arabidopsis*, the model legume *Medicago truncatula* (*Medicago*) is a eudicot long day plant. Exposure of *Medicago* to extended cold periods (vernalisation) followed by long days accelerates flowering, as seen in winter annual *Arabidopsis*. Given that *Arabidopsis* and *Medicago* have similar responses to these environmental cues, but are evolutionarily distant, we are interested in analyzing the genetic control of flowering in *Medicago* to extend and expand knowledge of flowering regulation. First, in a candidate gene approach and using transgenic over expression *Medicago* lines and *Tnt1*-insertion mutants, we have identified an FT-like (FTLa) gene that promotes flowering in *Medicago* in response to long day induction. FT genes encode mobile flowering signals or florigens in different plants. Interestingly, unlike *Arabidopsis* FT, the *Medicago* FTLa transcript is not regulated diurnally and does not appear to be regulated by upstream CONSTANS genes. Second, taking a forward genetic approach, we are characterising a dominant early flowering mutant of *Medicago* (spring) that no longer requires vernalisation to flower rapidly in long day conditions. Current progress in these projects and towards a model for flowering control in *Medicago* will be presented.

08042

ARABIDOPSIS TSO1 REGULATES CELL CYCLE EXIT AND PROMOTES DIFFERENTIATION IN FLORAL ORGAN DEVELOPMENT

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Although much is known about how floral organ identities are specified, little is known about how floral organs grow and differentiate into their final shape, size, and morphology. *Arabidopsis tso1-1* mutant flowers failed to develop differentiated floral organs. Besides abnormal sepals, the inner three whorls of *tso1-1* flowers possess a mass of callus-like undifferentiated tissues. In addition, inflorescence meristems of *tso1* mutants are often enlarged and split from one into several inflorescence meristems. *TSO1* encodes a protein containing two cysteine-rich DNA-binding domains. Based on sequence similarity between *TSO1* and its homologs in *Drosophila* and *C. elegans*, *TSO1* is likely a component of a novel chromatin complex dREAM, which is shown in animal cells to regulate cell cycle exit and terminal differentiation. To test if *TSO1* also regulates cell cycle exit, two cell cycle marker lines: pCYCB1::GUS for G2/M transition, and pTSO2::GUS for the S phase, were used to show that the cell cycle control is strongly affected in *tso1-1* plants. Chromatin Immunoprecipitation and an inducible *TSO1* (*TSO1-GR*) were employed to show that *TSO1* is a direct and negative regulator of several S-phase-specific and E2F regulated genes. Based on these results, a model is proposed that explains how *Arabidopsis TSO1* may repress cell cycle entry and promote organ differentiation in *Arabidopsis*.

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08043

TRANSCRIPTIONAL ACTIVATION OF *WOX8* IS REQUIRED FOR APICAL-BASAL AXIS IN THE EARLY EMBRYOS.

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Axis formation is one of the fundamental events in early development. In the plant embryogenesis, the main body axis is already evident from the zygotic polarity and its asymmetric cell division, which generates a small cytoplasmic apical cell and a large vacuolated basal cell. The apical cell massively proliferates to generate all aerial plant body, whereas the basal cell produces only the root tip and the extra-embryonic tissue. Thus the zygotic polarity correlates with the apical-basal body axis of mature plants, and these morphological characteristics are conserved in most seed plants. In spite of the importance, how the zygotic polarity appears and how its daughter cells obtain the different fates are unknown. The *Arabidopsis* homeobox gene *WOX8* (*WUSCHEL RELATED HOMEOBOX8*) is expressed in the zygote, and the expression is inherited only in the basal cell derivatives. Our analysis revealed that no known axis regulators, but novel transcription factors regulate the *WOX8* expression asymmetry by binding to *WOX8* cis-elements. Loss of these factors disturbed the axis patterning in the early embryos. These defects were partially rescued by the *WOX8* over-expression, indicating that these transcription factors are the novel regulators of axis formation via *WOX8* and yet-unidentified factors. Based on these results, the mechanism regulating plant axis development will be discussed.

08044

MORE CELL OR MORE CYCLE?: ENDOCYCLE CONTROL IN PLANT DEVELOPMENT

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What controls "size" is a fundamental question in biology but intrinsic mechanisms that mediate this control still remain largely unknown. Many cell types in plants increase their nuclear DNA content or ploidy through endoreduplication in which cells replicate their chromosomal DNA without intervening mitoses. Previous studies demonstrate strong correlations between ploidy levels and cell/organ sizes, and we are trying to understand how the endocycle progression is developmentally regulated and how an increase in ploidy modifies plant growth. We have recently identified several loss-of-function and gain-of-function mutants in *Arabidopsis* and through the characterisation of these mutants and their corresponding genes, we have started to uncover the mechanisms underlying the endocycle transition and/or progression (Ishida et al. 2009, Breuer et al. 2009, Ishida et al. 2010). Based on our progress, I will discuss how the cell cycle progression links with plant growth and development.

Ishida et al. (2009) SUMO E3 ligase HIGH PLOIDY 2 regulates endocycle onset and meristem maintenance in *Arabidopsis*. *Plant Cell*

Breuer et al. (2009) The trihelix transcription factor AtGTL1 regulates ploidy-dependent cell growth in the *Arabidopsis* trichome. *Plant Cell*

Ishida et al. (2010) Auxin modulates the transition from the mitotic cycle to the endocycle in *Arabidopsis*. *Development*

08045

FLUORESCENCE IMAGING-BASED SCREEN IDENTIFIES EARLY ENDOSOMAL TRAFFICKING COMPONENTS IN *ARABIDOPSIS THALIANA*

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The endocytic vesicle trafficking is crucial for regulating activity and localization of plasma membrane components, but the process is still poorly genetically defined in plants. Membrane proteins of PIN-FORMED (PIN) family exhibit polar localization in plant cells and facilitate cellular efflux of the plant hormone auxin, thereby regulating multiple developmental processes. PIN proteins undergo constitutive endocytosis and GNOM ARF GEF-dependent recycling and their localization is under extensive regulation by developmental and environmental cues. We designed a fluorescence imaging-based screen to identify *Arabidopsis thaliana* mutants defective in internalization of proteins including PINs from the plasma membrane. We identified *BFA-visualized endocytic trafficking defective* (*ben*) mutants that do not efficiently accumulate PIN1-GFP in intracellular compartments following inhibition of exocytosis by fungal toxin brefeldin A (BFA). *BEN1* encodes an ARF GEF homologue from the functionally uncharacterized BIG class. *BEN1* localizes to early endocytic compartments distinct from GNOM-positive endosomes. Whereas *ben1* and *ben2* single mutant showed moderate defects in cell polarity, BFA sensitivity and developmental defects, combination of these two mutations significantly enhanced these defects. Our results highlight important roles of *BEN1* and *BEN2* in early endosomal trafficking.

08046

ROLES OF ARABIDOPSIS PHOSPHATIDYLINOSITOL 3-KINASE COMPLEX IN POLLEN DEVELOPMENT

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Phosphatidylinositol 3-kinase (PI3K) is involved in various biological pathways in plants, such as autophagy and root hair growth. We

have previously shown that each component of Arabidopsis PI3K complex, AtVps30(AtAtg6), AtVps34, and AtVps15, is required for pollen germination. However, roles of PI3K in pollen tube growth have been obscure, because GFP-AtVps30 and GFP-AtVps34 proteins did not necessarily exhibit polarized localization to the tip of pollen tubes. Microscopic observations revealed that pollen grains of *atvps30* and *atvps15* were normal in appearance, while *atvps34* pollen grains in part exhibited abnormal morphology, as recently reported by Lee et al. (2008). We propose that reduced PI3K activity in *atvps30* and *atvps15* disturbs pollen germination signaling, possibly mediated by ROS production. By contrast, complete loss of PI3K activity in *atvps34* may affect pollen development as well as pollen germination. We also suggest possible involvement of phosphatidylinositol-3-phosphate 5-kinase (AtFab1) in pollen development.

08047

REGULATORY NETWORKS IN POLLEN DEVELOPMENT

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The formation of functional pollen relies upon the coordinated expression of genes from the maternal anther tissues and the developing gametophytes. These expression networks depend on both temporal and spatial regulation to produce functional gametophytes and then complete formation of pollen wall development and subsequently to release the pollen. A number of key regulators associated with pollen formation in Arabidopsis have now been characterised, however understanding of these regulators is still in their infancy.

Many of these genes show expression within the anther tapetum and mutant analysis has shown that the tapetum is critical for functional pollen development. A number of these mutants that show abnormal tapetal development have been analysed by microarray analysis (Xu et al.; Yang et al., 2007). We have overlaid these microarray data from key regulators in tapetal development onto our Arabidopsis regulatory network to identify clusters of related regulatory function. These regulatory clusters have been analysed for their roles in pollen development and to conduct functional analyses on these network components.

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08049

MUTATION IN ALKALINE α -GALACTOSIDASE FAMILY GENES AFFECTS PLANT GROWTH AND DEVELOPMENT IN ARABIDOPSIS

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Alkaline α -galactosidases belong to a recently identified glycosyl hydrolase family found only in plants. The protein is widely distributed in different species and shows apparent gene redundancy. Our previous works showed alkaline α -galactosidases capable of hydrolyze a variety of glycosides possessing non-reducing terminal and side-chain α -galactosyl residues. We hypothesize different alkaline α -galactosidases may play different roles in the turn-over, recycling and remodeling of cellular components in a plant during leaf senescence, stress response, seed germination and remobilization of photosynthetic assimilates. In this study, we present preliminary data from studying of three *Arabidopsis* alkaline α -galactosidase paralogs located on different loci (At1g55740, At3g57520 and At5g20250). We analyzed the protein sequences, and characterized homozygous T-DNA insertion mutants. We also examined the expression profiles of these transcripts and total proteins, as well as the total alkaline α -galactosidase enzyme activities during leaf senescence. All these paralogs expressed differentially in time and quantity, and their transcript levels were increased as senescence progressed. Total proteins and enzyme activities also showed similar patterns. All T-DNA insertion mutants tested showed enhanced plant growth and early flowering.

08050

BIOCHEMICAL ANALYSIS OF TARGET PROTEINS UBIQUITINATED BY ATL31 IN ARABIDOPSIS THALIANA

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Carbon and nitrogen availability is one of the most important factors to regulate plant development. To utilize limited resource of carbon and nitrogen efficiently, plants enable to sense and respond to balance of carbon (C) and nitrogen (N) metabolites, called C/N response.

We previously showed a novel ubiquitin ligase ATL31 has essential role to regulate C/N response in *Arabidopsis thaliana*. In this study, we tried to isolate target protein ubiquitinated by ATL31. As a result of immunoprecipitation and MS analysis used epitope-tagged ATL31, we found several candidates of target protein for the ubiquitination. Details of the associated proteins and biochemical function of ATL31 will be discussed.

08051

DUAL REGULATION OF EXPRESSION OF ETT/ARF3 BY ASYMMETRIC LEAVES2 (AS2) AND AS1 FOR ESTABLISHMENT OF THE MEDIAL-LATERAL LEAF POLARITY IN ARABIDOPSIS THALIANA

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Spatial and temporal regulation of gene expression is the most important process for plant development. Leaves are developed from a shoot apical meristem that contains a group of stem cells locating at the summit of a plant body. The structure of leaves basically forms along three axes, namely the proximal-distal, medial-lateral and adaxial-abaxial axes. The developmental process of leaves is a good model system for studying three-dimensional differentiation in a plant. It is, however, still unknown how spatial and temporal regulation of the differentiation is coordinated. The AS2 gene of *Arabidopsis thaliana*, which encodes a member of the AS2/LOB family, and the AS1 gene that encodes a myb transcription factor are key regulators for the proper leaf elongation along three axis(1, 2, 3, 4). We have

reported that AS2 and AS1 regulate multiple leaf phenotypes such as the proximal-distal differentiation through the repression of class 1 KNOX genes(4). We have also showed that AS2 and AS1 negatively regulate expression of ETT/ARF3, KAN2, YAB5, which are thought to be abaxial determinants and act as transcriptional regulators of multiple genes(3). ETT/ARF3 and ARF4 are closely related members of the ARF family and known to be targets of trans-acting siRNA, tasiR-ARF, which in turn is regulated by miR390. Here we report that AS2 and AS1 directly regulate ETT expression by binding to the ETT promoter region. In addition, our genetic and molecular analysis also suggested that AS2 and AS1 indirectly repressed the expression of both ETTIN/ARF3 and ARF4 through the miR390 and tasiR-ARF pathway. We will discuss mechanisms of spatial and temporal regulation of both ETTIN/ARF3 and ARF4 by AS2 and AS1, which might be required for the establishment of adaxial-abaxial and medial-lateral polarities of leaves. (1) Matsumura et al., Plant J. (2009); (2) Ueno et al., Plant Cell (2007); (3) Iwakawa et al., Plant J. (2007); (4) Ikezaki et al., Plant J. (2009).

08052

THE ARABIDOPSIS RWP-RK MOTIF-CONTAINING PUTATIVE TRANSCRIPTION FACTOR RKD4 FUNCTIONS IN EMBRYONIC PATTERN FORMATION

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RWP-RK motif-containing putative transcription factors occur widely in higher plants, but their biological functions are described only for leguminous NIN proteins in nodule formation. The *Arabidopsis* genome encodes fourteen RWP-RK motif-containing proteins, including nine NLP proteins (NLP1-9) with longer polypeptides and five RKD4s (RKD1-5) with shorter polypeptides. By a systematic screening of T-DNA insertion mutants of *RKD1-5*, we found that loss-of-function *rkd4* mutants germinated at a low rate and that many of germinated individuals lacked primary roots and bore abnormally shaped cotyledons. Once germinated, however, these individuals produced adventitious roots and normal leaves, and flowering *rkd4* plants were indistinguishable from wild-type, indicating that RKD4 functions are required specifically in embryos. Detailed inspection of the *rkd4* embryogenesis revealed that abnormality was observed already in zygotic cell elongation and continued in later cell division patterns. Expression patterns of DR5rev-GFP and PIN1-GFP markers indicated that both auxin transport and response were severely compromised in *rkd4* embryos. Analysis of the *RKD4* promoter-GFP lines revealed that *RKD4* expression occurs throughout early embryos, and then becomes restricted to the basal part of the embryo and suspensor. Overexpression of *RKD4* in embryos by the *RPS5A* promoter resulted in abnormal expression of DR5rev-GFP and cell division patterns. Consistent with the postulated role of RWP-RK motif-containing proteins as transcription factors, RKD4:GFP fusion proteins were localized to the nuclei of onion epidermal cells and the amino-terminal region of RKD4 activated reporter gene expression in yeast. Taken together, these results indicate that RKD4 controls pattern formation in early embryogenesis presumably through transcription of genes required for normal auxin transport and/or perception.

08053

LBD18/ASL20 REGULATES LATERAL ROOT FORMATION IN COMBINATION WITH LBD16/ASL18 DOWNSTREAM OF ARF7 AND ARF19 IN ARABIDOPSIS

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The LATERAL ORGAN BOUNDARIES DOMAIN/ASYMMETRIC LEAVES2-LIKE (LBD/ASL) genes encode proteins harboring a conserved amino acid domain, referred to as the LOB (for lateral organ boundaries) domain. While recent studies have revealed developmental functions of some LBD genes in *Arabidopsis thaliana* and in crop plants, the biological functions of many other LBD genes remain to be determined. In the present study, we have shown that the *lbd18* mutant evidenced a reduced number of lateral roots and that *lbd16 lbd18* double mutants exhibited a dramatic reduction in the number of lateral roots compared with *lbd16* or *lbd18*. Whereas the numbers of primordia of *lbd16*, *lbd18*, and *lbd16 lbd18* mutants were similar to those observed in the wild type, the numbers of emerged lateral roots of *lbd16* and *lbd18* single mutants were reduced significantly. *lbd16 lbd18* double mutants exhibited additively reduced numbers of emerged lateral roots compared with single mutants. This finding indicates that LBD16 and LBD18 may function in the emergence of lateral root formation via a different pathway. Using a steroid regulator-inducible system in which the nuclear translocation of LBD18 can be regulated by dexamethasone, we have shown that LBD18 overexpression rescued lateral root formation in *lbd18* and *lbd16 lbd18* mutants without inducing any other phenotypes. Furthermore, we demonstrated that LBD18 overexpression can stimulate lateral root formation in *arf7 arf19* mutants with blocked lateral root formation. Taken together, our results suggest that LBD18 functions in the emergence of lateral roots, in conjunction with LBD16, downstream of ARF7 and ARF19. Biochemical and developmental mechanisms of LBD18 in lateral root formation are under our investigation.

08054

TOWARD AN UNDERSTANDING OF LEAF SIZE REGULATION - RELATIONSHIP BETWEEN SALICYLIC-ACID RESPONSE AND COMPENSATION IN LEAF DEVELOPMENT

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Compensation is a phenomenon observed in mutants and transgenics in which aberrant leaf-cell expansion occurs in response to a severe defect in cell proliferation in leaf primordia. This suggests that there are some intrinsic mechanisms which coordinate cell proliferation and cell expansion during leaf development. To elucidate the mechanism of compensation, we isolated several *extra-small sisters* (*xs*) mutants that have a specific defect in cell expansion. Through the genetical analyses between *xs* mutants and a compensation-exhibiting mutant, *angustifolia3* (*an3*), we found that some of *xs* mutations clearly suppress aberrant cell enlargement in *an3*. For further analysis, we focused on *xs2* that is the strongest suppressor among them. Then, we produced double mutants between *xs2* and the other compensation-exhibiting strains, *erecta* (*er*) and KIP-RELATED PROTEIN 2 over-expresser (KRP2ox). Interestingly, *xs2* mutation suppressed aberrant cell enlargement in *er* as well as *an3*, but not in KRP2ox, indicating that compensation occurs via *XS2*-dependent or independent manner. We revealed that *XS2* encodes a member of cation calcium exchanger proteins. To gain more insights why defect in cation calcium exchanger causes smaller cell phenotype, we performed the microarray analysis. Interestingly, genes involved in salicylic-acid (SA) signaling were expressed at a much higher level in *xs2* than WT. Moreover, exogenous SA supply clearly suppressed aberrant cell enlargement both in *an3* and *er*. These indicated that the excessive SA-mediated response caused by *xs2* mutation is somehow involved in the suppression of compensated cell. We will discuss on possible mechanisms behind compensated cell enlargement, based on the above analyses.

08055

REGULATORY ROLES OF LIGHT ON THE FUNCTION OF THE PLANT SHOOT APICAL MERISTEM

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Plants cannot move from their physical location. Therefore, they need to have a flexible growth control mechanism to adapt to environmental change for survival. One of the major environmental factors controlling plant growth and development is light. It is well known that light influences post-meristematic processes such as photo-morphogenesis, shade avoidance and phototropism, but little is known about the effect of light on leaf initiation. In this study, we studied effects of light on the shoot apical meristem and leaf initiation.

In a tomato shoot apex culture, we found that apices grown in the dark stopped producing leaf primordia even though in the presence of sucrose. Light reversed the inhibitory effects of darkness and restarted leaf initiation. The apices cultured in the presence of photosynthesis inhibitors produced new leaf primordia in the light. These results suggest that light acts as a morphogenetic signal which is independent of photosynthesis.

In our previous studies, we showed that auxin is the major regulator of leaf initiation. Inhibition of polar auxin transport blocks leaf formation at the tomato shoot apex, resulting in pin-like naked stems (NPA-pins). Micro-application of auxin to the NPA-pins restores leaf formation (1). Interestingly, in contrast to NPA-pins in the light, local auxin treatment did not induce leaf formation to NPA-pins in the dark. It suggests that reconstruction of local auxin gradient is not sufficient for leaf initiation in the dark. We postulate a role for cytokinin in the signal transduction chain between light and organ initiation. Based on these data, regulatory roles of light on organ initiation at the shoot apical meristem will be discussed.

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08056

INFLORESCENCE DEFICIENT IN ABSISSION (IDA) IS INVOLVED IN SEPARATION OF CORTEX AND EPIDERMAL CELLS DURING LATERAL ROOT EMERGENCE

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Small peptides can act as signaling molecules that coordinate development, growth and differentiation. *INFLORESCENCE DEFICIENT IN ABSISSION (IDA)* encodes a putative peptide ligand necessary for the regulation of floral organ abscission in *Arabidopsis*. IDA is expressed specifically in the abscission zone at the base of the organ to be shed, and it is dependent on the two receptor-like kinases (RLK) HESA (HAE) and HESA-LIKE 2 (HSL2) to exert its function (Butenko et al., *Plant Cell* 2003; Stenvik et al., *Plant Cell*, 2008; Cho et al., *PNAS*, 2008). The *ida* and *hae hsl2* mutations block the final cell separation step. Ethylene is known to promote abscission, but IDA-HAE-HSL2 represents an ethylene-independent signaling pathway. Recently we found, however, that *IDA* is auxin-inducible in the root. During lateral root (LR) development *IDA* is expressed in cortex and epidermal cells overlying lateral root primordia. These cells have to separate to allow LR emergence. Mutation in *IDA* not only leads to total deficiency in floral organ abscission, but also to a delay in LR emergence, suggesting that IDA plays a similar role during these two cell separation events. Consistent with this, mutation in a putative IDA receptor results in a similar defect in LR emergence.

08057

REGULATION OF LATERAL ROOT FORMATION BY STRESS RELATED MITOGEN ACTIVATED PROTEIN KINASES

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Lateral root formation in *Arabidopsis* is strictly regulated with lateral roots emerging regularly spaced along the primary root and with roughly equal amount of lateral roots opposite each xylem pool. Initiation of lateral root formation starts with an asymmetric division and is dependent on polar auxin transport. In *Arabidopsis* these first divisions occur only in pericycle cells opposite the xylem poles and local auxin accumulation has been shown to precede these first divisions. Several regulatory genes for lateral root initiation have been identified through genetic screens. The best studied of these are the negatively regulating Aux/IAA protein Solitary Root (SLR; IAA14) and its two downstream acting Auxin Response Factors ARF7 and ARF19, which positively regulate lateral root formation. How the lateral root initiation signaling cascade is modulated under stress conditions has not been reported. Here we report on a set of stress related mitogen activated protein (MAP) kinases that regulate lateral root initiation. Double loss-of-function mutant combinations result in increased numbers of lateral root primordia with distorted spacing. These double mutants do not form any lateral roots up to 12 days post germination but later excessive numbers of lateral root emerge leading to aberrant root architecture. Overexpression of gain-of-function mutants results in the complete inhibition of lateral root initiation, similar to the slr-1 phenotype. The use of reporter lines combined with time lapse confocal image analysis shows normal auxin responsiveness in both the double loss-of-function and gain-of-function MAPK mutant lines. Together with genetic evidence our data support a role for the MAPK cascade acting upstream of the IAA14/ARF7/19 module in the regulating lateral root initiation.

08058

ARABIDOPSIS SYNAPTOTAGMINS: NON-REDUNDANT FUNCTIONS IN PLANT DEVELOPMENT

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Synaptotagmins are calcium sensors that regulate synaptic vesicle exocytosis and endocytosis. Thought to be exclusive to animals, they have recently been characterized in *Arabidopsis*, in which they comprise a five gene family (SYT A, B, C, D and E). We have shown that *Arabidopsis* SYTA regulates endocytosis and movement protein-mediated trafficking of plant virus genomes through plasmodesmata. Our studies suggest that distinct virus movement proteins transport their cargos to plasmodesmata for cell-to-cell spread via an endosome recycling pathway. SYTA has also been reported to have a role in responses to osmotic stress and calcium-dependent freezing tolerance. To better understand these roles of SYTA, and the potential roles of the other *Arabidopsis* SYTs, in biotic and abiotic stress, we are examining the spatial and temporal regulation of SYT gene expression using appropriate promoter::GUS reporter genes. We find that SYTA is ubiquitously expressed in *Arabidopsis*, and that SYTE appears to have a similar pattern of expression. In contrast, SYTB is highly expressed in stems, siliques, flowers and anthers, while SYTC is specifically expressed in stomatal guard cells. The detailed patterns of expression for SYTA, SYTB, SYTC and SYTE will be presented, and the consequences in terms of their roles in the responses of *Arabidopsis*

to biotic and abiotic stresses will be discussed.

08059

FIBRE GROWTH PATTERNS WITHIN THE ARABIDOPSIS INFLORESCENCE STEM DISSECTED THROUGH INTEGRATION OF GROWTH KINETIC PROFILING, LASER SCANNING CONFOCAL MICROSCOPY AND LASER CAPTURE MICRODISSECTION

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Fibres in many woody species achieve great length through an intrusive mode of cell elongation. This requires extreme anisotropic cell wall expansion as well as disruption of cell-cell adhesion within the intruded cell stories. Mature Arabidopsis fibres resemble those of woody species, and it has been suggested that Arabidopsis fibres may also grow intrusively. We have characterized the mode of growth of Arabidopsis inflorescence stem fibres through several approaches, all of which are anchored in an empirically defined developmental gradient of fibre elongation. Specific developmental stages were identified within individual plants by time-lapse imagery of growing inflorescence stems, and subsequent derivation of plant-specific growth kinetic profiles. Confocal laser scanning microscopy-based morphometric comparison of cell lengths revealed that fibres increase in length relative to diffusely growing cell types throughout the course of inflorescence stem elongation. We utilized sixty cell wall-related antibodies in an immunostaining screen of transverse whole-mount sections from each fibre elongation zone to detect potential fibre-specific epitopes that might be associated with the unique mode of fibre elongation. Hierarchical clustering of the immunolabeling (Ab) patterns across three stages of development revealed unique Ab pattern for fibres which were most distinctive when fibre elongation is maximal. While resembling the Ab pattern of the xylem, the pattern in the interfascicular region also displays extensive similarity to the pattern of a region of small diameter cells within the phloem. We are also investigating the transcriptional profiles of fibres and adjacent cell types in the inflorescence stem through laser capture microdissection of discrete cell types over the three stages of fibre development, with the goal of integrating these cell type-specific gene expression patterns with the immune-epitope and growth morphology cellular phenotypes.

08060

A GENOMIC FRAMEWORK OF POLYCOMB GROUP FUNCTION

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Polycomb repressive complex (PRC) function has been found to be responsible to maintain the differentiation status of a cell in an epigenetic manner and is evolutionary conserved throughout multicellular organisms. Different PRC2 complexes play an important role during the plant life cycle by silencing target genes through trimethylation of histone 3 at lysine 27 (H3K27me3). For instance PRC2 function is key for the parent-of-origin dependent expression during early seed development.

While there are diverse histone methyltransferase SET domain proteins and Zinc finger proteins as part of the Arabidopsis PRC2, all various compositions of the complex in Arabidopsis are thought to share the WD40 protein FERTILIZATION INDEPENDENT ENDOSPERM (FIE). However, the postembryonic function of FIE as well as the cross-talk between other PRC2 components are difficult to study due to the embryonic lethality of the respective mutants caused by maternal gametophytic effects.

We have recently identified a mutant that allows bypassing of imprinting requirements during seed development, thus also circumventing the necessity of PRC2 function in the seed. Here we have used this mutant to circumvent the need for FIE during seed development and thus homozygous fie mutants could be recovered in the following generation. We took advantage of this unique possibility to study PRC2 function postembryonically and a genomic framework of polycomb function will be presented.

08061

ROLES OF AN AGL6-LIKE MADS BOX GENE, MOSAIC FLORAL ORGANS1, IN FLORAL DEVELOPMENT IN RICE

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Floral organ identity and meristem determinacy in plants are controlled by combinations of activities mediated by MADS box genes. *AGAMOUS-LIKE6* (*AGL6*)-like genes are MADS box genes expressed in floral tissues, but their biological functions are mostly unknown. Here, we describe an *AGL6*-like gene in rice (*Oryza sativa*), *MOSAIC FLORAL ORGANS1* (*MFO1/OsMADS6*), that regulates floral organ identity and floral meristem determinacy. In the flower of *mfo1* mutants, the identities of palea (prophyll-like organ) and lodicule (equivalent to petal) are disturbed, and mosaic organs were observed. Furthermore, the determinacy of the floral meristem was lost, and extra carpels or spikelets developed in *mfo1* florets. The expression patterns of floral MADS box genes were disturbed in the mutant florets. Suppression of another rice *AGL6*-like gene, *Osmads17*, caused no morphological abnormalities in the wild-type background, but it enhanced the phenotype in the *mfo1* background, indicating that *Osmads17* has a minor but redundant function with that of *MFO1*. Whereas single mutants in either *MFO1* or the *SEPALLATA*-like gene *LHS1* showed moderate phenotypes, the *mfo1 lhs1* double mutant showed a severe phenotype, including the loss of spikelet meristem determinacy. We propose that rice *AGL6*-like genes help to control floral organ identity and the establishment and determinacy of the floral meristem redundantly with *LHS1*.

Reference:

Ohmori *et al.* (2009) Plant Cell 21 (10): 3008-3025.

08062

WEIRD FINGERS: FUNCTIONAL ANALYSIS OF WIP DOMAIN PROTEINS

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WIP proteins form a plant specific subfamily of C2H2 zinc finger (ZF) proteins with 6 members in arabidopsis. Up to now, loss of function phenotypes have been described only for the founding member *WIP1/TRANSPARENT TESTA 1* (*TT1*) and *WIP2/NO TRANSMITTING TRACT* (*NTT*).

In this study, we used a complementation approach to functionally characterize the WIP domain which consists of four ZF motifs arrayed in tandem. Two WIP ZFs fit well to the C2H2 consensus pattern ($X_2CX_2CX_{12}HX_3H/C$). The other two ZFs contain an atypically extended

spacing of zinc coordinating residues. Mutations in each of the motifs lead to loss of function of the protein. SV40 type nuclear localisation signals (NLSs) were detected as part of the atypical ZF motifs. The importance of these sequences was confirmed by two new *tt1* alleles identified by TILLING, both carrying point mutations in one of the NLSs. These mutations shift the localisation pattern of corresponding GFP fusion proteins from nucleus to cytoplasm in transfected protoplasts and lead to a decreased seed pigmentation *in planta*. Based on promoter swap experiments we propose that WIP factors are able to fulfil closely molecular functions and mainly differ in their expression patterns and therefore in target specificity.

All WIP ZF motifs are conserved in the positions known to contact DNA in C2H2 ZFs. However, neither DNA binding nor other molecular functions have been shown for WIP proteins so far. In order to identify WIP target genes, we tested several promoters of flavonoid pathway genes in cotransfection experiments together with TT1. *BANYULS* promoter activity induced by the TT2-TT8-TTG1 ternary complex was found to be increased in the presence of TT1 whereas TT1 alone or in combination with only one of the regulators had no impact. Based on these observations we discuss molecular functions for WIP ZF proteins other than the supposed binding of DNA.

08063

A GENETIC DISSECTION OF HOMEODOMAIN PROTEIN TRANSPORT THROUGH PLASMODESMATA.

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Cell-to-cell communication is critical for cell fate specification and to coordinate development in multi-cellular organisms. A new paradigm for such communication in plants is the selective trafficking of transcription factors and other signaling proteins and RNAs through plasmodesmata (PDs). PDs are also critical for the transport of nutrients and protection against disease. We have taken an unbiased genetic strategy to dissect the mechanism of PD trafficking.

Plant KNOX homeodomain proteins were the first found to selectively traffic through PDs, and their trafficking appears to be important for their function in stem cell maintenance. A gain-of-function trafficking assay in *Arabidopsis* has been developed to demonstrate that the C-terminal region of KNOX proteins KNOTTED1, and that of its *Arabidopsis* homologs SHOOTMERISTEMLESS and KNAT1/ BREVIPEDICILLUS is necessary and sufficient for trafficking *in vivo*. This system provides a simple system to understand how proteins traffic cell-to-cell, and to isolate trafficking mutants.

As a proof of concept, a mutant with reduced trafficking has been identified, and was found to be defective in a chaperonin gene. This gene appears to be essential for PD trafficking of some but all non-cell-autonomous proteins, and biochemical evidence suggests a physical association between this chaperonin and homeodomain proteins. This discovery is exciting because proteins are thought to undergo partial unfolding during PD translocation. The chaperonin mutation enhances a weak STM allele, and affects STM trafficking in the meristem, suggesting that its function is significant and relevant to the normal function of STM. The mutants also display a leaf polarity phenotype, suggesting they also disrupt a mobile signal that is necessary for leaf polarity. A functional characterization of the role of chaperonins will further our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking in plants.

08064

BIM1, A COMPONENT OF BRASSINOSTEROID (BR) SIGNALING, IS REQUIRED FOR SPL8 FUNCTION

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As we have shown previously, the non-miR156 targeted SBP-box gene *SPL8* plays a role in *Arabidopsis* anther and ovule development resulting in a growth condition-dependent semi-sterile phenotype of the *spl8* mutant (Unte et al., 2003; Zhang et al., 2007). With the aim of a better understanding of *SPL8* function, we performed a yeast two-hybrid screen to identify putative *SPL8* interacting proteins. For one of the identified proteins, BIM1 a bHLH protein involved in brassinosteroide signaling and embryonic patterning (Yin et al., 2005; Chandler et al., 2009), the interaction could be confirmed *in planta* using bimolecular fluorescence complementation (BiFC). Although *bim1* single mutants did not display an obvious fertility problem, mutation of *BIM1* further reduced fertility of the *spl8* mutant. To further investigate whether *SPL8* function requires BIM1, we introduced a *35S::SPL8* transgene into a *bim1* mutant background. At the seedling stage, *35S::SPL8* transgenics display narrower and darker-green cotyledons. Strikingly, this cotyledon phenotype disappeared against the *bim1* mutant background, indicating that this ectopic *SPL8* function in seedlings requires BIM1 activity. Together, our data suggest that *SPL8* requires interaction with BIM1 for its proper function *in planta*.

08065

RGA MEDIATES ARABIDOPSIS TAPETUM DEVELOPMENT VIA REGULATING A PLASMA MEMBRANE ASSOCIATED PROTEIN, RGAT1

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The phytohormone gibberellin (GA) plays important roles in regulating many aspects of plant development including floral organ development, especially male fertility. GA derepresses its signaling pathway by promoting the degradation of DELLA proteins, a family of nuclear growth repressors. Through genetic analysis of various combinations of null mutants of DELLA proteins in *ga1-3*, RGA has been identified as the most important DELLA family member involved in floral organ development. To elucidate the early molecular events and immediate targets controlled by RGA during flower development, we performed whole-genome microarray analysis to identify genes in response to the steroid-inducible activation of RGA in *ga1-3 rgl2 rga 35S::RGA-GR*. Among the genes that were regulated by RGA, *RGAT1* encoding an unknown protein was up-regulated by induced RGA activity within 4 hours. Further expression analyses demonstrated that *RGAT1* was expressed in the tapetum and may act as one of the immediate targets of RGA during the stamen development. Over-expression of *RGAT1* caused the delay of anther tapetum degradation, resulting in inviable pollen and subsequent low fertility with short siliques. *RGAT1-GFP* fusion protein localized to the plasma membrane. These results suggest that RGAT1 may be activated by RGA and play a negative role in modulating GA signaling pathway during flower development.

08066

THE ARABIDOPSIS *IN VIVO* POLLEN TUBE DEFECT 1 GENE ENCODES A PUTATIVE ALKALINE PHYTOCERAMIDASE THAT IS IMPORTANT FOR POLLEN TUBE AND FEMALE GAMETOPHYTE INTERACTION

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In flowering plants, the interaction between pollen tube and female gametophyte plays a critical role for the successful fertilization. Here, we identified an *Arabidopsis* mutant, *in vivo pollen tube defect 1* (*ipod1*), from *Ds* transposon insertion lines. The mutant displays an obvious phenotype of shorter siliques and a high frequency of aborted fertilization. Genetic analysis and molecular characterization demonstrated that the phenotype is caused by a single *Ds* insertion. Heterozygous *ipod1* mutants exhibit a modest reduction in seed set compared with wild-type plants. Reciprocal crosses proved that the mutation results in defects of male gametophytic function. Further phenotypic analysis demonstrated that pollen tube growth in pistil is abnormal while *in vitro* growth is not affected. Both genetic complementation experiments and additional T-DNA insertion allele analysis showed that the mutant phenotype is caused by the disruption of a putative alkaline phytoceramidase, which is partially colocalized with the Golgi apparatus and endoplasmic reticulum. Ceramidase carries a central role in sphingolipid metabolism in eukaryotic cells, which regulates cell proliferation, differentiation, apoptosis and different kinds of stress responses. *iPOD1* gene is mainly expressed in reproductive organs. These data suggest that *iPOD1* functions in the interaction between pollen tube and female gametophyte.

08067

CELL LINEAGE DETERMINES TAPETAL FATE IN THE ARABIDOPSIS ANTER

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The characteristic plasticity of plant form results from the formation, in meristems, of pluripotent cells that later acquire fates through local signals. This cannot be the case for reproductive tissues which contain small but consistent numbers of cells. Fate in animal reproductive tissues is generally established in founder cells that follow a fixed number and pattern of division, but mutations in the plant *EXCESS MICROSPOROCYTES1 (EMS1)* signaling pathway - required for tapetum formation in the *Arabidopsis* anther — are reported to result in precursor cells switching into meiocyte development, exhibiting plasticity typical of meristem-derived cells. Here we show tapetal fate to become determined very early in a group of founder cells and the *EMS1* pathway to be required only for patterned proliferation and putatively the final differentiation of this lineage. Different fate-establishment mechanisms must thus exist in plants for either 'plastic' somatic development, or for the formation of regularly-patterned reproductive tissues.

08068

GRP23 INTERACTS WITH GIP2 AND GIP3 TO DETERMINE CELL DIVISION PATTERN DURING EMBRYOGENESIS IN ARABIDOPSIS

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Pattern formation is a fundamental process in development. During embryogenesis in plants, embryonic patterning is reflected by the stereotyped cell divisions and auxin gradient established early in development by the asymmetric distribution of auxin influx/efflux carriers. Molecular mechanisms controlling cell division pattern and the distribution of auxin polar transporters are being deciphered recently through genetic approaches. Nevertheless, key regulators governing embryonic pattern formation remain to be revealed. In *Arabidopsis*, *grp23* mutant exhibited defects in early embryo development, and twenty percent of mutant embryos displayed aberrant cell division patterns. Here, we report two *GRP23* interacting proteins, *GIP2* and *GIP3*. They partially colocalize with *GRP23*. *GIP2* is a WD-40 protein, which likely involves in the ubiquitin-mediated proteosome pathway. The orthologue of *GIP3* is essential for cell polarity and cytokinesis in yeast. In addition, the *gip2* and *gip3* mutants also display embryo or gametophyte lethal phenotypes. Further functional studies of these genes may provide mechanistic insights into the determination of cell division patterns during plant embryogenesis.

08069

CCG, A PUTATIVE TRANSCRIPTIONAL REGULATOR, PLAY AN IMPORTANT ROLE IN THE POLLEN TUBE GUIDANCE IN ARABIDOPSIS.

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Unlike most animals and many lower plant species, the sperm cell of flowering plants cannot migrate unassisted and must be transported by the pollen tube to the female gametophyte to complete fertilization. Previous study showed that pollen tube guidance is controlled by the seven-celled female gametophyte, the embryo sac. The central cell-expressed CCG gene is required for micropylar pollen tube guidance in *Arabidopsis* (Chen et al., *Plant Cell*, 2007). CCG encodes a nuclear protein with an N-terminal conserved zinc β -ribbon domain that is functionally interchangeable with that of TFIIB in yeast. This suggests that CCG might act as a transcription regulator for pollen tube guidance. We compared the expression pattern of *A. thaliana* Ler and *ccg* mutant and found that 1093 genes are up-regulated, while 618 genes are down-regulated in *ccg* mutant. The down-regulated genes are involved in diverse biological processes which include defense response process, hormone response. By yeast two-hybrid screening, we identified a CCG interacting protein, CIP1. Whether CIP1 also functions in pollen tube guidance is being investigated. The functional study of the genes regulated by CCG and the proteins interacting with CCG will be instrumental to understand the underlying mechanisms in the pollen tube guidance.

08070

TAPPING INTO THE MECHANICAL LINK OF EXPANSINS IN LEAF MORPHOGENESIS

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Expansins are key cell wall-loosening agents for cell expansion. Although widely implicated in many aspects of plant development, only three out of 38 expansin genes in *Arabidopsis* has been functionally characterised. The fact that expansins are encoded by a large gene family poses problems for classical reverse genetic approaches. Furthermore, their role in the control of organ growth is still unclear with often contradictory or weak correlations between expression patterns and growth rates.

My research aims are three-fold: firstly, to identify which expansins are involved and where they are expressed during leaf development. This provides the basis for targeted knock-down of specific expansins to provide evidence for their functional role. Secondly, to understand the cellular behaviour underlying leaf morphogenesis, I am using expansin in a chemically-inducible system as a tool to answer what happens to leaf form when cells are restricted from expansion or are subjected to increased expansion during growth, and how the processes of cell division and cell expansion are coordinated. Lastly, to explore atomic force microscopy (AFM) application for quantitative analysis of cell wall mechanics, in turns, to test the *in vivo* outcome of altered expansin gene expression.

Data will be presented on progress towards these three aims, with special reference to the development of mechanical-based models

on the control of leaf form.

08071

ATHB12, AN ARABIDOPSIS HOMEOBOX GENE, NEGATIVELY CONTROLS THE ELONGATION OF INFLORESCENCE STEMS.

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Homeobox genes play crucial roles in numerous developmental processes including embryogenesis and organ development. HD-Zip family of homeobox genes has been found only in plants, so it is thought that HD-Zips have plant-specific roles in the plant developmental processes. ATHB12 belongs to the HD-Zip class I and its expression was highly induced by abiotic and biotic stresses. In the previous studies, we found that ATHB12 expression was mainly detected in stem, petiole and leaves after ABA treatment. Inducibility to ABA was analyzed using transgenic tobacco plants harboring serial 5' deletions of the ATHB12 promoter fused to the beta-glucuronidase (GUS) gene. GUS activity was checked by fluorometric assay and real-time quantitative PCR. The longest promoter (2.1-kb) produced the highest enzyme activity, suggesting that the promoter region between 2.1-kb and 1.5-kb contains an essential element(s) required for ABA-induction of ATHB12. A T-DNA insertion mutant of ATHB12 was isolated and found to contain an insertion -293 base pairs upstream of the transcription start site. The mutant had lower expression of ATHB12 in the stems and longer inflorescence stems compared to the wild type. T-DNA insertion mutant also had a higher germination rate on ABA-containing media. In contrast to the athb12 mutant, ATHB12 overexpressor showed the retardation of stem elongation at early stem development. Moreover, ABA treatment induced ATHB12 expression in inflorescence stem and inhibited the stem growth, similarly to the phenotype of ATHB12 overexpressor. Our data suggest that an ABA-inducible gene, ATHB12, might act as a negative regulator of stem growth against the stresses.

08072

LONG-DISTANCE TRAFFICKING OF ARABIDOPSIS FLOWERING LOCUS T RNA IS SUFFICIENT TO TRIGGER FLORAL INITIATION

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The concept that mRNA may act as a systemic information molecule is one of the most exciting discoveries in recent plant biology. However, reports that demonstrate the functional significance of non-cell autonomous RNAs remain limited. Here we show that the RNA of Arabidopsis FLOWERING LOCUS T (FT) acts in a non-cell autonomous manner to regulate flowering. Arabidopsis cleft-grafting experiments showed that FT RNA undergoes long-distance movement from the stock to the scion apex. In addition, FT RNA was sufficient to cause a cell-autonomous RNA to move over long distances, suggesting that FT RNA contains the motifs required for RNA trafficking. The FT RNA itself acts as a part of the systemic floral signaling, because when FT protein was sequestered in companion cells, the movement of FT RNA alone was sufficient to promote floral initiation. To examine the long-distance movement of FT RNA occurs in natural condition, we investigated whether FT RNA is present in the phloem sap. RTPCR analysis showed that the RNA of FT homologs were detected in the broccoli phloem sap, which suggests that FT RNA is a phloem mobile RNA. Taken together, our results support the notion that the FT RNA acts as a systemic signal to regulate floral initiation. Thus, both FT RNA and protein act redundantly to provide a dosage-dependent input that regulates floral initiation.

08073

SYNTHESIS OF VERY LONG CHAIN FATTY ACIDS CONTROLS CELL PROLIFERATION IN A NON-CELL-AUTONOMOUS MANNER

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Very long chain fatty acids (VLCFAs*) are fatty acids that consist of 20 or more carbons. VLCFAs and their derivatives (i.e., VLCFA-ester and ketones) are essential for plant growth, especially for synthesis of cuticle, triacylglycerols, ceramides and phospholipids. However, it remains unknown whether they function in controlling cell division and/or differentiation during continuous development of plants.

pasticcino2 (*pas2*) mutants exhibit pleiotropic phenotypes in aerial tissues, such as organ fusion, leaf curling and severe defects in overall growth. Previously Bach *et al.* (2008)* reported that *PAS2* encodes 3-hydroxy-acyl-CoA dehydratase that is an essential and limiting enzyme in VLCFA synthesis. We found that *Arabidopsis* seedlings treated with an inhibitor of VLCFA synthesis displayed similar phenotypes to those of *pas2* mutants; i.e., activation of cell division in the shoot apical meristem (SAM) and hypocotyls. These phenotypes were not observed in plants with defects in cuticle formation or ceramide synthesis. Moreover, we revealed that VLCFA syntheses in the epidermis is prerequisite for controlling plant growth by limiting over-proliferation in the SAM and young leaf primordia. Taken together, we hypothesize that VLCFA synthesis in the epidermis constitutes a homeostatic system at the shoot apex and guarantees continuous organ formation in *Arabidopsis*.

*Bach *et al.* (2008) PNAS, 105, 14727-14731

08074

GORGON, A NOVEL MISSENSE MUTATION IN THE SHOOT MERISTEMLESS GENE, IMPAIRS SHOOT MERISTEM HOMEOSTASIS IN ARABIDOPSIS

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In higher plants, nearly all above ground organs are derived from the shoot meristem. We isolated the recessive *Arabidopsis* mutant *gorgon* (*gor*), which displays continuous enlargement of the shoot meristem during postembryonic development. We mapped the *gor* mutation and identified a missense mutation in *SHOOT MERISTEMLESS* (*STM*), a homeobox gene required for shoot meristem formation and maintenance. In contrast to *gor*, known loss-of-function mutants of *STM* display severe reduction of the meristem, suggesting that *gor* represents a novel *STM* allele whose effect on shoot meristem development is opposite to that of other loss-of-function alleles. Transformation of a wild-type genomic fragment of *STM* into the *gor* background completely rescued the *gor* phenotype whereas the same fragment but with the *gor* mutation recapitulated the mutant phenotype when introduced into a loss-of-function allele of *STM*. These results demonstrate that the missense mutation in *STM* is responsible for the *gor* mutant phenotype. We analyzed the relationship between the *gor* and the other regulators of the shoot meristem. Based on the results, the significance of the *gor* mutation in the regulation of shoot development will be discussed.

08075

THE ANAPHASE-PROMOTING COMPLEX (APC) AFFECTS HORMONE-REGULATED DEVELOPMENT IN ARABIDOPSIS

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Initially identified for its role in regulating mitotic progression, the evolutionarily conserved Anaphase-Promoting Complex (APC) E3 ubiquitin ligase serves to target specific proteins for degradation and is associated with multiple pathways affecting cell biology, including genomic stability and longevity. Composed of eight to 13 highly conserved proteins in eukaryotes little is known of the role of the APC, and its individual subunits, in plants. The first APC subunit to be examined crystallographically, *APC10* is believed to play a direct role in targeting substrates for ubiquitination processes. The present study, featuring a phenotypic screen of over-expressing and knock-down *APC10* transgenic Arabidopsis plants, supports a role for the APC in hormone regulation of plant development, including altered embryo, seedling, mature plant and flower morphology, histology and growth rate. Analysis of transcript levels of auxin and ethylene response genes indicates a coincident effect on pathways regulated by the SCF, another E3 ligase essential to cell processes. A mechanism for the role of APC in auxin regulation of plant development is proposed.

08076

FRUIT-LOOP MODULATES FRUIT DEVELOPMENT IN ARABIDOPSIS

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The Arabidopsis fruit is composed by stigma, style, ovary and gynophore. The ovary consists of two valves, and their margins separate them from the replum which is the outer part of the septum, a tissue that separates the ovary in two halves. Fruit patterning and development are regulated by a network of modulators which, with clockwork precision, guide the correct temporal and spatial expression of each player. Findings that reveal new nodes and functions of this complex network provide a deeper understanding about fruit development. One of these new nodes is represented by the mutant *fruit-loop* (*flop*) which promotes increased replum size. It also activates *BP/KNAT1*, a homeobox transcription factor that is also expressed in the replum and, together with PNY/RPL, defines it by repressing other non-replum genes.

08077MOLECULAR ANALYSIS OF THE *TWISTED* MUTANT

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The correct temporal and spatial coordination of the division and elongation of cells is important for plant growth. Helical growth pattern is the result of a continuously tilted growth axis as cells grow. Different factors regulate helical growth such as auxins, microtubules, and microtubule associated proteins. *Twisted* (*twt1-D*) is a gain-of-function mutant obtained by activation-tagging using the *En-1* transposon system (Marsch-Martinez et al., 2002). In this mutant all organs are twisted with a more severe phenotype in the siliques. Moreover, this mutant shows a reduction in leaf vein formation and an open reticulum pattern. Furthermore, a change in auxin distribution is suggested by a change in the DR5:GUS auxin response marker. The latest results will be presented.

08078

NOVEL FUNCTION OF NUCLEUS-ENCODING CHLOROPLAST PROTEIN IN THE NUCLEUS

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In plant life cycle, light is one of the most important factors. Light not only energy source for photosynthesis but also play pivotal roles as environment stimuli. Key players in light signal transduction include phytochromes, which act as photoreceptor.

Here we used a one-step co-IP method to isolate the interactomes that form during different light conditions with the phytochromeB in a phyB-overexpressing transgenic Arabidopsis line. One of the interaction partners is nucleus-encoded chloroplast protein. Though chloroplast protein, we can see the nucleus localization image by GFP fusion. We also test photoresponses in loss-of function mutant. Fluence-rate response curves for inhibition of hypocotyls growth revealed that loss-of-function mutant were hyposensitive.

Therefore, we suggest that chloroplast protein also have another function in nucleus.

08079

POLARITY CONTROL IN STAMEN DEVELOPMENT IN RICE

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Floral organs are considered to be modified leaves. The morphology of the stamen, however, differs apparently from that of the leaf. The stamen consists of a laterally symmetrical anther and a radially symmetrical filament. Regulatory mechanisms of these symmetrical patterns in the stamen remain unclear.

We found a novel rice mutant, named *rod-like lemma1* (*rol1*), in which adaxial-abaxial polarity in the floral organs was compromised. Using this mutant, we obtained a new insight into the relationships between the stamen patterning and adaxial-abaxial polarity in rice. In *rol1* mutant, defects in anther patterning were observed from an early developmental stage. Morphological analysis revealed that the polarity defect is closely associated with formation of a theca consisting of two pollen sacs and suggested that the theca was a developmental unit of the anther. Next, we investigated spatial expression patterns of the marker genes for the abaxial or adaxial identity. The results revealed that a marked change occurred in the expression patterns of both marker genes during anther development,

although complementary expression pattern of the two marker genes was maintained. After this change, the expression patterns of the two marker genes seemed to be associated with theca development. Based on these observations, we hypothesized that a polarity along the adaxial-abaxial axis may be rearranged in the anther primordium at the early stage of stamen development, and that after the rearrangement, subsequent anther development may proceed according to a new polarity formed in a theca. Morphological analyses of anther development in *rol1* mutant support this hypothesis. In contrast to the anther, the filament is likely to develop by abaxialization.

08080

AUXIN-INDUCIBLE LBD/ASL MEMBERS REGULATE LATERAL ROOT FORMATION

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Lateral root (LR) formation is important for the construction of root system in vascular plants. Auxin promotes LR formation, which is initiated from asymmetric cell divisions of the protoxylem pericycle in most dicot roots. In *Arabidopsis*, two Auxin Response Factors (ARF7 and ARF19) and several Aux/IAAs regulate LR formation through auxin-mediated gene expression. It has been shown that *Lateral Organ Boundaries domain 16/Asymmetric Leaves2-like 18 (LBD16/ASL18)* and *LBD29/ASL16* genes act for LR formation as direct targets of ARF7/19 (Okushima et al., 2007, *Plant Cell*). From detailed expression analysis using LBD16-GFP, we observed that LBD16/ASL18 was mainly expressed at LR initiation sites in three cell files of protoxylem pericycle and that the expression started before the first anticlinal cell divisions. In addition to *LBD16/29*, the other three auxin-inducible LBD/ASL members (*LBD17/ASL15*, *LBD18/ASL20* and *LBD33/ASL24*) were expressed at LR initiation sites but no obvious phenotypes were observed in each single knockout mutant of *LBD16* and other *LBD/ASLs*, strongly suggesting functional redundancy of these LBD/ASL members in LR formation. We will also present the phenotypes of the transgenic plants expressing LBD16-SRDX.

08081

THE SUBUNITS OF CHROMATIN ASSEMBLY FACTOR-1 ARE INVOLVED IN THE ESTABLISHMENT OF THE ADAXIAL-ABAXIAL POLARITY OF LEAVES ON THE AS2 AND AS1 BACKGROUNDS IN ARABIDOPSIS THALIANA

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Loss-of-function mutations in the ASYMMETRIC LEAVES1 (AS1) and AS2 genes affect leaf morphology in *Arabidopsis thaliana*. These mutants show similar phenotype, such as the formation of asymmetrically lobed and downwardly curled leaves. On certain mutant backgrounds, the as1 and as2 mutants show the defects in the adaxial-abaxial polarity. The AS1 gene encodes a protein containing two myb repeats. The AS2 gene encodes a plant-specific protein with a domain containing a cysteine repeat and a leucine-zipper-like sequence. Though the molecular structures of AS1 and AS2 are well characterized, these molecular functions are poorly understood. The single mutants that show the abaxialized and filamentous leaves on the as1 and as2 mutant backgrounds tend to exhibit the pointed and flattened leaves. In this study, we investigated whether the other mutants exhibiting the pointed and flattened leaves show the defects in the adaxial-abaxial polarity of leaves on the as1 and as2 backgrounds. We found that the mutations in genes for the subunits of Chromatin Assembly Factor-1 (CAF-1), FASCIATA1 (FAS1) and FAS2, affect on the leaf polarity synergistically with as1 and as2 mutations. Mutants of the FAS1 and FAS2 show the pointed and flattened leaves. Double mutants, as1 fas1, as1 fas2 and as2 fas2, exhibited the abaxIALIZED and filamentous leaves. These results suggest that the function of CAF-1 is required for the establishment of the adaxial-abaxial polarity of leaves. FAS1 and FAS2 are functional homologues of human CAF-1 p150 and p60 subunits. CAF-1 is a histone chaperone that facilitates chromatin formation and the maintenance of specific chromatin states and CAF-1 is required for DNA replication, DNA repair and homologous recombination. We will discuss the relationship between the function of CAF-1, AS1 and AS2 and the adaxial-abaxial polarity of leaves.

08082

SEED DORMANCY IN ARABIDOPSIS REQUIRES BINDING OF MULTIPLE ISOFORMS OF THE DOG1 PROTEINS

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DELAY OF GERMINATION 1 (DOG1) was identified as a major determinant of natural variation for seed dormancy between the accessions Ler and Cvi of *Arabidopsis thaliana*. The gene was cloned and encodes a protein of unknown function. *DOG1* is predominantly expressed in seeds. Mutant alleles of *DOG1* are completely non-dormant implicating that *DOG1* is absolutely required for the induction of seed dormancy.

DOG1 is alternatively spliced into five different transcripts, resulting in three protein isoforms. We have taken a transgenic complementation approach to check the functionality of these isoforms and found that none of the single isoforms driven by the native promoter was able to complement the non-dormant phenotype of the *dog1* mutant. However, transformants that can produce all three isoforms do complement, suggesting that multiple isoforms would be necessary for the *DOG1* function.

A yeast-two-hybrid assay revealed that all three forms of the *DOG1* protein from alternative splicing are able to bind to each other. Using a combination of truncated *DOG1* proteins and alanine-scanning, we identified a single alanine-substitution that significantly reduced binding-efficiency. We tested if the *DOG1* genomic fragment with this substitution was able to complement the *dog1* mutant, and found that transformants with the substitution-mutation are less dormant than the ones with the wild-type genomic fragment. All together, our data suggest that self-binding of multiple isoforms is necessary for the full function of *DOG1* protein in dormancy induction in *Arabidopsis*.

08083

CRITICAL AMINO ACID CHANGES CONVERT A FLORAL PROMOTER INTO REPRESSOR

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Phosphatidylethanolamine binding protein (PEBP) is a ubiquitous protein family showing distinctive functionality, among which, FLOWERING LOCUS T (FT) and TERMINAL FLOWER 1 (TFL1) are two representatives of the *Plantae*. Though sharing the same origin and structural topology, FT and TFL1 are opposite in function, in which FT promotes while TFL1 inhibits flowering. By means of exonic swapping, a BC surface encoded by two interim segments of their exon four sitting close to the previously identified anion binding pocket was found to be critical to their respective promoting and repressing ability, but the residues involved and their exact way of interaction still remain a mystery. To better understand the machinery underlying plant PEBPs and to figure out by how, the interplay of this antagonistic pair

can trigger the plant reproductive switch, as a pilot, we adopted random mutagenesis to introduce point mutation into the FT sequence. Then by overexpressing and assessing the functional retention of around 36000 mutated versions of FTs under the *Arabidopsis thaliana* background, here we report a successful identification of 20 sets of critical amino acid changes that exert a profound impact on the floral promoting ability of FT, among which, 13 sets of these overexpressors can reproducibly show the late flowering phenotype and growth indeterminacy, those are typical to that of TFL1, but not to FT. Further, as more than 80% of these mutations involved a significant while consistent change in side chain properties, linkage has implied and this maybe the first clue on showing by how, this orthologous pair of plant PEBPs be determined and functions as either a floral promoter or repressor, by which modulating such critical time of switch.

08084

CDR1 SINA E3 LIGASE REGULATES THE SPEED OF MITOTIC CELL CYCLE PROGRESSION

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All eukaryotic cells replicate their nuclear DNA in a conserved manner, whereby the parent molecule is unwound and each DNA strand becomes the template for nascent DNA synthesis. Here we show that in *in silico* analysis for genes coexpressed with DNA replication genes, *COEXPRESSED WITH DNA REPLICATION 1 (CDR1)* gene was identified as a novel cell cycle regulator. *CDR1* gene is highly coexpressed with DNA replication genes such as PCNAs, MCMs, DNA primase, and ETG1. Plants lacking the *CDR1* gene had large leaves due to increase of cell number. Mitotic cell cycle progression was significantly faster in *CDR1*-deficient leaf. Conversely, overexpression of this gene caused severe cell cycle defect, indicating that *CDR1* arrests mitotic cell cycle progression. *CDR1* encoded SINA E3 ligase protein that has been mainly associated with ubiquitin-dependent proteolysis. *CDR1* gene was strongly expressed in dividing tissues such as shoot apex. This expression was controlled by E2F transcription factors, as shown by upregulation in E2Fa/DPa overexpressing plants and the presence of E2F binding cis-element in the *CDR1* promoter. Interestingly, *CDR1* protein was primarily localized in the nucleus, while the protein was regulated posttranscriptionally through 26S proteasomal degradation. Based on mutant characterization and gene structure, we postulate that *CDR1* mediates mitotic cell cycle regulation through the degradation of cell cycle regulators.

08085

A ROLE FOR LORELEI, A PUTATIVE GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN, IN ARABIDOPSIS THALIANA DOUBLE FERTILIZATION AND EARLY SEED DEVELOPMENT

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In plants, double fertilization requires successful sperm cell delivery in the female gametophyte followed by migration, recognition and fusion of the two sperm cells with two female gametes. We isolated a null allele (*Ire-5*) of *LORELEI* (*At4g26466*), which encodes a putative glycosylphosphatidylinositol (GPI)-anchored protein implicated in pollen tube reception by the female gametophyte. Although most *Ire-5* female gametophytes do not allow pollen tube reception, in those that do, early seed development is delayed. A fraction of *Ire-5*/*Ire-5* seeds underwent abortion due to female gametophyte defect(s). The aborted seeds contained endosperm but no zygote/embryo, reminiscent of autonomous endosperm development in the pollen tube reception mutants *scylla* and *sirene*. However, unpollinated *Ire-5*/*Ire-5* ovules did not initiate autonomous endosperm development and endosperm development in aborted seeds began after central cell fertilization. Thus, the egg cell likely remained unfertilized in aborted *Ire-5*/*Ire-5* seeds. The *Ire-5*/*Ire-5* ovules that remain undeveloped due to defective pollen tube reception did not induce synergid degeneration and repulsion of supernumerary pollen tubes. In ovules, *LORELEI* is expressed during pollen tube reception, double fertilization and early seed development. Arabidopsis genome contains three *LORELEI-Like-GPI-anchored protein (LLG)* genes (*LLG1-At5g56170*, *LLG2-At2g20700* and *LLG3-At4g28280*). Null mutants of *LLG1*, the closest relative of *LORELEI* among three Arabidopsis *LLG* genes are fully fertile and did not enhance reproductive defects in *Ire-5*/*Ire-5* pistils suggesting that *LLG1* function is not redundant with that of *LORELEI* in the female gametophyte. Our results show that, besides pollen tube reception, *LORELEI* also functions during double fertilization and early seed development.

08086

A PRO-RICH EXTENSIN-LIKE RECEPTOR KINASE REGULATES ARABIDOPSIS ROOT HAIR GROWTH

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Root hairs are tip-growing tubes that emerge from the root trichoblast cell. During the past decade, Arabidopsis root hairs have been a model system for studying the mechanisms of cellular morphogenesis. In plants there are more than 1000 protein kinases and hundreds of receptor-like protein kinases (RLKs) which are involved in diverse development processes. However, few RLKs have been implicated in root hair morphogenesis. ROOT HAIR SPECIFIC10 (RHS10) is a root hair cell-specific RLK with Pro-rich extensin-like regions. Loss of RHS10 (*rhs10*) grew longer hairs than did wild type and its overexpression (RHS10ox) greatly inhibited hair growth. The defect in root hair growth of RHS10ox transformants could not be rescued by exogenous auxin, ACC (the ethylene precursor), or separation of the root from the medium. To localize the RHS10 gene in the genetic pathway for root hair morphogenesis, genetic crossings between *rhs10* (and RHS10ox line) and other known root hair-morphogenetic mutants were carried out. A GFP fusion of RHS10 localizes mainly to the plasma membrane in the root hair cell. These results suggest that the RHS10 kinase may function as an RLK to relay certain signaling cue from the cell wall so as to modulate the root hair growth rate.

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08087

POSSIBLE REGULATION OF ORGAN ELONGATION AND CHLOROPHYLL ACCUMULATION BY CIRCADIAN CLOCK PROTEINS LHY AND CCA1 IN ARABIDOPSIS

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Circadian clock in higher plants regulates various phenomena. Two related myb proteins, LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and several other proteins are key regulators of the clock-controll processes in Arabidopsis. Under constant light (LL), *lhy;cca1* which exhibited severe defects in clock function, shows late flowering, semi-dwarf and dark green leaf

phenotypes. Mutations in genes encoding a MADS-box protein SHORT VEGETATIVE PHASE (SVP) or a clock protein EARLY FLOWERING 3 (ELF3) partially suppressed the late flowering phenotype of *lhy;cca1* under LL. However, molecular mechanisms underlying the semi-dwarf or dark green leaf phenotypes of *lhy;cca1* under LL have not been elucidated.

To reveal the regulation of organ elongation by circadian clock, genetic screening of enhancers with shorter hypocotyls and petioles than those of *lhy;cca1* was performed. 8 enhancers were further characterized and named *petanko1-8* (*pta1-8*). Genetic mapping showed that *pta1*, *pta5* and *pta8* were novel mutant alleles of brassinosteroid (BR) biosynthesis genes *ROTUNDIFOLIA 3* (*ROT3*), *DWARF 4* (*DWF4*) and *DWARF 1* (*DWF1*), respectively. Interestingly all of these mutations enhanced not only the semi-dwarf but also late flowering phenotypes of *lhy;cca1* under LL, suggesting that BR signaling may play key roles in the phenotypes of *lhy;cca1* under LL.

Recently, we have found that both chlorophyll content and a/b ratio in *lhy;cca1* varied with photoperiodic conditions, whereas both of them were constant in wild type. To understand the molecular mechanism underlying the clock-controll regulation of chlorophyll, genetic analysis were performed and several mutations that suppressed *lhy;cca1* phenotypes were identified. A hypothetical models explaining the regulation of LHY and CCA1 will be discussed.

08088

EXPRESSION ANALYSIS OF THE DROOPING LEAF (*DL*) GENE THAT PROMOTES MIDRIB FORMATION IN RICE AND ITS APPLICATION TO IMPROVEMENT OF PLANT ARCHITECTURE.

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The *DROOPING LEAF* (*DL*) gene regulates midrib formation in the leaf and carpel specification in the flower in rice (*Oryza sativa*). Midrib is a strong structure formed in the central region of the leaf. Loss-of-function mutant of *DL* fail to form midribs. In addition, severe mutations in *DL* cause homeotic transformation of carpels into stamens in the flower. Thus, *DL* has critical functions in rice development. Consistent with its mutant phenotype, *DL* is expressed in the central region of the leaf primordia, where midrib should be formed, and in the carpel primordia in flower development.

The *dl* mutants that lack the midrib show drooping leaf phenotype, suggesting that the midrib is responsible for the erectness of the leaf. Leaf erectness is one of the agronomically important traits in rice, because this characteristic is associated with an increase in light capture for photosynthesis and dense planting.

Here, we focus on the regulatory mechanism of *DL* expression and correlation between *DL* function and midrib formation. First, we found five conserved non-coding sequences (CNSs) in 5' upstream region and two introns (intron 1 and 2) of the *DL* orthologs by phylogenetic footprinting analysis. Second, we performed GUS reporter analysis in rice by referring to the five CNSs, to identify cis-regulatory regions for *DL* expression. As a result, we found several cis-regulatory regions that required for precise expression of *DL* in the leaf. Based on the results of expression analysis, we expressed precisely an active version of *DL* protein (DL-VP16). The transgenic rice carrying DL-VP16 produced more erect leaves, the midrib of which was larger than that of wild-type leaves. This result is consistent with our hypothesis that the size of midrib depends on the function of *DL* and raises a possibility that manipulation of *DL* function can improve rice plant architecture for agronomical use.

08089

EMF1-INTERACTING PROTEINS THAT MAINTAIN ARABIDOPSIS VEGETATIVE DEVELOPMENT

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EMBRYONIC FLOWER (*EMF*) 1 gene is necessary for the maintenance of vegetative development via epigenetic repression of flower homeotic genes. To investigate the molecular mechanism of *EMF1*-mediated vegetative development, we identified the proteins that physically interacted with *EMF1* in yeast cells, *in vitro* and in plant cells. The function of three *EMF1*-Interacting Proteins (*EMF1IPs*), *EMF1IP1*, 6 and 9, predicted to be a WNK kinase, a B-box zinc-finger protein and a DnaJ-domain protein, respectively, were investigated. The heterodimers between *EMF1* and *EMF1IP1*, 6 or 9 were localized in the nucleus. Interestingly, *EMF1* interacted with *EMF2* via *EMF1IP9* in the nucleus, forming an *EMF1*-*EMF1IP9*-*EMF2* protein complex. Expression patterns of *EMF1IP1*, 6 and 9 were similar to that of *EMF1*. T-DNA insertion mutants of *EMF1IP1* and *EMF1IP9* flowered early. Like early-flowering plants with impaired *EMF1* activity, these mutants expressed floral organ identity genes, i.e., *PISTILLATA* and *AGAMOUS*, before flowering. Taken together, our results suggest *EMF1IP1* and *EMF1IP9* acting in partnership with *EMF1* to maintain vegetative development in Arabidopsis.

08090

FUNCTIONAL ANALYSIS OF *FON1*-LIKE GENES IN RICE DEVELOPMENT

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The proper differentiation and maintenance of stem cells are critical for the organ formation at shoot and flower meristems in plants. In *Arabidopsis thaliana*, CLAVATA (CLV) signaling, including CLV1, which encodes a leucine-rich repeat receptor like kinase, a small-peptide CLV3 and the homeodomain-containing transcription factor WUSCHEL (WUS) plays a central roles in these processes. We revealed that *FLORAL ORGAN NUMBER1* (*FON1*) and *FON2* closely related to *Arabidopsis CLV1* and *CLV3* regulate the maintenance of the reproductive meristems in rice, a model monocot (Suzuki et al., 2004, 2006). Unlike the *clv* mutants in *Arabidopsis*, however, no abnormalities were observed in the vegetative phase even in the severe *fon1* and *fon2* mutants (Nagasawa et al., 1996; Suzuki et al., 2004, 2006). These results suggest that a regulatory mechanism redundant to *FON1*-*FON2* pathway is present to maintain the vegetative meristem in rice.

In this study, we focused on the genes encoding LRR receptor kinase, closely related to *FON1*. First, we identified five *FON1*-like genes (*FOL1*, 2, 3, 4, and 5) by a BLAST search using the *FON1* sequence as a query. We then analyzed the phylogenetic relationship of *FON1*-like proteins among several species. As a result, phylogenetic tree indicates *FOL1*, 2 and 3 are classified into the BAM1 (for BARELY ANY MERISTEM) and BAM2 clade, while *FOL4* and 5 are classified into the BAM3 clade. We also undertook some experiments to characterize the roles of these genes. From spatial expression analysis, it is suggested that these genes seem to have functionally diversified.

08091

PHENOTYPIC ANALYSES OF DOUBLE KNOCKOUT MUTANT FOR *ATL31* AND *ATL6*, WHICH ENCODE C/N REGULATORY UBIQUITIN LIGASE IN *ARABIDOPSIS THALIANA*

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In higher plants, metabolisms of sugar (C) and nitrogen (N) are mutually regulated. To clarify the C/N regulator, we identified novel *Arabidopsis* C/N regulatory gene *ATL31*. ATL family encodes RING-type ubiquitin ligase and consists of 80 members in *Arabidopsis*. Among them, ATL6 is the most similar to the ATL31 at the amino acid level. C/N stress assay demonstrated that plants overexpressing the *ATL31* and *ATL6* are insensitive to C/N stress conditions, whereas the single knockout mutants are hypersensitive. To investigate the functions of both genes, we generated double knockout mutant *atl31 atl6*. The double mutant showed the transient chlorosis of true leaves and promotion of primary root elongation. Here we report to characterize the *atl31 atl6* mutant by phenotypic analyses. How both genes contribute to C/N response in plants will be discussed.

08092

STUDIES ON ARABIDOPSIS UBIQUITIN LIGASE ATL31 AND THE INTERACTING PROTEINS

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Plants sense balance of carbon (C) and nitrogen (N) metabolites to regulate metabolism and development, which is called C/N response. However, mechanism of the C/N response remains unclear. We identified the novel C/N response regulator ATL31, an ubiquitin ligase, and demonstrated that the ubiquitin ligase activity is essential for the C/N response. Ubiquitin ligases recognize specific proteins to be degraded by the ubiquitin-proteasome system. To identify target proteins of the ATL31, we tried to isolate the interacting proteins by combination of immunoprecipitation and MS analysis. As a result, candidates for the interacting protein were identified. We are now investigating the detailed interactions between the ATL31 and the interacting proteins by biochemical analyses. Here, we will report these results.

08093

WEREWOLF AND CAPRICE DETERMINE THE CELL FATE BY COMPETING WITH EACH OTHER IN THE ARABIDOPSIS ROOT EPIDERMIS

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WEREWOLF (WER), a MYB-type transcription factor is known to play a pivotal role in specifying the fates of root epidermal cells of *Arabidopsis* based on the previous molecular genetic studies. However, it is difficult to fully understand how WER functions in this genetic pathway because the over-expression of WER under the control of cauliflower mosaic virus 35S (CaMV 35S) promoter failed to induce any discernible phenotype in the wild-type background. In this study, we have identified several enhancer trap promoters inducing distinctive hairless phenotypes by using the GAL4-pUAS:WER trans-activation system. We show that the ectopic expression of WER, exhibiting a strong hairless phenotype, positively regulates the expression of *GLABLA2* (*GL2*) and *AtMYB23*, genes specifying the non-hair cell fate, as well as the expressing *CAPRICE* (*CPC*), a gene specifying hair cell fate. WER directly enhances the expression of *GL2* by the interaction with specific cis-elements located in the *GL2* promoter. WER negatively regulates the expression of *ENHANCER OF GLABLA3* (*EGL3*) and *AtMYC1*. We also have found that WER requires GL3 and EGL3, bHLH proteins for its proper function while it does not require *TRANSPARENT TESTA GLABLA1*, a WD40 protein. Furthermore, we show that the ectopically expressed CPC by a GAL4-UAS system, exhibiting strong hairy phenotype, suppresses the phenotype induced by WER whereas *p35S:CPC* does not. Both WER and CPC interact with GL3 and EGL3 proteins in vivo. These results suggest that WER and CPC determine the cell fate in a dosage sensitive manner by competing with each other in the regulation of *GL2* expression in *Arabidopsis* roots.

08094

RLF, A CYTOCHROME B_5 -LIKE HEME/STEROID BINDING DOMAIN PROTEIN, CONTROLS LATERAL ROOT FORMATION INDEPENDENTLY OF ARF7/19-MEDIATED AUXIN SIGNALING IN ARABIDOPSIS THALIANA

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Lateral root (LR) formation is important for the establishment of root architecture in higher plants. Recent studies have revealed that LR formation is regulated by an auxin signaling pathway that depends on auxin response factors ARF7 and ARF19, and auxin/indole-3-acetic acid (Aux/IAA) proteins including SOLITARY-ROOT (SLR)/IAA14. To understand the molecular mechanisms of LR formation, we isolated a recessive mutant *rlf* (*reduced lateral root formation*) in *Arabidopsis thaliana*. The *rlf-1* mutant showed reduction of not only emerged LRs but also LR primordia. Analyses using cell-cycle markers indicated that the *rlf-1* mutation inhibits the first pericycle cell divisions involved in LR initiation. The *rlf-1* mutation did not affect auxin-induced root growth inhibition but did affect LR formation over a wide range of auxin concentrations. However, the *rlf-1* mutation had almost no effect on auxin-inducible expression of *LATERAL ORGAN BOUNDARIES-DOMAIN16/ASYMMETRIC LEAVES2-LIKE18* (*LBD16/ASL18*) and *LBD29/ASL16* genes, which are downstream targets of ARF7/19 for LR formation. These results indicate that ARF7/19-mediated auxin signaling is not blocked by the *rlf-1* mutation. We found that the RLF gene encodes At5g09680, a protein with a cytochrome B_5 -like heme/steroid binding domain. RLF is ubiquitously expressed in almost all organs, and the protein localizes in the cytosol. These results, together with analysis of the genetic interaction between the *rlf-1* and *arf7/19* mutations, indicate that RLF is a cytosolic protein that positively controls the early cell divisions involved in LR initiation, independent of ARF7/19-mediated auxin signaling.

08095

POLAR-LOCALIZED MAB4 SUBFAMILY PROTEINS RETAIN PIN PROTEINS IN THE PLASMA MEMBRANE

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The local, asymmetric distribution of the phytohormon auxin controls many developmental processes. This auxin distribution is established by polar auxin transport that is dependent on polar-localized auxin efflux carriers, PIN-FORMED (PIN) proteins. Recently, a NONPHOTOTROPHIC HYPOCOTYL 3 (NPH3)-like protein, MACCHI-BOU 4/ENHANCER OF PINOID (MAB4/ENP), has been reported to regulate

polar auxin transport through the control of subcellular localization of PIN1 proteins in cotyledon development. *MAB4* has four paralogues, named *MAB4/ENP LIKE (MEL) 1, MEL2, MEL3 and MEL4*. *mel1 mel2 mel3 mel4* quadruple mutants exhibit abnormal gravitropism like *pin2* mutants, although each single mutant does not exhibit any phenotypes in root gravitropism. In root tips of quadruple mutants, PIN abundance was severely reduced and PIN2 polarization was weakened. To investigate the mechanism by which the *MAB4* subfamily genes regulate PIN2 localization, we used pharmacological approaches in PIN2-GFP expressing mutants. When treated with BFA or wortmannin, PIN2 protein accumulated in induced intracellular compartments of *mel1 mel2 mel3 mel4* roots, as well as wild-type roots. Furthermore, when BFA was washed out, the BFA compartments were disappeared and PIN2-GFP was normally reverted to the plasma membrane in the mutants. These results suggest that the *MAB4* subfamily genes are not involved in intracellular trafficking of PIN2 proteins. Meanwhile, the *MAB4* subfamily proteins are localized at the cell periphery with polarity in plant cells. The *MAB4* polarity is almost identical to PIN polarity. However, peripheral *MAB4* localization did not change by treatment with BFA, indicating that the *MAB4* subfamily proteins do not exist in intracellular compartment and that their functional domain is limited to cell periphery. Taken together, it is suggested that the *MAB4* subfamily proteins would retain PIN proteins with polarity in the plasma membrane.

08096

AMINO ACID SEQUENCES THAT ARE REQUIRED FOR LOCALIZATION OF ASYMMETRIC LEAVES2 (AS2) TO THE SUB-NUCLEOLAR BODY ADJACENT TO THE NUCLEOLUS

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ASYMMETRIC LEAVES2 (AS2) gene is one of key regulators of morphogenesis of leaves in *Arabidopsis thaliana*. It encodes a plant-specific nuclear protein with a AS2/LOB domain that consists of the C motif containing four cysteine residues, the single conserved glycine residue and the leucine-zipper-like sequence in its amino-terminal half. The AS2/LOB domain includes a short stretch of basic residues (RRK) in the C-motif. The AS2 protein localizes to a sub-nuclear body that is adjacent to nucleoli and we designated this body as the AS2 body. In order to identify the signal in AS2 that is required for the localization of AS2 to the AS2 body, we have made DNA constructs that encoded mutant AS2 proteins with various deletions and amino acid substitutions in the AS2/LOB domain such as the cysteine residues in the C motif, the basic stretch RRK and the deletion of the leucine zipper like sequence. We have investigated sub-nuclear localization of these mutant proteins. We also have examined whether the localization to the AS2 body might be required for the function of AS2 in morphogenesis of leaves. We will present latest results of these experiments.

08097

IDENTIFICATION OF NOVEL GENES INVOLVED IN FT FUNCTION OR TRANSPORT

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Flowering of *Arabidopsis* is influenced by seasonal changes in day length, so that plants flower early in long days of summer and late under short days of winter. In *Arabidopsis*, *FLOWERING LOCUS T (FT)* is a component of the photoperiodic pathway and its transcription is activated under long days. FT protein is expressed in the leaf vascular tissue and then moves through the phloem to the shoot apex, where it causes changes in gene expression that reprogram the shoot apical meristem to form flowers instead of leaves. Recent studies demonstrated that the *FT* homolog *TSF* also participates in the photoperiodic flowering pathway redundantly with *FT*. To deepen our knowledge of the function and transport of FT, we performed different genetic and genomic approaches. First, we have generated a mutant collection from the *ft tsf* double mutant, which is insensitive to day length and very late flowering under long days, and from *GAS1:FT ft tsf* plants which are early flowering. We have already identified many early and late flowering mutants in both genetic backgrounds. The genes affected in the most promising mutants will be identified using Illumina next generation sequencing. Second, we investigated the global effects of FT on gene expression in leaves by studying which genes are specifically affected by FT and/or TSF by comparing Col-0 with *ft tsf* and *GAS1:FT tsf ft* genotypes. Our Tiling Array experiment showed that several differentially expressed genes affected by FT in leaves encode proteins which potentially can modify cellular membranes. Further studies are being performed in order to test the contribution of these candidate genes to FT function or transport.

08098

EARLY IN SHORT DAYS 6 (ESD6), A FLORAL REPRESSOR IN ARABIDOPSIS, ENCODES A RING MOTIF-CONTAINING E3 UBIQUITIN LIGASE

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Flowering time control must be tightly regulated as it is essential for reproductive success in plants. Both promotive and repressive factors are involved in the control of the floral transition. We have isolated a recessive mutation, named *early in short days 6 (esd6)*, causing an acceleration of flowering. Besides their flowering phenotype, *esd6* mutant plants also displayed complex pleiotropic alterations of both vegetative and reproductive development. Mutant plants are smaller than wild type and *esd6* leaves showed slightly reduced size in comparison to wild type leaves. Moreover, *esd6* primary root showed a significant decrease on elongation with lower production of adventitious roots. *esd6* flowers and siliques also displayed some developmental abnormalities including a reduction in size in comparison to wild type plants.

Genetic analyses performed by combining *esd6* and mutants affected in flowering inductive pathways suggest that flowering inhibition mediated by ESD6 occurs through both *FLC*-dependent and independent pathways. *ESD6* has been cloned by a map-based approach and encodes HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1), an E3 ubiquitin ligase previously demonstrated to negatively regulate the low-temperature responses. Progress in understanding the role of *ESD6 / HOS1* in the repression of floral initiation will be presented.

08099

MAPK CASCADE REGULATES THE HOMEOSTASIS OF STEM CELL POPULATION IN ARABIDOPSIS SHOOT APICAL MERISTEM

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Arabidopsis shoot stem cells generate all aerial parts of plants throughout the whole lifespan. The constant population size of the shoot

stem cells is tightly controlled by a negative feedback mechanism between CLV1 and CLV2-CRN/SOL2 receptors and the homeodomain WUS transcription factor expressed in organizing center. Since *CLV1*, *CLV2* and *WUS* have been identified through the genetic dissection, MAPK cascade has been proposed based on the precedents in other systems. Here we present that the synthetic CLV3 peptide activates MAPK cascade in the shoot apical meristem (SAM), which immediately represses *WUS* expression. The loss-of-function study of *MAPK Kinase (MKK) 7, 9 and 10* displays the enlarged shoot meristem and the increased floral organ number, whereas constitutive overexpression of *MKK7, 9 and 10* causes the abnormal growth in the shoot apex. Remarkably, MKK10 is able to interact with MKK7 or MKK9, and its expression domain is localized in the central zone of the SAM. Our data suggest that MAPK cascade acts as a signaling network in CLV-WUS pathway, dynamically regulating the balance of the stem cell population.

08100

THE TWO HOMOLOGOUS BAH-PHD-CONTAINING PROTEINS SHL AND EBS ARE INVOLVED IN CHROMATIN-MEDIATED REPRESSION OF FLOWERING IN ARABIDOPSIS

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Chromatin remodelling plays a crucial role in the establishment and maintenance of gene expression patterns that control plant development. We are studying the role of two *Arabidopsis* homologs, *EARLY BOLTING IN SHORT DAYS (EBS)* and *SHORT LIFE (SHL)*, in the chromatin-mediated repression of flowering. Both loci encode plant specific nuclear proteins with identical modular architecture characterised by the presence of two domains (BAH and PHD) frequently found in transcriptional regulators involved in chromatin remodelling processes. We have shown that *EBS* is required to repress the floral integrator *FT*. As for *EBS*, *SHL* shows a ubiquitous pattern of expression and is necessary to delay flowering, mainly under non-inductive conditions. However, genetic and molecular analyses indicate that *SHL* has partially redundant but also independent roles from *EBS* in the control of flowering, and in contrast to *EBS*, *SHL* appears to be required for *SOC1* but not for *FT* repression. *EBS* and *SHL* proteins can bind in vitro histone H3 peptides that carry lysine 4 di or trimethylation; also, both proteins bind H3K4me3 in pull down assays with *Arabidopsis* histone extracts. Site-directed mutagenesis of conserved residues within the PHD domain abolish the binding of both proteins to these modified histones, suggesting that the PHD motifs in *EBS* and *SHL* are responsible for the recognition of this histone modification. Moreover, *ebs* and *shl* mutations cause increased levels of histone acetylation in the chromatin of their target genes, indicating that these two BAH-PHD-containing proteins are required to maintain an inactive chromatin conformation in the loci regulated by them. Altogether, these observations suggest that *EBS* and *SHL* participate in the modulation of the expression of the floral integrators *FT* and *SOC1* through a mechanism involving chromatin remodelling.

08101

KOMPEITO REGULATES CALLOSE AND CALLOSE SYNTHASE ACCUMULATION AND IS REQUIRED FOR POLLEN WALL PATTERNING IN ARABIDOPSIS

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Fertilization is a key event for sexually reproductive plants. Pollen-stigma adhesion, the first step of male-female interaction during fertilization, requires proper pollen wall patterning. Callose, a β -1,3-glucan synthesized by Callose Synthase (CalS), is an essential polysaccharide required for pollen development and pollen wall. It is not clear how CalS activity and callose synthesis are regulated. Here we report the isolation of *kompeito-1 (kom-1)* mutant defective in pollen wall patterning and pollen-stigma adhesion in *Arabidopsis thaliana*. Callose was not accumulated in meiocytes and microspores in *kom-1*, which is very similar to that of the male sterile *callose synthase 5 (cals5)* mutant. *KOM* gene encodes a member of a recently-identified subclass of rhomboid proteins that lack active site residues. *KOM* localizes to the Golgi apparatus in plants, and both *KOM* and *Cals5* genes were highly expressed in meiocytes. A 220 kDa *Cals5* product, detected in wild type floral buds using anti-*Cals5* antibodies, was greatly reduced in *kom-1* and absent in *cals5*. These results suggest that *KOM* is required for the accumulation of *Cals5* and may regulate meiocyte-specific callose synthesis in *Arabidopsis*.

08102

LSH4 AND *LSH3*, TWO MEMBERS OF THE ALOG GENE FAMILY IN *ARABIDOPSIS THALIANA*, ARE ACTIVATED IN SHOOT ORGAN BOUNDARY CELLS BY THE TRANSCRIPTION FACTOR CUP-SHAPED COTYLEDON1

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In higher plants, shoot organs are initiated on the flank of the shoot apical meristem. During this process, boundary cells are specified between organ primordia as well as between primordia and the shoot meristem, separating the cellular fates of them. In *Arabidopsis thaliana*, the *CUP-SHAPED COTYLEDON* genes *CUC1*, *CUC2* and *CUC3*, which encode transcription factors of the NAC family, play central roles in boundary cell specification. These genes are expressed in boundary cells of various shoot organs, and mutations in these genes cause defects in boundary functions, resulting in fusions of adjacent shoot organs and/or lack of shoot meristem initiation. Here we identified *LIGHT-DEPENDENT SHORT HYPOCOTYL 4 (LSH4)* and *LSH3* as putative transcriptional targets of *CUC1*. These genes are members of the ALOG (*Arabidopsis LSH1* and *Oryza G1*) family, which is conserved in land plants but not in animals or fungi. During embryogenesis, *LSH4* and *LSH3* are expressed in the boundary cells between cotyledon primordia, showing a similar pattern with that of *CUC* genes, and their expression is dependent on *CUC1* and *CUC2*. Moreover, induction of *CUC1* activity by using a glucocorticoid-receptor system results in transcriptional activation of the *LSH* genes even in the presence of a protein synthesis inhibitor. The *LSH4* protein fused with GFP is localized to nuclei of the boundary cells when expressed under of its own promoter. Ectopic expression of *LSH4* and *LSH3* causes developmental defects including formation of extra flowers, extra floral organs or chimeric floral organs within a flower. Our data suggest that spatial regulation of *LSH4* and *LSH3* expression is required for proper morphogenesis, and *CUC1* and *CUC2* are partly responsible for this regulation.

08103

CONTROL OF ENDOREDUPLICATION OF TRICHOME BY RPT2A, A SUBUNIT OF THE 19S PROTEASOME IN ARABIDOPSIS

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The ubiquitin/26S proteasome pathway plays a central role in the degradation of short-lived regulatory proteins to control many cellular events. The *Arabidopsis* knockout mutant *rpt2a*, which contains a defect in the AtRPT2a subunit of the 26S proteasome regulatory particle, showed enlarged leaves caused by increased cell size that correlated with increased ploidy caused by extended endoreduplication. To clarify the role of RPT2a in endoreduplication control, trichome development was genetically examined in further detail. RHL1 and GL3 encode proteins that have a role in the positive regulation of endocycle progression in trichomes. The *rhl1* mutants are stalled at 8C and have trichomes with only a single branch. The *rpt2a* mutation did not alter the *rhl1* mutant phenotype, and trichomes of double *rpt2a rhl1* mutants resembled that of single *rhl1* mutants. On the other hand, the *rpt2a* mutation suppressed the *gl3* phenotype (stalled at 16C, two trichome branches), and trichomes of the double *rpt2a gl3* mutant resembled those of the wild type (WT) plants. Together, these data suggest that RPT2a functions to negatively regulate endocycle progression following completion of the third endoreduplication step mediated by RHL1 (8C-16C).

08104

ATML1 AND PDF2 ARE ESSENTIAL IN ARABIDOPSIS EMBRYO DEVELOPMENT.

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The epidermis of shoot organs in angiosperms develops from the outermost layer of the shoot apical meristem. In *Arabidopsis thaliana*, a pair of homeobox genes, *MERISTEM LAYER1* (*ATML1*) and *PROTODERMAL FACTOR2* (*PDF2*), play a role in regulating the expression of L1-specific genes. *atml1-1 pdf2-1* double mutants show striking defects in the differentiation of shoot epidermal cells. However, because *atml1-1* and *pdf2-1* have a T-DNA inserted downstream of the respective homeobox sequences, these alleles may not represent null mutations. *ATML1* has two start sites of transcription. We confirmed that *atml1-1 pdf2-1* mutants contain *atml1-1* transcripts from these two start sites and *pdf2-1* transcripts. The *atml1-2* allele has a T-DNA inserted within the homeobox sequence and shows normal growth and morphology. The cross between *atml1-2* and *pdf2-1* generated no seedlings of the double mutant. Ovaries of plants that are homozygous for one and heterozygous for the other mutant allele contained arrested embryos, which exhibited aberrations in the cell division plane in the protoderm. The seedlings homozygous for *atml1-2* and heterozygous for *pdf2-1* also showed abnormal development of cotyledons frequently, suggestive of a dominant negative effect of the *pdf2-1* allele. These results indicate that *ATML1* and *PDF2* act redundantly in the maintenance of shoot epidermal cells and at least one copy of these genes is essential for survival of the embryo.

08105

SYSTEMIC AND LOCAL ROOT DEVELOPMENT TO LOCALIZED PUTRESCINE SUPPLY IS DEPENDENT ON HIGH-AFFINITY UREA TRANSPORTER

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Although urea is the most used nitrogen (N) fertilizer worldwide, the mechanism of urea uptake from the soil solution into roots is not well understood. Previous analyses showed that the *Arabidopsis* gene AtDUR3 encodes a proton/urea co-transporter that is up-regulated in N-deficient roots (Liu et al., 2003). Its functional role in planta, however, has not yet been determined. We have shown that promoter activity of AtDUR3 increased in epidermal cell layer in response to nitrogen deficiency, and AtDUR3 protein was accumulated at plasma membrane of root hair. Short-term isotope labeled urea uptake was compared between wild-type and T-DNA insertion lines for AtDUR3, we found that high-affinity urea transport was almost lost in mutants. This result suggests that major contribution of AtDUR3 on high-affinity urea transport (Kojima et al., 2007). AtDUR3 could be found not only plants but also in fungus. ScDUR3, budding yeast urea transporter, was shown to transport not only urea but also some polyamines. Polyamines are known to modulate several biological processes, for example, cell division, fruit ripening, flowering and development in plant. Uptake studies with isotope-labeled polyamines in different plant species showed that polyamine transport into the plant root is not simple diffusion but a carrier-mediated process. However, the molecule responsible for polyamine transport is still elusive. Here, we have investigated the possibility of polyamine transport by AtDUR3 and the effect of polyamine application on development of root system. We found that polyamine supply to whole plant inhibited primary root elongation, and localized putrescine application to lateral roots on segmented agar plates decreased the size of the non-treated root system and increased tertiary root development, suggesting a combined, systemic and local, response to putrescine supply. A reverse genetic approach revealed that AtDUR3 is partially responsible for this response.

08106

CALMODULIN DIRECTLY BINDS TO ASYMMETRIC LEAVES1 (AS1) AND INHIBITS ITS FUNCTION IN ARABIDOPSIS

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Calmodulin (CaM), ubiquitous calcium-binding protein, regulates diverse cellular functions by modulating the activities of a variety of enzymes and proteins. In this study, we isolated a CaM binding transcription factor, AS1 (Asymmetric Leaves1), by using HRP-conjugated CaM probe from *Arabidopsis* cDNA expression library. AS1 is well known as a Myb-related protein that is required for leaf development in *Arabidopsis*. Two Ca²⁺-dependent CaM binding domains (CaMBDs) in N terminus of AS1 protein was identified by gel overlay assays. In vivo CaM binding ability was confirmed by yeast split-ubiquitin assay.

CaM overlay assays using mutated CaMBDs showed that three amino acids, Trp⁴⁹ in CaMBDI and Trp⁸²and Phe¹⁰⁴ in CaMBDII, play a pivotal role in CaM binding. We also showed that CaM directly inhibited the binding of AS1-AS2 complex to the two specific cis-regulatory motifs in the KNAT1 promoter by electrophoretic mobility shift assays (EMSA).

These results suggest that Ca²⁺/CaM may be involved in leaf development via the direct interaction with AS1.

08107

IDENTIFICATION OF FACTORS THAT REGULATE MERISTEMATIC ACTIVITY IN ARABIDOPSIS THALIANA

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Plant architecture is greatly determined by the activity of the shoot apical meristem (SAM). In a tightly regulated process, part of the SAM is maintained as undifferentiated cells and can self-renew while other cells differentiate to become lateral organs. The *SHOOT MERISTEMLESS* (*STM*) gene from the model plant *Arabidopsis thaliana* has a key role in the control of the activity of the SAM. Despite its described function in this process, little is known about the transcriptional regulation of *STM*. To better characterize the regulation of *STM*, we are analyzing the *STM* gene promoter. We previously showed a well conserved region between species in the 5' regulatory region of *STM*, named the K-box, which is necessary for the permanent *STM* downregulation in late stages of leaf development in simple leafed species but not for the downregulation of *STM* during leaf initiation. We are characterizing another well conserved region, the RB-box. To assess the role of this region in *Arabidopsis* we have generated promoter-deletion fusions of the *STM* promoter. We have also generated several combinations deleting the K and RB boxes. These regions appear to act as repressor elements of the *STM* transcription. To look for cis-activator elements of the transcription of *STM*, we have made internal deletion-promoter analysis and found a region, F3, required for the activation of the expression of *STM* in the shoot apical meristem. We are performing yeast one-hybrid assay using the K-box, RB-box and F3 elements to determine the factors that bind to these regions. The role of these factors in the control of shoot apical meristem function will be discussed.

Uchida N, Townsley B, Chung K-H, and Sinha N (2007) Regulation of SHOOT MERISTEMLESS genes via an upstream-conserved noncoding sequence coordinates leaf development PNAS 104:15953-15958

08108

IDENTIFICATION OF THERMOSPERMINE-RESPONSIVE GENES IN ARABIDOPSIS THALIANA

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The *acaulis5* (*acl5*) mutant of *Arabidopsis thaliana* shows over-proliferation of xylem tissues and a severe defect in stem elongation. *ACL5* encodes thermospermine synthase. Thermospermine is a structural isomer of spermine. To know the effect of *acl5* at the molecular level, we performed cDNA microarray experiments using *acl5* seedlings and found that a lot of genes involved in xylem differentiation are up-regulated in *acl5*. These include the HD-Zip III class homeodomain proteins, all members of the vascular-related NAC domain (VND) transcription factors, Xylem cysteine proteases (XCP1 and XCP2), and the TDIF receptor (TDR). We also examined the effect of exogenous application of thermospermine on *acl5* sprms double mutants, which produce neither thermospermine nor spermine and identified the genes that are up-regulated or down-regulated by thermospermine. The results are summarized in this presentation.

08109

ROLE OF KRP GENES IN SHOOT APICAL MERISTEM AND LEAF DEVELOPMENT IN ARABIDOPSIS

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Cell division plays an important role to achieve the appropriate architecture of plants during development and adaptation. The Kip-related protein (KRP) family negatively regulates the cell cycle through the control of cyclin-dependent kinases activity. To investigate KRP function during plant development, we generated and characterized transgenic *Arabidopsis* overexpressing KRPs. *KRP* overexpression driven by the 3SS promoter resulted in remarkable effects of consistent phenotypes, including dwarfism, leaf serrations and altered flower morphology. The stability of *KRP3* and *KRP6* depended on 26S proteasome activity, although they were expressed in SAM, leaf primordia, and root tissues. Interestingly, *KRP3* overexpression triggered distinct modification of SAM structure, whereas *KRP6* overexpression affected preferentially on the leaf structure through the change of the balance between cell number and cell size. Our results suggest that KRPs allow plants to have diverse regulatory mechanisms of cell division during plant development.

08110

ISOLATION AND CHARACTERIZATION OF BROCCOLI VEF GENES

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Polycomb group (PcG) genes regulate major developmental processes in *Arabidopsis*. *Arabidopsis* proteins EMBRYONIC FLOWER2 (EMF2), VERNALIZATION2 (VRN2), and FERTILIZATION INDEPENDENT ENDOSPERM2 (FIS2) share a VEF domain also found in the *Drosophila* PcG protein, Su(z)12. The small family of VEF-domain-containing genes exists throughout angiosperm. Phylogenetic analysis showed that *AtEMF2*, being the prototype of plant VEF genes, gave rise to *AtVRN2*, while *AtFIS2* and another VEF gene were derived from *AtVRN2*. However, *FIS2*-like sequence is only found in *Arabidopsis*, not poplar and rice although their genomes have been sequenced. Here, we identified five broccoli VEF genes including two putative *BoFIS2*. Like *AtEMF2*, *BoEMF2* is composed of an N-terminal domain, an E5-10 domain, a C2H2 domain, an E15-17 domain and a VEF domain. Like *AtVRN2*, *BoVRN2* lacked the E5-10 domain. Like *AtFIS2*, *BoFIS2* lacked both E5-10 and E15-17 domains and acquired two unique domains, an AtFIS2 S-rich repeat like domain and a C-terminal alanine rich domain. Real time PCR analyses showed similar expression pattern between *Arabidopsis* and broccoli VEF genes: constitutive expression of *EMF2* and *VRN2*, and preferential expression of *FIS2* in reproductive organs. An *EMF2*-knockdown broccoli exhibited early flowering and curly leaf phenotype. These data suggested that *FIS2* is likely to be conserved at least in Brassicaceae and the BoVEFs are likely to play similar roles in broccoli as AtVEFs in *Arabidopsis*. Further study on biochemical and biological function of BoVEFs will help to verify this hypothesis. Phylogenetic study including the 5 BoVEF proteins is consistent with prior model of VEF gene evolution (Chen et al., 2009), indicating the divergence of VRN2/FIS2 from EMF2 occurring prior to broccoli and *Arabidopsis* speciation.

08111

A HOMOLOGUE OF YEAST MITOCHONDRIAL TRANSLOCATOR SUBUNIT TIM50 MODULATES ENDOREDUPLICATION IN DARKNESS.

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Endoreduplication is a kind of cell cycle that increases nuclear DNA content (ploidy) without cell and nuclear division and is important for plant development. Here we screened mutants showing increased polyploidy from RIKEN *Arabidopsis* full length cDNA Overexpressor lines (FOX lines) and found that one of the FOX line F07144 showed increase in polyploidy in darkness and the corresponding gene encoded a homologue of yeast mitochondrial translocator subunit TIM50. Tim50 maintains the permeability barrier of mitochondria by closing the translocation pore in presequence regulated manner. We checked sub-cellular localization of expressed protein from F07144 line using GFP markers and found that this protein was localized in mitochondria, so this protein can be designated as *AtTIM50*. GFP fused *AtTim50* degrades very rapidly and this degradation were stabilized by MG132, a proteasome inhibitor. Additionally T-DNA insertional mutant showed reduction in polyploidy and decrease in hypocotyls elongation and also showed low ATP levels than that of wild type plants. Histological analysis using TIM50 promoter GUS showed that expression pattern is specific in highly endoreduplicating organs like root, hypocotyls, and root hairs. Here, we demonstrate that *AtTIM50* modulates endoreduplication through cellular ATP-levels.

08112

HOW SEED PLANTS GOT THEIR LEAVES: A ROLE FOR YABBYs IN THE EVOLUTION OF THE SEED PLANT LEAF

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In seed plants, leaves are born on radial shoots but unlike shoots they are determinate organs with dorsiventral polarity and laminar growth. YABBY genes are found only in seed plants and in all cases studied, are expressed primarily in lateral organs and in a polar manner. Despite their simple expression patterns, *Arabidopsis* plants lacking all YABBY gene activities have a wide range of morphological defects in all lateral organs as well as the shoot apical meristem (SAM). Here we show that leaves lacking all YABBY activities are initiated as dorsiventral appendages but fail to properly activate lamina developmental programs. In particular, a marginal leaf domain is not established, the activation of most CINCINNATTA class TCP genes (CIN-TCPs) does not commence, and SAM-specific programs are reactivated. Altered distribution of auxin signalling and the auxin efflux carrier PIN-FORMED1 (PIN1), highly reduced venation, initiation of multiple cotyledons, and gradual loss of the SAM accompany these defects. The complex YABBY mutant phenotype can be interpreted as a failure to establish a lamina growth pattern (e.g. TCP maturation/determination program), with leaves developing with a mixture of shoot-like characteristics (shoot-like PIN1 expression and ectopic *WUSCHEL* expression) and YABBY-independent leaf characteristics. We suggest that YABBY functions were recruited to shape modified shoot systems into flat plant appendages by translating organ polarity into lamina specific programs that include marginal auxin flow and activation of a maturation schedule directing determinate growth.

08113

CHARACTERIZATION OF SUPPRESSOR OF ELF3 20, A NOVEL DELETION OF CRY2 THAT SUPPRESSES THE FLOWERING PHENOTYPE OF THE CLOCK MUTATION ELF3 IN ARABIDOPSIS UNDER CONTINUOUS LIGHT

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LATE ELONGATED HYPOCOTYL (LHY), CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and EARLY FLOWERING 3 (ELF3) play key roles in the control of plant morphology, flowering time and circadian rhythms in *Arabidopsis thaliana*. In our previous works, we demonstrated that double loss-of-function mutations in *LHY* and *CCA1* (*lhy;cca1*) delayed flowering under continuous light (LL) but accelerated it under short-day (SD) or long-day (LD). The late flowering phenotype of *lhy;cca1* was partially suppressed by *elf3* under LL, suggesting that ELF3 is required for *lhy;cca1*-dependent delay of flowering. This result also indicated that *lhy;cca1* may cause late flowering through both ELF3-dependent and -independent pathways under LL. However, molecular mechanisms underlying the roles of LHY and CCA1 in the control of flowering under light/dark cycles and LL, respectively, have not been fully understood. Also there has been less information on downstream factors of ELF3 for the control of flowering.

Here we report a genetic screen to identify suppressors of *elf3-1* mutant under LL with *elf3-1* seeds mutagenized by heavy ion beam. Seven suppressors of *elf3-1* have been identified and named *suppressor of elf3 1, 3, 5, 7, 14, 15* and *20* (*sel1, 3, 5, 7, 14, 15, and 20*). All these mutants were further characterized and mapped to different regions in *Arabidopsis* genome. Among these mutants *sel20* appears to have a unique and very interesting flowering phenotype compared to other suppressors isolated. In fact, *sel20* suppressed the early flowering phenotype of *elf3-1* under LL but not under LD and SD. Through the mapping analysis of *sel20*, we identified a novel deletion allele of *CRY2* gene encoding a blue-light receptor. Possible roles of *CRY2*, *ELF3*, *LHY* and *CCA1* in the LL-dependent delay of flowering will be discussed.

08114

A GENETIC FRAMEWORK FOR TISSUE-SPECIFIC INTEGRATION OF GA SIGNALING BY THE GRAS TRANSCRIPTION FACTOR SCARECROW-LIKE 3 IN THE ARABIDOPSIS ROOT

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The *Arabidopsis* root growth is progressed by continuous cell division in the root apical meristem and rapid cell elongation toward maturation region. It is reported that coordination of these developmental processes are largely dependent on the interplay between hormones and genetic programs. Recent studies have revealed that GA signaling in the root endodermis is required for coordinated cell elongation for root growth. In addition, together with the GRAS transcription factors SHR/SCR that play a key role in the asymmetric cell

divisions for ground tissue formation, GA signaling controls the timing and extent of formative divisions to generate the endodermis and the middle cortex in the root. However, molecular components that link the two pathways, GA/DELLA and SHR/SCR pathways, remain unknown. Here, we provide lines of evidence that SCARECROW-LIKE 3 (SCL3), which also belongs to the GRAS transcription factor family, acts as a molecular link that integrates GA signaling tissue-specifically in the root endodermis. Our genetic analysis reveals that RGA, a major repressor of GA signaling, is the direct upstream of SCL3. Interestingly, SCL3 acts as a positive regulator in GA signaling to maintain GA homeostasis. The endodermis-confined GA signaling controls coordinated cell elongation, and also the timing and extent of formative divisions to fine-tune post-embryonic root development.

08115

ANALYSIS OF *GIR1* (GLUCOSE INSENSITIVE ROOT 1) REVEALS THAT A ROLE OF THE CHLOROPHYLL COPPER TRANSPORTER PAA1 IN SUGAR SIGNALING

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It has been known that root growth of the *Arabidopsis* seedlings is substantially inhibited under high glucose conditions. Thus, we screened ~1500 activation tagging plants for insensitive root phenotype under high glucose conditions (MS agar plates with 7% glucose). We identified one line, SAL54, with strong insensitivity in the root, which is recessive and named *gir1* (glucose insensitive root growth 1). Under normal conditions, *gir1* mutants exhibited a short root phenotype. Thus, we characterized developmental defects of *gir1* with a variety of tissue-specific markers, including markers for stem cell specification and maintenance. Root meristem size was drastically reduced, and cell division potential, monitored by CYCB1;1-glucuronidase (GUS) reporter, was also severely reduced, although no patterning defects were found in *gir1*. To investigate the molecular basis of glucose signaling in root growth, we isolated the *GIR1* gene by TAIL-PCR, and found a T-DNA insertion in the PAA1 locus, which is previously known as a P-Type ATPase that transports copper (Cu) to the chloroplast stroma being located chloroplast envelope. We also confirmed that root growth of *gir1* was complemented with Cu supplement in MS agar plates. In addition, another recessive T-DNA insertion allele from the SIGNAL database exhibited the identical phenotype. Thus, we renamed our mutants, paa1-t1 (T-DNA insertion 1) and paa1-t2 (T-DNA insertion 2), respectively. To elucidate the role of PAA1 in the sugar-mediated root growth, we treated paa1 mutants with mannose, an analogue of glucose, and observed insensitive phenotype as in glucose treatment. Unlike in wild-type, expression levels of HEXOKINASE 1 (HXK1) and ABA-INSENSITIVE 4 (ABI4) under glucose conditions showed no change in *gir1*, suggesting that PAA1, a copper transporter, other than its role in green tissues containing chloroplasts, functions also in glucose signaling-mediated cell proliferation of root meristem in non-green tissues.

08116

SYSTEMATIC AND MOLECULAR GENETIC ANALYSES FOR ARABIDOPSIS TERMINAL FLOWER 1

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Arabidopsis TERMINAL FLOWER 1 (TFL1) is a key regulator to control the flowering and inflorescence architecture. The TFL1 belongs to CETS (CEN, TFL1, SP) family and shares 59% amino residues with FLOWERING LOCUS T (FT). Despite of the sequence similarity, both TFL1 and FT had antagonistic functions; while FT promotes flowering, TFL1 represses it. These proteins are believed to be regulators of bZIP transcription factors, FD and FD PALALOG (FDP). It is proposed that FT activates the FD-dependent transcription. However, little is known about the molecular roles of TFL1 in the FD/FDP-dependent transcription. Here, we hypothesized two distinct models about the TFL1 roles: (1) in the protein trafficking or (2) in the transcriptional repression. In our mathematical analysis, we suggested that the transcriptional-repression model is more suitable to explain the TFL1 roles in the inhibition of floral pathway. To experimentally confirm this hypothesis, we made transgenic plants over-expressing TFL1 proteins fused with a transcriptional activator or a repressor domain. The flowering time and inflorescence architecture of these transgenic plants were observed. Genetic analyses between *tfl1* and *fd* are also in progress. We will report the phenotypes of these plants and expressions of target genes, and then discuss about the molecular mechanisms underlying the FD/FDP-dependent transcription controlled by FT and TFL1.

08117

CARROT SOMATIC EMBRYOGENESIS FOR ANALYSIS OF DESICCATION TOLERANCE AND ABSCISIC ACID SIGNAL TRANSDUCTION

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Carrot (*Daucus carota*) somatic embryogenesis has been extensively used as an experimental system to study plant embryogenesis. In maturing zygotic embryos, abscisic acid (ABA) is involved in acquisition of desiccation tolerance and dormancy. On the other hand, somatic embryos contain low levels of endogenous ABA and show desiccation intolerance and lack dormancy, but tolerance and dormancy can be induced by exogenous application of ABA. In ABA-treated carrot embryos, some ABA-inducible genes (*ECPs* and *CAISEs*) are expressed. Most of the genes encode late embryogenesis abundant (LEA) proteins, whereas one of them encodes a glucose and ribitol dehydrogenase. The expression of the *ECP* and *CAISE* genes was detected in maturing seeds, embryogenic cells, and ABA-treated somatic embryos in which exhibit desiccation tolerance induced by endogenous or exogenous ABA. The higher enzymatic activity of the glucose dehydrogenase was observed in embryogenic cells and somatic embryos, and the activity was enhanced by ABA-treatment in somatic embryos. These results indicate that ABA-induced desiccation tolerance in carrot somatic embryos may be induced by the LEA proteins and the glucose and ribitol dehydrogenase encoded by these ABA-inducible genes. We isolated the *Daucus carota* bZIP1 (*DcBZ1*) gene encoding a G-box binding factor-type basic region/leucine zipper (GBF-type bZIP) factor from carrot somatic embryos. During the development of zygotic and somatic embryos, increased expression of *DcBZ1* was commonly detected in the later phase of development. The recombinant *DcBZ1* protein showed specific binding activity to the two ABA-responsive element-like motifs in the promoter region of the carrot ABA-inducible gene. Our findings suggest that *DcBZ1* is involved in ABA signal transduction in embryogenesis.

08118

EXPRESSION AND FUNCTIONAL ANALYSIS OF TWO KINDS OF THE VP1/ABI3 FACTORS IN CARROT EMBRYOGENESIS

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Somatic embryogenesis has been extensively used as an experimental system to investigate the development of zygotic embryogenesis. In the carrot (*Daucus carota*) system in particular, numerous physiological, biochemical, and molecular biological studies have examined

somatic embryogenesis. In zygotic and somatic embryos, abscisic acid (ABA) is involved in acquisition of desiccation tolerance and dormancy. Generally, the VP1/ABI3 factor functions as a transcriptional factor on seed specific ABA signal transduction. It has been reported that most plant species have only one *VP1/ABI3* in genome. We isolated two carrot homologs of the VP1/ABI3 factor, *C-ABI3-1* and *C-ABI3-2*. The expressions of *C-ABI3-1* and *C-ABI3-2* was observed in embryogenic cells, somatic embryos, and developing seeds, but not in non-embryogenic cells (NC), seedlings, nor true leaves. Each gene exhibited similar expression profile during somatic embryogenesis and seed development. In the transgenic NC showing ectopic expression of *C-ABI3-1* or *C-ABI3-2*, the expression of some seed specific ABA-inducible genes was successfully induced by ABA-treatment. The gene set regulated by *C-ABI3-1* was different from that regulated by *C-ABI3-2*. These suggest that *C-ABI3-1* and *C-ABI3-2* may function collaboratively on ABA signal transduction in carrot embryos. Furthermore, the interaction between *C-ABI3-1* and *C-ABI3-2* on transcriptional regulation was analyzed by trans-activation assay in carrot or *Arabidopsis* cells. *C-ABI3-1* and *C-ABI3-2* are likely to effect indirectly to each transcription.

08119

THE ROLE OF CLASS I TCP GENES IN DETERMINING LEAF SHAPE AND SIZE

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The growth and development of leaves determines their final shape and size, and is a fundamental feature of plants, responding to environmental change and adapting to suit the physiological requirements of leaves. Using the LeafAnalyser and Leafpredictor software we have designed and built, we have quantified leaf shape and size variation in *Arabidopsis* with a library of more than 3,500 leaves. We have used this library to assess leaf shape and size in candidate plant lines, and aim to identify and characterize genes that have important roles in determining the final shape and size of leaves. Our interest has specifically fallen on INTERNODE SHORT1 (INS1) and INTERNODE SHORT 2 (INS2) members of the TCP family (TB1, CYC and PCFs), which are expressed in early leaf development affecting leaf shape. TCP genes are known to be involved in the regulation of a cell-cycle arrest front, travelling from the leaf tip to the base, and may influence the final shape and size of leaves at this early stage of development. My current research uses a combination of molecular genetics and morphometrics to examine the role of a small sub- family of class I TCP genes in leaf development (TCP8, TCP22, TCP23). Single and double insertion lines are being phenotypically characterised, their leaf shape and size analysed, and histological assays conducted to characterise cell number, shape and size.

08120

GENOME-WIDE EXPRESSION ANALYSIS OF AGE-DEPENDENT

CALLUS FORMATION ABILITY IN ARABIDOPSIS HYPOCOTYL EXPLANTS

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The transition from the juvenile to the mature phase of shoot development in plants is accompanied by changes in vegetative morphology and an increase in reproductive potential. Here, we found a novel phenomenon is apparent in the ability of callus formation in *Arabidopsis*. Interestingly, mature (5-week-old) hypocotyl explants exhibited better callus-forming potential than that of juvenile (1-week-old), determined by callus growth rates. Moreover, a fusion of DR5::GUS, a synthetic auxin response element, was more inductive in mature than in juvenile explants after auxin treatment, indicating that the sensitivity to auxin was one of factors accounting for the age-dependent callus formation capacity. Gene expression profiling indicated that age-dependent callus formation ability was associated with changes in phytohormone (auxins and cytokinins) homeostasis, epigenetic mechanism and the cell cycle regulation. Besides, we identified two groups of genes involved in age-dependent callus formation ability: (1) positive regulatory and (2) negative regulatory categories. Indeed, vim1, belonging to the positive regulatory category, reduced the efficiency of callus formation in mature explants, but not in juvenile. The results also suggest that these transcriptional factors not only involve in developmental programs, but also play important roles in regulating age-dependent callus formation ability. Taken together, the investigation will help to better understand the molecular regulatory mechanism of age-dependent callus formation.

08121

IDENTIFICATION OF A HEAT SHOCK TRANSCRIPTION FACTOR AFFECTING MALE GAMETOPHYTE DEVELOPMENT

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The haploid male gametophyte generation represents a vital role in fertility and plant life cycle. Despite a long-term research on the field of plant sexual reproduction, the knowledge of transcription factors playing role in male gametophyte development is still very limited. Hence the wide-scale genetic analyses of transcription factors using a plentiful collection of T-DNA insertion mutant lines make a good strategy. Exploiting microarray technologies and bioinformatic analyses, we selected 27 genes encoding putative transcriptional factors expressed specifically during early stage of male gametophyte development. We performed phenotype screening of respective 36 T-DNA insertion lines for aborted or defective pollen grains by both light and UV microscopy. Several structural abnormalities were found showing a significant impact of knocked out transcription factor genes on cellular processes.

Subsequently we focused on a heat shock transcription factor (At1g77570) since the members of transcription factor family are known to be involved in stress response and developmental processes. In addition to pollen subcellular disorder of mutant plants, the reduced ability of pollen tube growth was confirmed by *in vivo* and *in vitro* experiments. Moreover, selected heat shock transcription factor caused segregation ratio distortion and significantly reduced the allele transmissibility via both male and female gametophyte. Transient expression revealed the subcellular localization of the heat shock transcription factor in the nucleus and particularly in the nucleolus suggesting its possible involvement in regulation of mRNA/rRNA transcription.

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08122

MOBILE MIRNA165/6 TARGET HD-ZIP III IN THE ROOT STELE PERIFERY FOR PROPER XYLEM PATTERNING

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A fundamental aspect of developmental biology is information exchange between cells resulting in proper cell identity. In *Arabidopsis*, the root xylem pattern is very consistent: radially, xylem forms in an axis with protoxylem at either end and metaxylem in the center. How is this pattern determined? We have identified a mutant, *phb-7d*, harboring a mutation in the microRNA165/6 (miR165/6) target site of the class III homeodomain leucine zipper (HD-ZIP III) gene *PHABULOSA* (*PHB*), which leads to an expanded expression domain of *PHB* now encompassing not only the central, but also the peripheral stele. This mutant develops metaxylem in the place of protoxylem. In contrast, multiple mutants in *HD-ZIP III* genes form protoxylem in the place of metaxylem. Hence, the HD-ZIP III transcription factors act together to determine the xylem cell type. We show that their activity domain is determined by the movement of miR165/6 from the endodermal cell layer. Therefore, we describe a bi-directional signaling pathway where stele-produced SHORT-ROOT protein moves out to the endodermis to activate *miR165/6*, which then acts non-cell autonomously to restrict *HD-ZIP III* from the stele periphery, ultimately leading to proper xylem patterning in the stele.

08123

FLOWERING TIME CONTROL OF ARABIDOPSIS CYTOPLASMIC N-ACETYLTRANSFERASE (ATATF) HOMOLOGS VIA THE AUTONOMOUS PATHWAY

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Protein acetylation is an efficient way to regulate many DNA-templated processes and to influence protein stability, protein-protein interactions in eukaryotes. Although some nuclear acetyltransferases mediate the developmental process in plant, the biological function(s) of cytoplasmic ones remain to be understood. To elucidate the function and regulatory role of the cytoplasmic N-acetyltransferase genes, we conducted molecular analyses of a putative *Arabidopsis* N-acetyltransferase 1 gene (AtATF1) orthologous to *Escherichia coli* RimL-type N-acetyltransferase gene. The transient assays and expression pattern analyses of GFP-tagged AtATF1 both in *Arabidopsis* protoplasts and in root tips, respectively, revealed that AtATF1 was localized in the cytoplasm. To investigate the physiological effect of the ATF deficiency in *Arabidopsis*, we used RNAi to simultaneously silence three paralogous genes, AtATF1, AtATF2, and AtATF3. Interestingly, the RNAi mutant exhibited severely late flowering under long-day and short-day conditions, although late flowering was slightly reversed by gibberellin or vernalization treatment. The transcription levels of FLOWERING LOCUS C (a central floral repressor) and its paralogs, MADS AFFECTING FLOWERING 4 and 5 were increased. In contrast, we observed decreased transcript levels of FLOWERING LOCUS T and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 in the RNAi mutant. Notably, the RNAi mutant also had downregulated transcription of FLOWERING LOCUS D, but not three LDL (Lysine-Specific Demethylase1-LIKE) homologs, the autonomous pathway floral factor. Collectively, it can be suggested that the cytoplasmic AtATF may function as a floral regulator in the flowering time via the autonomous pathway.[Supported by a grant from BK21 program and EB-NCRC at Gyeongsang National University]

08124

ANALYSIS OF DOWNSTREAM GENES REGULATED BY MASTER REGULATORS OF VASCULAR DIFFERENTIATION

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The NAC transcription factors, VND6 and SND1/NST3, have been shown to be master regulators for differentiation of xylem vessel cells and fiber cells, respectively. However, how these processes are regulated by VND6 and SND1 is unclear. To investigate downstream genes regulated by VND6 or SND1, we established novel in vitro transgenic systems using *Arabidopsis* suspension cultured cells in which VND6 or SND1 was overexpressed. We confirmed that the in vitro culture systems could reflect processes of xylem cell differentiation in planta. Then, we performed microarray experiments with the suspension cultured cells we had established. We identified a number of downstream genes. Based on these results, we will discuss the gene regulation mechanism of xylem cell differentiation.

08125

IDENTIFICATION AND EXPRESSION PROFILING OF AMBIENT TEMPERATURE-RESPONSIVE MIRNAS IN ARABIDOPSIS

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Flowering is the primary trait affected by ambient temperature changes. Plant microRNAs (miRNAs) are small non-coding RNAs playing an important regulatory role in plant development. In this study, to elucidate the mechanism of flowering-time regulation by small RNAs, we identified six ambient temperature-responsive miRNAs (miR156, miR163, miR169, miR172, miR398 and miR399) in *Arabidopsis* via miRNA microarray and northern hybridization analyses. We also determined the expression profile of 120 unique miRNA loci in response to ambient temperature changes by miRNA northern hybridization analysis. The expression of the ambient temperature-responsive miRNAs and their target genes was largely anticorrelated at two different temperatures (16 and 23°C). Interestingly, a lesion in *short vegetative phase* (*SVP*), a key regulator within the thermosensory pathway, caused alteration in the expression of miR172 and a subset of its target genes, providing a link between a thermosensory pathway gene and miR172. The miR172-overexpressing plants showed a temperature-independent early flowering phenotype, suggesting that modulation of miR172 expression leads to temperature insensitivity. Taken together, our results suggest a genetic framework for flowering-time regulation by ambient temperature-responsive miRNAs under non-stress temperature conditions.

08126

MAB4 SUBFAMILY GENES INVOLVED IN AUXIN-REGULATED ORGANOGENESIS

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In higher plants, phytohormone auxin plays a crucial role in organ formation. Auxin is accumulated asymmetrically in the shoot apical meristem (SAM) dependently on polar auxin transport system. Previously, several *Arabidopsis* mutants displaying severe defects of organogenesis were identified; *pin-formed1* (*pin1*), *pinoid* (*pid*), and *monopteros* (*mp*). PIN1 is an auxin efflux carrier, localized in the plasma membrane with polarity in the SAM. PID is a Ser/Thr kinase that controls PIN1 polarity and MP is a transcription factor that mediates auxin signaling in organ formation. In addition, we have identified *MACCHI-BOU 4* (*MAB4*) as a factor involved in organ formation. *MAB4* encodes a NONPHOTOTROPIC HYPOCOTYL 3 (NPH3)-like protein and has several paralogues named *MAB4/ENP-LIKE (MEL)* genes. *mab4 mel1 mel2* triple mutants develop pin-like inflorescences as *pin1*, *pid* and *mp* mutants. To examine the function of *MAB4* subfamily genes in organogenesis, we performed expression analysis of PIN1-GFP and DR5-GFP in *mab4 mel1 mel2* inflorescences. In the L1 layer of the mutant SAM, PIN1-GFP was normally localized in the upper side of the plasma membrane toward the prospective organ primordia. However, in inner cells of the mutant, polarized PIN1-GFP in the plasma membrane was severely reduced compared with the wild-type SAM. Expression of DR5-GFP was expanded in the L1 layer of the mutant SAM. These results indicate that in the mutant auxin could not flow downwards from the L1 layer due to the defect of PIN1 localization in inner cells, suggesting the function of *MAB4* subfamily genes as an auxin sink in organogenesis. Now, we are analyzing the relationship between *MAB4* subfamily genes, *PID* and *MP*. Together with these results, we will discuss the molecular mechanism of auxin-regulated organ formation.

08127

SCARECROW-LIKE 23, A GRAS TRANSCRIPTION FACTOR, PLAYS A ROLE IN THE ARABIDOPSIS VASCULAR DEVELOPMENT OF THE SHOOT SYSTEM, INTERACTING WITH SHORT-ROOT

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SHORT-ROOT (SHR) and SCARECROW (SCR) are key regulators of the stem cell maintenance and radial patterning in the *Arabidopsis* root. It is relatively well known about the molecular basis of radial patterning processes in the root regulated by the module of SHR/SCR transcription factors. However, SHR function in the shoot system remains elusive, even though its loss-of-function mutant phenotype in the shoot is evidently obvious and *SHR* is also expressed in the shoot. In an attempt to elucidate the molecular mechanisms by which SHR controls in the shoot system, we isolated a SHR-interacting protein, SCARECROW-LIKE 23 (SCL23), which also belongs to the GRAS transcription factor family. SCL23 is most closely related to SCR, which is also known to interact with SHR, among the GRAS members. Interestingly, *SCL23* is, however, not expressed in the root. Expression of *SCL23* is found primarily in the vasculature and meristem of the shoot. In addition, *scl23* mutants only suppress the phenotype of *shr* mutant shoots, suggesting that SCL23 plays a role in the vascular development of aerial part in conjunction with SHR.

08128

GROWTH OF CRYPTIC BRACT IN ARABIDOPSIS

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Bracts are defined as modified leaves subtending flowers or inflorescence, which are commonly found on various plant species. Interestingly, Brassicaceae species, including *Arabidopsis*, usually lack bracts. However, previous investigations on *Arabidopsis* showed that it possesses the ability to develop so-called cryptic bracts, suggesting the involvement of a unique mechanism suppressing bract growth in Brassicaceae to modify inflorescence architecture. Here we show that the outgrowth of cryptic bracts in *Arabidopsis* is associated with every flower in the triple mutant where SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1), SHORT VEGETATIVE PHASE (SVP) and AGAMOUS-LIKE 24 (AGL24) are lost. Our results have excluded the possibility that the growth of bracts may rely on the activity of JAGGED, a factor thought to be sufficient to induce the proliferation of lateral organ tissues. Further detailed examination revealed that the initiation of a bract in the *soc1 agl24 svp* triple mutant starts with the protrusion of the abaxial part of a floral primordium; and is associated with an altered pattern of polarized auxin distribution and ectopic expression of auxin biosynthesis genes. In addition, we found that LEAFY (LFY) physically interacted with SOC1 and AGL24; while svp loss-of-function mutant synergistically enhanced bract initiation in lfy partial loss-of-function mutant.

08129

INTERACTIONS AMONG GENES INVOLVED IN GYNOECIUM DEVELOPMENT IN ARABIDOPSIS

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INTERACTIONS AMONG GENES INVOLVED IN GYNOECIUM DEVELOPMENT IN ARABIDOPSIS

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The gynoecium is the female reproductive organ of the flowering plants. In *Arabidopsis thaliana*, the gynoecium is composed of two carpels that are fused along their margins. The margin of the carpels contain meristematic tissue that gives rise to internal organs in the ovary including ovules, septum and transmitting tract which are required for successful pollination and fertilization. The CUP-SHAPED COTYLEDON genes CUC1 (At3g15170) and CUC2 (At5g53950) which encode NAC domain transcription factors, are involved in gynoecium development through promoting the formation of the meristematic tissue of the carpel margins. CUC1 and CUC2 are expressed on the adaxial side of carpel margins and the *cuc1 cuc2* double mutant often fails to form the organs derived from marginal meristematic tissue, resulting in a severe reduction of ovules and the septum. Interaction between CUC1 and CUC2 and other genes involved in gynoecium remains to be investigated. Two carpel genes SPATULA (At4g36930) and CRABS CLAW (At1g69180), each encoding transcription factors of bHLH and YABBY proteins, respectively, are required for preventing fusion of the carpel margins and septum formation. We are analyzing the genetic relationship of these two genes with CUC1 and CUC2. The *cuc1 spt* and *cuc2 spt* double mutants showed severe defects in ovules and septum formation. In addition, each of *cuc1* and *cuc2* mutations suppressed the split of carpel margins in *spt*, suggesting that CUC1 and CUC2 activity was responsible for this phenotype. Genetic interactions between CUC and CRC also represent severe reduction of ovule and septum development. Results of expression studies in *spt*, *crc* and *cuc1cuc2* mutants will also be presented.

08130

THERMOSPERMINE AND NORSPERMINE ARE NOVEL SUPPRESSORS OF XYLEM DIFFERENTIATION.

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Polyamines are ubiquitous organic polycations in prokaryotic and eukaryotic cells. Thermospermine is a structural isomer of spermine first discovered in thermophilic bacteria and is essential for stem elongation in *Arabidopsis thaliana*. The *acaulis5* (*acl5*) mutant of *Arabidopsis* is a loss-of-function mutant of thermospermine synthase and exhibits excessive differentiation of xylem tissues and very short inflorescent stem. In this study, we examined the external effect of thermospermine on plant growth. Exogenously supplied thermospermine suppressed xylem differentiation of liquid-grown *Arabidopsis* seedlings. Furthermore, thermospermine remarkably blocked tracheary element differentiation of isolated mesophyll cells of *Zinnia elegans*. We noted the C3C3 arrangement of carbon chains in thermospermine (C4C3C3), which is not found in spermine (C3C4C3), and examined whether it could be functionally replaced with norspermine (C3C3C3) or not. Norspermine significantly suppressed xylem differentiation in both *Arabidopsis* seedling and *Zinnia* cell cultures, whereas spermine had no such effect. Together with effects of thermospermine and norspermine on marker gene expression, our results clearly indicate that thermospermine and norspermine function as suppressors of xylem differentiation and the C3C3 arrangement is essential for their biological activity.

08131

THE RAB GTPASE FUNCTIONS IN AUTOPHAGY AND CONTRIBUTES TO TRACHEARY ELEMENT DIFFERENTIATION IN ARABIDOPSIS
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The tracheary elements (TEs) of the xylem serve as the water-conducting vessels of the plant vascular system. To achieve this, TEs undergo cell death, during which the cell contents are completely removed, and thickened and lignified secondary cell walls are formed. Cell death of TEs is a typical example of developmental programmed cell death that has been suggested to be autophagic. However, little evidence of autophagy in TE differentiation has been provided. The present study demonstrates that the small GTP binding protein plays a role in TE differentiation through its function in autophagy. Differentiating wild type TE cells were found to undergo autophagy in an *Arabidopsis* culture system. Both autophagy and TE formation were significantly stimulated by overexpression of a constitutively active mutant, and were inhibited in transgenic plants overexpressing a dominant negative mutant or RNAi, a brassinosteroid insensitive mutant *bri1-301*, and an autophagy mutant *atg5-1*. Taken together, our results suggest that autophagy occurs during TE differentiation, and that Rab GTPase, as a component of autophagy, regulates TE differentiation.

08132

TENUIPETALA-D REVEALS A ROLE OF PUX1 IN CELL DIVISION CONTROL IN ARABIDOPSIS THALIANA
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Organ growth is one of the fundamental and crucial developmental processes in multicellular organisms, but little is known about how organs achieve their final size and shape. Here we analyzed *tenuipetala-D* (*tep-D*), which displayed characteristic flowers with narrower petals than those of wild-type. *tep-D* was isolated by activation-tagging screen and insertion was linked with *tep-D* mutant phenotypes. Several genes near the insertion were moderately activated in *tep-D* and we focused one of the genes, *PLANT UBX DOMAIN-CONTAINING PROTEIN 1* (*PUX1*). *PUX1* was previously suggested to regulate organ growth via the negative regulation of an AAA-ATPase CELL DIVISION CYCLE 48 (CDC48) activity. Loss-of-function plants of *PUX1* displayed slightly larger organs and overexpression of *PUX1* repressed organ growth in a dosage-dependent manner. Phenotypes of organ size were attributable to mainly alterations in cell number in these *PUX1* mutants and *tep-D*. In consistent with the cellular phenotype, expression of *CYCLIN B1;1* (*CYCB1;1*), one of the marker genes of cell division, was reduced in *tep-D*. In contrast, accumulation of *CYCB1;1* protein was dramatically increased, suggesting that cell cycle progression in *tep-D* might be delayed due to some defect in M phase, where *CYCB1;1* is degraded by anaphase-promoting complex/cyclosome (APC/C) E3 ubiquitin ligase complex under the control of spindle checkpoint mechanism. Furthermore, it is known that Clb2, the yeast cyclin B, fails to be degraded in *cdc48* mutant in yeast due to spindle checkpoint response. Taken together, *PUX1* may regulate cell division and organ growth by modulating an activity of CDC48, which has multiple functions in cellular activities.

08133

MACCHI-BOU2 REGULATES EMBRYO PATTERNING AND COTYLEDON DEVELOPMENT VIA MODULATION OF AUXIN-RESPONSIVE TRANSCRIPTION
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The asymmetrical distribution of auxin depending on polar transport has an instructive role in plant organogenesis. Although some of the factors regulating auxin-mediated organogenesis were identified, its overall process remains poorly understood. To clarify the molecular mechanisms underlying auxin-mediated organogenesis, we have analyzed the *macchi-bou 2* (*mab2*) mutant identified by a *pinoid* (*pid*) enhancer mutant screen. While *mab2* or *pid* single mutant seedlings showed only a mild phenotype in cotyledon positioning and/or number, the *mab2 pid* double mutant completely lacked cotyledons. The *mab2* single mutation disturbed cell division pattern and caused aberrant cotyledon development during embryogenesis. Expression analysis of auxin markers revealed that *mab2* mutation affected auxin response but not auxin transport at the developing cotyledons in their embryos. Furthermore, genetic analysis between auxin-insensitive mutants and *mab2* showed the synergistic interaction in cotyledon formation, indicating the contribution of *MAB2* on auxin response. *MAB2* encodes an *Arabidopsis* homolog of MED13, a putative regulatory module (CDK8 subcomplex) component of the Mediator complex, and is identical to *GRAND CENTRAL*. Mediator is a multicomponent complex evolutionarily conserved among the eukaryotes and CDK8 subcomplex associates with the Mediator to control the interaction of Mediator and RNA polymerase II. The *Arabidopsis* genome appears to contain corresponding homologs of the Mediator complex including CDK8 subcomplex. To investigate the relationship among *Arabidopsis* CDK8 subcomplex, we carried out the yeast-two hybrid assay. As a result, *MAB2* possess the ability to interact with other CDK8 subcomplex component in yeast cells. Thus, our results suggest that the *MAB2* gene functions as a key effector for the auxin-responsive transcription during embryogenesis.

08134

T-DNA SCREENING FOR GENES REQUIRED FOR GAMETOPHYTE AND SEED DEVELOPMENT IN ARABIDOPSIS
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The purpose of this project is to identify large numbers of *Arabidopsis* genes which have essential functions during reproductive stage. Especially we are focusing on genetic and molecular mechanisms governing female/male gametogenesis and subsequent embryogenesis. Activation tagging mutagenesis causes dominant activation mutation by activating nearby genes. Like other conventional T-DNA, however, it can cause recessive insertional mutations as well. If T-DNA is inserted in genes which have important function during gametogenesis, the mutant plants will show, most likely, about 50% ovule-abortion phenotype. If the mutants plants show about 50% seed abortion phenotype, it is possible that T-DNA is inserted in genes which are imprinted like *MEDEA* or its regulators. If T-DNA causes insertional mutation in genes which function during embryogenesis, the mutant plants will show about 25% seed abortion due to the zygotic lethality. More than 70,000 activation tagging T-DNA lines were generated. Transgenic plants have been screened for defective ovule and seeds based on above criteria. More than 200 mutant lines were first chosen. Among them, 10 lines showing 50% seed abortion phenotype were selected for further study and described below. This screen will tell us how these genes regulate gametogenesis and embryogenesis in near future.

08135

THE ARABIDOPSIS WUSCHEL IS A BIFUNCTIONAL TRANSCRIPTION FACTOR THAT ACTS AS A REPRESSOR IN STEM CELL REGULATION AND AS AN ACTIVATOR IN FLORAL PATTERNING

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Most transcription factors act either as activators or repressors, and no such factors with dual function have been unequivocally identified and characterized in plants. The *Arabidopsis* HOMEOBOX transcription factor WUSCHEL (WUS), which controls the maintenance of stem cell populations in shoot meristems, has been shown to act as a repressor for *ARABIDOPSIS RESPONSE REGULATOR* genes and as an activator of *AGAMOUS*. Both of these genes have been shown to be direct target of WUS. We demonstrate that WUS is a bifunctional transcription factor that acts mainly as a repressor but becomes an activator when involved in the regulation of the *AG* gene. The WUS-box, which is conserved among *WUSCHEL RELATED HOMEOBOX* genes, acts as repression domain and can convert transcriptional activators into dominant repressors when it fused to transcriptional activators. All the known activities of WUS are eliminated by mutation of the WUS-box, including the regulation of stem cell identity and size of floral meristem and the induction of *AG* expression. The mutation of the WUS-box is complemented by fusion of an exogenous repression domain, SRDX, with resultant formation of somatic embryos in roots and expansion of floral meristems as observed upon ectopic expression of *WUS*. Our results confirm that WUS acts as a repressor and not as an activator in the induction of stem cell identity and in maintenance of populations of pluripotent stem cells in shoot and floral meristems. By contrast, fusion of an exogenous activation domain, VP16, does not result in expanded floral meristems but induces carpeloid sepals with stigmatic papillae and staminoid petals in transgenic flowers, which similar to those induced by the ectopic expression of *AG*. Our results demonstrate that WUS acts mainly as a repressor and that its function changes from that of a repressor to that of an activator in the case of regulation of the expression of *AG*.

08136

THE DWARF PHENOTYPE OF THE *ACL5-1* MUTANT IS SUPPRESSED BY MUTATIONS IN RIBOSOMAL PROTEINS.

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The *acaulis5* (*acl5*) mutant is defective in the synthesis of thermospermine and exhibits a severe dwarf phenotype. Thermospermine is a structural isomer of spermine. To elucidate the role of thermospermine in stem elongation, we have analysed suppressor mutants of *acl5* (*sac*). We have previously shown that *SAC51* encodes a bHLH-type transcription factor and that *SAC52* encodes a ribosomal protein L10 (RPL10). *SAC51* mRNA has five upstream ORFs (uORFs) in its 5'-UTR and *sac51-d* has a point mutation in the 4th uORF. Our data have suggested that translation of the *SAC51* main ORF is negatively regulated by ribosome stalling at the 4th uORF in the absence of thermospermine. Furthermore, the genes responsible for *sac53-d* and *sac56-d* encode a receptor for activated C kinase (RACK1) and a ribosomal protein L4 (RPL4), respectively. These are also components of the ribosome. Based on our experiments, we suggest that *sac52-d* and *sac56-d* mutants result in an increase in the translation efficiency of the *SAC51* main ORF but *sac53-d* has a distinct effect for overcoming the deficiency of thermospermine.

SAC51 mRNA is known to be a target of non-sense mediated mRNA decay (NMD). We found that the *SAC51* mRNA is unstable in the absence of thermospermine. The experiments to examine whether the *sac53-d* mutation affects the *SAC51* mRNA stability or not are underway.

We will discuss the mechanism by which mutations in ribosomal proteins suppress the deficiency in thermospermine.

08137

FUNCTIONAL ANALYSIS OF *MIZ1*, A GENE ESSENTIAL FOR ROOT HYDROTROPISM

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Roots display tropisms that control the direction of growth, hereby avoiding environmental stresses such as drought. It has been hypothesized that roots display hydrotropism in response to moisture gradients, which could permit water acquisition more effectively than any other tropism. However, there have been surprisingly few studies on root hydrotropism when compared with other tropisms such as gravitropism and phototropism. Recently, we established experimental system for the induction of hydrotropic responses in *Arabidopsis* roots, which had enabled us to use molecular genetics as a tool. Genetic screens based on the inability to develop hydrotropic root curvature allowed us to isolate a series of ahydrotropic mutants termed "*mizu-kussei* (*miz*)". We recently succeeded in identifying the mutated genes for *miz1*, which was the first ahydrotropic mutant whose responsible gene has ever been determined. Physiological and morphological analyses of the mutants showed that *miz1* had normal gravitropic responses, root elongation growth and root cap organization, while it completely lacked hydrotropic response. *MIZ1* encodes a protein of unknown function with a conserved domain at its C-terminus, which we termed MIZ domain. The genes encoding MIZ domain are found only in the genomes of land plants, and the MIZ domain has no clear similarity to any characterized peptide sequence. To analyze the localization and functional roles of *MIZ1*, we

generated transgenic plants that express the GFP-fused MIZ1 in addition to MIZ1 over-expressing plants. In this presentation, results from physiological analyses of these transgenic plants will be reported.

08138

SEARCH FOR NEW IMPRINTED GENES BY REVERSE GENETICS

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Gene imprinting refers to the different allele-expression depending on parental origin. In flowering plants, imprinting is thought to be established during the formation of gametophyte. For example, *DEMENTER(DME)* is an active DNA demethylase which is expressed in the central cell of the female gametophyte, and there it hypomethylates maternally expressed imprinted genes as *MEDEA(MEA)*, *Fertilization-Independent Seeds 2(FIS2)* and *FWA*.

Still, we don't know how many other imprinted genes remains unknown and how they regulate plant development. In order to find maternally expressed imprinted genes which function during seed development, we took a reverse genetic approach. Potential target genes are selected into two steps. First, genes of the female gametophyte transcriptome and Ds insertion line screening result showing abnormal seed development were compared. Selected genes were also compared with the H3K27me3 microarray and DNA methylation profile, narrowing down genes which seems to be epigenetically regulated. Finally 23 genes were selected and their Salk line mutants from ABRC are being used for further study. By this screening we hope to find new imprinted genes and their downstream genes which function during the reproductive stage.

08139

MIR156 VIA SPL REPRESSION IS RESPONSIVE TO AMBIENT TEMPERATURE CHANGE IN FLOWERING-TIME REGULATION IN ARABIDOPSIS

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Microrna(miRNA) is non-protein-coding small RNA with 21-22 nt length, which regulate gene expression at post-transcriptional levels through mRNAs cleavage or translational repression. Plant miRNA is considered as important regulator in plant development. Recently, it had been reported that miRNA is involved in response to biotic/abiotic stress in plants. Our lab had identified a subset of ambient temperature-responsive miRNAs in *Arabidopsis thaliana* (Lee et al., 2010). Here, we demonstrate the role of miR156/SPL as flowering-time regulator in response to ambient temperature change. Up-regulation of miR156 expression at 16 °C and flowering-time phenotype in different ambient temperature showed that miR156 is capable to respond to ambient temperature change in regulation of flowering-time. Pri-miR156a, b, and d expression levels were dramatically decreased at 16 °C relative to 23 °C. But no difference was observed in miRNA biogenesis genes expression at different ambient temperature, suggesting that up-regulated mature miR156 expression in 16 °C was result from acceleration of miR156 biogenesis. Consistent with miR156 expression, the transcript level of their target genes *SPL 3, 4, 5, 6* and *9* was significantly reduced at 16 °C. 5' RLM-RACE results showed *SPL* mRNA was negatively regulated by miR156-mediated mRNA cleavage. These results indicate that miR156 via *SPL* repression plays an important role in regulation of flowering-time in response to ambient temperature change in *Arabidopsis*.

08140

ROLE OF *ILT3* IN THE CONTROL OF FLOWERING TIME BY AMBIENT TEMPERATURE IN ARABIDOPSIS

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Plants show remarkable developmental plasticity to survive in a continually changing environment such as photoperiod and temperature. In contrast to light and vernalization, little is known about thermosensory pathway that mediates the response to ambient temperature. Here, we identified one ecotype that does not respond to a change in ambient temperature by natural variation approach. Furthermore, we confirmed that flowering times of T-DNA alleles of the loci were not changed by ambient temperature, and renamed it *ILT3 (INSENSITIVE TO LOW TEMPERATURE 3)*. The genetic interactions of *ilt3* with other floral repressor mutants showed that *svp* mutations appeared to be largely epistatic to *ilt3* mutations, and the temperature insensitivity was also maintained in double and triple mutants. The early flowering of *ilt3* mutants at 23°C or 16°C was resulted from upregulation of *FT*, *TSF* and *SOC1*, suggesting that *ILT3* is a floral repressor. ChIP (chromatin immunoprecipitation) analysis showed that *ILT3* and *SVP* bound to the genomic regions of *FT*, *TSF* and *SOC1*. Interestingly, *ILT3* interacts with *SVP* *in vivo* and *in vitro*. These data suggest that *ILT3* is another component in response to ambient temperature, and *ILT3* and *SVP* act within the thermosensory pathway.

08141

IDENTIFICATION OF TWO NOVEL GENES AFFECTING THE CONTROL OF THE FLORAL TRANSITION IN ARABIDOPSIS THALIANA

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The floral transition is one of the main event in the life of higher plants. The timing of this transition has to be regulated in order to get a better reproductive success. In *Arabidopsis thaliana*, four main pathways that control the time of flowering have been described and many genes have already been identified. However, we identified mutants from the riken arabidopsis genome information database, related with two genes, *FLORAISON TARDIVE1 (FLA1)* and *FLA2*, that showed a late flowering phenotype and that have never been described as late-flowering.

FLA1 is described as a C2 domain-containing protein with an enzymatic domain. *fla1* mutants flowered later only in long day conditions (LD). This phenotype indicates that *FLA1* belongs to the photoperiodic pathway. *FLA1* expression is constant over the time. *GI* or *CO* expression was not affected by mutation of *fla1* suggesting that *FLA1* acts in a *CO*-independent pathway inside the photoperiodic pathway. Moreover, *fla1* mutants displayed resistance to an inhibitor of the tryptophan biosynthesis pathway, indicating that the putative phosphoribosyl transferase domain of *FLA1* might be effective.

FLA2 seems to have a transcriptional factor activity. In both LD and SD, *fla2-1* and *fla2-4* were late-flowering. These observations indicate that FLA2 belongs to the autonomous pathway. Moreover, *fla2-1* mutation negatively regulates most of the autonomous pathway genes. *fla2-1/flc* double mutant flowered as early as *flc* mutant suggesting that FLA2 is upstream of FLC. FLA2 has previously been described as tubby-like protein 4, TLP4 and belongs to a family of tubby domain containing proteins. Nine of the eleven members of this family displayed an altered flowering phenotype in LD suggesting that the tubby domain might play a role in the regulation of the floral transition.

08142

EXPRESSION ANALYSIS OF THE AQUAPORIN GENES IN CARROT SOMATIC EMBRYOGENESIS

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In higher plants, developing seeds are dehydrated in the maturation phase, and subsequently rehydrated during germination. Plant aquaporins facilitate water flux across biomembranes as water channels, and are involved in various physiological phenomena in several plant tissues. In order to reveal physiological roles of aquaporins during plant embryogenesis, we analyzed the expression of aquaporin genes in carrot (*Daucus carota*) somatic embryogenesis as a model experimental system for studying the development of zygotic embryogenesis. Carrot genes encoding plasma membrane aquaporins (PIP_s; *DcPIP1;1*, *DcPIP1;2*, and *DcPIP1;3*) were isolated. The higher expression of three *DcPIP1s* was detected in seedlings, true leaves, and tap roots, whereas lower expression was detected in embryogenic cells, somatic embryos, and developing seeds. In somatic embryos, the expression of *DcPIP1s* was downregulated by abscisic acid (ABA) treatment. The histochemical analyses of the *DcPIP1;1* and *DcPIP1;2* promoters were performed in the transgenic carrots (*DcPIP1;1::GUS* and *DcPIP1;2::GUS*). The promoter activities of *DcPIP1;1* and *DcPIP1;2* were observed in epidermis and cambium layers of somatic embryos, and vascular bundles and root tips of seedlings. Our results suggest that PIP-type aquaporins have main physiological roles in germinating embryos. The lower expression of *PIP1s* may be maintained by a developmental program or increased endogenous ABA during seed development, and the water permeability of cells may be inhibited in dormant embryos.

08143

SEL1 (SEEDLING LETHAL 1), A PENTATRICCOPEPTIDE REPEAT (PPR) PROTEIN, IS REQUIRED FOR CHLOROPLAST DEVELOPMENT AND CHLOROPLAST GENE EXPRESSION IN ARABIDOPSIS

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Chloroplasts are the site of photosynthesis and other essential metabolites biosynthesis, including amino acids, fatty acids and secondary metabolites. It has been known that many seedling lethal mutants are affected in chloroplast function or development. Therefore, development of functional chloroplast is essential for plant development. To understand the molecular mechanisms underlying chloroplast development, we have isolated a T-DNA insertion mutant, dubbed *sel1* (*seedling lethal 1*), that exhibited pigment defective and seedling lethal phenotype, in which a pentatricopeptide repeat (PPR) gene is disrupted. The PPR is a degenerate 35-amino acid repeat motif which is thought to interact with RNA. Most of PPR proteins are predicted to be targeted to mitochondria or chloroplasts, and involved in post-transcriptional regulation of organellar gene expression. Confocal microscopic analysis showed that the SEL1-GFP fusion protein is localized in the chloroplast. Transmission electron microscopic analysis showed that the chloroplast development is severely impaired in *sel1* mutants. Western blot analysis revealed that *sel1* mutants could not accumulate plastid-encoded proteins involved in photosynthesis. In addition, transcript profiles of chloroplast genes revealed that *sel1* mutants have global defects in chloroplast gene expression. These results suggest that SEL1 may play an essential role in the regulation of chloroplast gene expression during chloroplast development. The possible role of SEL1 in chloroplast development and chloroplast gene expression will be discussed. (YJ Pyo and A Kim contributed equally) [This work was supported by the Crop Functional Genomics Center funded by the Ministry of Science and Technology of the Korean government (grant no. CG2152) and the Second Phase of the BK21 program (YBRI) of Korea]

08144

ROLE OF ATTERC, A SEEDLING-LETHAL GENE, IN EARLY CHLOROPLAST DEVELOPMENT IN ARABIDOPSIS

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Biogenesis of chloroplast from proplastid is one of the important events for early seedling development. T-DNA insertional mutation (*atterc*) of AtTerC, an *Arabidopsis thaliana* gene whose product shares sequence similarity with bacterial tellurite resistance C (TerC), shows pigment-deficient and seedling lethal phenotype under normal growth condition. AtTerC protein is localized in the thylakoid of the chloroplast as shown by localization of an AtTerC-GFP fusion product in protoplasts and by immunoblot analysis of subfractions of chloroplasts. Transmission electron microscopic analysis showed that the ultrastructure of prothylakoid and thylakoid membrane was deformed in etioplast and chloroplast in mutants. Western blot analysis revealed that thylakoid membrane proteins, which are related to photosynthesis, were not accumulated in *atterc* mutant. However, the failure of accumulation and the significant loss of photosynthetic proteins in mutants were not resulted from transcriptional differences between genotypes, because similar transcripts levels of relevant genes were observed in wild-type and in *atterc* mutants. Polysome profiling analysis demonstrated that there was no significant difference in the distribution of ribosomes between the mutant and the wild-type, suggesting that the impaired protein accumulation observed in the thylakoid of *atterc* mutants is caused by accelerated degradation of proteins rather than defects in protein synthesis. Taken together, these results suggest that AtTerC plays a crucial role in prothylakoid biogenesis and thylakoid formation in early chloroplast development. (KC Kwon and SJ Lee contributed equally.) [This work was supported by the Crop Functional Genomics Center funded by the Ministry of Science and Technology of the Korean government (grant no. CG2152) and the Second Phase of the BK21 program (YBRI) of Korea]

08145

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF MYB FAMILY TRANSCRIPTION FACTORS REGULATING CUTICULAR WAX BIOSYNTHESIS.

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Cuticle layer covering most epidermal cells of aerial plant surfaces consists of cutin polymer and wax, and protects from loss of

water, pathogens and insects. While WAX INDUCER1(WIN1), a AP2/ERF family transcription factor (TF), is known to activate cutin and epidermal wax biosynthesis genes, however, other regulators are hardly known. To identify novel factors involving to wax biosynthesis, we screened transgenic plants expressing chimeric repressor of various TFs and found that the chimeric repressor of MYB TF, named WAX REGULATOR1(WAR1), induces adhesion of organs due to loss of wax. SEM observation of the stem epidermis revealed that epicuticular wax crystal was decreased in WAR1-SRDX plant which is expressed WAR1 fused the repression domain under control of the 35S promoter. Microarray experiment revealed that WAR1-SRDX and WIN1-SRDX plants had similar transcriptomes. These results indicate that WAR1 directly or indirectly regulates wax biosynthesis as similar manner as WIN1. Now, we are performing the analysis of redundant transcription factors of WAR1 and WIN1.

08146

NOVEL INSIGHT OF PLANT CUTICULAR WAX AS LUBRICANT OF ELONGATING PETALS

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Petals are most important flower organs to fascinate pollinator, and show the most diversity among the organs. In the process of flower development in *Arabidopsis* and other Brassica family, growth of petal primordia delay until stamen primordia develop to have stalked at base and locules appear at stage 8. Then petal primordia restart to elongate through narrow space between the developed stamens and sepals. In order to understand the elongation process of petal primordia, we isolated and analyzed unique mutants of *Arabidopsis* showing petals are not straightly extended and folded twice at the center, *folded petals (fop)*. Three non-allelic mutants were identified. Sections of floral bud at different stages showed the folding of petals occur after a tip of petal primordium touched the stamens. When sepals were removed from a floral bud in early stage, petals did not show the folded form. There was no obvious difference in size and shape of mature petals between mutants and wild type. These results suggest that the growing petal of *fop* mutants could not pass smoothly through the narrow space between stamens and sepals, and the caught of the petal to these floral organs causes the folding in the mutant flowers. We cloned *FOP1* and *FOP2* genes, and found that *FOP1* and *FOP2* encode a protein homologous to bacterial lipid biosynthesis enzyme, and an ABC transporter, respectively. It is suggested that *FOP2* is involved in secreting wax. *FOP1* and *FOP2* were expressed in mainly flower organs. These results indicate that wax/cutin have a novel role as lubricant which decrease the physical friction between elongating petals and stamens or sepals.

08147

HORMONAL REGULATION OF LATERAL ROOT DEVELOPMENT IS MODULATED BY OVEREXPRESSION OF *MIZ1*

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Plant roots function to acquire nutrients and water essential for their life. To achieve this task, roots show tropisms that determine the direction of their growth. Of these tropic responses, hydrotropism is a directional growth that occurs in response to moisture gradient. Root usually displays positive hydrotropism growing toward the higher moisture, which probably contributes to effective water uptake. Previously, we identified *MIZU-KUSSE11 (MIZ1)* as an essential gene for root hydrotropism. While *MIZ1* and its homologues are widely conserved in terrestrial plants, neither physiological nor molecular characteristics of them have been reported. To gain the insight into the function of *MIZ1*, we generated and analyzed *MIZ1*-overexpressed *Arabidopsis* plants. Unexpectedly, *MIZ1*-overexpressed plants were defective in lateral root development and root growth. We found that *MIZ1*-overexpressed roots showed hypersensitivity to cytokinin but showed normal sensitivities to other plant hormones. *MIZ1*-GFP fusion protein localized in pericycle cells but not in lateral root primordia (LRP) at an early developmental stage. Application of cytokinin induced the localization of *MIZ1*-GFP in LRP, suggesting *MIZ1* potentially inhibits lateral root development downstream of cytokinin action. We also found the application of exogenous auxin recovered lateral root formation in *MIZ1*-overexpressed plants. This result suggested that alteration of auxin level is critical step to inhibit formation of lateral roots in *MIZ1*-overexpressed plant. Collectively, it is possible that *MIZ1* regulated auxin accumulation downstream of cytokinin signaling in LRP.

08148

SPLICING FACTOR VARIANT CONTROLS *ARABIDOPSIS* GROWTH IN RESPONSE TO NUTRIENT CONDITIONS

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Cell proliferation is one of important factors to modulate plant growth. To elucidate cell cycle control, we analyzed *Arabidopsis* *segregation distortion 5 (sd5)* that exhibited reduction in cell number resulting in dwarf phenotype. The *sd5* phenotype depends on the environmental nutrient conditions, in particular that of sucrose - a reduction in the level of sucrose and that of the macronutrients recovers the *sd5* phenotype. The corresponding gene of *sd5* encodes a homolog of yeast *DIM1*, a component of the U5 spliceosome. Higher organisms such as mammals and plants possess two *DIM1* homologs, although only a single *DIM1* gene has been found in the yeast genome. SD5 interacts with Prp6, a subunit of the U5 spliceosome. *Arabidopsis* has two homologs, SD5 and AtDIM1, which show opposite expression patterns and also have a dependency on the nutrients and over-expression of *AtDIM1* enhances the *sd5* phenotype on sucrose. Thus, SD5 has antagonistic functions to the authentic *DIM1* to adapt to nutrient conditions. RNA immunoprecipitation analysis revealed that specific mRNAs interacted with SD5 and this expression was reduced in the *sd5* mutant. These observations indicate that SD5 is involved in a subset of mRNA processing events.

08149

THE MITOCHONDRIAL ATP MODULATES HYPOCOTYLS ELONGATION THROUGH ENDOREPLICATION IN DARK-GROWN SEEDLINGS

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Endoreduplication is one of the special cell cycle which is defined as successive chromosomal DNA replication without cell division and is correlated with cell size. To reveal molecular mechanisms of endoreduplication, *segregation distortion 3* (*sd3*) was isolated from RIKEN *Ds* insertional mutants collection. *sd3* showed short hypocotyls and decreased polyploidy under the dark. Corresponding gene of *SD3* encodes a protein with high similarity to yeast Translocase on the Inner Mitochondrial membrane 21 (TIM21), which is a component of the TIM23 complex. Indeed, SD3 protein fused to GFP was localized in mitochondria. *SD3* over-expression increased polyploidy level and hypocotyl size. Additionally, expression levels of several subunits of the respiratory-chain complexes III and IV were up-regulated in *SD3* over-expressing plants. Furthermore, *SD3* over-expressing plants showed high ATP levels whereas *sd3* showed low ATP levels. These results suggest that mitochondrial activity and ATP levels modulate hypocotyls elongation. Moreover, treatment of seedling with mitochondrial electron transport inhibitor antimycin A resulted in short hypocotyls, decrease in polyploidy levels and ATP levels. These phenotypes are similar to *sd3* phenotypes. Here we propose that hypocotyl is elongated as a result from enhanced endocycle in response to mitochondrial ATP levels in dark-grown seedlings.

08150

A NOVEL CHLOROPLAST FUNCTION IN BALANCING THE ADAXIAL AND ABAXIAL DOMAIN SIZES IN LEAF PRIMORDIA

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In many land plants, the cell characters are different between the adaxial and abaxial domains within the same leaf. Among several genes regulating adaxial and abaxial differentiation, *PHABULOSA* (*PHB*) and *FILAMENTOUS FLOWER* (*FIL*) are known as ones being expressed specifically in the adaxial or abaxial domains, respectively, during leaf primordial development. Though the precise regulation of their expression pattern is thought to be a basis for leaf development, its molecular mechanism is still far from completely understood.

Recently we isolated a novel *Arabidopsis* mutant, *enlarged fil expression domain2* (*enf2*), in which leaf primordia show larger *FIL* expression domain than that of wild type. On the other hand, the expression domain of *PHB* is smaller in *enf2* mutant than in wild type. To know the temporal pattern of *FIL* expression, we measured the ratio of the *FIL* expression domain size to the whole leaf primordium at various developmental stages. And we found that the ratio is as high as 90% even in wild type as well as in *enf2* at very early stages. In later stages, the ratio decreases in wild type, but it does not in *enf2*. Therefore, *enf2* is thought to be defective in regulating the balance between the adaxial and abaxial domain sizes rather than the establishment of adaxial-abaxial polarity.

Interestingly, the responsible gene, *ENF2*, encodes a chloroplast-targeted protein. Chloroplast development of *enf2* was found to be impaired in the leaf primordia from electron microscopy observations. In addition, when chloroplast development of wild type plant is inhibited by lincomycin treatment, the expression pattern of *FIL* and *PHB* resembles that of *enf2* mutant. Our results indicate that early chloroplast development, in which *ENF2* has a primary role, is involved in balancing the adaxial and abaxial domain sizes.

08151

MUTATIONS IN ASYMMETRIC LEAVES2 AND A DEAD-BOX RNA HELICASE REQUIRED FOR THE RNA PROCESSING SYNERGISTICALLY IMPAIR THE ESTABLISHMENT OF THE LEAF POLARITY IN ARABIDOPSIS THALIANA

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Leaves are flat and lateral organs and developed from shoot apical meristem along three axes: the proximal-distal, medial-lateral and adaxial-abaxial axes. Mutations in the *ASYMMETRIC LEAVES2* (*AS2*) gene of *Arabidopsis* result in various defects in the establishment of all three axes. *AS2* is involved in the regulation of the polarity determination of leaves. *AS2* gene encodes a protein with a plant specific domain (*AS2/LOB* domain) and expresses shoot apical meristem and adaxial side of developing leaf primordia. To understand molecular roles of *AS2*, we have screened and isolated several mutants that showed the abnormalities in the polarity determination of leaves in the *as2* mutant background. A mutation #16, one of these mutants we isolated, generated filamentous leaves in the *as2* background and pointed leaves in the wild-type background. The gene corresponding to #16 was predicted to encode a DEAD box RNA helicase, which might function in processing of pre-ribosomal RNA. Pre-rRNA in fact accumulated in the #16 mutant. To confirm whether the defect in the adaxial-abaxial polarity in *as2* #16 double mutant was affected by the defects in processing of pre-rRNA, we analyzed double mutant of *as2* and mutant of another gene that also encodes a protein having a role in processing of pre-rRNA. This double mutant plants also generated filamentous leaves. These results suggest the genetic interaction between an *AS2* pathway and the processing of pre-rRNA in the polarity determination of leaves.

08152

ANALYSIS OF *RRD1*, *RRD2*, AND *RID4*, TEMPERATURE-SENSITIVE MUTANTS OF ARABIDOPSIS THAT FORM FASCIATED ROOTS UNDER THE RESTRICTIVE TEMPERATURE.

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rrd1, *rrd2*, and *rid4* are temperature-sensitive mutants of *Arabidopsis* that were isolated by screening with adventitious root formation as an index phenotype, and characterized by forming fasciated lateral roots at the restrictive temperature. Temperature-shift experiments with a semi-synchronized lateral rooting system showed that lateral root primordia of these mutants develop into fasciated roots when exposed to the restrictive temperature at the initial stage. Detailed observation of arising primordia indicated that expansion of the area where cell division is reactivated to form a root primordium results in the fasciation phenotype.

In *rrd1*, *rrd2*, and *rid4*, besides root development, several aspects of development and growth were found to be also temperature-sensitive. Of particular note, embryogenesis was severely affected by each of the three mutations at the restrictive temperature. Double mutant analysis showed that any combinations of the mutations strikingly aggravate the embryo phenotype and eventually cause

embryonic lethality even at the permissive temperature, suggesting functional relations among *RRD1*, *RRD2*, and *RID4*.

Positional cloning revealed that *RRD1* and *RID4* encode a poly(A)-specific ribonuclease-like protein and a pentatricopeptide protein, respectively. Sequence analysis of the *rrd2* genome detected a mutation in a gene encoding another pentatricopeptide protein, to which the temperature sensitivity of *rrd2* might be attributable. Based on all these findings, we will discuss possible roles of *RRD1*, *RRD2*, and *RID4* in plant development.

08153

TRANSCRIPTION FACTOR COMPLEXES IN ARABIDOPSIS ROOT DEVELOPMENT

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Whereas most plant cells differentiate to generate the specialized cell types within the different organs, stem cells remain undifferentiated and retain the potential to divide and generate new cells for sustained growth.

We have previously shown that two members of plant-specific gene families, double AP2-domain *PLETHORA* (*PLT*) transcription factors and GRAS family transcription factor *SCARECROW* (*SCR*), are key players in root growth and maintenance of the stem cell niche in *Arabidopsis*: Ectopic expression of *PLTs* can induce formation of ectopic root structures and a combination of loss of function data for *PLTs* and *SCR* indicate a role for both genes as master regulators for root development.

However, the molecular mechanisms by which those factors may interact were largely unknown.

To address this question, we screened protein interactors of both *PLT* and *SCR* proteins, and identified several candidate regulatory factors of *PLTs* and *SCR*. A third family of plant-specific transcription factor is associated with both *SCR* and *PLT* proteins *in vitro* and *in vivo*. Genetic and histological analyses support the idea that these factors together with *PLT* and/or *SCR* proteins fine tune the progression of differentiation in plants. Our progress towards characterizing the interacting proteins and their role(s) in regulating *PLT* and *SCR* networks will be presented.

08154

TISSUE SPECIFIC CIS-REGULATORY ELEMENT OF *DME* PROMOTER

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In *Arabidopsis*, *DME* DNA glycosylase is expressed in the central cell of the female gametophyte, and activates maternal allele of *MEDEA* gene through demethylation process. *DME* is also expressed in vegetative tissues including cells flanking the root and shoot apical meristem region. However, neither *DME* regulatory mechanism nor its role in vegetative tissues is known so far. To investigate cis-acting regulatory elements in the 5' flanking region of *DME*, we generated the constructs including sequentially deleted promoter region fused to GUS. Until the promoter was deleted to -39bp region, GUS expression pattern was not changed both in vegetative and reproductive tissues. When we deleted whole known promoter sequence, GUS signal was not detected in vegetative region but in the central cell of ovule. This signal was greatly reduced in +350bp *DME*:GUS transgenic plants. and disappeared in +427 *DME*:GUS plants. This region(-135 to +615) was divided into 5 fragments and ligated with minimal CaMV 35S promoter fused to GUS. The expression analysis is now on going. When we narrow down the proper cis-element, we will do yeast one-hybrid to get regulator of *DME*.

08155

MUTATION AT THE NUCLEOLIN LOCUS ENHANCES THE DEFECTS IN THE ADAXIAL-ABAXIAL POLARITY OF LEAVES IN ASYMMETRIC LEAVES1 (AS1) AND AS2 MUTANTS OF ARABIDOPSIS THALIANA

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Plant leaves are developed along proximal-distal, adaxial-abaxial and medial-lateral axes. ASYMMETRIC LEAVES1 (AS1) and AS2 in *Arabidopsis thaliana* are involved in the establishment of the adaxial fate of leaves by negatively regulating the expression of abaxial determinants as well as class 1 KNOX genes in the adaxial domain of the leaf primordia. We have shown that AS1 and AS2 proteins are co-localized at the periphery of nucleolus in subnuclear bodies (AS2 bodies). Recently it was shown that several ribosomal proteins and the factors involved in biogenesis of small RNAs regulated the leaf adaxial-abaxial patterning and also localized in nucleolus or at the periphery of nucleolus. Therefore, it seems that the structure and/or functions of nucleolus might be important for the determination of adaxial-abaxial polarity. However, the role of nucleolar function in the leaf development has not been clear yet. Recent works suggest that nucleolus has important functions in the cell cycle control, the aging, and the stress responses besides the well-known ribosome biosynthesis. Therefore, it is worthwhile to demonstrate the roles of nucleolus for these events. Nucleolin is known to be a major nucleolar protein that is involved in various nucleolar functions. We found that the mutation at AtNuc-L1 locus strongly enhanced the defects in the adaxial-abaxial polarity of leaves in each mutant of as1 or as2. We will discuss the role of the nucleolin in the determination of adaxial-abaxial polarity regulated with AS1 and AS2 in leaves.

08156

GENOMIC DISTRIBUTION AND FUNCTIONAL ANALYSES OF PUTATIVE G-QUADRUPLEX-FORMING SEQUENCES IN ARABIDOPSIS THALIANA

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Guanine-rich DNA sequences often form G-quartets, planar arrays of four guanines stabilized by monovalent cations (K^+ and Na^+) that interact to form a G-quadruplex. G-rich sequences (repeats of TTTAGGG) in the telomerase form a G-quadruplex that interferes in the elongation reaction by telomerase. Recent bioinformatics analysis revealed that G-quadruplex-forming sequences exist ubiquitously in human, fission yeast, and prokaryotic genomes, and are enriched in promoters and near translation start sites. Ligand binding of a G-quadruplex may increase or decrease transcription of downstream genes. These findings support the current hypothesis that the G-quadruplex may be a novel type of ubiquitous regulatory element. Nonetheless, little is known about this unique sequence in plants. Our analysis *in silico* revealed that the Arabidopsis genome possesses approximately 1,200 G-quadruplex sequences that are distributed on all the five pairs of chromosomes. We next examined whether putative G-quadruplex sequences affect levels of gene transcription. Microarray analysis showed that the expression of 17% of those genes analyzed near G-quadruplex sequences was changed by berberine treatment. Microarray analysis also revealed that G-quadruplex ligand berberine treatment on wild-type plants significantly downregulated those genes with G-quadruplex sequence on the coding strand and downstream of the transcribed region. Furthermore, berberine treated asymmetric leaves1 (as1) and asymmetric leaves2 (as2) mutants specifically exhibited filamentous leaves, whereas wild-type (Col-0) plants showed no morphological changes. Berberine treatment also led to higher transcription levels of class 1 KNOX genes and some abaxial genes in as1 and as2 than those in wild type. These results indicate that berberine inhibited the adaxialization of leaves in as1 and as2 mutant plants. We discuss functional significances of the G-quadruplex on leaf development.

08157

REGULATION OF AUXIN TRANSPORT DEPENDENT LEAF VASCULAR CONTINUITY BY VAN3 ARF-GAP

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Dicotyledonous plants produce closed, reticulated venations in leaves and cotyledons. Elegant physiological experiments performed by Sachs proposed the auxin signal flow canalization hypothesis to explain the complex network of leaf vasculature. This hypothesis assumes the positive feedback effect of auxin on the direction of intercellular auxin flow, which was probed by observing the subcellular localization of PIN auxin efflux carrier (Paciorek et al., 2005 and Sauer et al., 2006). Furthermore, recent study by Scarpella et al. (2006) indeed provides evidence for an auxin transport-driven mechanism in leaf venation patterning. However, the molecular mechanism that governs auxin canalization during venation formation has not been demonstrated experimentally.

Here we identify the molecular components that regulate auxin transport-dependent leaf vascular development. We applied genetics and cellular techniques to the *VAN3* which encode ARF GTPase activating protein (ARF-GAP), regulator of vesicle budding and found that auxin influences the polarity of its own flow via *VAN3* dependent vesicle transport. We also found that subcellular localization of *VAN3* is dynamically changed during leaf vascular development. These findings suggested that *VAN3* function as one of the molecular components of auxin canalization process during leaf vascular development. Based on these results, we will discuss how venation continuity and PIN polarity are integrated by *VAN3* ARF-GAP.

08158

AHL16, AN AT-HOOK PROTEIN REGULATES THE FLORAL TRANSITION IN FLC-DEPENDENT AND INDEPENDENT MANNERS

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The *Arabidopsis* genome encodes 29 AHL (AT-hook motif nuclear localized) proteins, which contains an AT-hook DNA binding motif and an unknown PPC (plants and prokaryotes conserved) domain. AT-hook motif proteins are shown to bind to nuclear matrix attachment regions of DNA. We recently showed that one of the AT-hook motif proteins, GIANT KILLER controls multiple downstream targets of the floral homeotic protein AGAMOUS. Here, we show that knocked down of another AT-hook protein, AHL16 in Landsberg erecta causes global development defects, including extremely late flowering, as well as altered phyllotaxis and reduced fertility. The expression level of FLC (FLOWERING LOCUS C) is dramatically increased in the AHL16 knocked-down lines compared with in the wild-type plants. Expression microarray assay showed that lot of transposable elements were de-repressed in the transgenic plants. In consistent with this, we found that the transposable element in intron 1 of FLC was jumped out in the transgenic plants. ChIP assay also showed that a higher level of H3K4 trimethylation, an activation mark, was found at FLC chromatin in the transgenic plants at Day 5 after germination. In Day 15 wild-type plants, FLC expression level is greatly reduced, accompanied with the reduction of trimethylated H3K4 and the enriched trimethylated H3K27, a repression mark, at FLC chromatin. In contrast, FLC is still highly expressed in Day 15 transgenic plants and H3K4 trimethylation but not H3K27 trimethylation is highly enriched. While the vernalization treatment reduced the FLC expression level, the flowering time of the treated transgenic plants is only slightly accelerated. FWA and FLC-like genes including MAF3, 4 and 5 are identified to be the other targets increased in transgenic plants and the expression level of FWA is not decreased by the vernalization treatment. Thus, AHL16 regulates the floral transition in *Arabidopsis* by FLC- dependent and independent pathways.

08159

EXPRESSION OF SHORT INTERNODES/STYLISH FAMILY GENES IN AUXIN BIOSYNTHESIS ZONES OF AERIAL ORGANS IS DEPENDENT OF A GCC-BOX-LIKE REGULATORY ELEMENT

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We have recently been able to show that STYLISH1 (*STY1*), which belongs to the *Arabidopsis SHI/STY* transcriptional activator family, regulates auxin biosynthesis by directly binding to, and activating, the promoter of the auxin biosynthesis gene *YUC4* (Eklund et al., 2010; Sohlberg et al., 2006). *STY1* is expressed at the tip of aerial organs, as well as at organ initiation sites. We suggest that *STY1* plays an important role in auxin peak formation at these sites, and that the up-stream regulators of *STY1* expression therefore must play a crucial role during auxin regulated plant development and we are now aiming to identify putative upstream pathways. Because *STY1* appears to have redundant or overlapping functions with other members of the *SHI/STY* gene family we have approached this question by searching for common promoter elements. We have identified a GCC-box in their 5'UTR region and modulation of this sequence resulted in abolished *STY1* expression in aerial organs as well as in the lateral root primordial, suggesting that this promoter sequence is crucial. We are currently searching for putative regulators binding to this promoter sequence.

08160

SOR12, A NEGATIVE REGULATOR OF CYTOKININ MEDIATED DELAY OF LEAF SENESCENCE

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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 inhibition of hypocotyl and root elongation and shoot induction.

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.

08161

MOLECULAR CLONING OF MADS-BOX GENES FROM LILIU FORMOSANUM AND ECTOPIC EXPRESSION IN ARABIDOPSIS TO CHARACTERIZE THE FUNCTION IN FLORAL DEVELOPMENT

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Mechanism of floral development is under the control of a complex genetic system. The ABCDE model for floral development was proposed and since then many studies have been performed on model species, such as *Arabidopsis thaliana* in order to confirm this hypothesis. Floral formation involves the development of four whorls of organs, sepals, petals, stamens, and pistils, that are specified by a set of major flower organ identity genes that include APETALA1 (AP1), APETALA2 (AP2), APETALA3 (AP3), PISTILLATA (PI), AGAMOUS (AG) and several AGAMOUS-LIKE (AGL) genes. Based on the sequence and functional similarity, the MADS box genes play a central role involved in floral development. Here we isolate many MADS-box genes from Formosan lily, a strategy combining RT-PCR with degenerate primer and 5'-RACE or 3'-RACE was used. The deduced amino acid sequence show that full-length LFAG1 gene revealed the MIKC structure and a high homology in the C-function genes among AG and other orthologues. The sequence of LFGLO1 is homologous to B-function gene GLO1 by blast analysis. In addition, the sequence of LFAGL9 showed sequence homology to E-function gene. Spatial expression data showed LFAG1, LFGLO1 and LFAGL9 transcripts exclusively in floral organ by RT-PCR analysis. Functional analysis was carried out in *Arabidopsis* by ectopic express these three genes that driven by the CaMV35S promoter. Phenotypic and expression analysis in these transformed plants will be discussed. Knowledge obtained from this study will help us understanding of the function of lily MADS-box genes and those of orthologs from other plant species would contribute to the elucidation of molecular regulation during floral transition and floral formation.

08162

ROLES OF ANR1-FAMILY MADS-BOX TRANSCRIPTION FACTORS IN REGULATION OF LATERAL ROOT DEVELOPMENT IN ARABIDOPSIS THALIANA

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Nitrogen is one of the macro-nutrients required for plant growth. In addition, it is well known that supply of nitrogen source significantly modifies plant root system architecture. Plasticity of root architecture is considered crucial for adaptation to limiting or excessive supplies of nitrogen source. When roots encounter localized source of nitrate, lateral root growth is stimulated under a nitrate-dependent signalling pathway. Previous studies suggested a MADS-box transcription factor, ANR1, as a key regulator in this signalling pathway. In this study, we characterized a family of ANR1 homologues, AGAMOUS-LIKE 21 (AGL21) and AGL17, in *Arabidopsis thaliana*. The *agl21* mutants showed reduced lateral root growth compared to the wild-type plants when nitrate concentrations were 0.03 - 0.1 mM or when 0.1 mM glutamine was substituted for nitrate as a nitrogen source. The overexpression of *AGL21* was able to restore the growth of lateral roots in the *agl21* mutant, and further stimulated lateral root growth more than the wild-type plants both in the presence and absence of nitrate. Consistent with AGL21's role in regulating lateral root growth, the *AGL21* promoter directed expression of a GFP reporter gene specifically to the root tips of the primary and lateral roots. To investigate their individual roles in root development or redundancy among the family members, *agl21 anr1* double mutant and *agl21 agl17 anr1* triple mutant were generated. The results of the phenotypes of multiple knockout plants and cell-specific localization of *AGL17* and *ANR1* expression will be presented.

08163

ESTABLISHMENT OF THE LASER ABLATION SYSTEM IN SHOOT APICAL MERISTEM (SAM) OF ARABIDOPSIS

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Classical surgical experiments at shoot apical meristem (SAM) clearly suggested that some signaling system(s) is responsible to phyllotaxis determination and abaxial-adaxial axis-dependent tissue development in developing leaves. Recent molecular genetic approach clarified the presence of several intercellular signaling molecules working between SAM and leaf primordia, but the signaling mechanism, especially timing and pathway, is not fully elucidated yet. In order to examine these issues, microsurgical techniques would be a powerful system in addition to the elegant molecular biological techniques. Modern microsurgical system with laser-beam was developed by Cris Kuhlemeier and his group by using tomato. We started to establish laser ablation system for *Arabidopsis*, because many important mutants and gene expression markers are available.

First, we set up a microscope system which ablates single cell with a fine beam of UV laser at 2-3 μm in diameter. Second, we developed a system to ablate vegetative SAM of *Arabidopsis* seedlings of 2-3 days after germination. Based on these preparations, we are examining the effect of ablating cells at central zone (CZ) and peripheral zone (PZ) by *CLV3promoter::GFP* and *FILpromoter::GFP* lines.

08164

SHI GENES AFFECT REPRODUCTIVE DEVELOPMENT IN THE MOSS *PHYSCOMITRELLA PATENS*

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Auxin plays important roles during reproductive organ development in *Arabidopsis*, by affecting e.g. floral organ initiation, apical-basal patterning of the gynoecium, ovule development, pollen formation/release and the pollination process. The *Arabidopsis SHI/STY* family genes encode positive regulators of auxin biosynthesis genes, and several are expressed in e.g. the apical part of developing gynoecia controlling style development, in the ovules and at different stages of pollen development. The moss *Physcomitrella patens* has two *SHI/STY* homologues, *PpSHI1* and *PpSHI2*. We have produced single disruptants of these genes in moss, by homologous recombination. Both mutants exhibit similar vegetative phenotypes including reduced internode elongation, decreased caulinema/chloronema ratio and an increased number of axillary hairs and both mutants show reduced auxin biosynthesis rates and levels in the leafy shoots (Eklund et al., 2010, Development 137:1275-1284). Here we present a closer characterization of the reproductive organs of the moss *shi* mutants, which exhibit shorter female archegonia compared to wild-type. The archegonia of the mutant lines also appear defective in their capacity to open the tip of the tube to allow fertilization. Further, examination of the expression pattern of the reporter lines *pPpSHI1:PpSHI1-GFP* and *pPpSHI2:PpSHI2-GUS* reveals a specific expression in archegonia, antheridia and ovules, which indicates that auxin plays an important role during female and male organ development not only in angiosperms but also in the haploid moss gametophyte.

08165

ROLES OF CYTOKININS IN CAMBIUM ACTIVITY DURING SECONDARY DEVELOPMENT IN ARABIDOPSIS ROOT

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Our previous discoveries have suggested the role of cytokinin in regulating cambial functions in *Arabidopsis* (Mahonen et al. 2000; Mahonen et al. 2006a). We and the others have been able to show that cytokinin signalling induces cambial growth and cytokinins are major hormonal regulators required for cambial development (Niemenen et al 2008, Matsumoto-Kitano et al 2008). To identify genes involved in cambium development and activity and to identify the components downstream of cytokinin signalling, a genome-wide gene expression profiling in combination with Fluorescence Activated Cell Sorting (FACS) was performed. Sorting of cambial cells was based on the procambium/cambium specific marker gene *ARR15::GFP* (Mahonen et al. 2006a) and RNAs from the cambial cells representing three different developmental zones from either cytokinin treated or non-treated *Arabidopsis* roots were used for whole genome chip hybridization. Gene expression data obtained were compared against databases representing gene expressions in various other *Arabidopsis* root cell types and a total of 528 gene expressions were defined as "cambium enriched", from which a group of genes seemed to be cytokinin responsive. These genes are the potential targets of cytokinin signaling in cambium development. Since the role of most of them in vascular development is uncharacterized, we are currently analyzing the identified genes functionally. We are now characterizing corresponding T-DNA insertion knock-out lines and creating over expression lines of the genes of interests.

08166

FUNCTIONAL ANALYSIS OF MADS-BOX GENES IN REGULATING FLORAL TRANSITION OF *CRYPTOMERIA JAPONICA*

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Cryptomeria japonica is one of the most commercially important coniferous tree species in Japan. However, pollinosis caused by *C. japonica* has become serious medical and social problems. Therefore, biotechnological control of flowering is expected as a means of regulating pollen dispersal of *C. japonica*. Studies of *Arabidopsis* have led to the identification of many genes involved in flowering. On the other hand, little is known about the genetic factor of the transition to flowering in conifers. In previous study, the eleven MADS-box genes of *C. japonica* were classified into five clade. In this study, we describe the effect of these MADS-box genes on flowering time in transgenic *Arabidopsis* and discuss the possibility of involvement of these MADS-box genes on flower development in *C. japonica*.

To determine the effect of MADS-box genes of *C. japonica* on flowering time, six kinds of MADS-box genes under the control of CaMV 35S promoter were transformed into *Arabidopsis* plants. Two of six transgenic *Arabidopsis* lines flowered earlier than wild type plants under long day conditions. One belonged to SOC1 clade (*CjSOC1*), the other belonged to AP1/SEP clade (*CjAP1/SEP*). Transformants expressing exogenous genes at high level flowered earlier than those at low level. These results suggest that these two genes participate in the regulation of flower development.

To examine whether *CjSOC1* and *CjAP1/SEP* participates in the regulation of flower development in *C. japonica*, we examined seasonal change of expression of these two genes in shoots treated with gibberellin to induce bud formation artificially. Expression of *CjSOC1* increased in a week after gibberellin treatment. On the other hand, expression of *CjAP1/SEP* increased in one month after treatment, and then flower buds were visible. Our results suggest that these two genes could function to promote flowering in *C. japonica*.

08167

MOLECULAR DISSECTION OF CLV3 SIGNALLING.

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In *Arabidopsis*, the CLAVATA (CLV) pathway operates in the regulation of the size of the stem cell population in the shoot apical meristem. CLV3 functions as a small peptide ligand to negatively regulate the expression of WUSCHEL transcription factor through

two major receptor complexes of CLV1 and CLV2-SUPPRESSOR OF LLP1-2 (SOL2)/CORYNE (CRN). In addition to these two genetically independent pathways, our recent genetic study has identified a new member of receptor, CLV3 PEPTIDE INSENSITIVE1 (CLI1) as the third signalling route of the CLV3 signalling. Our current project aims to understand the precise molecular details of the CLV3 signalling mediated by these receptor-like proteins in a target cell. We are conducting biochemical studies on these receptor functions. Our various mutational screenings for the CLV3 synthetic peptide insensitivity identified a number of potential candidates. Here, we present current progresses in our studies towards understanding the molecular basis of CLV3 peptide signalling.

08168

OVEREXPRESSION OF A *BRASSICA RAPA NGATHA* GENE IN *ARABIDOPSIS THALIANA* NEGATIVELY AFFECTS CELL PROLIFERATION DURING LATERAL ORGAN AND ROOT GROWTH

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In an effort to elucidate biological functions of transcription factors of *Brassica rapa* L. (ssp. *pekinensis*), an *NGATHA* homologue, *BrNGA1*, that belongs to the B3-type transcription factor superfamily was identified and expressed in *Arabidopsis thaliana* under the control of the CaMV 35S promoter. *Arabidopsis* plants overexpressing *BrNGA1*, named *BrNGA1ox*, displayed markedly reduced organ growth compared to the wild type: lateral organs, such as leaves, flowers, and cotyledons, were small and distinctively narrow, and their root growth was severely retarded as well. Reduced sizes of *BrNGA1ox* organs were mainly due to reduction in cell numbers. Kinematic analysis on leaf growth revealed that both the rate and duration of cell proliferation declined during organogenesis, which was consistent with the reduced expression of cyclin genes. Reduction in organ growth was strongly correlated with the small size of meristematic cell pools in the shoot and root meristems. Taken together, these data indicate that *BrNGA1* acts as a negative regulator of cell proliferation and may do so, in part, by regulating the size of the meristematic cell pool.

08169

GENETIC INTERACTION BETWEEN *GRF* AND *CUC* GENES IN ORGAN SEPARATION OF *ARABIDOPSIS THALIANA*

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Double and triple mutations in *CUP-SHAPED COTYLEDON* (*CUC*) genes of *Arabidopsis thaliana* caused defects in the separation of cotyledons and floral organs as well as in the formation of shoot apical meristem (SAM). *grf1 grf2 grf3 grf4* quadruple mutation in *GROWTH-REGULATING FACTOR* (*GRF*) gene family also displayed weak but similar defects in the separation of cotyledons and SAM formation. The present study shows that the *grf* quadruple mutant developed fused floral organs as well. Construction and analysis of various combinations of *cuc grf1/2/3* quadruple mutants revealed that *cuc grf* quadruple mutants synergistically increased the fusion of cotyledons and floral organs, indicating that *CUC* and *GRF* genes genetically interact in regulating organ separation processes, probably through the same pathway. However, neither *CUCs* affected gene expression level of *GRFs* nor *GRFs* did that of *CUCs*, indicating that they may not act in a linear fashion. Taken together, the present study suggests that *CUC* and *GRF* proteins may form a functional complex involved in regulating the organ separation process.

08170

FUNCTIONAL ANALYSIS OF *GRF-INTERACTING FACTOR* GENE FAMILY IN REGULATING FLORAL ORGAN DEVELOPMENT OF *ARABIDOPSIS THALIANA*.

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Previously, the *GRF-INTERACTING FACTOR* (*GIF*) transcription coactivator gene family comprising three members was characterized as a positive regulator of cell proliferation in *Arabidopsis thaliana*. In this study, we present evidence that the *GIF* gene family is also required for the indeterminacy of the inflorescence meristem and normal development of floral structure, including ovule development. First, we found that *gif1/2/3* triple mutant completely consumed the inflorescence meristem later in its life cycle. Second, carpels of *gif1/2/3* triple mutant are not fused at all, exposing ovules. Third, the second and third whorls developed fewer organs, frequently showing petaloid and stamenoid structures. Finally, mutant ovules showed abnormal development of inner and outer integuments. These *gif1/2/3* phenotypes resemble those of *ant*, *crc* and *seu* mutants. Function of *GIF* genes in floral organ development will be discussed with respect to *ANT*, *CRC* and *SEU*.

08171

PX1 IS REGULATOR OF VASCULAR DEVELOPMENT POSSIBLY INVOLVED IN SPLICING

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Research during past years has established plant hormone cytokinin as a crucial regulator of *Arabidopsis* vascular development. Protein AHP6, a member of cytokinin transduction cascade has been demonstrated to play a critical role during protoxylem formation. In order to find a molecular link between cytokinin signaling and possibly novel pathways in vascular development, we performed a genetic screen, using AHP6::GFP as marker of cytokinin activity in protoxylem. Among others, *px1* mutant has been isolated. Besides its aberrant protoxylem formation, *px1* shows a pleiotropic phenotype, including defective gravitropism, abnormal cotyledonal development or altered hormonal responses. Rough mapping and deep sequencing of the mutant genome revealed that PX1 codes for a weak allele of an embryonic lethal gene possibly involved in splicing. Based on its orthologs from animal systems, we explore role of alternative splicing in vascular development and propose PX1 as one of its important factors.

08172

GENETIC ANALYSIS OF THE CLV SIGNALING PATHWAY IN THE SAM

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The shoot apical meristem (SAM) is the fundamental structure located at the growing tip and gives rise to all aerial parts of plant tissues and organs. In *Arabidopsis thaliana*, the CLAVATA (CLV) signaling pathway regulates the stem cell pool in the SAM, in which a small

peptide ligand, CLV3, is perceived by two major receptor complexes, CLV1 and CLV2-CORYNE (CRN) /SUPPRESSOR OF LLP1 2 (SOL2), to restrict the expression of *WUSCHEL* (*WUS*), a transcription factor promoting the stem cell pool. Recent genetic studies have revealed that these two complexes comprise two independent pathways of the CLV signaling (Mueller *et al.* 2008). However, it is still possible that there are additional signaling routes for the CLV3 recognition, since the SAM defect of *clv1 crn* is weaker than that of *clv3*.

In order to identify novel molecular components operating in the CLV signaling pathway, we have performed mutational screens for insensitivity to the synthesized CLV3 peptide (MCLV3). A total of 14 mutants, designated *clv3 peptide insensitive* (*cli*) mutants, were isolated for maintaining the SAM, among which, *cli1* showed the strongest resistance to the MCLV3. Furthermore, when grown under the MCLV3-free condition, *cli1* exhibited the typical *clv*-like phenotypes such as enlarged SAM and increased number of floral organs. Positional cloning revealed that *CLI1* encodes a receptor-like kinase. Further genetic studies suggest that *CLI1* transmits the CLV3 signal in the SAM, independently of two known CLV3 signaling pathways, namely, the CLV1 and the CLV2-CRN/SOL2 pathways. Our current progress will be presented.

08173

TDIF PEPTIDE SIGNALING REGULATES VASCULAR STEM CELL PROLIFERATION VIA THE WOX4 HOMEOBOX GENE IN *ARABIDOPSIS*

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Indeterminate property of plant growth and development depends on stem cells which reside in specific proliferative tissues called meristems. Vascular tissues continue to grow in the radial direction due to the activity of the vascular meristem. In the lateral meristem, cell division and differentiation of undifferentiated procambial cells are well controlled to maintain vascular tissue organization. We have found that cell-cell signaling through a ligand-receptor pair, the TDIF peptide and TDR/PXY LRR-RLK, controls this process in *Arabidopsis* (Hirakawa *et al.*, 2008). However, the molecular mechanism of the intracellular pathway downstream of TDIF-TDR, which regulates both proliferation and xylem-differentiation of the procambial cells, is largely unknown.

To identify components of this pathway, we searched for genes whose expression levels were altered by TDIF. Of them, *WOX4* was rapidly up-regulated in response to TDIF application in a TDR-dependent manner. Genetic analysis indicates that *WOX4* acts in the same genetic pathway as TDIF and *TDR* to control proliferation but not xylem-differentiation of the procambial cells. Observation of mutant phenotypes in the mature plants clearly shows that the TDIF signaling is required for the maintenance of the vascular meristem. Our results suggest that the TDIF signaling diverges into at least two pathways in its intracellular signal transduction to control the proliferation and differentiation of vascular stem cells, which contributes to the maintenance of the vascular meristem.

08174

#27. MUTATION AFFECTS LEAF POLARITY AND LEAF VENATION PATTERNS IN *ARABIDOPSIS THALIANA*.

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In dicot plants, leaf primordia are derived from the shoot apical meristem and develop along proximal-distal, adaxial-abaxial, and medial-lateral axes. The establishment of adaxial-abaxial polarity is required for lateral expansion of leaf blade. In *Arabidopsis thaliana*, class III HD-ZIP, *YABBY* and *KANADI* transcription factor families are involved in establishment of adaxial-abaxial polarity through several pathways. The loss-of-function mutations in the *ASYMMETRIC LEAVES2* (*AS2*) genes of *Arabidopsis thaliana* cause pleiotropic phenotypes in leaves, such as downward curling of leaves, reduced complexity of leaf venation pattern, generation of lobes, and weak defect in the leaf adaxial-abaxial polarity. *AS2* encodes a protein with plant specific AS2/LOB domain. *AS2* transcripts are accumulated in the adaxial domain of young leaves. Our previous study suggest that *AS2*, directly or indirectly, represses the expressions of class 1 *KNOX* genes and abaxial factor genes, *ETTIN*, *KANADI2* and *YABBY5*. To identify new components that function together with *AS2* in leaf development, we analyzed enhancers of the *as2-1* mutant. #27 *as2-1* and #43 *as2-1* mutants formed filament-like leaves associated with defect in the adaxial-abaxial leaf polarity. #27 mutation also caused weak defects in leaf venation patterns. We will also present genetic analysis between #27 and other mutants to discuss genetic relationship among them.

08175

A ROOT PREFERENTIALLY EXPRESSED PHOSPHATIDYLINOSITOL/PHOSPHATIDYLCHOLINE TRANSFER (*PIT*) GENE REGULATES *ARABIDOPSIS* ROOT HAIR GROWTH BY INFLUENCING VARIOUS SUBCELLULAR COMPONENTS

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Root hairs are the critical site where plants absorb water and nutrients from soil. The establishment of root hair architecture is a polarized growth procedure in which many subcellular components have been documented to participate. *COW1*, a previously described *Arabidopsis* *PIT* (phosphatidylinositol/phosphatidylcholine transfer) gene, was shown to control root hair growth by regulating membrane trafficking, Ca²⁺ signaling, and cytoskeletal structure. In addition, according to our phylogenetic and gene expression studies in specific tissues, we found that *COW1* is the only *PIT* gene that is preferentially expressed in *Arabidopsis* roots. To further investigate the function of *COW1* and search for co-effectors during root hair development, we compared the transcript profiles of wild type and *cow1* mutant. This analysis was accomplished using a method for efficiently enriching a large quantity of root hair tissues from 4 day old seedlings growing on 1% MS plates. The resulting root hairs were processed for mRNA and subsequently synthesized for cDNA for hybridization on microarray chips. The gene array result indicated that blocking *COW1* gene changed the expression of 105 genes (more than two fold), 89 of which were down-regulated and 16 of which were up-regulated. Importantly, among these genes, majority of them are annotated to be membrane-related cellular components. This suggested that the function of *COW1* requires the involvement of many other membrane-related proteins. Moreover, to validate our microarray result, we identified several *Arabidopsis* knock-out lines that were null mutants of a few of the co-effector genes. These plants mimicked the root hair morphological defects of the *cow1* mutant. Taken together, our root hair specific microarray data provides a precious resource to identify genes that are involved in the genetic regulation of root hair development by interacting with *COW1*.

09001

ARABIDOPSIS PROTEIN QUANTIFICATION BY TARGETED MASS SPECTROMETRY

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The identification and quantification of a protein or a set of proteins is essential for functional analysis and can serve to untangle signaling networks. Detection of proteins by chromatographic or electrophoretic methods combined with immunochemical techniques is limited by the necessity for specific antibodies and by the methodical difficulties inherent in quantifying the detected proteins. Mass spectrometry on the other hand, has made major advances in the past and can be used to detect specific proteins in complex protein mixtures by Selected Reaction Monitoring (SRM). Highly abundant proteins can be quantified by developing measurement routines trained using existing public data sets. However, for low abundant proteins such data sets barely exists. Additionally it is difficult to detect peptides of these proteins in a complex protein mixture. For these reasons quantitative information of low abundant proteins is rare, despite the fact that it is crucial for understanding basic biological processes. We established a procedure allowing us to detect and quantify low abundant proteins, such as signal transduction components, by using recombinant 15N-labelled peptides as standards for SRM measurements of complex extracts from *Arabidopsis* seedlings. By this means we were able to obtain quantitative data on the plant photoreceptor phytochrome A in a spatiotemporal manner under different activation states.

09002

MICROARRAY ANALYSIS OF mRNA TRANSLATION STATE

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Novel modalities of genome-wide RNA analysis have yielded datasets for several of the events that shape the fate of individual transcripts, from transcription and splicing all the way to RNA turnover by degradation. In between, regulation at the level of translation, which occurs in response to environmental perturbation and is increasingly recognized as a factor affecting plant development. The molecular machinery that controls how efficiently an mRNA is translated is not well understood and the corresponding signaling pathways are largely unknown. Our work is guided by the hypothetical concept that the *Arabidopsis* transcriptome is organized into regulons of translational control. We employ microarray analysis of translation state, in conjunction with traditional gene expression assays and computational modeling, to attribute molecular functions to individual components of the translation machinery. For example, the h subunit of eIF3 (At1g10840) helps to ameliorate the inhibitory effects of short upstream open reading frames that are present in the 5' leader of many *Arabidopsis* mRNAs. These genome-wide datasets also reveal unexpected correlations between mRNA sequence features and translation state that shed light on heretofore unsuspected functions of the eIFs. A challenge for the future consists of integrating data on translation state across different conditions with datasets from other 'ribonomics' modalities in such a way that trends can be visualized and interpreted.

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09003

HORMONE ANALYSIS IN ARABIDOPSIS AND RICE USING A HIGHLY-SENSITIVE AND HIGH-THROUGHPUT PLANT HORMONE MEASUREMENT SYSTEM

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We have developed a highly sensitive and high-throughput method for the simultaneous analysis of 43 molecular species of cytokinins, auxins, ABA, and gibberellins (GAs) using an automatic liquid handling system for solid phase extraction, UPLC-ESI-qMS/MS, and chemical derivatization with bromocholine. Our current method needs less than 100 mg (usually 10 to 100 mg fresh weight) of plant tissues to determine phytohormone profiles and enables us to analyze simultaneously more than 180 plant samples. Application of this method to plant hormone profiling enabled us to draw organ-distribution maps of hormone species in *Arabidopsis* and rice and also to identify interactions among the 4 major hormones in the rice GA-signaling mutants, *gid1*, *gid2*, and *slr*. Combining the results of hormone profiling data with transcriptome data in the GA signaling mutants allows us to analyze relationships between changes in gene expression and hormone metabolism. We are now trying to identify key genes regulating hormone levels in rice using QTL analysis. We have measured hormone contents of Sasanishiki X Habataki BILs. The data will be presented.

09004

FAST (FLUORESCENCE-ACCUMULATING-SEED TECHNOLOGY) PROVIDES A RAPID AND ANTIBIOTIC/HERBICIDE-FREE METHOD FOR IDENTIFYING TRANSFORMED SEEDS OF ARABIDOPSIS THALIANA

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The generation of transgenic plants has contributed extensively to the progression of plant science. Establishing homozygous transgenic lines is time-consuming and laborious, and using antibiotics or herbicides to select transformed plants may adversely affect the growth of some transgenic plants. Here we show a novel technology, which we have named FAST (Fluorescence-Accumulating-Seed Technology), that overcomes these difficulties. Although this technology was designed for use in *Arabidopsis thaliana*, it may be adapted for use in other plants. The technology is based on the expression of a fluorescent co-dominant screenable marker FAST, under the control of a seed-specific promoter, on the oil body membrane. The FAST marker harbors a fusion gene encoding either GFP or RFP with an oil body membrane protein (oleosin 1) that is prominent in seeds. The marker protein was only expressed in a specific organ (i.e. in dry seeds) and at a specific time (i.e. during dormancy), which are desirable features of selectable and/or screenable markers. This technique provides an immediate and non-destructive method for identifying transformed dry seeds. It identified the heterozygous transformed seeds among the T1 population and the homozygous seeds among the T2 population with a false-discovery rate of <1%. The FAST marker reduces the length of time required to produce homozygous transgenic lines from 7.5 to 4 months. Furthermore, it does not require sterilization, clean-bench protocols or the handling of large numbers of plants. We further generated ten purpose-designed variations of the FAST vectors. This technology should greatly facilitate the generation of transgenic *Arabidopsis* plants.

Shimada, T. L. et al. (2010) *Plant J.*, 61: 519-528.

09005

ISOLATION AND CHARACTERIZATION OF SUMO BINDING PROTEINS FROM ARABIDOPSIS

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Reversible conjugation of the small ubiquitin modifier (SUMO) peptide to proteins (sumoylation) plays important roles in many cellular processes including DNA repair, subcellular localization, transcriptional regulation, cell cycle progression, or ubiquitination antagonism in animal and yeast. Sumo binding itself seems to provide some biological function. In plants, sumoylation is also involved in abiotic stress responses (phosphate deficiency, heat, low temperature, and drought) and biotic stress (defense reactions against pathogen infection), and flowering time. With more than 200 SUMO target proteins characterized in animals and yeasts, only very few proteins (AtMYB30, PHR1, ICE1, FLD, GTEs) have been identified as SUMO targets in plants. To identify and characterize the complexity of plant sumo targets, and their biological function, we have developed two independent proteomic-based approaches. The first includes *Arabidopsis* plants over-expressing AtSUMO1 fused to the 6His- 3Flag tag. After protein extraction from the transgenic plants and Ni affinity chromatography followed by 2D SDS-PAGE, MALDI-TOP mass spectrometry is used to identify gel-excised proteins. A second system was designed that uses affinity chromatography with His-GST-AtSUMO1 as the bait to obtain putative targets from *Arabidopsis* total protein extracts. SUMO binding protein complexes are then purified and identified by EI Mass spectrometry. To date, we have identified more than 20 novel SUMO binding proteins. As a control, these proteins were shown to interact with AtSUMO1 by yeast split-ubiquitination assays and split-luciferase complement assays in plants. SUMOylation pattern and functions of these novel SUMO targets will be discussed.

09006

RIKEN SCINES: A SEMANTIC-WEB DATABASE INTEGRATING PHENOME OF ARABIDOPSIS THALIANA

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Arabidopsis thaliana is a popular experimental model plant, and its phenome data are especially important for basic and applied plant science. It is desired to produce databases focusing phenome of this plant, with standardized description of phenotypes with links to other omic information, but improvement of such databases has not been sufficiently realized.

RIKEN BASE has established the database integration system named "RIKEN SciNeS" (<https://database.riken.jp/>), into which various kind of omics databases related to *Arabidopsis* have been integrated. SciNeS is based on semantic web technology. Thus we can define novel terminology using ontologies and can describe the meaning of relationships among data in a machine-readable format. It is useful for studies of combined heterogeneous data over omics, such as phenotypes and genotypes.

To realize such database integration, we first focused three databases of *Arabidopsis* mutant lines, namely FOX hunting line, *Ds* transposon line, and activation tagging line, which are integrated into SciNeS. They include phenotypic information mainly about morphological traits, described with terms independently defined by each database. We defined ontology of those phenotypes using existing ontologies including PO (Plant Ontology), and PATO (the ontology of phenotypic qualities), and published them as the novel database (<http://scines.org/item/ria143i>), with links to relating records of other databases in SciNeS.

We proceed with development of a novel integrated database comprehending *Arabidopsis* phenome more widely, including phenotypes about morphology of vegetative and reproductive organs, biotic and abiotic stress tolerance, and biochemical characteristics. As a part of this attempt, we have surveyed published information comprehensively, listed descriptions of phenotypes with related information such as experimental conditions, and proceed with development of the novel phenotype ontology.

09007

NEW CLASSES OF STOCKS AND EXPANSION OF STOCK HOLDINGS AT ABRC

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The Arabidopsis Biological Resource Center (ABRC) endeavors to maintain the most comprehensive and relevant collections of Arabidopsis seed and DNA stocks, to best serve the plant research community. In this spirit, we are presently expanding our holding in two areas. First, we are acquiring new classes of stocks for distribution. Second, we are expanding our seed and DNA holding to related plants, especially Brassica. The following new classes of stocks are available from ABRC: a) Cell culture lines; the Arabidopsis T87 cell culture, derived from the Columbia ecotype. Due to shipping limitations, these are currently distributed only within the USA. b) Protein chips; generated by S.P. Dinesh-Kumar et al, contain ~5,000 spotted proteins; it is hoped that this resource will be widely used in continued genomics research. c) Antibodies; Initial steps have been taken to start the acquisition and distribution of antibodies. Aliquots of custom-made antibodies are being obtained from a select group of researchers, as a first stage of an antibody donation campaign that will begin in 1/2011.

With the sequencing of *Brassica* species, these resources offer an excellent platform for comparative genomic, transcriptomic, proteomic and metabolomic studies. Some *Brassica* resources are already available at the Center, including 52 stocks of *B. napus*, *B. rapa* and *B. oleracea*. In addition, several researchers have committed to deposit *Brassica* resources at the ABRC in the next few years.

An independent catalog that makes the community aware of these resources is also part of our ongoing endeavors.

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09008

LILLY POLLEN PROTOPLAST SYSTEM FOR FUNCTIONAL ANALYSIS OF PLANT AQUAPORINS

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Aquaporins facilitate water flux across biomembranes, and are involved in various physiological phenomena in several plant tissues. Generally, the water-flux activity of exogenously expressed aquaporins is measured in *Xenopus laevis* oocytes or yeasts. The heterogeneous

systems are likely to be not optimal for analysis of plant aquaporins. We analyzed the water-flux activity of plant aquaporins using lily (*Lilium longiflorum*) pollen protoplasts. Large protoplasts with a uniform diameter of approximately 95 µm were isolated from lily nearly mature pollen grains. Furthermore, no plasma membrane aquaporin (PIP) was detected in lily pollen. For ectopic expression of PIPs in the lily pollen protoplasts, we made the plasmid constructs in which *Arabidopsis thaliana* *AtPIP1;1*, *AtPIP2;1*, *Daucus carota* (carrot) *DcPIP1;1*, or *DcPIP2;1* were put under the control of the strong pollen-specific promoter, maize *Zm13* promoter. The PCR amplified DNA fragments were introduced into the pollen protoplasts by electroporation, and then 45%-60% of protoplasts were transformed successfully. The pollen protoplasts expressed *AtPIP2;1* or *DcPIP2;1* increased significantly their volumes in hypotonic solution (350 mM mannitol) compared with the vector control. By contrast, the volume changes of the protoplasts expressed *AtPIP1;1* or *DcPIP1;1* were similar to that of the vector control. These results suggest that *AtPIP2;1* and *DcPIP2;1* show higher water-flux activity in plant cells, whereas *AtPIP1;1* and *DcPIP1;1* do not. Thus, we propose the lily pollen protoplasts as a simple and useful experimental system to analyze the function of plant aquaporins.

09009

DEVELOPMENT OF NEW METHODS TO IDENTIFY TRANSCRIPTION FACTORS THAT INTERACT WITH PROMOTER REGION

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To identify transcription factors (TFs) that would interact with 5' upstream region of a gene, yeast one-hybrid screening (Y1H) is often used. For Y1H, we need to identify cis-regulatory region and multimerize it for placing them in upstream of reporter gene. In addition, large-scale screening is required because non-TF genes mostly occupy cDNA library. To overcome such problems, we constructed novel cDNA library that only contains TF genes. Results of our test experiments showed that several transcription factors were identified with efficiency more than 100-fold even though whole 500-1000bp promoter region was directly used as a probe. However, false-positive or false-negative result was sometimes observed in the yeast experimental system. Therefore we are now trying to develop new method using plant instead of yeast. We will report the results of comparison of these methods to identify upstream transcription factors.

09010

ABRC GENOMICS AND PHENOMICS STOCKS AND ACTIVITIES, 2010

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The Arabidopsis Biological Resource Center (ABRC) maintains seed stocks relevant to genome exploration and functional genomics. Sequence-indexed insertion collections are distributed, as early-generation segregating progeny of transformants and some as confirmed, homozygous lines. Included are T-DNA lines from 1) SALK Institute, J. Ecker lab, 2) Syngenta Biotechnology, 3) GABI-Kat lines, 4) the Wisconsin Ds-Lox population, and 5) the Saskatoon population. We are presently making the homozygous lines available as full single-line and pooled sets. In addition, the transposon lines from Cold Spring Harbor Laboratory and John Innes Centre, as well as the RNAi lines from the Chromatin Functional Genomics and AGRIKOLA consortium are available. Various resources for genome-based investigation of natural variation are also included in the collections.

Most of the DNA resources can be directly applied to genomics, notably: 1) the majority of the SSP ORF/cDNAs available in a Gateway™ Entry vector; 2) the Gateway™ compatible yeast two hybrid bait and prey clones from M. Vidal and J. Ecker; 3) the split ubiquitin vectors and clones from W. Frommer; and 4) the organelle-targeted, multi-color GFP vectors from A. Nebenfuehr; 5) expression constructs from S. P. Dinesh-Kumar, S. Clouse, and J. Doonan; and 6) versatile destination and other vectors.

The number of full length entry cDNA clones at ABRC is over 32,000, representing approximately 16,889 loci and includes donations from SSP (J. Ecker, A. Theologis, R. Davis), Salk (J. Ecker), TIGR (C. Town), Peking/Yale (X. W. Deng), J. Callis, members of the Assocomics consortium and CNRGV (C. Lurin).

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09011

FORWARD CHEMICAL GENETICS IN ARABIDOPSIS WITH A NOVEL "TAGGED" AND FLUORESCENT SMALL MOLECULE LIBRARY

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A current problem in the application of forward chemical genetics is the elucidation of target proteins of bioactive compounds. As one approach to addressing this, we have created a library of fluorescent small molecules "tagged" with chemical handles that enable affinity chromatography of target proteins without a priori knowledge. Additionally, the use of a fluorophore tag enables other applications. For instance, fluorophores in bioactive compounds can be utilized for detection *in vivo* and *in situ* to localize organelle compartments of the bioactive small molecules, or associated proteins. Often, downstream target identification efforts can be hampered by low affinity interactions. To address this, our design strategy enables, "tagged" hits to be converted into a form containing a photo-reactive diazirine group that can be activated to produce a covalent bond to the target in addition to the fluorescent labeling.

Our "tagged" library was created utilizing the highly reactive and stereospecific ligand stabilized Copper (I)-catalyzed Click Reaction to produce 32,000 unique reactions from 4,000 terminal acetylene drug-like building blocks and eight novel azide fluorophores. The reaction mixtures were screened for inhibitors of cell expansion and growth in etiolated *Arabidopsis* seedlings to yield a handful of compounds that induce novel phenotypes. These compounds are currently under investigation. Our research provides proof-of-principle for tagged bioactive compounds that induce strong phenotypes in *Arabidopsis*. These tagged small molecules should facilitate target identification and accelerate chemical genetics based discoveries.

09012

REFERENCE GENES FOR MORE ACCURATE NORMALIZATION OF QRT-PCR DATA IN ARABIDOPSIS

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Quantitative reverse transcription (qRT)-PCR analysis is a powerful tool for quantifying gene expression levels. For the accurate determination of gene expression level by qRT-PCR, genes stably expressed under given conditions, the so called 'reference genes', for normalization constitute a prerequisite. Here, we experimentally recommended sets of reference genes that are suitable for gene quantification in qRT-PCR analysis. We validated the expression stability of the potential candidate genes identified by Czechowski et al. (2005) under six experimental conditions (time-course, photoperiods, ambient temperature, diurnal expression, different tissues, and defined growth stages). We found that expression of the putative reference genes was considerably more stable than that of ACTIN (ACT), TUBULIN (TUB), POLYUBIQUITIN (UBQ10) and ELONGATION FACTOR-1a (EF-1a), four commonly used housekeeping genes for reference genes. Based on the systematic validation in this study, we recommend starting pool of reference genes for more accurate normalization in qRT-PCR analysis.

09013

COMPREHENSIVE TRANSCRIPTOME ANALYSIS REVEALS HYDROCARBON BIOSYNTHESIS PATHWAYS ACTIVE IN OIL-PRODUCING GREEN ALGA *BOTRYOCOCCUS BRAUNII*

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Botryococcus braunii produces long-chain liquid hydrocarbon compounds resembling petroleum through photosynthesis. *Botryococcus* strains are classified into different races, depending on the type of hydrocarbons they produce. Strains of two major races, Race A and Race B, produce alkadiene / alkatriene and triterpene, respectively. Because little genetic information existed for this species, here we obtained, via 454 sequencing, novel datasets of approximately 200,000 each complementary DNA reads for representative Race A and Race B strains. This allowed us to retrieve candidate genes for majority of enzymes involved in the alkadiene / alkatriene biosynthesis, namely, the enzymes for biosynthesis of fatty acids and very long fatty acids (VLFAs). Comparison of the transcriptome profiles for Race A and Race B revealed transcripts for enzymes involved in the glycerolipid metabolism and VLFA elongation were significantly more abundant in Race A, suggesting that the substrates for the VLFA biosynthesis in the Race A strain are likely to be derived from glycerolipids. Regarding the triterpene biosynthesis, candidate genes were retrieved for four out of the seven steps of reactions in the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway, while no genes were retrieved for the mevalonate pathway, suggesting that liquid hydrocarbons are biosynthesized through the MEP pathway in Race B.

09014

ARABIDOPSIS DELETION MUTANT COLLECTION PRODUCED BY HEAVY-ION BEAM IRRADIATION

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The Heavy-ion beam has a high linear energy transfer (LET). LETs of γ -ray and X-ray are 0.2 keV/ μ m and 2 keV/ μ m, respectively. By contrast, LETs of heavy-ion beams in the RIKEN RI-beam factory have a range from 22.5 keV/ μ m to 4,000 keV/ μ m. The high-LET irradiation would physically induce DNA double-strand breaks. Thus, heavy-ion beam induced mutants tend to have a deletion mutation. In this study, we created *Arabidopsis* deletion mutant collections by heavy-ion beam irradiation to develop tools for functional genomics. Dry seeds were irradiated with $^{12}\text{C}^{+}$ (22.5 keV/ μ m, 30 keV/ μ m, or 290 keV/ μ m), $^{40}\text{Ar}^{17+}$ (290 keV/ μ m), or $^{56}\text{Fe}^{24+}$ ions (640 keV/ μ m). In total, 73,691 M_2 plants derived from 8,888 M_1 plants were screened. Through the screening, around 600 of morphological mutants, including pale green, variegated, early flowering, and narrow-leaved mutants were obtained. We previously found that the most effective LET (LET_{max}) for albino-mutant induction is 30 keV/ μ m for *A. thaliana* (Kazama et al., 2008). With LET_{max}-irradiation, the albino mutant incidence was about three times greater than that with other LET irradiations. To confirm the effectiveness of LET_{max} on any other mutant induction, mutation rate for the *elongated-hypocotyl* (*hy*) and *globrous* (*gl*) mutants was calculated as described in Koornneef et al. 1982. The *hy* and *gl* mutation rate was 1.04% after LET_{max}-irradiation, while those were less than 0.55% after the other LET-irradiation. The mutation rate after LET_{max}-irradiation was three times greater than that after X-ray irradiation (0.32%), and was slightly greater than that after fast neutron irradiation (0.84%) and EMS treatment (0.87%). These results suggest that the LET_{max}-irradiation is the most effective in *Arabidopsis* mutagenesis.

09015

LET-DEPENDENT EFFECT OF HEAVY-ION BEAM IRRADIATION ON MUTATION INDUCTION IN RICE

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Heavy ion beams have high linear energy transfer (LET) and produce more localized ionization than other radiation such as X-rays and γ -rays for the same dose. Therefore, a heavy ion particle is postulated to cause double strand break of DNA and induce mutation with low dose irradiation. LET of ion beams is an important factor affecting mutagenesis. In *Arabidopsis*, the lethality rate and flowering rate after ion beam treatment are affected by LET. Furthermore, there is an optimum LET value for mutation induction (Kazama et al. 2008). We examined the effect of LET on mutation induction in rice. Imbibed seeds of rice (*Oryza sativa* L. cv. Nipponbare) were exposed to C or Ne-ion accelerated to 135MeV/u by RIKEN Ring Cyclotron at RI-Beam Factory. The dose range and the LET range of the beam were 5 to 40Gy and 22.5 to 100keV/ μ m respectively. The LET values were calculated at the surface of the seeds. M_1 plants were grown in the paddy field and harvested individually. Survival rates and seed fertilities were surveyed in M_1 plants. The mutation rate was estimated by the frequency of M_1 plants that produced CDM (chlorophyll-deficient mutants) in their progenies. The survival rate did not decrease at lower than 15Gy irradiation with any LET values, and decreased as the LET value increase at more than 20Gy irradiation. In over all the seed fertility decreased as the dose increased, and there was no significant difference with increased LET values. Mutation rates were low at any dose of irradiation with more than 80keV/ μ m. The irradiations at 15Gy with 50-70keV/ μ m were most effective for CDM induction in both ions. These results show that increase of mutation rates is achieved by controlling LET in adequate dose irradiation. In this study, we isolated several mutant lines such as salt-tolerant, bronzing, earliness and dwarf. These mutants could be important as genetic resources

for research in plant functional genomics.

09016

LET DEPENDENT EFFECT ON DNA DAMAGE IN IRRADIATION OF HEAVY-ION BEAM TO ARABIDOPSIS THALIANA

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The linear energy transfer (LET) is important factor for mutation induction by heavy-ion beam, and we reported that the LET of heavy-ion beam affects the mutation rate (Kazama et al. 2008). In *Mesorhizobium loti*, symbiotic bacterium, irradiation of iron ion showed a tendency to induce larger deletions than that of carbon ion (Ichida et al. 2008). As LET of the iron ion (640 keV/μm) has higher than that of the carbon ion (23-40 keV/μm), we postulate that the LET may also influence the deletion size in the mutated genes. In the present study, therefore, we analyzed the mutations induced by heavy-ion beam with different LETs. Dry seeds of *A. thaliana* (Col-0) were irradiated with ¹²C⁶⁺ (23 keV/μm, 30 keV/μm, and 290 keV/μm), ⁴⁰Ar¹⁷⁺ (290 keV/μm), or ⁵⁶Fe²⁴⁺ (640 keV/μm). For detection of mutations, *elongated hypocotyl* (*hy*) and *globrous* (*gl*) mutants were screened in M₂ generation, and then responsible genes of the mutants were sequenced. We analyzed the mutations in genes of the 31 mutant lines. Mutation sites on the genes contained base substitution, deletion, and insertion. Moreover, chromosomal rearrangement was also observed. The heavy-ion beam irradiation predominantly induces deletion-type mutation (26 lines/31 lines). In carbon ion irradiation with 23 and 30 keV/μm, all of the deletion sizes were less than 1 kbp. On the other hand, large deletion sizes, such as 7 kbp, 33 kbp, and 80 kbp, were observed in ion beam irradiated with 290 and 640 keV/μm. These results suggest that deletions induced by heavy-ion beam have a tendency to increase the size with increasing LET. Taken together, we discuss a possibility of deletion size controlled mutagenesis by selecting appropriate LETs.

09017

COMPARATIVE PHOSPHOPROTEOMICS IN PLANTS

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Knowledge of phosphorylation events and their regulation is crucial to understand the functional biology of plants. In the case of the well-characterized model plant *Arabidopsis*, more than several thousand phosphorylation sites have been identified by recent large-scale phosphoproteome studies. However, transferring information on phosphorylation from *Arabidopsis* to other agronomically important plant species still remains a considerable challenge, because of limited evidence that conserved residues are modified in the same manner in different plant species.

To investigate the conservation of phosphoproteomes in plants, we performed comparative analyses of *Arabidopsis* and rice phosphoproteomes. For this purpose, we identified 5,143 and 6,919 unique phosphopeptides from *Arabidopsis* and rice cells, respectively. Despite of the phylogenetic distance and the use of different cell types, more than 50% of the phosphoproteins identified in *Arabidopsis* and rice, which possessed ortholog(s), had an orthologous phosphoprotein in the other species. Moreover, nearly half of the phosphorylated orthologous pairs were phosphorylated at equivalent sites. Further comparative analyses against *Medicago* phosphoproteome, utilizing recent published data, also showed similar results. These data provide direct evidence for conserved regulatory mechanisms based on phosphorylation in plants.

09018

FLUORESCENT-ACTIVATED MALE GERM UNIT SORTING IN ARABIDOPSIS THALIANA

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The study of molecular processes that regulate male gametogenesis, pollen tube growth and double fertilization, hold great promise to understand key aspects of cell biology, such as cell fate specification and epigenetic reprogramming, cell cycle transitions, and apical growth. The first genome-wide expression profile of purified *Arabidopsis* pollen was only possible using FACS and microarray technology. However, mature pollen encloses two differentiated cell types (two sperm cells embedded within a vegetative cell), which contribute differentially to the overall expression profile observed in total.

We have been developing methods to isolate sperm cells and vegetative nuclei by FACS, using transgenic *Arabidopsis* pollen expressing fluorescent proteins under the control of cell-type specific promoters. We further hypothesized that combining both transgene expression in the same plant would allow their co-purification from the same pollen sample for total RNA and genomic DNA isolation. The latter has already been possible from extracted nuclei counterstained with intercalating DNA dyes, but to date, transgenic lines with strong and specific expression of fluorescent proteins in both the sperm cells and vegetative nucleus were lacking. Furthermore, avoiding intercalating dyes would be preferable for studies of chromatin dynamics and DNA methylation patterns.

Here, we report a method to purify sperm cells and vegetative nuclei together, by developing i) an adapted sperm extraction buffer containing relatively high sucrose content that can sustain intact and viable sperm cells and vegetative nuclei, and ii) transgenic *Arabidopsis* pollen with sperm cells expressing GFP driven by the sperm-specific promoter AtMGH3, and the vegetative nucleus expressing H2B-mRFP under the control of ACT11 promoter. We anticipate that this method will finally allow to profile the (epi)genomic divergences between the vegetative cell and the male germline in the same genetic background.

09019

NEW INNOVATIVE TECHNOLOGY FOR MUTATION BREEDING USING HEAVY-ION BEAMS

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The RIKEN Nishina Center, RI Beam Factory (RIBF) is the one of the biggest facilities to accelerate heavy ions in all over the world since 1986. Heavy-ion beam consists of particles of vary widely in mass, from helium to uranium and beyond. There are two significant

differences between photon (γ -ray, X-ray) and ion; ion has both mass and charge, by contrast photon has neither one. As the result, they provide different linear energy transfer (LET); 0.2 to 2 keV/ μ m for γ -ray and X-ray, by contrast large and vary widely for ion beams, e.g. 23 keV/ μ m for C ion and 640 keV/ μ m for Fe ion. High-LET radiation causes more localized, dense ionization within cells than low-LET radiation. Then ion beam irradiation is expected to produce double-stranded DNA breaks. It is still uncertain whether the repair systems are inactivated, or merely ion-beam lesions are less repairable. Mutations induced by ion beam irradiation at the molecular level have been most extensively studied in mammalian cells. The frequency of deletion is higher for ion beam irradiation than for γ -rays. We found that the ion beam is an excellent tool for mutation breeding. There are the advantage of ion beam mutagenesis, low dose with high survival rates, high mutations rates and a wide variation. The irradiation treatment given to the various plant materials is short, only seconds or a few minutes, but is enough to induce mutation. A new variety can be obtained by selecting a mutant with a modification to the target characteristic while retaining the existing valuable ones. The time span for breeding can be shortened significantly to two to three years. We already put 18 new cultivars on the market in Japan, USA, Canada and EU since 2002. An international heavy-ion plant research consortium has been organized with 125 national user groups and 15 international institutes.

09020

SYSTEMATIC ELUCIDATION OF FUNCTIONS FOR RICE TRANSCRIPTION FACTORS DEDUCING FROM GAIN-OF-FUNCTION PHENOTYPES IN TRANSGENIC RICE PLANTS OVEREXPRESSING INDIVIDUAL TRANSCRIPTION FACTOR cDNAs

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Transcription factors (TFs) are master switches of the downstream target genes. Therefore the functional analysis of TFs is valuable for the elucidation of transcriptional regulation and expression networks of the target genes. Although much effort has been made to identify functions of TFs, most of them remain to be clarified. Transgenic rice plants overexpressing individual rice TF cDNAs (RTF-OX rice plants) are expected to be highly useful bio-resources for estimating functions of individual TFs and identifying expression profiles of the target genes. The number of loci for TFs has been estimated to be approx. 2,500 in the rice genome, and about 75% of them are supported by corresponding full-length (FL-) cDNA clones. We have been producing RTF-OX rice plants by using the rice FL-cDNAs encoding potentially functional TFs. Initially, the FL-cDNAs with functional protein-coding sequences (CDSs) were selected by inspecting individual CDSs, and used as templates to construct Gateway entry clones and also binary Gateway expression plasmids for the respective TF cDNAs to be introduced into rice via *Agrobacterium*. So far, 1629 transgenic rice plants, individually overexpressing 229 TF cDNAs, have been produced. Among the RTF-OX plants, we observed such altered phenotypes as increased plant height, wide leaf, semi dwarf, small grains, and lesion mimic, etc. Such gain-of-function phenotypes are expected to be informative in deducing the TF functions. The latest status on the production of RTF-OX transgenic rice plants, interesting phenotypes found among the transgenic plants, the linkage between altered phenotypes and TF overexpression, etc. will be reported.

09021

DYNAMIC REARRANGEMENT INDUCED BY FE-IONS IN ARABIDOPSIS THALIANA

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Heavy-ion-beam irradiation can be performed with select the Linear Energy Transfer (LET) or ion species in RIKEN RI-beam factory. Previous studies reported that Fe-ion (LET: 640 keV/ μ m) induced larger size of deletions than C-ion (LET: 22.5 keV/ μ m) in the microbe *rhizobium*. It may be possible that Fe-ion could cause large deletions also in higher plant. In this study, we identified DNA mutations caused by Fe-ion irradiation. *Arabidopsis* dry seeds were irradiated with Fe-ion at a dose of 50 Gy. We screened mutants which were easily-distinguishable by morphology and leaf colors: *pg* (*pale green*) mutant, *nl* (*narrow leaves*) mutant, *hy* (*elongated hypocotyl*) mutant, and *gl* (*glabrous*) mutant. As a result, from 29,176 M₂ plants, we obtained 41 *pg* mutants, two *nl* mutants, two *hy* mutants, and one *gl* mutant. Among *pg* mutants, we found a mutant which had downward-pointing siliques. We considered this as *bp* (*brevipedicellus*) mutant and tried to amplify a DNA fragment containing the responsible gene *BP1*. However, no PCR product was obtained. The result of genomic tiling array showed that this mutant had at least three large deletions whose sizes were 80.5 kbp, 84.5 kbp, and 91.0 kbp. Sequencing of the genomic DNA fragment containing *AN* (*ANGUSTIFOLIA*) gene in one of the *nl* mutant showed that a 178-bp sequence of the genomic fragment was tandemly repeated. Sequencing of cDNA of the *AN* gene revealed a 82-bp insertion fragment. While the deletion sizes of mutants yielded by C-ion (LET: 22.5-50 keV/ μ m) were within the range between 1 bp and 1 kbp, we obtained larger size of deletion by Fe-ion. Moreover, since no tandem duplication was observed in C-ion mutants, it may be caused by the high LET of Fe-ion. These results suggest that the adjustment of ion species is effective for the control of mutation induction.

09022

ARABIDOPSIS NATURAL ACCESSIONS VARIOUSLY RESPOND TO MANNITOL TREATMENT

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RIKEN BioResource Center (BRC) takes over the preservation and distribution project of the Sendai Arabidopsis Seed Stock Center (SASSC) and starts distribution of the Arabidopsis accession seeds under the National Bio-resource Project (NBRP) supported by Japanese government (http://sassc.epd.brc.riken.jp/sassc/create_search_panel2.php?mode=general). It has been speculated that the natural accessions of Arabidopsis adapt themselves to the environment of their habitat by natural mutations in their genome. Therefore the natural accessions must be useful for elucidating the relationship between environmental adaptations and natural variations.

In this study, we focused on the osmotic stress by mannitol to find that natural accessions showed various responses to the mannitol treatment. We expect such data can be applied for association mapping and/or QTL analysis. A database that contains these phenotype data is under construction.

09023**AN ARABIDOPSIS SINGLE CELL SYSTEM TO STUDY PLANT CELL DIVISION**

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Numerous compounds such as phytohormones, nutrients and signaling peptides control mitotic activity. In plant tissues, cells are embedded in a complex environment and specific signals cannot be isolated. Therefore we have developed an *in vitro* system in which cellular organization is simplified to dissect the signaling network driving cell proliferation. Our model is a monolayer of Arabidopsis isolated cells resuming mitotic division after protoplasting. These cells express a transgene coding for the histone 2B fused to the yellow fluorescent protein which marks the nucleus. Protoplasts prepared from cell suspension cultures are seeded and immobilized in 96 well plates. This configuration is compatible with time-lapse imaging, in both transmission and fluorescent channels, and in multiple optical sections. Software algorithms have been developed that determine the number, the duration and the spatial repartition of all individual mitotic events in given microscopic fields and across multiple days. The robustness of the system was established through the study of the effects of known mitotic inhibitors and growth promoting compounds. The comparison of automated versus manual annotations of cell division validated our analytical pipeline. We present experimental results highlighting the potential of this single Arabidopsis cell system for compound and genetic screens.

09024**GATEWAY VECTORS FOR PLANT GENOME ANALYSIS**

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The Gateway technology (www.invitrogen.com) has been developed to facilitate the transfer of DNA segments between plasmids by site-specific recombinational cloning. We have constructed a large collection of Gateway-compatible destination vectors for a wide range of gene function analyses in transgenic plant cells. Using MultiSite recombination Gateway cassettes, plant binary destination vectors have also been created in which two or three segments can be transferred contiguously or in independent expression unit, in a single LR clonase *in vitro* reaction. Our destination vectors carry one of three plant selectable markers coding for resistance to kanamycin (*nptII*), hygromycin (*hpt*) or glufosinate ammonium (*bar*), and are available in small high copy number plasmids.

To further streamline the construction of recombinant genes, we have built series of reference Gateway entry clones carrying promoters, terminators, and reporter open reading frames most commonly used in plant research. This collection obeys simple engineering rules: the genetic elements (parts) are designed in a standard format; they are interchangeable, fully documented, and can be combined at will according to the desired output. We recently added to our resources a novel series of Gateway binary vectors optimized for the transformation of monocotyledonous species that covers most applications in this field.

The Gateway entry clones and destination vectors can be obtained on line (<http://www.psb.ugent.be/gateway>). This web site provides recombinational cloning instructions, as well as experimentally verified sequences, maps and Vector NTI files for each plasmid.

Karimi et al. (2002) GATEWAY vectors for Agrobacterium-mediated plant transformation. *Trends Plant Sci.* 7:193-195.

Karimi et al. (2005) Modular cloning in plant cells. *Trends Plant Sci.* 10:103-105.

Karimi et al. (2007) Building blocks for plant gene assembly. *Plant Physiol.* 145:1183-1191.

10001**NATURAL VARIATION IN SEASONAL ADAPTATION**

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The importance of understanding natural variation in *Arabidopsis* populations has been highlighted in recent years. The role of natural variation in adaptation remains of key interest. Here, we report natural variation in growth rate in populations from the Edinburgh area, which are genetically more similar than global accessions. We examined growth of these genotypes in the field from autumn and spring conditions. We found genetically determined differences in growth rate and fitness, and evidence that some genotypes perform consistently better as winter annuals and others as summer annuals. By growing these genotypes under controlled conditions, we have been able to identify photoperiod and temperature as environmental variables to which different genotypes may be adapted.

10002**EVOLUTION OF SELF-COMPATIBILITY IN ARABIDOPSIS BY A MUTATION IN THE MALE SPECIFICITY GENE**

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Ever since Darwin's pioneering research, the evolution of self-fertilisation (selfing) has been regarded as one of the most prevalent evolutionary transitions in flowering plants. A major mechanism to prevent selfing is the self-incompatibility (SI) recognition system, which consists of male and female specificity genes at the S-locus and SI modifier genes. Under conditions that favour selfing, mutations disabling the male recognition component are predicted to enjoy a relative advantage over those disabling the female component, because male mutations would increase through both pollen and seeds whereas female mutations would increase only through seeds. Despite many studies on the genetic basis of loss of SI in the predominantly selfing plant *Arabidopsis thaliana*, it remains unknown whether selfing arose through mutations in the female specificity gene (*SRK*), male specificity gene (*SCR/SP11*), or modifier genes and whether any of them rose to high frequency across large geographic regions. Here we report that a disruptive 213-bp inversion in the *SCR* gene (or its derivative haplotypes with deletions encompassing the entire *SCR-A* and a large portion of *SRK-A*) is found in 95% of European accessions, which contrasts with the genome-wide pattern of polymorphism in European *A. thaliana*. Importantly, interspecific

crossings using *A. halleri* as pollen donor reveal that some *A. thaliana* accessions retain the female SI reaction, suggesting that all female components including SRK are still functional. Moreover, when the 213-bp inversion in *SCR* was inverted and expressed in transgenic plants, the functional SCR restored the SI reaction. The inversion within *SCR* is the first mutation disrupting SI shown to be nearly fixed in geographically wide samples, and its prevalence is consistent with theoretical predictions regarding the evolutionary advantage of mutations in male components.

10003

PENTATRICOPEPTIDE REPEAT GENES RELEVANT TO MALE DEVELOPMENT ARE THE 'ARMS' EQUIPPED AGAINST PARASITIC MITOCHONDRIA

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Whereas the vast majority of genes are subject to purifying selection during evolution, limiting diversity, there are a few genes known to show diversifying selection. These genes include those implicated in plant-pathogen interactions such as R genes and chitinases. Studies on the agronomically important crop trait cytoplasmic male sterility (CMS) have suggested the semblance of a host-parasite system between the nucleus and mitochondria. Key players in CMS systems are the fertility restorer (*Rf*) genes required for the development of a functional male gametophyte in plants carrying a mitochondrial CMS gene. In the majority of cases, *Rf* genes encode pentatricopeptide repeat (PPR) proteins. We show that all angiosperms for which extensive genomic sequence data exist contain multiple PPR genes related to *Rf* genes. These *Rf*-like (*RFL*) genes show a number of characteristic features compared to other PPR genes, including chromosomal clustering and unique patterns of evolution, notably rampant gene conversion and high rates of non-synonymous to synonymous mutations, suggesting diversifying selection. The highest probabilities of diversifying selection were seen for amino acid residues 1, 3 and 6 within the PPR motif. PPR proteins are known to bind RNA and mapping the selection data to a predicted consensus structure of an array of PPR motifs suggests that these residues are likely to form base-specific contacts to the RNA ligand. We suggest that the selection patterns on *RFL* genes reveal a molecular 'arms-race' between the nuclear and mitochondrial genomes that has persisted throughout most of the evolutionary history of angiosperms.

10004

GENOME-WIDE ASSOCIATION IN *ARABIDOPSIS THALIANA*: RESULTS FROM AN INCREASED SAMPLE SIZE.

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Arabidopsis thaliana is ideally suited for genome-wide association (GWA) studies as it occurs as naturally inbred lines that can be genotyped once, and phenotyped repeatedly for diverse traits in essentially an infinite number of environments. To realize this potential, ~half a million SNPs were identified in a global population with high-density microarrays, and more than 1000 lines were genotyped for 216K of these SNPs with an Affymetrix SNP array. In March 2010, the initial GWA scans of phenotypes using 96 -194 genetically diverse lines were documented¹. The results were promising, with many trait associations distinctly matched to candidate genes, including numerous a priori expectations and plausible novel candidates. However, many traits also exhibited a more complex pattern of association spanning up to several hundred kb, making it difficult to identify candidate genes (though several obvious causal genes were found within these regions). The initial findings highlighted the need for appropriate sample sizes, as well as for accession selection to lessen the confounding effect of population structure. Following on from our initial findings I will highlight results from a larger population size and sub-sampling within.

¹Atwell et al, 2010. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. Nature, March 11th.

10005

DIVERGENT ALLELES OF DISEASE RESISTANCE GENE LEAD TO HYBRID INCOMPATIBILITY IN *ARABIDOPSIS THALIANA*

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Hybrid necrosis is a phenomenon that is often observed in intra/inter-specific plant crosses and characterized by elevated immune responses. This phenomenon has been suggested to serve as an effective postzygotic gene flow barrier in plant species (1). Here we report cases of hybrid necrosis that result from the incompatible epistatic interaction involving a complex disease resistance (*R*) gene cluster. The *R* gene cluster that carries *RECOGNITION OF PERONOSPORA PARASITICA 1 (RPP1)-like* *R* genes on chromosome 3 is highly divergent both in sequence and number in different accessions. We identified a single *R* gene in the cluster that is sufficient to cause hybrid incompatibility in one case. However, this particular *R* gene allele does not manifest necrotic symptoms in the other cases involving this cluster. Furthermore, epistatically interacting loci of this *R* gene cluster differ in all cases. This indicates that the *R* gene cluster evolved under different evolutionary trajectory among different accessions yet carries numerous potential alleles that would contribute to gene flow barrier. Population genetic structure of this cluster both in local and global accessions as well as investigation on incompatible substitutions will be presented at the meeting. This study will shed light on our understanding of evolutionary forces that lead to hybrid incompatibility and speciation.

Reference

1. Bomblies K and Weigel D. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. Nat Rev Genet. 2007 May;8(5):382-93.
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10006

EPISTATIC INTERACTION BETWEEN AN ALLELE OF A BACTERIAL BLIGHT-RESISTANCE GENE XA1 AND AN ALLELE OF A PUTATIVE TRANSCRIPTIONAL REPRESSOR HWC1 CAUSES HYBRID WEAKNESS OF RICE

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Hybrid weakness, sometimes known as hybrid necrosis, has been described for various plant taxa with different ecologies and life strategies in both cultivated and wild species (Bomblies and Weigel 2007). The cause of hybrid weakness or inviability is explained by the

Bateson-Dobzhansky-Muller model as epistatic incompatibilities between loci (Orr 1996). Previous studies revealed that a causal gene of a hybrid necrosis in *Arabidopsis* is a resistance-gene homolog (Bombliis *et al.* 2007). In rice, hybrids between a Peruvian rice cultivar 'Jamaica' and most temperate-japonica rice cultivars show weakness. This hybrid weakness is induced by the 'Jamaica' allele of *HYBRID WEAKNESS C 1* (*HWC1*) and an allele of *HYBRID WEAKNESS C 2* (*HWC2*) (Akemine and Amemiya 1963, Ichitani *et al.* 2001, Ichitani *et al.* 2007, Kuboyama *et al.* 2009). We identified both causal genes using a map-based strategy. Results show that *HWC1* resembles known transcriptional co-repressors, *LEUNIG* of *Arabidopsis* and *STYLOSA* of *Antirrhinum*, and chromosomal location of *HWC2* corresponded to a NB-LRR-type bacterial-blight-resistance gene, *Xa1*. The hybrids showed ectopic cell death around the root apical meristem, induction of oxidative stress, and elevated production of a phytoalexin. These results indicate that a cause of this hybrid weakness is an autoimmune response between a transcription factor and a resistant gene. A nonsynonymous substitution at a specific site of *HWC1* can induce hybrid weakness. In *HWC2*, the smallest difference between the causal allele and of a non-causal-allele was only five nucleotide substitutions including two nonsynonymous substitutions in mRNA. Consequently, a slight genetic alteration such as a nucleotide substitution can produce a strong reproductive barrier between plants.

10007

GENETIC BASIS OF PLANT RESPONSE TO DIFFERENT ENVIRONMENTAL SCENARIOS USING NATURAL VARIATION IN ARABIDOPSIS THALIANA

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Responses to contrasting environments in plants can lead to different fitness levels. These responses can be quantified at several levels such as growth, pigmentation and flowering time, which are determined by genetic and environmental factors. Plants have evolved several mechanisms of adaptation to changing light and temperature scenarios but sensing mechanisms of these factors remains partially unknown. A set of *Arabidopsis* accessions have been tested in a combination of increased light intensity and reduction of temperature. Eri-1 (Eringsboda-1 from Sweden) showed the highest anthocyanin content in this scenario. In order to reveal the genetic basis of plant's response to this environment, we tested a mapping population derived from Eri-1 (Sweden) and Ler (Poland) accessions. Quantitative Trait Loci (QTL) of the responses of flowering time, growth and pigmentation were detected. Among all the mapped QTL, 3 interacting clusters of QTL for all the quantified traits have been detected. Phenotypes of selected NILs (near isogenic lines) in this environmental scenario confirmed the effects of these QTL. These QTL will be pursued by using a fine-mapping approach to unravel genetic and molecular determinants underlying the genotype by environment interaction.

10008

THE EVOLUTION OF A PLANT BRANCHING HORMONE.

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The pattern of branch outgrowth is a key determinant of the flexible plant body plan, and varies among and within species. In angiosperms, branches are produced from axillary meristems, which either remain dormant or grow out. This decision is affected by several hormones, either repressing or promoting outgrowth.

Strigolactones, originally identified as germination stimulants for parasitic plants, and more recently as signals in the formation of mycorrhizal symbioses, have now been shown to repress branching in rice, pea and *Arabidopsis*. *MAX1* in *Arabidopsis* encodes a cytochrome P450 family member, which is required for the synthesis of the strigolactone-related signal, and forms part of a biosynthetic and signal transduction pathway containing at least three other genes in *Arabidopsis*.

While other known components of the strigolactone signalling pathway are conserved throughout the land plants, genes closely related to *MAX1* are present in all plant genomes published except the 'basal' land plant, moss, the only fully sequenced plant without branching in the sporophyte generation. These findings suggest that *MAX1* is an evolutionarily later addition to pathway. We test this hypothesis by investigating the ability of homologues from a range of species to complement *Arabidopsis max1* mutants. We also investigate whether the loss of mycorrhizal symbiosis in *Arabidopsis* has freed strigolactone signalling from a coevolutionary constraint imposed by the fungal partner, and allowed diversification of the endogenous signal in this species, by analysing the complementation of *max1* with homologues of the putative strigolactone receptor *MAX2* from other species. We complement these molecular and genetic approaches with physiological investigation of the actions of strigolactones in non-angiosperm species, including spruce and *Selaginella* species.

10009

REFUNCTIONALIZATION OF THE ANCIENT RICE BLAST DISEASE RESISTANCE GENE *PIT* BY THE RECRUITMENT OF A RETROTRANSPOSON AS A PROMOTER

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The plant genome contains a large number of disease resistance (*R*) genes that have evolved through diverse mechanisms. Here, we report that a long terminal repeat (LTR) retrotransposon contributed to the evolution of the rice blast resistance gene *Pit*. *Pit* confers race-specific resistance against the fungal pathogen *Magnaporthe grisea*, and is a member of the nucleotide-binding site-leucine-rich repeat (NBS-LRR) family of *R* genes. Compared to the nonfunctional allele *Pit*^{Nlb}, the functional allele *Pit*^{K59} contains four amino acid substitutions and has the LTR retrotransposon *Renovator* inserted upstream. Pathogenesis assays using chimeric constructs carrying the various regions of *Pit*^{K59} and *Pit*^{Nlb} suggest that amino acid substitutions might have a potential effect in *Pit* resistance; more importantly, the upregulated promoter activity conferred by the *Renovator* sequence is essential for *Pit* function. Our data suggest that transposon-mediated transcriptional activation may play an important role in the refunctionalization of additional 'sleeping' *R* genes in the plant genome.

Reference:

Hayashi *et al.* (2009) Plant J 57: 413-425.

10010

USING GENOME-WIDE NATURAL DIVERSITY OF ARABIDOPSIS THALIANA TO INVESTIGATE GENETIC POLYMORPHISM OF PLANT GROWTH

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The aim of this project is to elucidate the genetic basis of plant growth using a multi-parent mapping population derived from 8 *Arabidopsis* accessions collected in diverse habitats. From these crosses, 532 Recombinant Inbred Lines (RILs) have been phenotyped for growth-related traits such as: relative growth rate of rosette size, leaf production rate, root elongation rate, etc... High heritabilities and large transgressions for each of these traits have been observed. However, using this data set only one QTL for root growth has been mapped on chromosome 5. Furthermore, residual heterozygosity is still present in most of the RILs and segregation of growth-related traits could be detected within some of them. Indeed, set of heterogeneous inbreed families (HIFs) showing obvious growth segregation have been selected and these HIFs have been genotyped with 250 000 SNPs spread over the genome to highlight co-segregation between variation of growth and allelic values at defined marker position(s). Detailed characterization and fine mapping of the respectively loci is underway and includes determination of metabolites and enzyme activities in these selected lines. This will enable us to link variation of growth with variation in primary metabolism.

10011

FUNCTIONAL DIVERSIFICATION OF THE TUBBY-LIKE PROTEIN GENE FAMILIES (TULPs) DURING EUKARYOTIC EVOLUTION

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The Tubby-like protein (TULP) is ubiquitous among multicellular organisms but has extremely diversified functions. Although functional analyses of certain mammalian and model plant TULPs are available and phylogenies separately based on animal or plant TULPs have been reconstructed, the origin and evolutionary history of TULPs remain obscure. The existence of TULP in unicellular organisms is also uncertain. In this study, 145 TULP homologs were identified from four eukaryotic supergroups: Excavata, Chromalveolata, Archaeplastida (Plants), and Opistokonta (Fungi and Animals). In addition to the tubby domain, many TULPs also contain different motifs (F-box, WD40 repeat, or SOCS box) in their N-terminus regions, leading to their groupings into three classes. TULPs with no N-terminal modifications (class II) locate at the basal positions on the phylogeny. In a comparison of the three phosphatidylinositol 4,5-bisphosphate (PIP2) binding sites at K330, R332, and R363 in mouse TUBBY, our data show that PIP2 binding sites are conserved in class II TULPs, especially those that are animal derived, but not in TULPs from classes I and III. Our experimental data further demonstrate that only class II TULPs can be targeted to the plasma membrane. These results suggest functional divergence and differentiation of TULPs and Mutations at the three PIP2 binding sites indicate the function of class II TULPs differs from those of classes I and III. Functional divergence and differentiation of TULPs should have occurred after duplications and structural modifications around 1100 MYA as eukaryotic organisms started diversifying.

10012

MOLECULAR AND GENETIC ANALYSIS OF BIOMASS-HETEROSIS IN *ARABIDOPSIS THALIANA*

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The molecular basis of heterosis is analysed in the C24 x Col-0 cross of *Arabidopsis thaliana* accessions that exhibits strong mid-parent heterosis of vegetative growth / biomass accumulation (up to 161% at high light). Using QTL-mapping in combination with metabolite profiling and gene expression profiling, loci responsible for the heterosis have been identified and heterosis-associated gene expression / metabolite composition has been analysed. Differences in mRNA and metabolite levels of parents and hybrids indicate additive, dominant, and overdominant effects with different prevalence at early and late developmental stages. In order to identify genomic regions involved in biomass heterosis QTL, generation means, and mode-of-inheritance classification analyses were performed using a modified North Carolina Design III: 429 recombinant inbred lines (RILs) and 140 introgression lines (ILs) were backcrossed to the two parental accessions and analysed for biomass accumulation at 15 days after sowing. Mid-parent heterosis of shoot dry weight in the RILs ranged from -31% to 99%. Treating mid-parent-heterosis and augmented dominance effect as quantitative traits, ten genomic positions involved in heterosis were detected, that explain between 2.4% and 15.7% of the phenotypic variation. While overdominant gene action was prevalent in heterotic QTL, the results suggest that a combination of dominance, overdominance and epistasis is involved in biomass heterosis in this *Arabidopsis* cross. A strong heterotic biomass QTL region of chromosome 4 that co-locates with a cluster of 23 metabolic heterotic QTL was narrowed down to 14 genes by using subILs and segregating IL families. This genomic region is further investigated by comparative sequence analysis (C24 vs. Col-0) and the candidate genes are studied using k.o. mutants subjected to crosses and by transformation of alleles into the opposite genotype.

10013

NATURAL VARIATION OF BRANCHING PATTERN IN THE *ARABIDOPSIS* MULTI-PARENT RIL POPULATION

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Shoot branching is a major determinant of plant architecture governing many aspects of plant shape, light perception, and resource allocation. Shoot branching is highly regulated by endogenous and environmental cues. Shoot branching involves the formation of axillary buds in the axil of leaves and subsequent outgrowth of the buds. Most *Arabidopsis* accessions have one side branch per axil in the elongated stems. However, in the *Arabidopsis* Multi-Parent RIL (AMPRIL) population derived from a set of 8 diverse accessions of *Arabidopsis thaliana*, we observed that some lines have no side branch in all axils of stem branch. The novel branching pattern was named as Reduced Stem Branch (RSB). RSB phenotype is stably heritable and is only presented in later flowering plants. Using backcross segregating population and bulked segregant analysis (BSA), we show that a total of 8 putative QTL contributes to natural variation for the traits. They were confirmed using a type of near-isogenic line called a heterogeneous inbred family (HIF). Furthermore, we fine mapped a QTL (RSB2) to a 30Kb region on the bottom of chromosome 2, which included 8 candidate genes. The cloning and characterization of the branching-related QTL would be helpful in elucidating molecular mechanism of shoot branching.

10014

DIFFERENTIAL PLASTIC RESPONSES OR LOCAL ADAPTATIONS? INSIGHTS FROM PHENOTYPING NATURAL POPULATIONS

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Recent studies with natural accessions of *Arabidopsis thaliana* have provided insights into the genetic variation underlying phenotypic differences in lab or common garden experiments. However, there has been essentially no information on phenotypic variation in natural settings. To address this lacuna, we have selected ca. 20 populations representing various types of habitats out of our recently identified sites near Tubingen in Germany (Bombliet et al., PLoS Genetics 2010) for monitoring. Field phenotypic variations were directly measured from sets of individuals from these natural populations, and were contrasted with phenotypic data from progenies of those individuals in a common garden condition. We will present these phenotypic properties in the context of ecological responses to local environments and discuss plasticity and GxE interaction with comparison of field and common garden phenotypic measurements. Also we will address parent-progeny regression in a specific site to estimate the heritability in our phenotypic traits.

10015

VARIATION IN GROWTH AND ITS RESPONSE TO ELEVATED CO₂ AMONG ARABIDOPSIS THALIANA ECOTYPES
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It is known that effects of elevated CO₂ on growth vary among species. To study mechanisms underlying this variation, 44 ecotypes of *Arabidopsis thaliana* from various latitudes and altitudes were grown under ambient and elevated (800 ppm) CO₂ concentrations. Biomass determined at 38 days after sowing considerably varied among ecotypes: under both CO₂ concentrations, biomass varied by ca 2.7 fold. Enhancement of biomass by elevated CO₂ also varied from 1.43 to 3.16 fold, indicating a significant variation in the CO₂ response among ecotypes. The variation in relative growth rate was mainly ascribed to net assimilation rate rather than leaf area ratio. Enhancement ratio of biomass by elevated CO₂ was significantly correlated with photosynthetic nitrogen use efficiency.

10016

GENOMIC BASIS OF LOCAL ADAPTATION IN A. HALLERI FROM CROSS-SPECIES MICROARRAY ANALYSIS

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Adaptation to the local environment is the major driving force in evolution. Especially for plant species, gradients in environmental characters cause adaptation to different habitats, resulting in ecotypic differentiations as well as speciation. Although the genetic basis of local adaptation is one of the major topics in evolutionary biology, it is difficult to conduct such genetic studies on most wild species due to the lack of knowledge about their genome. *Arabidopsis halleri* ssp. *gemmifera* is most closely related species to *A. thaliana* and it demonstrates phenotype differentiation attributed to altitudinal gradients. Two ecotypes grow in the lowland and highland habitats on Mt. Ibuki in Japan, providing a favored study system for elucidating the mechanisms of local adaptation along altitude. To uncover genetic basis of local adaptation, we conducted cross-species genomic microarray analysis in two ecotypes of *A. halleri* ssp. *gemmifera* using AtMap1, tilling array for *A. thaliana*. In these results, we found eighteen genes whose signal intensities significantly differentiated between lowland and highland types. These genes encoded several metabolic enzymes, disease resistance proteins, kinase family proteins, and a transcription factor. In this presentation, we will discuss the functions of these genes for different environmental factors, such as temperature and wind.

10017

ISOLATION AND CHARACTERIZATION OF EMBRYO DORMANT ARABIDOPSIS ACCESSIONS

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Seed dormancy, defined as the failure of intact seeds to germinate under favorable conditions, is thought to be an important adaptive trait in many plant species. Seed dormancy is established during seed maturation phase and subsequently released by after-ripening (dry storage of matured seeds). In this study, we investigated the effect of after-ripening on embryo dormancy release using freshly harvested and 4-week after-ripened Cape Verde Islands-0 (Cvi-0) seeds. Because Cvi-0 is a dormant accession, both fresh and after-ripened seeds germinated less than 5 % at 23 °C in the light after imbibition. However, when embryos were separated from seed coat after imbibition and incubated for further period, almost all of embryos isolated from after-ripened seeds germinated (or started growth), while those of fresh seeds did not resume growth. This result suggested that embryo dormancy is released by after-ripening within 4 weeks in Cvi-0 and seed dormancy of after-ripened Cvi-0 largely depend on the seed coat. Next, we tried to identify hyper-“embryo dormant” accessions among 34 dormant wild-type accessions. When 4-week after-ripened seeds were analyzed, 9 accessions were classified into strong embryo dormant (Emd) type whose embryos germinated less than 10 % after separation from seed coat. On the other hand, 15 accessions including Cvi-0 were classified into seed-coat dormant (Scd) type whose embryos germinated more than 90 % after separation from seed coat. Interestingly, Emd accessions exhibited distinct response to exogenously applied nitrate and gibberellin. In addition, comparison of phytohormone levels indicated that there are at least two types of Emd accessions; Type I accumulates comparable amount of abscisic acid (ABA) to Scd accessions, and Type II accumulates increased amount of ABA relative to Scd accessions. These results suggested that embryo dormancy is determined not only by endogenous ABA level but also by combination of multiple factors.

10018

ANALYSES OF DNA VARIATION IN THE PHERES GENES OF ARABIDOPSIS SPECIES

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Signature of natural selection has been suggested for an imprinted gene, Medea (MEA) in *Arabidopsis* species. The Pheres (PHE) genes are among the known genes imprinted in endosperm cells of the plant *Arabidopsis thaliana*. To test whether an evolutionary arms race has driven rapid sequence evolution in this component of the imprinting system, we studied DNA variation in the two *A. thaliana* PHE genes, which are closely linked, and in the homologues in its close relative, *A. lyrata*. In *A. thaliana*, strongly reduced divergence between the PHE1 and PHE2 loci in a short region (about 100bp) of the 5' flanking region of the genes suggests gene conversion. The conversion events tend to occur from PHE1 to PHE2, causing high diversity in PHE2. In *A. lyrata*, the PHE1 gene is duplicated, but the sequences cannot be classified into two clear clusters, and three similar sequences were found in a single individual plant; both results again suggest gene conversion between the duplicates. A short 5' flanking region is conserved among all the *A. lyrata* PHE1 sequences, suggesting purifying

selection. The *A. lyrata* PHE2 locus diversity is high in the 5' flanking region, with divergent haplotypes. These results suggest natural selection affect diversity patterns of the 5' flanking region of both PHE loci.

10019

DIVERSITY OF ARABIDOPSIS THALIANA ATPRX53 AND ATPRX54 PEROXIDASE GENES IN NATURAL POPULATIONS AT THE NORTHERN LIMITS OF THE SPECIES RANGE

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In natural populations of *Arabidopsis thaliana*, occupying northern limits of the species range (Karelia), the level of genetic diversity was evaluated using RAPD-markers (82 RAPD loci were tested). Considerable genetic diversity revealed was not typical of self-pollinating plant species. It was demonstrated that genetic differentiation among the populations was rather high, pointing to the low level of gene flow in the isolated insular populations. It was suggested that the high level of *Arabidopsis* population polymorphism in Karelia could be associated with extreme growing conditions at the northern limits of the species range. It is well known that class III peroxidases play an important role in plant protection against biotic and abiotic stresses. We analyzed DNA polymorphism in the *AtPrx53* and *AtPrx54* genes and found several alleles of studied genes in Karelian accessions. The alleles of *AtPrx53* belong to one of two haplotypes, identified previously among 20 ecotypes (Kupriyanova et al., 2007). Only one accession possesses *Dj*-like haplotype from 6 Karelian accessions. We did not find recombinant alleles despite high level of RAPD-polymorphism in Karelian accessions and high level of recombination in peroxidase genes *AtPrx53/54* (Kupriyanova et al., 2010). The total number of all studied by us *AtPrx53* alleles is 26. Among them 17 belong to *Col* and 10 belong to *Dj*-like haplotypes (including *TSU-1* allele submitted to TAIR by D. Weigel). Two of the amino acid substitutions (*Phe/Ser180* and *Asp/Asn270*) responsible for the difference in electrophoretic mobility of *AtPrx53* allozymes from Karelia accessions as in other ecotypes. The revealed pattern of polymorphism at the *AtPrx53* and the strong haplotype structure in the absence of detectable recombination across *AtPrx53* may be interpreted as evidence of balancing selection on this locus or other nearby sites.

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10020

ENDOGENOUS STEROID HORMONES IN *PHYSCOMITRELLA PATENS*

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Genetic studies have revealed that brassinosteroids (BRs) are essential plant hormones for growth and development of seed plants. However, little is known about the occurrence and physiological roles of BRs in moss. The biosynthesis genes of sterols are highly conserved in lower to higher plants. However, the genome of the moss *Physcomitrella patens* comprises no genes with high similarity to BR biosynthesis genes of seed plants. In order to know whether *P. patens* produces BRs, we analyzed endogenous BRs in the protonema using GC-MS. Furthermore, we also analyzed endogenous animal steroid hormones using the same material although their roles and biosynthesis in plants are unknown. As the result, a small amount of castasterone that is a biological active form in seed plants and a series of the precursors such as 6-dexoxocastasterone were identified in *P. patens*. In addition, animal steroid hormones including progesterone were also identified. These findings may imply that not only BRs but also animal steroid hormones had been utilized as physiologically active steroids before the branching-off from the common ancestor into moss and seed plants.

10021

EVIDENCE OF LOCAL HIGH-ALTITUDE ADAPTATION IN NATURAL ARABIDOPSIS THALIANA POPULATIONS

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To understand the genetic basis accounting for ecological adaptation, it is necessary to integrate molecular knowledge with ecological data by employing multidisciplinary approaches. Here, we investigate genetic variation responsible for high-altitude adaptation in *Arabidopsis thaliana* by using the methods of population genetics and molecular biology. Diversifying selection in some life-history traits was found and putative altitudinal adaptive or non-adaptive traits in various developmental phases were identified from a quantitative-trait experiment. However, we found that detected phenotypic clines were existent only in an intraregional sample, our collected wild populations, suggesting a local adaptation to high altitude. Analysis of effects of several environmental factors, including cold treatment (vernalization), photoperiod, ambient temperature and thermoperiod, in two of putative adaptive traits, flowering time (FT) and plant height at FT (PH) suggested a mutual contribution from gene-environments interaction during high-altitude adaptation. Furthermore, we investigated the genetic variation of two regulatory genes controlling flowering time in *A. thaliana*, FRI and FLC, and their effects on the natural variation of FT and PH, which reveals the pleiotropic effects of FLC and functional epistatic interaction between FLC and FRI on both traits. Our results demonstrate a comprehensive example by using natural variation as a tool to explore potential ecologically important questions. Moreover, the report shows us the importance of pleiotropic effects of regulatory genes and their epistatic interaction within the pathway in generating phenotypic variation along environmental clines during natural adaptation.

10022

STUDY OF THE CAUSAL AMINO-ACID SUBSTITUTIONS IN *HWC2* FOR A HYBRID WEAKNESS IN RICE

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In rice, hybrids between a Peruvian rice cultivar 'Jamaica' and most of temperate-japonica rice cultivars show weakness, and this phenomenon was explained by epistatic incompatibility between the 'Jamaica' allele of *HWC1* (*Hwc1-1*) and an allele of *HWC2* (*Hwc2-1*) (Akemine and Amemiya 1963). *HWC2* encodes NB-LRR protein. Therefore, the gene products of *Hwc2-1* might recognize the gene products of *Hwc1-1* and induce autoimmune responses, such as hypersensitive cell death. In our study, we induced artificial nucleotide substitutions to the *HWC2* and tried to clarify the causal amino-acid substitutions in *HWC2*. We introduced five patterns of nucleotide substitutions to genomic clones of *HWC2*, and examined amino-acid residues of *Hwc2-1*, alanine 496, glutamine 1527, or lysine 1529, because non-causal alleles of *HWC2* had substitutions in these amino-acid residues. All transgenic plants carrying modified *HWC2* that had an amino-acid substitution in these residues did not show weakness. This result indicates that the interaction between *Hwc2-1* and *Hwc1-1* is highly specific.

In addition, we surveyed *Tos17*, a retrotransposon, mutant panel and obtained several insertion mutant lines of *Hwc2-1*. These lines were test-crossed with 'Jamaica', and phenotypes of obtained mutants' hybrids were observed. Although most of *Tos17* insertion lines tested produced normal F1 plants in the test cross, hybrids between NF3032 and Jamaica showed alleviated phenotype of hybrid weakness. NF3032 has a *Tos17* insertion at 1 bp from the stop codon of *HWC2*, and this mutation substitutes one amino-acid residue at carboxyl-terminal. The alleviated phenotype of this mutant suggests that even a slight carboxyl-terminal change of *Hwc2-1* can reduce the function of *Hwc2-1*.

10023

NUCLEAR HORIZONTAL GENE TRANSFER BY THE PARASITIC WITCHWEED, *STRIGA HERMONTHICA*

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Horizontal gene transfer (HGT) represents the incorporation of genetic material from one organism into another that is not its offspring. In plants, the majority of reported cases of HGT are limited to plant-microbe genetic exchanges, mitochondrial transfer, or the translocation of mobile elements among related species. Parasitic plants are known to be vectors of mitochondrial HGT, but it has been unclear whether they also mediate nuclear HGT. Here we present evidence for an HGT event moving a nuclear monocot gene into the genome of the eudicot parasite witchweed (*Striga hermonthica*), which infects many grass species in Africa. Analysis of expressed sequence tags revealed that the genome of *S. hermonthica* contains a nuclear gene, designated *ShContig9483*, that is widely conserved among grass species but is not found in other eudicots. Phylogenetically, this gene clusters with sorghum genes, the monocot host of the parasitic weed. The sequence similarity extends to the 5' and 3' untranslated regions of the open reading frame, indicating the high conservation of the sequence is not due to purifying selection. The high conservation of sequence between *S. hermonthica* and sorghum outside the ORF suggest that transfer was a relatively recent event. Indeed, a sequence highly similar to *ShContig9483* was found in *Striga gesnerioides* but not in *Orobanche minor* from a closely related genus. These data suggest that incorporation of the *ShContig9483* fragment occurred before speciation of *S. hermonthica* and *S. gesnerioides* but after differentiation of the genera *Striga* and *Orobanche*. Together these data strongly suggest that nuclear genes from crops can be captured by a parasitic weed in nature.

10024

IDENTIFICATION AND CHARACTERIZATION OF GENES CONTROLLING VEGETATIVE BIOMASS ACCUMULATION IN *ARABIDOPSIS THALIANA*

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Growth related parameters (biomass, leaf area, growth rate) and metabolite content were recorded in large recombinant inbred line (RIL) and introgression line (IL) populations derived from *Arabidopsis thaliana* accessions Col-0 and C24, which were genotyped using single nucleotide polymorphism (SNP) and simple sequence length polymorphism (SSLP) markers. The data were subjected to correlation and quantitative trait loci (QTL) analyses and seven QTL for biomass and 157 metabolic QTL for 84 metabolites could be identified. Using introgression lines 6 of 7 biomass and 55% of the metabolite QTL could be validated. In addition, the IL analysis revealed a further four regions with an effect on biomass. Detailed analyses of the individual ILs indicated complex situations, e.g. on chromosome 1 with potentially two QTL of opposing effects located very closely to each other. Two biomass QTL coincide with significantly more metabolic QTL than expected for a random distribution. Colocalised biomass/metabolite QTL data provide an excellent basis for the detection of functionally relevant variation in known genes with metabolic function and for the identification of genes with unknown roles in the control of growth and metabolism. One genomic region on chromosome IV, where QTL from several traits colocalised, was narrowed down to 14 genes by using subILs and segregating IL families. We are screening C24 cosmid libraries with these candidates to obtain the corresponding C24 genomic sequence and use C24 genome re-sequencing data provided by D. Weigel and co-workers (MPI-DB, Tuebingen, Germany) for comparison with Col-0. Binary cosmid clones are selected for complementation studies in k.o. and RNAi lines.

11001

UNTANGLING EARLY STEPS IN PHYTOCHROME A SIGNALLING

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Plants use phytochromes to sense red (R) and far-red (FR) regions of their light environment. Phytochrome A (phyA) is the primary photoreceptor regulating deetiolation and early seedling development. After photoactivation phyA translocates to the nucleus, where it orchestrates a set of transcription factors to steer development. A small protein, FHY1 and to a certain degree its homolog FHL are essential for the translocation of the activated photoreceptor. Light-dependent phyA:GFP nuclear translocation was undetectable in *fhl/fhy1* protoplasts. In the *fhl/fhy1* background, the native phyA is probably exclusively cytoplasmic, enabling us to identify the abrogation of gravitropism and the R enhancement of phototropism in blue light (B) as exclusively cytoplasmic phyA responses. Since phyA is rapidly degraded in B, these responses are likely to be initiated within the first six hours. A further component of the phyA signal transduction pathway, PKS1, is a membrane-associated protein which can interact with phyA and PHOT1. It was also shown to be involved in B responses such as phototropism and to act negatively in gravitropism. Therefore PKS1 and its homologue PKS4 might be involved in transducing these cytoplasmic phyA responses. Although PKS1 expression was shown to be dependent on phyA, its transcriptional regulation appears not to be altered in *fhl/fhy1* in B. We also investigated the mechanism by which FHY1 facilitates phyA nuclear translocation and identified cytoplasmic phyA/FHY1 interacting proteins, among them also PKS1. The implication of these findings in regard to early phyA signalling events will be discussed.

11002

A TISSUE-SPECIFIC RNA INTERFERENCE STRATEGY TO STUDY THE ROLE OF *ARABIDOPSIS MINICHROMOSOME INSTABILITY 12 (MIS12)*

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The kinetochore is a large protein complex that assembles at centromeres to ensure proper chromosome alignment and segregation during cell division. In humans and maize, the MIS12 protein is localized to a central position of the kinetochore serving as a bridge between the inner kinetochore proteins (DNA binding) and the outer kinetochore proteins (microtubule binding). AtMIS12 is constitutively localized to the centromere. Analysis of a T-DNA insertion line indicates that *AtMIS12* is essential for cell division. In order to further investigate the function of *AtMIS12* and other essential kinetochore genes, we have developed a tissue-specific RNA interference (psRNAi) system. Petals are good targets for tissue-specific RNAi because they are large, visible organs that are not required for growth or reproduction. A portion of the *Arabidopsis PISTILLATA (PI)* promoter confers early petal-specific expression. We found that when this domain was used to drive a *GUS* RNAi transgene, *GUS* expression was knocked down specifically in petals. We have also tested this novel psRNAi system to knockdown the expression of *AtMIS12*. Plant lines expressing the *PI:AtMIS12* RNAi transgene exhibit an array of defective petal phenotypes. The petal phenotype has proven to be heritable and stable in the T1 and T2 generations. Significant decreases in petal length and significant increases in cell size correspond well to the measured levels of the *PI:AtMIS12* RNAi transgene. Cytological analyses revealed that the petal defects are caused by cell cycle arrest at a prometaphase-like stage. More detailed studies combining FISH and immunolocalization will be used to determine whether the *psMIS12* RNAi lines fail during metaphase alignment or anaphase onset. We hope to further use the system to study the pathway of kinetochore assembly and the role of the spindle checkpoint in organogenesis.

11003

COORDINATION OF PLASTID PROTEIN IMPORT AND NUCLEAR GENE EXPRESSION BY GUN1-GLK1 MEDIATED PLASTID-TO-NUCLEUS RETROGRADE SIGNALING PATHWAY

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Expression of nuclear-encoded plastid proteins and import of those proteins into plastids are indispensable for plastid biogenesis. One possible cellular mechanism that coordinates these two essential processes is retrograde signaling from plastids to the nucleus. However, the molecular details of how this signaling occurs remain elusive. Using the *ppi2* mutant of *Arabidopsis thaliana*, which lacks the *atToc159* protein import receptor, we demonstrate that the expression of photosynthesis-related nuclear genes is tightly coordinated with their import into plastids. Down-regulation of photosynthesis-related nuclear genes is also observed in mutants lacking other components of the plastid protein import apparatus. Genetic studies indicate that the coordination of plastid protein import and nuclear gene expression is independent of proposed plastid signaling pathways such as the accumulation of Mg-protoIX and the activity of ABI4. Instead, it may involve GUN1 and the transcription factor *AtGLK*. The expression level of *AtGLK1* is tightly correlated with the expression of photosynthesis-related nuclear genes in mutants defective in plastid protein import. Furthermore, the activity of GUN1 appears to down-regulate the expression of *AtGLK1* when plastids are dysfunctional. Based on these data, we suggest that defects in plastid protein import generate a signal that represses photosynthesis-related nuclear genes through repression of *AtGLK1* expression, but not through activation of ABI4.

11004

A CHEMICAL GENETIC APPROACH TO BRASSINOSTEROID RECEPTOR TRAFFICKING

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Brassinosteroids (BRs) are a class of plant hormones that are essential for growth and development. Despite the vast progress in the BR research, very little is known about the subcellular compartmentalization and trafficking of BR signaling complexes and their relevance for BRs physiological responses. Therefore, we initiate a chemical screen to discover small synthetic molecules that will interfere with the trafficking of BR receptor complex. We screened 10000 chemical compounds library (DIVERSet™, ChemBridge Corp.) to find inhibitors or activators of BR action based on hypocotyl elongation in light. We identified 113 activators and 196 inhibitors of the hypocotyl elongation. In a secondary screen, we checked the effect of those chemicals on the localization of BRI1-GFP in *Arabidopsis* roots, by using a Confocal Laser scanning Microscope (CLSM). 10 chemical compounds affected the localization of BRI1, and their effect on BR responses and different *Arabidopsis* endomembrane markers is being currently investigated.

11005

TWO DUAL-TARGETED PROTEINS ANTAGONISTICALLY REGULATE WRKY53 DEPENDENT LEAF SENESCENCE IN ARABIDOPSIS

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The communication between chloroplasts and the nucleus is a key determinant for many developmental processes in a plant cell and could be, amongst others, mediated by proteins with dual localization in both compartments. Two proteins were recently shown to be located in chloroplasts and nucleus. One protein having a kinase domain with similarities to HPT kinase is AD protein. The GFP fusion AD protein was shown to be located in plastids and the nucleus (Miao et al., 2008). Phosphorylation of AD protein was observed to increase its DNA binding activity at the *WRKY53* promoter region and activating *WRKY53* gene expression, which *WRKY53* protein was identified as an upstream regulator of leaf senescence activating the expression of many downstream genes associated with senescence (Hinderhofer and Zentgraf, 2001; Miao et al., 2004, 2007). While the AD protein is required for activation of *WRKY53* gene expression, another dual-located protein Whirly1 (Krause et al., 2005; Grabowski et al., 2008) was showed act as a repressor of *WRKY53* gene expression. Whirly1 mutants showed accelerated senescence and enhanced transcript levels of the *WRKY53* gene and of downstream genes *WRKY33*, *SAG101* and *SAG12*. Transgenic *Arabidopsis* plants overexpressing recombinant Whirly1 constructs targeting the protein either to the plastids (pWh1) or to the nucleus (nWh1) or to both compartments (pnWh1) were characterized for the level of *WRKY53* gene expression and senescence. The results indicate that the nuclear isoform of Whirly1 can directly repress *WRKY53* gene expression. In comparison to wildtype plants, the partial mutant with Whirly1 accumulating exclusively in plastids had an enhanced level of the *WRKY53* transcript. This suggests that the plastid form of Whirly1 is involved in retrograde signalling positively affecting the expression of *WRKY53* in the nucleus.

11006

SYSTEMS BIOLOGY AS A MEANS TO UNDERSTAND ARABIDOPSIS LEAF SENESCENCE

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Leaf senescence is a programmed event responding to a wide range of external and internal signals including those caused by development, age and environment. Senescence requires de novo gene expression and protein synthesis and is controlled in a tightly regulated manner. Identification of the genes that control senescence has been complicated by the complex combination of signalling pathways that appear to be involved in senescence. Cross talk exists between senescence and stress or pathogen responses and also the hormonal and nutrient signals that are implicated in the control of senescence.

We are using Arabidopsis as a model, taking a systems biology approach, to identify the genes involved with the control of leaf senescence. Extensive high resolution timelapse microarray analysis has been analysed using a variational Bayesian State Space modelling method and transcriptional networks that pinpoint potential regulatory genes have been generated. Mutant analysis with potential hub genes has shown that many of these appear to have a role in the senescence process and we are formulating a few senescence related network models to test further. We are using various clustering techniques together with promoter motif analysis to characterise the global changes in gene expression during senescence. This analysis is being used to group potentially co-regulated genes. In addition, cross talk between stress related pathways and senescence is being elucidated by the use of mutants, stress treatments and comparative gene expression analysis.

11007

A MEMBRANE-BOUND RECEPTOR KINASE, RPK1, POSITIVELY CONTROLS AGE-DEPENDENT LEAF SENESCENCE AND CELL DEATH IN ARABIDOPSIS

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Leaf senescence has been regarded as a genetically programmed cell death (PCD) that is modulated by multiple internal and environmental cues, but the critical components underlying this process have not yet been elucidated. Using a PCR-based subtractive hybridization, we found that a membrane-bound receptor kinase RPK1 is expressed predominantly along leaf aging. Two stable knock-out mutants, rpk1-3 and rpk1-4 exhibited the extended leaf longevity with significant delays in cell death process during natural leaf senescence, while there is no noticeable alteration in the overall developmental process including the timing of leaf emergence and growth. On the contrary, the phenotype of ecdysone-agonist inducible RPK1 and RPK1-GFP transgenic plants indicated that RPK1 promotes onset of leaf senescence. Furthermore, inducible expression of RPK1 in transgenic leaves resulted in the induction of various senescence-upregulated genes, suggesting its positive role as an upstream component in age-dependent senescence and cell death pathway in Arabidopsis. In particular, oxidative stress-induced artificial senescence and cell death was dramatically impaired in the rpk1 mutants. Attempts are now being made to understand molecular and genetic functions toward an age-induced membrane-bound receptor kinase, RPK1, in controls of age-dependent senescence, and the data will be discussed in detail.

11008

THE ARABIDOPSIS PHOSPHATIDYLINOSITOL PHOSPHATE 5-KINASE PIP5K3 AT2G26420 IS A KEY REGULATOR OF ROOT HAIR TIP GROWTH

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Phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂] functions as a site-specific signal on membranes to promote cytoskeletal reorganization and membrane trafficking. Localization of PtdIns(4,5)P₂ to apices of growing root hairs and pollen tubes suggests that it plays an important role in tip growth. However, its regulation and mode of action remain unclear. We found that *Arabidopsis thaliana* PIP5K3 (*for Phosphatidylinositol Phosphate 5-Kinase 3, At2g26420*) encodes a phosphatidylinositol 4-phosphate 5-kinase, a key enzyme producing PtdIns(4,5)P₂, that is preferentially expressed in growing root hairs. T-DNA insertion mutations that substantially reduced the expression of PIP5K3 caused significantly shorter root hairs than in the wild type. By contrast, overexpression caused longer root hairs and multiple protruding sites on a single trichoblast. A yellow fluorescent protein (YFP) fusion of PIP5K3, driven by the PIP5K3 promoter, complemented the short-root-hair phenotype. PIP5K3-YFP localized to the plasma membrane and cytoplasmic space of elongating root hair apices, to growing root hair bulges, and, notably, to sites about to form root hair bulges. The signal was greatest in rapidly growing root hairs and quickly disappeared when elongation ceased. These results provide evidence that PIP5K3 is involved in localizing PtdIns(4,5)P₂ to the elongating root hair apex and is a key regulator of the machinery that initiates and promotes root hair tip growth.

11009

COMPARATIVE PHOTOSYNTHETIC ANALYSES OF THREE ARABIDOPSIS ECOTYPES

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In this study we have selected three *Arabidopsis* ecotypes, which are commonly used for generation of mutant lines, have distinct geographic distribution and plant morphology, namely Columbia Col-0 (Columbia USA), Landsberg erecta Ler-0 (Germany) and Wassilewskija Ws-4 (Russia). We have performed a detailed photosynthetic characterization using various biophysical, biochemical, electron microscopy and physiological methods. Similar levels of photosynthetic pigments, protein complexes and activities were determined in the three ecotypes. Nevertheless, Ws-4 displayed 50% lower levels of phosphorylation for the photosystem II reaction center D1 and D2 proteins as compared to Col-0 and Ler-0. These results were obtained using western blotting with phosphothreonine antibodies, and confirmed by quantitative mass spectrometry (LC-MS). Another remarkable difference was observed by electron microscopy in the ultrastructure of thylakoid membranes, namely fewer and shorter grana stacks in Ws-4 than in the other two ecotypes. Finally, Ws-4 is the ecotype with the largest shoot biomass as well as seed production. The significance of these differences will be discussed in the context of plant photoacclimation and repair cycle of photosystem II complex.

11010

PLASTID REPLICATION IN LEAF EPIDERMIS: INSIGHTS FROM THE ATMINE1 MUTANT OF ARABIDOPSIS

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In the leaf epidermis, chloroplasts are generally smaller and poorly developed, containing lower amount of chlorophyll, as compared with the mesophyll chloroplasts. Although extensive studies on the mechanism for plastid replication have been conducted with a major focus on mesophyll chloroplasts, recent studies have also pointed the tissue-dependent mode of plastid replication and the relevancy

of stromule formation to this process, especially in non-photosynthetic tissues. Here, we report the detailed morphology of immature chloroplasts and their stromules in the leaf epidermis, the intraplastidic localization of chloroplast division regulators AtMinE1 and AtFtsZ1-1, and its association with plastid replication and development in *Arabidopsis thaliana*. Using the functional AtMinE1-yellow fluorescent protein fusion, we demonstrate that AtMinE1 concentrates at the constricting plane of the dividing plastids, and simultaneously diffuses throughout the stroma, in the epidermis of leaves and petals, against the expectation of the Min system model of *Escherichia coli*. Our data also show that the localization of AtMinE1 is highly dependent on its expression level. In the knockdown *atminE1* mutant expressing the plastid-targeted cyan fluorescent protein, the epidermal chloroplasts exhibited the enhanced emanation of stromules and the severe heterogeneity of plastid size, which is totally different from the phenotype observed in the *atminE1* mesophylls. Further examination of plastid morphology in conjunction with the visualization of AtFtsZ1-1 indicates that the FtsZ1 ring-mediated stromule fission is responsible for the plastid heterogeneity in the *atminE1* epidermis. These results imply a difference, or a developmental transition, in the process of plastid replication between epidermal and mesophyll chloroplasts.

11011

EXTRACELLULAR NUCLEOTIDES ELICIT CYTOSOLIC FREE CALCIUM OSCILLATIONS IN ARABIDOPSIS

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In recent years, extracellular ATP (eATP) has been recognized as a cellular signaling compound. An extensive literature demonstrates that eATP is involved in numerous cellular processes in mammals. In contrast, recognition of eATP as a signaling molecule is relatively recent in plants. The addition of eATP is known to induce a rise in the level of cytosolic free calcium in plant cells. To expand our knowledge about the function of extracellular nucleotides, the effects of several nucleotide analogs on cytosolic calcium changes were studied, using transgenic Arabidopsis expressing aequorin or the calcium sensor Yellow Cameleon. Exogenously applied CTP caused elevations in cytosolic calcium levels that displayed distinct time- and dose-dependent kinetics compared to ATP or GTP. The inhibitory effects of purinoceptor antagonists and calcium influx inhibitors on nucleotide-induced cytosolic calcium elevations were distinct between the pyrimidine and purine nucleotides. These results suggest that distinct recognition systems may exist for the respective types of nucleotides. Interestingly, a mutant lacking the heterotrimeric G-protein beta-subunit (AGB1) exhibited a remarkably higher cytosolic calcium elevation in response to all tested nucleotides in comparison with wild type. The data suggests a role for AGB1 in negatively regulating extracellular nucleotide signaling and points to an important role for the G proteins in modulating the cellular effects of extracellular nucleotides. The addition of extracellular nucleotides induced multiple temporal cytosolic calcium oscillations that were attenuated by a vesicle trafficking inhibitor, indicating that the oscillations likely require ATP release via exocytotic secretion. The results reveal new molecular details concerning extracellular nucleotide signaling in plants and the importance of fine control of extracellular nucleotide levels to mediate specific plant cell responses.

11012

PLASTID STUDY OF TOMATO FRUITS USING THE TECHNIQUES OF ARABIDOPSIS RESEARCH

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We are analyzing the differentiation mechanism of plastids in tomato using the experimental methods as follows.

First, to analyze the comprehensive proteome involved in chloroplast to chromoplast differentiation, we established methods to isolate and identify chromoplast proteins of Micro-Tom fruits at four developmental stages (mature green, yellow, orange and red). We identified approximately 440 plastid proteins using LC-MS/MS. When we compared the chromoplast proteome data in Micro-Tom with the data in a bell pepper, we found common proteins. Second, we are trying to collect natural variations and mutants that have various colors of fruits such as white, black and orange, and then stop their ripening at the intermediate stage. We compare the chromoplast proteome in these fruits with that of Micro-Tom fruits at four developmental stages using two-dimensional gel electrophoresis, and find specific proteins related to chromoplast development, ripening, and the fruits' color. Third, we are trying to recreate specific protein mutants (found during the aforementioned proteome analysis) using RNA interference. Finally, we are making approximately 150 T-DNA tag lines and looking for mutants that change the fruits' color and inhibit fruit ripening.

At this conference we will report on proteins important to differentiation into chromoplast.

11013

ECHIDNA A NEW COMPONENT OF THE VESICULAR TRAFFICKING MACHINERY REQUIRED FOR CELL ELONGATION IN ARABIDOPSIS

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Our knowledge of the subcellular mechanisms underlying cell elongation in plants remains fragmentary. Here we report on the isolation of an *Arabidopsis* cell-elongation mutant named *echidna*. *ech* mutant plants displays defects in cell elongation in several tissues including root tissues. ECH is a membrane protein, well conserved among eukaryotes but of unknown function. ECH displays similarities with yeast Tvp23 and ECH can partly replace Tvp23 function in yeast. ECH colocalizes mainly with the V-ATPase subunit VHA-a1, syntaxin SYP41 and partly with RabA2a. Functional ECH-EYFP co-localises with the endocytic tracer FM4-64 rapidly after its application suggesting an early endosomal/TGN (trans-golgi network) localisation of ECH. In the *ech* mutant background, the Sec-GFP marker accumulates inside the cells indicating defect in secretion. Interestingly, the GFP fusions of VHA-a1 and RabA2a are partly mislocalised in the *ech* mutant being found on vacuole-like structures in root cells in the elongation zone. Additionally, VHAa1-GFP also localises to cell plates in *ech* mutant roots. Concanamycin A treatment phenocopies in many ways *ech* phenotype, suggesting that VHA-a1 mislocalization is greatly responsible for the mutant phenotype. Furthermore, EM ultrastructure analysis unravels a decrease of TGN association to Golgi apparatus. Taken together these results suggest that ECH is important for TGN integrity and confirm that the loss of maintenance of its organelle is crucial for cell elongation.

11014

IDENTIFICATION OF CO₂-BINDING CARBONIC ANHYDRASES THAT MEDIATE CO₂ SIGNAL TRANSDUCTION IN GUARD CELL

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Stomatal guard cells sense many stress and environmental signals and regulate CO₂ influx into leaves for photosynthetic carbon fixation and plant water loss. The continuing rise in atmospheric CO₂ causes leaf stomata to close, but the CO₂-binding proteins and early signal transduction mechanisms that trigger CO₂-induced stomatal movements and CO₂ sensing have remained largely unexplored. Our recent study shows that two β-carbonic anhydrases, βCA1 and βCA4, are required for [CO₂] regulation of stomatal movements and that CA-mediated catalysis is an important mechanism for βCA-mediated CO₂-induced stomatal closing (1). Moreover, the βCA-mediated stomatal CO₂ responses are not, in first order, linked to leaf photosynthesis and can function in guard cells. Patch clamp analyses indicate that CO₂/HCO₃⁻ transfers the signal to anion channel regulation. These findings together with *ht1-2* epistasis analysis demonstrate that βCA1 and βCA4 function very early in the CO₂ signaling pathway. βCA1 localizes in chloroplasts and also close to the plasma membrane, while βCA4 localizes to the plasma membrane. We will present protein interactors of βCA1 and βCA4 at the plasma membrane using several independent techniques. Interactions of βCA1 and βCA4 with the identified guard cell membrane proteins provide a mechanism for βCA-mediated CO₂ signaling in guard cells.

1. Hu, H., Boisson-Dernier, A., Israelsson-Nordstrom, M., Bohmer, M., Xue, S., Ries, A., Godoski, J., Kuhn, J. M., Schroeder, J. I. (2010). Carbonic anhydrases are upstream regulators of CO₂-controlled stomatal movements in guard cells. *Nature Cell Biology* 12(1): 87-93.

11015

DISSECTION OF POLAR LOCALIZATION AND ENDOCYTIC DEGRADATION MECHANISMS OF BORON TRANSPORTERS

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Boron (B) is essential for plant growth, but is toxic when present in excess. In the roots of *Arabidopsis thaliana* under B limitation, a boric acid channel, NIP5;1, and a boric acid/borate exporter, BOR1, are required for efficient B uptake and subsequent translocation into the xylem, respectively. However, upon high B supply, BOR1 activity is repressed through vacuolar trafficking presumably to avoid B toxicity. When expressed under the control of their native promoters, GFP-NIP5;1 and BOR1-GFP localized preferentially in outer (distal) and inner (proximal) plasma membrane (PM) domains, respectively, of various root cells under B limitation (Takano et al. 2010 PNAS). The polar localization of the boric acid channel and boric acid/borate exporter indicates the radial transport route of B toward the stele. In this presentation, we would like to compare the mechanisms of the polar localization and endocytic degradation of these transporters based on mutational analyses and localization analyses in the PM and the endosomes.

11016

ARABIDOPSIS FORWARD GENETICS SCREEN IDENTIFIES DPT3, A NOVEL REGULATOR OF PIN POLARITY

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Auxin is the hormone that coordinates plant development and, by a directional flow through the plant tissues, it mediates the polarity of tissues and organs. The directional auxin flow (known as polar auxin transport; PAT) is achieved by the subcellular localization of auxin influx and efflux carriers. Plasma membrane localized PIN proteins have been identified as auxin efflux carriers (Petrášek et al., 2006) and their polar localization determines the direction of auxin flow (Wiśniewska et al., 2006).

In order to shed light on how PIN proteins are delivered to the correct side of the cells and to identify novel regulators of PIN polarity, we EMS-mutagenized transgenic PIN2::PIN1:HA, an agravitropic line that show basal localization of PIN1:HA in the epidermal cells. We identified 4 novel Arabidopsis mutants that show positive gravitropic response and changed basal-to-apical polarity switch of PIN1:HA in the epidermal cells.

Here we present novel insights into the regulation of PIN polarity by detailed analysis of dpt3 (defective polar targeting3) mutant. Phenotypic analysis, immunolocalizations and hormone measurements clearly indicate that DPT3 regulates PIN polarity by a complex regulation of hormonal signalling.

11017

FUNCTIONAL CHARACTERIZATION OF ARABIDOPSIS GLUTAMATE RECEPTOR HOMOLOGS

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In animals, ionotropic glutamate receptors are ligand-gated ion channels and function as neurotransmitter receptors that mediate synaptic excitation in the central nervous system, and thus play a crucial role in long-term memory. The *Arabidopsis* genome encodes 20 glutamate receptor homolog genes (AtGLRs) that are divided into 3 phylogenetically distinct subfamilies. Several studies with pharmacological blockers and genetic mutants have suggested that *Arabidopsis* glutamate receptor homologs have a role in light signaling, calcium fluxes, cell division and survival in roots, carbon and nitrogen metabolism, ABA biosynthesis, responses to aluminum or environmental stress, calcium homeostasis, and resource allocation. Despite all these findings, direct evidence for the role of AtGLRs has not yet been provided. Using a heterologous expression system, we have identified AtGLRs that form calcium-permeable ion channels. Our data indicate that these AtGLRs are localized to the plasma membrane and physically interact with each other. Analysis of atglr knockout mutants demonstrates that these proteins function in calcium-mediated cellular signaling and growth processes. Further progress will be discussed.

11018

ERMO1/GNOM-LIKE1 AND ERMO2/SEC24A ARE REQUIRED FOR MAINTENANCE OF ENDOPLASMIC RETICULUM IN ARABIDOPSIS THALIANA

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The endoplasmic reticulum (ER) forms diverse complex structures with the largest surface area among organelles in eukaryotic cells. They are composed of tubules, sheets, and three-way junctions, resulting in a highly conserved polygonal network. These morphology supports various cellular functions of the ER, although the molecular mechanisms underlying the formation and maintenance of these structures remain poorly understood. In this study, we used a transgenic *Arabidopsis thaliana* plant (GFP-h) in which the ER was fluorescently labeled. We isolated two mutants so-called *ermo1* and *ermo2*, for "endoplasmic reticulum morphology", that showed defects in ER morphology (1). The cells of both mutants developed a number of ER-derived spherical bodies, ~1 μm in diameter, in addition to the typical polygonal network of ER. The spherical bodies were distributed throughout the *ermo1* cells, while they formed a large aggregate in *ermo2* cells. Our results demonstrate that these mutants are defective in a novel mechanisms that were unique to higher plant, rather than typical pathways such as interaction with cytoskeletons. We identified the responsible gene for *ermo1* to be *GNOM-LIKE1* (*GNL1*) and the gene for *ermo2* to be *SEC24a*. Both of *GNL1/ERMO1* and *SEC24a/ERMO2a* and their homologs were thought to be involved in ER-Golgi protein trafficking in various eukaryotes. In conclusion, we hypothesized that *ERMO1/GNL1* and *ERMO2/SEC24a* are transporting some specific proteins that were crucial for organizing ER morphology.

(1) Nakano, R. T. et al. (2009) *GNOM-LIKE1/ERMO1* and *SEC24a/ERMO2* Are Required for Maintenance of Endoplasmic Reticulum Morphology in *Arabidopsis thaliana*, *Plant Cell*, 21: 3672-3685.

11019

R1R2R3-MYB PROTEINS ACTING AS TRANSCRIPTIONAL REPRESSORS FOR G2/M PHASE-SPECIFIC GENES

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In plant cell cycle, a suit of genes have roles in entry into or progression through M phase and are specifically expressed at G2/M phase. Most of such G2/M phase-specific genes contain common cis-acting elements, called mitosis-specific activator (MSA) elements. The MSA elements bind to the R1R2R3-Myb transcription factors, which can be classified into two evolutionarily conserved subclasses, A-type and C-type Myb. We have previously shown that two A-type Myb proteins act as positive regulator of cytokinesis through transcriptional activation of many G2/M phase-specific genes including *KNOLLE* with an essential function for cell plate formation in *Arabidopsis thaliana*. Here, we report three R1R2R3-Myb proteins, MYB3R1, MYB3R3 and MYB3R5, acting redundantly as transcriptional repressors for the G2/M phase-specific genes. The *myb3r1 myb3r3 myb3r5* triple mutation resulted in elevated levels of G2/M phase-specific transcripts in *Arabidopsis thaliana*. Such up-regulation was preferentially observed in developmentally old organs with reduced activity for cell division rather than meristems or young organs that contain plenty of dividing cells, suggesting repressing roles of these R1R2R3-Myb proteins in quiescent cells at post-mitotic stages. When promoters from several G2/M phase genes were fused to GUS, mutations of their MSA elements resulted in similar up-regulation of GUS gene in developmentally old organs. CYCB1;1-GUS, which normally shows specific expression in G2/M phase cells, was up-regulated uniformly irrespective of cell cycle stages in root tips and young leaves of *myb3r1 myb3r3 myb3r5* triple mutant. These results suggested that the MSA element may have repressor function during G1 and S phases and in post-mitotic stages, which may be accomplished by binding with MYB3R1, MYB3R3 and MYB3R5.

11020

ARABIDOPSIS DYNAMIN-RELATED PROTEINS DRP2B AND DRP1A PARTICIPATE IN CLATHRIN-COATED VESICLE FORMATION DURING ENDOCYTOSIS.

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Endocytosis performs a wide range of functions in animals and plants. Clathrin-coated vesicle (CCV) formation is an initial step of endocytosis, and in animal cells is largely achieved by dynamins. However, little is known of its molecular mechanisms in plant cells. To identify dynamin-related proteins (DRPs) involved in endocytic CCV formation in plant cells, we compared the behaviors of two structurally different *Arabidopsis* DRPs, DRP2B and DRP1A, with those of the clathrin light chain (CLC), a marker of CCVs, at the plasma membrane by variable incidence angle fluorescent microscopy (VIAFM). DRP2B shares domain organization with animal dynamins while DRP1A is plant-specific. We show that green fluorescent protein (GFP)-tagged DRP2B and DRP1A co-localized with CLC tagged with monomeric Kusabira Orange (mKO) in *Arabidopsis* cultured cells. Time-lapse VIAFM observations suggested that both GFP-DRP2B and GFP-DRP1A appeared and accumulated on the existing mKO-CLC foci, and disappeared at the same time as or immediately after the disappearance of mKO-CLC. Moreover, fluorescently-tagged DRP2B and DRP1A signals co-localized and assembled/disassembled together at the plasma membrane in *Arabidopsis* cells. A yeast two-hybrid assay showed that DRP2B and DRP1A interacted with each other. An inhibitor of clathrin-mediated endocytosis, tyrphostin A23, disturbed the localization of DRP1A at the plasma membrane, but had little effect on the localization of DRP2B, indicating that DRP1A and DRP2B have different molecular properties. These results suggest that DRP2B and DRP1A participate together in endocytic CCV formation in *Arabidopsis* cells despite the difference of their molecular properties.

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MIRO1, AN ARABIDOPSIS MIRO GTPASE, INFLUENCES MITOCHONDRIAL MORPHOLOGY DURING POLLEN TUBE GROWTH AND EARLY EMBRYOGENESIS

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The regulation of mitochondrial morphology, intracellular distribution and inheritance are essential for plant growth and development. However, the molecular mechanisms involved are still unclear. Here, we show an analysis of the three *Arabidopsis* orthologs of the evolutionarily conserved Miro GTPases. Two of the genes, *MIRO1* and *MIRO2*, are transcribed ubiquitously throughout the plant tissues, and their gene products localize to mitochondria via their C-terminal transmembrane domains. While insertional mutations in the *MIRO2* gene do not have any visible impact on plant development, an insertional mutation in the *MIRO1* gene substantially impairs pollen germination and tube growth. It is also lethal during embryogenesis at the zygote to four-terminal-cell embryo stage. Laser confocal and transmission electron microscopy revealed that the *miro1* mutation causes abnormally enlarged mitochondrial morphology, leading to the disruption of continuous streaming of mitochondria in the growing pollen tube and the defective segregation of mitochondria in the dividing embryonic cells. Our findings suggest that mitochondrial morphology is influenced by *MIRO1* and plays a vital role during pollen

tube growth and embryogenesis.

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FUNCTIONAL ANALYSES OF THE PLANT AURORA KINASE IN CHROMOSOME DYNAMICS

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The proper segregation of chromosomes during mitosis is required for accurate distribution of genetic information by two daughter cells. Aurora kinases are serine/threonine protein kinases with essential roles in cell division through eukaryotes. Although functions of animal and yeast Aurora kinases have been analyzed in detail, those of plant Aurora kinases are unknown. In the *Arabidopsis thaliana* genome, the three deduced amino acid sequences showed high similarity to those from the animal Aurora kinase genes. The kinase domain of these three proteins has more than 60% identities to those of animal and yeast Aurora kinases. The three genes were designated AtAUR1 (*A. thaliana* Aurora kinase), AtAUR2, and AtAUR3, respectively. The localization of AtAUR3 was different from those of AtAUR1 and AtAUR2 in mitotic spindles. AtAUR3 localized at the nuclear periphery during interphase. At prophase, the dot-like condensed signals of AtAUR3 appeared when the chromosomes began to condense. At prometaphase, the signals moved to the metaphase plates along with the condensed chromosomes. At metaphase, the signals align in the center of the metaphase plates. However, at anaphase, in accordance with chromosome cohesion, AtAUR3 signals were almost ubiquitously observed on the whole chromosome. After cell division, the AtAUR3 signal returned to the nuclear membrane and the cytoplasm around the nucleus. Our live cell imaging analyses indicated that the plant Aurora kinase has dual roles; correction of aberrant kinetochore-microtubule attachment and dissociation of cohesin during chromosome alignment and segregation.

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FUNCTIONAL ANALYSIS OF AN *ARABIDOPSIS THALIANA* ABIOTIC STRESS-INDUCIBLE FACILITATED DIFFUSION TRANSPORTER FOR MONOSACCHARIDES

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Sugars play indispensable roles in biological reactions and are distributed into various tissues or organelles via transporters in plants. Under abiotic stress conditions, plants accumulate sugars as a means to increase stress tolerance. Here, we report an abiotic stress-inducible transporter for monosaccharides from *Arabidopsis thaliana* which is termed ERD six-like 1 (ESL1). Expression of *ESL1* was induced under drought and high-salinity conditions and with exogenous application of ABA. Promoter analyses using GUS and GFP reporters revealed that *ESL1* is mainly expressed in pericycle and xylem parenchyma cells. The fluorescence of *ESL1*-GFP fused protein was detected at tonoplast in transgenic *Arabidopsis* plants and tobacco BY-2 cells. Furthermore, alanine-scanning mutagenesis revealed that an N-terminal LxxLL motif in *ESL1* was essential for its localization at the tonoplast. Transgenic BY-2 cells expressing mutated *ESL1*, which was localized at plasma membrane, showed an uptake ability for monosaccharides. Moreover, the value of K_m for glucose uptake activity of mutated *ESL1* in the transgenic BY-2 cells was extraordinarily high and the transport activity was independent from a proton-gradient. These results indicate that *ESL1* is a low affinity facilitated diffusion transporter. Finally, we detected that vacuolar invertase activity was increased under abiotic stress conditions and the expression patterns of vacuolar invertase genes were similar to that of *ESL1*. Under abiotic stress conditions, *ESL1* might function coordinately with the vacuolar invertase to regulate osmotic pressure by affecting the accumulation of sugar in plant cells.

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ER MOTILITY AND F-ACTIN ORGANIZATION MEDIATED BY PLANT-SPECIFIC MYOSINS

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Plants exhibit an ultimate case of the intracellular motility involving rapid organelle trafficking and continuous streaming of the endoplasmic reticulum (ER). Although it was long assumed that the ER dynamics is actomyosin-driven, the responsible myosins were not identified, and the ER streaming was not characterized quantitatively. Here we developed software to generate a detailed velocity distribution map for the GFP-labeled ER. This map revealed that the ER in the most peripheral plane was relatively static, whereas the ER in the inner plane was rapidly streaming with the velocities of up to $\sim 3.5 \mu\text{m/sec}$. Similar patterns were observed when the cytosolic GFP was used to evaluate the cytoplasmic streaming. Using gene knockouts, we demonstrate that the ER dynamics is driven primarily by the ER-associated myosin XI-K, a member of a plant-specific myosin class XI. Furthermore, we show that the myosin XI deficiency affects organization of the ER network and orientation of the actin filament bundles. Collectively, our findings suggest a model whereby dynamic three-way interactions between ER, F-actin, and myosins determine the architecture and movement patterns of the ER strands, and cause cytosol hauling traditionally defined as cytoplasmic streaming.

Reference: Ueda, H. et al. (2010) *Proc. Natl. Acad. Sci.*, in press

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EXPRESSION OF NAI2 AND PYK10 IS SUFFICIENT FOR THE ENDOPLASMIC RETICULUM (ER) BODY FORMATION IN ARABIDOPSIS

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The endoplasmic reticulum (ER) body is an ER-derived organelle that is specific to Brassicales plants. In *Arabidopsis thaliana*, a basic helix-loop-helix type transcription factor NAI1 regulates the expression of PYK10, a beta-glucosidase of major ER body component, and NAI2, a novel ER body protein for ER body formation (1,2). The ER body is disappeared in the *NAI2* deficient mutants (2) and ER bodies become longer in the mutants of *PYK10* gene (3), suggesting that NAI2 and PYK10 are involved in ER body formation. We found that the co-expression of NAI2 and PYK10 is sufficient for ER body formation in onion (*Allium cepa*), a non Brassicales. These findings indicate that NAI2 and PYK10 are sufficient for the ER body formation. NAI1 regulates the expression of membrane of ER body 1 (MEB1) and MEB2.

MEB1 and MEB2 are integral membrane proteins that localize to the ER body membrane. The artificially induced ER bodies accumulate MEB2 in onion cells. These findings indicate that ER body components, NAI2 and PYK10, regulate ER body formation and localization of membrane proteins of ER body.

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PHOSPHOLIPASE A2 AFFECTS THE INTRACELLULAR TRAFFICKING OF PIN PROTEINS IN THE ARABIDOPSIS ROOT

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Phospholipase A2 (PLA2), which hydrolyzes a fatty acyl chain of membrane phospholipids, has been implicated in several biological processes in plants. However, its role in intracellular trafficking in plants has yet to be studied. Here, using pharmacological and genetic approaches, the root hair bioassay system, and PIN-FORMED (PIN) auxin efflux transporters as molecular markers, we demonstrate that plant PLA2s are required for the trafficking of PIN proteins to the plasma membrane (PM) in the *Arabidopsis thaliana* root. The PLA2 α and PLA2 β proteins co-localized with Golgi and endoplasmic reticulum markers, respectively. Impairments of PLA2 function by PLA2 α mutation, PLA2-RNAi, or PLA2 inhibitor treatments significantly disrupted the PM localization of PINs, causing internal PIN compartments to form. Conversely, supplementation with lysophosphatidylethanolamine (the PLA2 hydrolytic product) restored the PM localization of PINs in the pla2 α mutant. Suppression of PLA2 activity by the inhibitor induced a greater accumulation of trans-Golgi network vesicles. Root hair-specific PIN overexpression (PINox) lines grew very short root hairs due to lowered auxin levels in root hair cells, but PLA2 inhibitor treatments, PLA2 α mutation, or PLA2-RNAi restored the root hair growth of PINox lines by disrupting the PM localization of PINs and thus probably reducing auxin efflux. These results suggest that PLA2, likely acting in Golgi-related compartments, modulates the trafficking of PIN proteins.

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CDKS NEGATIVELY REGULATE A MAPK CASCADE INVOLVED IN PLANT CYTOKINESIS

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Cyclin-dependent kinases (CDKs) operate and coordinate multiple cell-cycle events. However, it remains unclear whether CDKs regulate events following anaphase including cytokinesis. Plant cytokinesis occurs in specific arrays of microtubules named the phragmoplast that is formed at the region between two daughter nuclei. The NACK-PQR MAPK pathway, which is composed of NACK1 kinesin-like protein (a tobacco homologue of *Arabidopsis* HINKEL), NPK1 MAPKKK, NQK1 MAPKK and NPK1/NTF6 MAPK in tobacco, is known as a key-regulator controlling plant cytokinesis. All these components are localized on the equator of phragmoplast and positively regulate the phragmoplast expansion followed by the formation of cell plates. NACK1 is an activator of this MAPK cascade and it controls the activation and localization of NPK1 by direct binding. Although NPK1 is specifically activated during cytokinesis, both NACK1 and NPK1 already accumulate at prophase and metaphase when NPK1 is inactive. Therefore, it is speculated that the interaction between NACK1 and NPK1 is repressed during these phases. Here we report that CDKs function as negative regulators of the NACK-PQR MAPK pathway during early M phase. Both NACK1 and NPK1 were highly phosphorylated at the conserved sites of CDK-phosphorylation *in vitro* and *in vivo* during early M phase. Levels of phosphorylated NACK1 and NPK1 dramatically decreased at late M phase when NPK1 was activated. The phosphorylation of these proteins depended on the activity of CDKs *in vivo*. In addition, the phosphorylation of NACK1 and NPK1 by CDKs inhibited the interaction between NACK1 and NPK1 that results in the NPK1 activation. These results suggest that CDKs repress the activation of NACK-PQR pathway until the onset of cytokinesis.

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AMSH3 IS A DEUBIQUITINATING ENZYME REQUIRED FOR VACUOLE BIOGENESIS

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AMSH (Associated Molecule with the SH3 domain of STAM) is an evolutionarily conserved deubiquitinating enzyme (DUB) that belongs to the MPN+ domain protein family. Deubiquitinating enzymes can counteract the activity of ubiquitininating enzymes, but the regulation of cellular processes and plant development through deubiquitination is not yet well understood in comparison to that of ubiquitination.

The *Arabidopsis thaliana* AMSH3 protein is a DUB that is essential for plant growth. *amsh3* null mutants are seedling lethal and accumulate ubiquitinated proteins. However, the rate of degradation of proteasomal substrates are indistinguishable from the wild-type, suggesting that the AMSH3 function is independent of the ubiquitin-proteasome pathway. Moreover, the *amsh3* mutants have vacuolar biogenesis defects accompanied by defects in endocytosis and also in vacuolar protein transport. To understand the molecular function of AMSH3 in the context of the mutant phenotype, we carried out a yeast-two-hybrid screen and immunoprecipitation experiment followed by LC/MS-MS and identified interacting proteins of AMSH3. Many of the proteins thus identified are proteins with known functions in intracellular trafficking, indicating a role of AMSH3 in this pathway. We further characterized the interaction of AMSH3 with an ESCRT (Endosomal Sorting Complex Required for Transport)-III component VPS2 that was isolated as an AMSH3-interactor in the yeast-two hybrid screen, and found out that among the three VPS2 proteins in *Arabidopsis* VPS2.1, but not its close homologues VPS2.2 and VPS2.3 interact with AMSH3.

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MICROPYLAR GUIDANCE1 IS ESSENTIAL FOR POLLEN TUBE RESPONSE TO THE GUIDANCE CUES FROM THE EMBRYO SAC IN ARABIDOPSIS

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MG1 is essential for pollen tube response to the guidance cue from the embryo sac in *Arabidopsis*
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In flowering plants, precise guidance of the pollen tube through the female reproductive tissues to the embryo sac is critical for sexual reproduction. Pollen tube guidance by which pollen tube find the entry to the embryo sac, is a common phenomenon in angiosperms and requires coordinated male-female gametophytic interactions, but the underlying molecular mechanism is poorly understood. The entire process of pollen tube guidance can be divided into two phases: the sporophytic phase and gametophytic phase, and the latter include funicular phase and micropylar phase. There are multiple guidance cues along the pollen tube pathway, and the pollen tube employs different mechanisms to perceive and respond to the cues. Although there are evidences that the synergid in ovule emits diffusible attractants, how the pollen tube responds to them is still unknown.

We identify a novel *Arabidopsis* mutant, (*mg1*) that is defective in micropylar pollen tube targeting. The *mg1* pollen tube can grow normally up the funiculus but can not enter the micropyle, displaying a wandering behavior outside the micropyle. *MG1* is expressed specifically in synergid cell and mature male gametophyte in the reproductive organs, and ubiquitously in other parts of the plant. *MG1* protein is conserved and essential for embryogenesis, because *mg1* mutant embryos are lethal. Furthermore, RNAi knock-down of *MG1* phenocopies the mutant phenotype. *MG1* has a dynamic subcellular localization that provides important clues of *MG1* function in pollen tube guidance. Through yeast two hybrid screening, a series of candidate interacting proteins of *MG1* is identified which may help to decipher the *MG1* molecular mechanism.

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SUBCELLULAR LOCALIZATION OF PHOSPHATIDYL SERINE IN *A. THALIANA*

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In *A. thaliana*, phosphatidylserine (PS) is relatively enriched in roots and flowers compared with photosynthetic tissues comprising ~5.9% and ~20.6% of extraplastidial membrane phospholipids, respectively. A base-exchange-type PS synthase (AtPSS1) is responsible for the synthesis of PS from phosphatidylethanolamine (PE) and serine. AtPSS1-EYFP is associated with the ER and nuclear membranes in root and anther cells. *atpss1* mutation causes abnormal morphology and partial inhibition of pollen development, suggesting that PS biosynthesis is required for normal pollen development. GUS expression driven by an *AtPSS1* promoter in transgenic *Arabidopsis* was prominent in anther and embryo.

To further understand the physiological significance of PS in plant development, we examined the subcellular localization of PS in *Arabidopsis* plants that expressed the PS biosensor EGFP-LactC2 (Yeung et al., 2008). In root cells, EGFP-LactC2 fluorescence was associated with the nuclear membranes, ER and punctate structures in root cells. In root hair, EGFP-LactC2 fluorescence was selectively associated with mitochondria that showed relatively weak Mito Tracker Red fluorescence. In pollen mother cells and tetrads, EGFP-LactC2 fluorescence was associated with the punctate structures. After a microspore release from tetrads, EGFP-LactC2 fluorescence was associated with nuclear membranes and then translocated to punctate structures during pollen mitosis. These results suggest that translocation of PS between different organelles and its exposure to the cytosolic side could play an important role in plant development.

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FUNCTIONAL DIVERSIFICATION OF *ARABIDOPSIS* EB1 PROTEINS

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Morphogenesis and division of eukaryotic cells are regulated by microtubule cytoskeleton and its regulators. End-binding 1 (EB1) proteins are evolutionarily conserved plus-end-tracking proteins that localize to growing microtubule plus ends where they regulate microtubule dynamics and interactions with intracellular targets. Animal EB1 proteins possess acidic C-terminal tails that might induce an autoinhibitory conformation. Although EB1 proteins with the same structural features occur in plants (EB1a and EB1b in *Arabidopsis thaliana*), a variant form (EB1c) is present that lacks the characteristic tail. We show that in *Arabidopsis* the tail region of EB1b, but not of EB1c, inhibits microtubule assembly in vitro. EB1a and EB1b form heterodimers with each other, but not with EB1c. Furthermore, the *EB1* genes are expressed in various cell types of *Arabidopsis*, but the expression of *EB1c* is particularly strong in the meristematic cells where it is targeted to the nucleus by a nuclear localization signal in the C-terminal tail. Reduced expression of *EB1c* compromised the alignment of spindle and phragmoplast microtubules and caused frequent lagging of separating chromosomes at anaphase. The *eb1a eb1b* roots exhibited abnormal skewing growth in response to a microtubule-disrupting drug oryzalin whereas the *eb1c* roots showed increased sensitivity to oryzalin at the cell division zone and complete rescue of the mutant phenotype required the tail region of EB1c. These results suggest that *Arabidopsis* has EB1s that are different in function, especially a plant-specific EB1 subtype has evolved to function preferentially on the spindle microtubules by accumulating in the prophase nucleus.

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THE PP2A REGULATORY SUBUNIT TAP46 CONTROLS CELL GROWTH AND METABOLISM IN PLANTS

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Tap42/d4, a regulatory subunit of protein phosphatases 2A (PP2A), is a downstream effector of the target of rapamycin (TOR) protein kinase that regulates cell growth in yeast in coordination with nutrient and environmental conditions. However, Tap42/d4 functions in higher eukaryotes differ from those in yeast, while the function of its homolog in plants, Tap46, is unknown. In this study, we have characterized the functions and phosphatase regulation of plant Tap46. Depletion of Tap46 in *Nicotiana benthamiana* and *Arabidopsis* resulted in growth arrest and acute plant death with morphological markers of programmed cell death (PCD). Tap46 interacted with PP2A and PP2A-like phosphatases PP4 and PP6, and Tap46 deficiency dramatically decreased cellular PP2A activities. In earlier stages of gene silencing before PCD occurs, reduced cellular translation activities and activation of autophagy were observed in both the VIGS and RNAi plants, as observed in TOR-silencing plants. Interestingly, Tap46 silencing in tobacco BY2 cells caused chromatin bridge formation at anaphase, indicating that plant Tap46 plays an unique role among Tap42/d4 family members in sister chromatid segregation. These findings suggest that Tap46, in conjunction with associated phosphatases, plays an essential role in plant growth and development, and that Tap46 has additional functions in plant, apart from being a TOR signaling component.

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PLANT AUTOPHAGY NEGATIVELY REGULATES CELL DEATH BY CONTROLLING SALICYLIC ACID SIGNALING DURING SENESCENCE AND THE INNATE IMMUNE RESPONSE

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Autophagy is an evolutionarily conserved intracellular process for vacuolar degradation of cytoplasmic components. Recent reverse genetic studies showed that autophagy-defective *Arabidopsis* plants (*atg* mutants) exhibited phenotypically accelerated leaf senescence, insufficient root elongation and reduced seed yields under nutrient-starved conditions. These results supported the notion that plant autophagy plays an important role in nutrient recycling especially in nutrient-starved conditions. However, several lines of evidence suggested that plant autophagy also has additional functions other than just recycling of proteins to serve as a source of amino acids. Irrespective of nutrient-rich conditions, autophagy defects in higher plants resulted in early senescence and excessive immunity-related programmed cell death (PCD). Until recently the mechanisms by which cells die in the absence of autophagy have been unclear.

We recently found a conserved requirement for salicylic acid (SA) signaling for these phenomena in *atg* mutants. The *atg* mutant phenotypes of accelerated PCD in senescence and immunity were SA signaling dependent but did not require intact jasmonic acid or ethylene signaling pathways. Application of an SA agonist restored the senescence/cell death phenotype in SA-deficient *atg* mutants but not in *atg npr1* plants, suggesting that the cell death phenotypes in the *atg* mutants are dependent on the SA signal transducer NPR1. We also showed that autophagy was induced by the SA agonist. These findings imply that plant autophagy operates a novel negative feedback loop modulating SA signaling to negatively regulate senescence and immunity-related PCD.

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HETERODIMER FORMATION AS MECHANISM OF REGULATION OF ALLENE OXIDE CYCLASE ACTIVITY IN ARABIDOPSIS THALIANA

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Jasmonic acid (JA) and its precursor 12-oxo-phytodienoic acid (OPDA) are major components of plant defence mechanisms against biotic and abiotic stresses, but are also involved in developmental processes. A crucial enzyme in biosynthesis of JA is the allene oxide cyclase (AOC), which is encoded by a small family of four genes in *A. thaliana*^[1]. These four genes exhibit an organ specific and overlapping expression pattern. Since the AOC of corn may form dimers^[2] and the AOC of *Arabidopsis* was found as trimers after crystallisation^[3], the question raised, whether the AOC isoforms can form heteromers resulting in an additional level of regulation in the biosynthesis of JA. The aim of this work is to investigate the possible interaction and formation of heteromers of the four *Arabidopsis* AOC proteins and the impact on enzymatic activity. This was done for *in vitro*- and *in vivo*-interaction using biochemical methods (crosslinking experiments and SDS-page analyses) and the bimolecular fluorescence complementation system (split-YFP), respectively.

Crosslinking experiments showed a multimerization of AOCs, which was identified as trimers using size exclusion chromatography. The multimerization was also observed in the split-YFP experiments. Here, all AOC isoforms were able to interact with each other, while each interaction showed its own "morphology".

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FUNCTIONAL ANALYSES OF PTAC10 IN PLASTID TRANSCRIPTION AND CHLOROPLAST BIOGENESIS.

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pTAC10 was identified as a component of the plastid transcriptionally active chromosome (pTAC) in *Arabidopsis*, but its function in chloroplasts has not been characterized. In this study, we investigated *in vivo* functions of pTAC10 using gene silencing and overexpression technology in *Nicotiana benthamiana*. NbTAC10, *Nicotiana benthamiana* TAC10, contains a S1 domain that is involved in nucleic acid binding. Confocal microscopy indicated that NbTAC10:GFP fusion protein was targeted to the chloroplasts and colocalized with the chloroplast (cp)-nucleoids. Induced expression of the recombinant proteins of the full-length NbTAC10 or its S1 domain condensed genomic DNA in *E. coli*, and arrested the bacterial growth. Interestingly, the NbTAC10:GFP proteins were localized in the vicinity of the condensed nucleoids in the growth-arrested *E. coli* cells. Silencing of NbTAC10 by using virus-induced gene silencing (VIGS) or cosuppression resulted in leaf yellowing and moderate plant growth defects. Based on northern blot analyses, NbTAC10 deficiency significantly perturbed the PEP (plastid-encoded monomeric RNA polymerase)-dependent transcript accumulation but not the NEP (nucleus-encoded phage-type RNA polymerases)-dependent transcript accumulation. Prolonged depletion of NbTAC10 resulted in chloroplast ablation with drastic size reduction and thylakoid degeneration. In contrast, NbTAC10-overexpressing plants showed no visible plant phenotypes, and their plastid transcription profiles and chloroplast biogenesis were also normal. These results and the previous finding suggest that TAC10 plays a critical role in PEP-dependent chloroplast transcription and biogenesis within the plastid transcriptionally active chromosome complex.

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FUNCTIONAL CHARACTERIZATION OF NBRABE1 IN NICOTIANA BENTHAMIANA

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Small GTP-binding proteins regulate diverse processes in eukaryotic cells such as signal transduction, cell proliferation, cytoskeletal organization, and intracellular membrane trafficking. The Rab family is a part of the Ras superfamily of small GTPases. There are at least 60 Rab genes in the human genome, and a number of Rab GTPases are conserved from yeast to humans. Different Rab GTPases are localized to the cytosolic face of specific intracellular membranes, where they function as regulators of distinct steps in membrane traffic pathways. Particularly, it has been reported that RabE isoforms regulate polarized secretion in yeast and mammals. In this study, we used virus-induced gene silencing (VIGS) to investigate the *in planta* functions of *Nicotiana benthamiana* RabE1 (NbRabE1). Depletion of NbRabE1 resulted in growth arrest and premature senescence in *N. benthamiana*. The NbRabE1 promoter drove strong reporter gene expression in the stomata guard cells and in young tissues containing dividing cells, such as shoot apical meristems, leaf primordia, developing vascular tissues, root tips, and lateral roots. In mature leaves, NbRabE1:GFP fusion proteins were predominantly localized in the nucleus and the plasma membrane of the guard cells. When expressed in leaf mesophyll cells, the dominant negative and constitutive active mutants of NbRabE1 were also localized in the nucleus and plasma membrane. NbRabE1 silencing led to severe defects in guard cell division, resulting in stomata with abnormal morphology. Thus RabE1 is critically involved in guard cell division in higher plants. In addition to the stomata defects, NbRabE1-silenced plants exhibited increased ploidy levels and reduced cell size in the leaf, showing the pleiotrophic effects of NbRabE1 silencing.

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THE V-ATPASE- ASSEMBLY AND QUALITY CONTROL OF A COMPLEX ENGINE

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The protein repertoire of a eukaryotic cell is largely organized in protein complexes and protein machines. Although detailed data on function and structure exist for individual complexes, the mechanisms underlying their assembly remain elusive. The V-ATPase is a highly conserved protein machine, which in yeast is built with the help of dedicated assembly factors. Here, we explore the role of the potential *Arabidopsis thaliana* assembly factor orthologues AtVMA12 and AtVMA22 in the assembly of the V-ATPase. In order to assess whether AtVMA12 and AtVMA22 are true assembly factors, we characterized T-DNA insertion lines. Phenotypic analysis revealed an embryolethality, which has also been observed for V-ATPase knock-out alleles. We were able to complement this phenotype using a genomic construct of AtVMA22-GFP as well as overexpression constructs of AtVMA12-RFP and AtVMA22-GFP. Transient expression revealed that AtVMA22 is recruited to the endoplasmatic reticulum (ER) by AtVMA12, proving an interaction between the two proteins. Inducible expression of an amiRNA against AtVMA22 led to retention of VHA-a3, a subunit of the tonoplast V-ATPase in ER-like structures, indicating that the AtVMA22 affects either assembly or trafficking of the V-ATPase.

To ask, whether function of the V-ATPase is related to assembly, we generated a mutant defective in proton pumping with otherwise normal V-ATPase assembly (VHA-a3M-GFP). While a functional V-ATPase is transported to the tonoplast, the VHA-a3M-GFP mutant was retained in the ER. However, genetic and pharmacological inhibition of V-ATPase activity led to transport of the VHA-a3M-GFP past the ER and to the tonoplast. These results indicate that assembly of the V-ATPase is linked to a quality control mechanism that requires activity of the V-ATPase in the ER.

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A NOVEL, HYDROPHILIC CATION-BINDING PROTEIN PCAP1 IS STABLY ASSOCIATED WITH PLASMA MEMBRANE AND IS INVOLVED IN REGULATION OF STOMATAL APERTURES

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A new type of Ca-binding protein, PCaP1 [plasma membrane (PM) associated cation-binding protein-1], was found in *Arabidopsis thaliana* (Ide *et al.* 2007). The orthologous proteins were found in several plant species. PCaP1 has no transmembrane domain and localizes in the PM via N-myristoylation. The protein binds Ca^{2+} , Cu^{2+} , PtdInsPs and CaM/ Ca^{2+} (Nagasaki *et al.* 2008a, b). The promoter-GUS reporter expression analysis revealed that PCaP1 is expressed in most tissues. The PCaP1 protein can be detected immunochemically in these tissues. The PCaP1 mRNA level is increased in response to excess Cu^{2+} and the flagellin oligopeptide flg22.

We investigated intracellular localization of PCaP1 by expressing a pPCaP1::PCaP1-GFP construct. PCaP1-GFP was clearly detected on the PM in the tissues. The localization was not changed even under stress conditions. In most tissues, the fluorescence was detected on the PM at even intensity indicating no polarity of the PCaP1 distribution. In guard cells, however, PCaP1 was detected in the outside half of the PM, which is surrounded by epidermal cells. There was no morphological change in the stomata of *pcap1* leaves. The stomatal apertures of *pcap1* leaves were higher than that of wild type leaves in the dark. Moreover, *pcap1* leaves did not close their stomata completely even treated with ABA. The results indicate the involvement of PCaP1 in regulation of the stomatal apertures. With these results and biochemical properties, we estimate that PCaP1 functions as a molecular switch to mediate the calcium signaling to the phosphoinositide signaling in the PM. PCaP1 may mask PtdInsP₂ at resting state. Elevated cytoplasmic Ca^{2+} level induced by physiological stimuli may trigger the interaction of Ca^{2+} /CaM complex with PCaP1. We will propose a biochemical model of role of PCaP1 in plant cells.

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ANALYSIS OF THE ATSEC23, COAT COMPONENTS OF COPII TRANSPORT VESICLE IN ARABIDOPSIS THALIANA.

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Among transport vesicle responsible for protein trafficking in eukaryotic cells, COPII coated vesicles mediate anterograde transport from endoplasmic reticulum (ER) to Golgi apparatus. The COPII coat complex is composed of small GTPase, Sar1 and 2 types of protein complexes (Sec23/Sec24 complex and Sec13/Sec31 complex). Sec23/Sec24 hetero-dimer recognizes and sorts specific cargo. Sec13/Sec31 hetero-tetramer concentrates cargo and curves ER membrane. By search of database, there are 7 types of Sec23p homologue. We named these Sec23 homologues ATSEC23A, ATSEC23B, ATSEC23C, ATSEC23D, ATSEC23E, ATSEC23F and ATSEC23G, respectively. Here, we report results of subcellular localization, expression site and interaction analysis for each ATSEC23.

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ANALYSIS OF THE ARABIDOPSIS RESPONSE REGULATOR 4 (ARR4) AND ITS INTERACTION WITH PHYTOCHROME B

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The *Arabidopsis* Response Regulator 4 (ARR4) acts as the output element of a two-component signalling pathway, an important phospho-transfer mechanism in higher plants (Stock *et al.*, 2000). After activation by phosphorylation, ARR4 interacts with the red/far-red light photoreceptor phytochrome B (PhyB) leading to the stabilization of its active form which results in enhanced photomorphogenic responses (Sweere *et al.*, 2001; Mira-Rodado *et al.*, 2007). Mutation of the conserved phosphorylation site within the receiver domain of ARR4 negatively affects photomorphogenesis (Mira-Rodado *et al.*, 2007). By using the Förster Resonance Energy Transfer (FRET) method we show that ARR4 and Phytochrome B also interact *in planta*.

Although the cross-talk between the two-component system and red light signalling is well understood, it is unclear in which subcellular compartment the phosphorylation of ARR4 and its interaction with PhyB take place since both proteins are found in the cytoplasm and the nucleus.

To further investigate these questions, transgenic *Arabidopsis* lines have been created overexpressing either wildtype ARR4, ARR4 fused to a nuclear localisation signal (NLS) or ARR4 fused to a nuclear export signal (NES). Initial results will be presented and discussed.

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LOCALIZATION, INTRACELLULAR DYNAMICS AND FUNCTION OF THE *ARABIDOPSIS* HISTIDINE KINASE AHK1

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The histidine kinase AHK1 has been suggested to act as an osmosensor that detects water stress at the plasma membrane and initiates various downstream responses to establish stress tolerance in *Arabidopsis thaliana* (1, 2, 3). It has also been demonstrated that AHK1 is a positive regulator of drought and salt stress and abscisic acid signaling (2, 3). However, until now little is known about the molecular mechanism that links AHK1 signal perception at the plasma membrane and the role of intracellular AHK1 dynamics to AHK1-dependent processes.

We present data indicating that a functional GFP fusion of AHK1 localizes to the plasma membrane and to vesicle-like compartments in transiently transformed tobacco epidermal leaf cells. Furthermore *in planta* FRET and yeast mbSUS interaction studies suggest that AHK1 forms homodimers. To gain further insight into the molecular function of AHK1 we have developed a single-cell system in tobacco that allows us to directly measure osmotic stress-induced changes in gene expression at single cell level as a result of AHK1-dependent activity and its intracellular dynamics.

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ANALYSIS OF TRANS-GOLGI NETWORK (TGN) DYNAMICS IN PLANTS

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In all eukaryotic 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 critical roles 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 platform 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 SYP43, 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-SYP43 under the control of the native promoter. The observation by confocal laser scanning microscopy showed that the TGN is a punctum-looking, BFA (brefeldin A)-sensitive and Wm (wortmannin)-insensitive compartment, which is partially stained with FM4-64 at 15 min. Next, we addressed the relations between the TGN and the Golgi apparatus by analyzing the transgenic plant expressing mRFP-tagged ST (sialyl transferase, trans-Golgi marker) with SYP43 tagged with GFP. The fluorescence patterns indicated that there are two types of the TGN; one mainly locates beside the Golgi apparatus and behaves together, the other is independent from the Golgi apparatus. Moreover, we found that the TGN independent from Golgi apparatus is generated form the TGN associated with Golgi apparatus. We will also discuss dynamic interactions between TGN and the Golgi apparatus.

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VISUALIZING THE ROLE OF THE TRANS-GOLGI NETWORK FOR EARLY ENDOSOMAL SYSTEM IN PLANT CELLS

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The trans-Golgi network (TGN) is known as a major sorting organelle. Recent work has suggested that the TGN also functions in early endosomal trafficking in plant cells. However, contribution of the TGN to the endocytic pathway still remains elusive. We tried to visualize endocytosis in plant cells using a Leu-rich repeat transmembrane receptor kinase, FLAGELLIN SENSITIVE2 (FLS2), as an endocytosis marker. We have succeeded in visualization of flg22-dependent endocytosis in leaves of *Nicotiana benthamiana* transiently expressing FLS2-GFP. To examine the compartments involved in endocytosis of FLS2, we also co-expressed several organelle markers. For information on molecular machineries of the endocytosis, we then focused on RAB GTPases, small GTPases regulating targeting and/or tethering of transport vesicles. We expressed GDP- or GTP-fixed mutants of RAB GTPases and observed its effect on endocytosis of FLS2. We present how RAB GTPases mediate endocytic transport of FLS2 through the TGN and late endosomal compartments.

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PLANT-UNIQUE POST-GOLGI TRAFFICKING PATHWAYS REGULATED BY ARA6 AND VAMP727

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Membrane trafficking plays integral parts in various cell activities in all eukaryotic cells. Especially, post-Golgi organelles play fundamental roles in various plant functions of higher order, where RABs and SNAREs play crucial regulatory roles in membrane tethering and fusion. Each organism has distinct set of RABs and SNAREs, and each molecule is expected to regulate a specific transport pathway. We are

studying molecular mechanisms of post-Golgi trafficking pathways with a special focus on plant-unique RAB and SNARE molecules.

A. thaliana has two types of RAB5 members, conventional RAB5 and plant unique ARA6. Distinct subcellular localization of ARA6 from conventional RAB5 suggested functional differentiation between these two RAB5 groups, but their precise functions remained unknown. On the other hand, VAMP727 is a plant-unique R-SNARE, which is characterized by an insertion of 20 amino acids in its N-terminal longin domain. We have already reported that VAMP727 forms a SNARE complex with VAM3, VTI11, and SYP51 on a subpopulation of PVCs closely associated with the vacuolar membrane (Ebine *et al.*, 2008). In this meeting, we will report our recent results on a regulatory role of ARA6 in VAMP727 complex formation.

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CHARACTERIZATION OF RAB11 COMPARTMENTS IN ARABIDOPSIS THALIANA

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Rab GTPase is one of the key regulators of membrane trafficking. Rab11, a broadly conserved Rab GTPase group in eukaryotes, seems to have diversified in a unique way in plants. Plant Rab11 has notable diversity, while yeast and animals possess a few members. In *Arabidopsis thaliana*, 57 Rab GTPases are encoded in its genome in total, 26 of which are classified into the Rab11 group (subclassified into RabA1-RabA6). It is already known that animal Rab11 plays an important role in regulating various cellular functions. On the other hand, the precise function of plant Rab11 remains largely unknown. To reveal the plant Rab11 function, we analyzed subcellular localization of 9 members belonging to the RabA1 subgroup (RabA1a-RabA1i). We generated transgenic plants expressing GFP/Venus-tagged RabA1s, which are driven by their own promoters. These transgenic plants showed that RabA1a, RabA1b, RabA1c and RabA1d are ubiquitously expressed through all tissues. On the other hand, RabA1e is expressed specifically in root hair cells and RabA1f and RabA1g are detected only in pollen. Detailed observation of GFP-RabA1e in root hair cells revealed that it localizes on dynamically moving punctate structures, some of which are adjacent to SYP42 and SYP43-labelled *trans*-Golgi network (TGN). RabA1e compartments also accumulate in the tip region of growing root hairs, where TGN does not exist. Furthermore, we found that RabA1e colocalizes with an R-SNARE protein that functions in the secretory pathway. These results suggest that RabA1e functions in secretion from the TGN to the PM in growing root hairs. We will also report the phenotype of mutants lacking RabA1 proteins.

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THE UGPASE IS ESSENTIAL FOR PLANT GROWTH AT VEGETATIVE AND REPRODUCTIVE STAGES IN ARABIDOPSIS THALIANA

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UDP-glucose pyrophosphorylase (UGPase) is an important enzyme for UDP-glucose, a precursor for the synthesis of the carbohydrate and cell wall components, such as a cellulose and callose. The *Arabidopsis* genome contains two putative genes encoding UGPase, *AtUGP1* and *AtUGP2*. In order to discriminate the role of UGPase in the vegetative and reproductive organs, we approached a reverse genetic and biochemical studies using the T-DNA insertion mutants, *atugp1* and *atugp2*. Despite significant decrease of UGPase activity both in the *atugp1* and *atugp2* single mutants, none was essential for a normal growth and reproduction. In contrast, the *atugp1/atugp2* double mutant displayed drastic growth defects and male sterility. These results suggest that the *AtUGP1* and *AtUGP2* genes are functionally redundant and the UGPase activity is essential for both vegetative and reproductive phases in *Arabidopsis*. Importantly, the size of the double mutant which grown on the extra-sucrose was recovered to the comparable size to the wild type. By contrast, its male sterility was not restored in the sucrose fed growth medium but in the UDP-glucose fed medium. These results suggest that UGPase controls differently for carbohydrate metabolism in vegetative and reproductive phases in *A. thaliana*.

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MOLECULAR STUDY ON ARABIDOPSIS CA²⁺-PERMEABLE MECHANOSENSITIVE CHANNEL CANDIDATES USING YEAST

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To investigate the mechanism of mechanosensing in plants, we have identified the *MCA1* and *MCA2* genes encoding Ca²⁺-permeable mechanosensitive channel candidates in *Arabidopsis thaliana* (Nakagawa *et al.*, PNAS **104**:3639-3644, 2007). *MCA1* and *MCA2* are 72.7% identical in amino acid sequence and can complement a low Ca²⁺ influx activity of the yeast mid1 mutant, although sequence similarity between *MCA1/2* and *Mid1* is low. There are homologs of *MCA1* and *MCA2* in various plants, though their functions are unknown. Although we reported previously the localization and function of *MCA1* and *MCA2* in *planta* (Yamanaka *et al.*, Plant Physiol. **152**:1284-96, 2010), we have been unable to detect *MCA1* and *MCA2* in *Arabidopsis* protein preparations by immunoblotting, probably because they are low in content or solubility. Therefore, we expressed *MCA1* and *MCA2* individually in yeast cells to determine their structural and functional features. As we reported before, *MCA1* is localized to the yeast plasma membrane as an integral membrane protein and is also present in the *Arabidopsis* plasma membrane. Here, we confirmed that the localization of *MCA2* is the same as that of *MCA1*. SDS-PAGE analysis showed that each of *MCA1* and *MCA2* formed a tetramer by disulfide bonding. We made a series of truncation mutants lacking the N-terminal or C-terminal region of *MCA1* and *MCA2*. One of these truncation mutant proteins expressed in yeast cells inhibited cell growth. Ca²⁺ uptake assays for these mutants suggested that a functional region of *MCA1* and *MCA2* is different each other in terms of Ca²⁺ permeability. In addition, An Asp (or Glu) to Ala substitution mutant of an EF hand-like motif changed Ca²⁺ uptake activity of yeast cells. These results suggest that *MCA1* and *MCA2* have a distinct function in yeast cells irrespective of the similarity of their amino acid sequences.

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ANALYSIS OF INTERACTION BETWEEN CLATHRIN LIGHT AND HEAVY CHAINS IN ARABIDOPSIS THALIANA

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Clathrin is a coat protein of a clathrin-coated vesicle (CVC), which sorts cargo proteins into various endosomes from *trans*-Golgi network and plasma membrane in mammalian, yeast and plant cells. The clathrin coat is composed of a microscopically visible structure with three-legged shape, called triskelion. The triskelion comprises three clathrin heavy chains (CHCs), each harboring a single clathrin light chain (CLC). CHCs are well conserved among eukaryotic cells while CLCs vary in similarity, suggesting the latter may have an organism-specific function. Amino acid sequences of *Arabidopsis thaliana* CLCs have approximately 60 % of similarity to those of mammalian cells. An interaction between CLC and CHC in *Arabidopsis thaliana* has not been analyzed in detail. In addition, *Arabidopsis* CVC is reported to be smaller in size than those of animals. In this study, the interaction of both types of *Arabidopsis* clathrin molecules was analyzed. Yeast two-hybrid and bimolecular fluorescent complementation analyses revealed *Arabidopsis* CLC actually interacted with CHC. Deletion analysis of CLC (1-258) showed an internal region of CLC (81-144) interacted with hub of CHC (1087-1705), suggesting the similar tendency of interaction of plant clathrin chains to that in animals. The analysis of the interaction of *Arabidopsis* CLC and CHC molecules will be discussed in this presentation.

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THE LON2 PROTEASE CONTRIBUTES TO CONTINUED MATRIX PROTEIN IMPORT INTO PEROXISOMES

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Peroxisomes are small, single membrane-bound organelles that compartmentalize oxidative reactions, including critical metabolic processes like fatty acid beta-oxidation, hydrogen peroxide production and decomposition, and conversion of the protoauxin indole-3-butryic acid (IBA) into the active auxin indole-3-acetic acid (IAA). Proteins enter the peroxisome post-translationally with assistance from the receptor peroxins (PEX5 and PEX7, which recognize peroxisome-targeting signal (PTS) sequences in proteins bound for the peroxisomal matrix. PTS2-type proteins are recognized by PEX7 and have their PTS cleaved upon import. After cargo delivery, PEX5 and PEX7 are retrotranslocated to the cytosol and reused in further rounds of import. We are characterizing the molecular functions of LON2, a peroxisomal AAA-ATPase/protease that contributes to the import and processing of matrix proteins. *lon2* mutants exhibit mild sucrose dependence following germination and strong resistance to the promotion of lateral roots by IBA, phenotypes that indicate peroxisomal defects. Additionally, *lon2* seedlings accumulate unprocessed PTS2 proteins and display matrix protein import defects as they age. Though LON2 is not directly responsible for PTS2 processing, it may assist in matrix protein delivery by degrading cleaved PTS2 peptides to free PEX7 for future rounds of import or by assisting in the dissociation of the PEX5-PEX7-cargo complex after peroxisome entry. We are elucidating the roles of LON2 in matrix protein import and the interaction between LON2 and the PEX5-PEX7 complex. We are conducting a forward genetic screen for *lon2* suppressors to recover potential LON2 targets and/or regulators, as well as biochemical assays to isolate LON2 interactors. These approaches will expand our understanding of the molecular mechanisms supporting continued peroxisomal matrix protein import and how peroxisome functions contribute to successful seedling development.

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UNRAVELLING THE FUNCTION OF THE TWO PLASMA MEMBRANE LONG COILED-COIL PROTEINS TMD1 AND TMD2 IN ARABIDOPSIS THALIANA

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Coiled coils are protein motifs that have widespread functionality in all organisms. They are found in rigid structures, cytoskeletal components and oligomerization domains. The coiled-coil motifs expose extended amphiphilic surfaces for protein-protein interactions, which make them ideal for coordinating spatial organization of cellular processes. Our lab has undertaken an extensive study to unravel the function of proteins with long coiled coil domains in *Arabidopsis thaliana*.

Using the ARABI-COIL database developed in our lab, we identified TMD1 and TMD2 (for Transmembrane Domain 1 and 2) in a search for proteins with long coiled-coil domains and a transmembrane domain. Domain analysis using bioinformatic tools identified a PREFOLDIN domain in both TMD1 and TMD2. In addition, TMD2 has a region containing SPECTRIN repeats. These domains point towards a chaperone-like function for these proteins in cytoskeletal organization. Using GFP-fusion proteins, we have shown that TMD1 is located at the plasma membrane while TMD2 was found at the plasma membrane, ER and plasmodesmata. Further research is underway to confirm these localizations and understand the topology of these putative tail-anchored proteins.

Using a yeast two-hybrid library screen we have identified Glutamyl t-RNA synthetase (GluRS) as a potential binding partner for TMD1. Other than their well known function, aminoacyl t-RNA synthetases have been demonstrated in additional cellular processes like splicing, viral assembly and apoptosis. In *Arabidopsis*, GluRS has been found in cell-wall fractions and is known to bind directly to a CC-NBS-LRR protein. These findings suggest an additional role for Glutamyl t-RNA synthetase in disease resistance. Currently, we are probing into the putative functional interaction between GluRS and TMD1. In addition, we are analysing *tmd1tmd2* double mutants to further understand the role of TMD1 and TMD2 in *Arabidopsis thaliana*.

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IDENTIFICATION OF SUBSTRATES OF CYCLIN DEPENDENT KINASES IN ARABIDOPSIS

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Analysis of the *Arabidopsis* genome reveals that ~2% of *Arabidopsis* genes encode for protein kinases or protein phosphatases. Thus, protein phosphorylation regulates a large number of cellular processes and the cell cycle represents an example where a regulation by phosphorylation is of key importance. Cyclin-dependent kinases (CDKs) change the activity of their substrates by phosphorylation and by that promote progression into the next cell cycle phase. A-type CDK (CDKA;1), a homolog of Cdc2/CDC28, is one of major cell cycle kinases in *Arabidopsis* and previous work has revealed that CDKA;1 activity is required for mitotic cell cycle control during sporophyte and gametophyte development as well as for the meiotic cell cycle. In spite of intense studies of CDKs only a limited number of CDK substrates are known in plants. Preliminary data suggest that CDKA;1-dependent phosphorylation might regulate not only cell cycle substrate but could have a role in coordinating cell proliferation and growth. Thus, the identification of CDK substrates will allow us to get deep insights into cell cycle regulation and general plant development. A powerful method has been developed in yeast to isolate and identify phosphorylated peptides using a bulky ATP analog. By this, kinase substrates can be labeled and be subsequently identified on a proteome-wide level. This approach requires that the ATP-binding pocket is enlarged and we have engineered CDKA;1 and CDKBs to accept a bulky ATP. In planta work shows that these gatekeeper mutants are functional. As a base for a proteome-wide substrate screen we have co-expressed and purified the different CDKs with cyclin partners in insect cells, and present here the basic properties of the

modified kinases.

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QUEST FOR THE BIOLOGICAL SIGNIFICANCE OF "BULBS", COMPLEX CONFIGURATION IN VACUOLAR MEMBRANE.

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The plant vacuole has a variety of roles and is essential for plant growth and development. We have previously identified "bulbs," a complex structure on the continuous vacuolar membrane (Saito et al., 2002). To obtain a clue to understand its biological significance and function, we have carried out several approaches including: 1) search for other markers that visualize bulbs and 2) reverse genetics. We have succeeded in detecting bulb-like structures by expressing soluble and peripheral membrane proteins. Bulbs are formed in more tissues than we already reported, in flowering organ or suspension culture cell. We also found that the numbers of bulbs are significantly decreased in two shoot gravitropism (sgr) mutants, which are known to have a defect in morphology of the vacuolar membranes in endodermal cells. In contrast, bulbs were still observed in various tissues of autophagy (atg) mutants, indicating that autophagy is not a main pathway for the biogenesis of bulbs. With the merit of the new marker line, which enables us to observe the process of the biogenesis of bulbs, we propose that at least two independent pathways would be needed for the formation of bulbs; one pathway is common to the formation of the transvacuolar strand, and the other is not.

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SCREENING FOR NOVEL REGULATORS OF ATRBOHD AND ATRBOHF, NADPH OXIDASES INVOLVED IN PRODUCTION OF REACTIVE OXYGEN SPECIES.

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Reactive oxygen species (ROS) produced by NADPH oxidases (NOX) have been shown to play crucial roles in various signaling pathways including regulation of biotic and abiotic defense responses, programmed cell death and development in plants. Plants possess multiple NOX family genes, and 10 *respiratory burst oxidase homolog (rboh)* genes (*AtrbohA-J*) have been identified in *Arabidopsis*. Though the activity of many animal NOX are regulated by various regulatory subunits, only the homologs of small GTPase, Rac, have been found among them in plants. Unlike many animal NOX, plant rboh proteins possess an extended N-terminus, which contains two Ca²⁺ binding EF-hand motifs. By applying a heterologous expression system with HEK293T cells, we showed that AtrbohC and AtrbohD show ROS-producing enzyme activities synergistically activated by binding of Ca²⁺ to the EF-hand motifs and phosphorylation (Takeda et al., *Science*, 2008; Ogasawara et al., *JBC*, 2008).

To gain further insight into the regulatory mechanisms for the ROS producing enzymes, we have screened for proteins that specifically interact with the N-terminal region of AtrbohD and AtrbohF using yeast two-hybrid assay. We so far identified 4 and 19 putative interacting proteins for AtrbohD and AtrbohF, respectively. We have also established a heterologous coexpression system, and have been analyzing their roles. Regulatory mechanisms of AtrbohD and AtrbohF by these putative interactors will be reported.

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CHLOROPLAST RH3 IS A DEAD-BOX RNA HELICASE THAT FUNCTIONS IN 50S RIBOSOME BIOGENESIS AND RH3 UPREGULATION SUPPRESSES DEFECTS DUE TO LOSS OF CLPR PROTEASE CAPACITY

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The *clpr2-1* mutant is a pale-green, developmentally delayed mutant due to reduction of the chloroplast ClpPR protease complex (1). Moreover, *clpr2-1* showed delayed processing of the dicistronic 23S-4.5S rRNA and comparative proteome analysis showed that the chloroplast-localized stromal DEAD box RNA helicase RH3 was more than 5-fold up-regulated (2). In this study, we determined the function of RH3 and tested whether RH3 overaccumulation is due to a lack of degradation by the Clp protease system or if it represents a compensatory response to overcome defects due to loss of Clp protease capacity. DEAD box RNA helicases comprise a large protein family in *Arabidopsis* and other plant species, and they are expected to contribute to RNA metabolism. However, the precise molecular functions of most plant DEAD box helicase proteins remain to be elucidated. In *Arabidopsis*, RH3 protein accumulation was under strong developmental control with the highest accumulation levels in green seedlings at early stages of development. RNA coimmunoprecipitation assay in maize stroma and sucrose gradient analysis showed that RH3 associated with 50S pre-ribosome particles, suggesting a role in rRNA assembly. Loss of *RH3* expression in *Arabidopsis* resulted in embryo lethality, whereas T-DNA insertion lines with a leaky allele (*rh3-4*) resulted in pale-green seedlings with a clear defect in ribosomal rRNA biogenesis. The double mutant for *rh3-4* and *clpr2-1* mutant had an albino phenotype and additive molecular phenotypes. These data suggest that RH3 functions in 50S ribosomal biogenesis, and that RH3 overaccumulation in *clpr2-1* is not because RH3 is a ClpPR substrate, but rather represents a compensatory response to suppress defects due to loss of ClpR protease capacity.

1. Rudella, et al., (2006) *Plant Cell* 18, 1704-1721
 2. Zybailov, et al., (2009) *Mol Cell Proteomics* 8, 1789-1810
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REGULATION OF ROS-PRODUCING ACTIVITY OF AN ARABIDOPSIS NADPH OXIDASE, ATRBOHF, BY BINDING OF CA²⁺ AND PHOSPHORYLATION.

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Reactive oxygen species (ROS) produced by NADPH oxidase have been shown to play many critical roles in plant signaling including stress responses and development. *Respiratory burst oxidase homolog (rboh)* genes have been identified as homologs of animal NADPH

oxidases in plants. Among 10 *rboh* genes in *Arabidopsis*, *AtrbohD* and *AtrbohF* have been shown to be involved in defense responses against pathogens and ABA signaling, but they may play different roles in various systems. We have been analyzing the regulatory mechanisms for activation of Atrboh proteins by a heterologous expression system using HEK293T cells, and showed that AtrbohD possess ROS-producing activity, that is synergistically activated by phosphorylation and binding of Ca^{2+} to its EF-hand motifs (Ogasawara et al., JBC, 2008). In the present study, we heterologously expressed AtrbohF and its various site-directed mutants to reveal its activation mechanisms. Ca^{2+} binding properties of its EF-hand motifs and their physiological relevance were also characterized. Similarities and differences of the ROS-producing activity and regulation between AtrbohD and AtrbohF will be discussed.

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A CALMODULIN REGULATED PROTEIN PHOSPHATASE (DSPTP1) DEPHOSPHORYLATES AND INACTIVATES THE ACTIVITIES OF MAP KINASES IN ARABIDOPSIS

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MAP kinases (MPKs) play roles as key signaling components in plant development, and abiotic and biotic stress signaling pathways. It was reported that calcium ion (Ca^{2+}) in addition to upstream MPK cascades is involved in the full activation of MPKs. However, there is no evidence how Ca^{2+} is involved in the activation of MPKs. We previously isolated and reported a calmodulin(CaM)-regulated dual-specificity protein phosphatase1 (DsPTP1) that might be involved in the regulation of the activities of MPKs in plants. 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 was able to dephosphorylate and inactivate the active MPK3, MPK4, and MPK6 that were activated by MEK1 and MEK2. Interestingly, the dephosphorylation activity of DsPTP1 on phospho-Tyr residue of MPKs was strongly inhibited by the addition of CaM, whereas the activity on phospho-Ser/Thr was not affected. Consistently, the activities of plant MPKs were inhibited in DsPTP1 overexpressing transgenic plant. In addition, the activities of MPKs lasted longer in CaM binding negative DsPTP1 overexpressing transgenic plant than in wild type plant, which indicated that CaM inhibited the activity of DsPTP1 and resulted in the activation of MPKs. This study implies that Ca^{2+} mediated signaling pathway can cross-talk with MPKs signal pathway via CaM regulated DsPTP1 that inactivate MPK signaling pathways.

11057

A KEY FACTOR FOR THE AUTOPHAGY OF CHLOROPLASTS; THE PRODUCTION OF RUBISCO-CONTAINING BODIES IS SPECIFICALLY LINKED TO LEAF CARBON STATUS BUT NOT NITROGEN STATUS IN ARABIDOPSIS

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Autophagy is an intracellular process for vacuolar degradation of cytoplasmic component, and considered to be important for nutrient recycling during starvation. We previously demonstrated that chloroplasts can be partially mobilized to the vacuole by autophagy via spherical bodies, named Rubisco-containing bodies (RCBs; Ishida et al., 2008 Plant Physiol. 148: 142-155). Although chloroplasts contain approximately 80% of total leaf nitrogen and represent major carbon and nitrogen source to recycle, the relationship between the RCB production and leaf nutrient conditions is unclear. We examined the effects of nutrient factors on the appearance of RCBs using transgenic *Arabidopsis* (*Arabidopsis thaliana*) expressing stroma-targeted fluorescent proteins. In excised leaves, the appearance of RCBs was suppressed by the presence of metabolic sugars which were added externally or by irradiation of the light. The light-mediated suppression of the RCB appearance was defused by inhibition of photosynthesis. In a diurnal cycle, the RCB production was negatively correlated to leaf carbohydrates content, mainly starch. In leaves of starch-related mutants, there were more RCBs in starchless mutants (*pgm*, *adg1*), and less RCBs in starch-excess mutants (*sex1*, *mex1*). In nitrogen-limited plants, leaf carbohydrates were accumulated and the RCB production was suppressed simultaneously. We propose close relationship between the degradation of chloroplast proteins via RCBs and leaf carbon status but not nitrogen status in autophagy. We also found that the response of RCBs and other autophagic bodies, containing cytoplasmic component other than chloroplasts, to nutrient conditions was not always the same. The appearance of autophagic bodies other than RCBs was not suppressed in the light and somewhat responded to nitrogen in excised leaves unlike RCBs. These results imply that the degradation of chloroplast proteins is specifically controlled in autophagy.

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MEMBRANE CORRALS: DIFFUSION BARRIERS DEFINE LARGE-SCALE SUB-COMPARTMENTS OF THE PLASMA MEMBRANE

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The lateral organization of the plasma membrane into specialized domains of specific composition is known to be important for many cellular processes, including signaling, nutrient transport and protein turnover. On a fine scale (<100 nm), this compartmentalization is defined by microdomains that house specific proteins in characteristic lipid environments.

Flotillins (a.k.a. reggies) are examples for proteins that form microdomains at the plasma membrane. Flotillins are ubiquitously expressed and found in all three kingdoms (archaea, bacteria, eukaryotes). First discovered in animals, flotillins share several common features with their counterparts in plants. The GFP-tagged *Medicago truncatula* flotillin MtFLOT2 localizes to a punctate pattern in the plasma membrane when expressed in *M. truncatula* or *Arabidopsis thaliana*. This localization is reminiscent of the distribution pattern of flotillins in animals, where they are suggested to function as scaffolds for signaling processes. Similar to flotillins in animals which accumulate at cell-cell contacts, the plant protein also accumulates at boundaries of neighboring cells.

To investigate domain formation and dynamics of flotillins in plants, we transiently expressed MtFLOT2-GFP in *Arabidopsis* mesophyll protoplasts. By using photo-bleaching methods we detected linear barriers for lateral diffusion of this protein. Co-labeling experiments indicated that these diffusion barriers were often coincident with cortical microtubules. In a corral-like manner, these barriers inhibited efficient exchange of flotillin clusters between adjacent membrane areas. We therefore conclude that lateral partitioning of the protoplast

plasma membrane can also occur on a large scale by the formation of membrane corrals that may be defined by cortical microtubules.

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AN R2R3-TYPE TRANSCRIPTION FACTOR (ATMYB77) IS A NOVEL SUBSTRATE OF MAP KINASE IN ARABIDOPSIS

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Mitogen-activated protein kinase (MAPK) cascades have emerged as a major signal transduction mechanism that connects diverse receptors or sensors to cellular and nuclear responses in eukaryotes. Although function and activation of some MPKs in response to stimuli have been studied in details, but substrates of these kinases were still poorly understood. Here, we provide that *Arabidopsis* MYB77 transcription factor, a R2R3-MYB protein, is a substrate of MPK3/6 *in vitro*. Using *in vitro* phosphorylation assays we have identified that MYB77 was not only phosphorylated by recombinant MPK3/6 but also phosphorylated by native MPK3/6 extracted from plant. To identify the phosphorylation sites of MYB77 by MPK, purified His-MYB77 protein was phosphorylated by MPK, desalting and passed over TiO₂ for improved phosphopeptide detection. This sample has been used to analysis for mass spectrometry. As results we identified four putative phosphorylation sites originated from 7 phosphopeptide peaks. By site-directed mutagenesis we generated 8 recombinant proteins for single, double and triple site mutations of MYB77. Using these mutant proteins as substrates in kinase assay we confirmed that Ser-62, Ser-151 and Thr-297 are the phosphorylation sites of MYB77 by MPKs. Our results suggest that MYB77 is a substrate of MPKs containing three phosphorylation sites in *Arabidopsis*.

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SGR9, A RING TYPE E3 LIGASE, MODULATES THE INTERACTION BETWEEN AMYLOPLAST AND F-ACTIN IN GRAVITY SENSING

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Gravitropism is initiated by sensing the directional change of gravity within specialized gravity sensing cells. In *Arabidopsis* shoot, the endodermal cell that contains amyloplasts is essential for gravity sensing. The amyloplast sedimentation toward the direction of gravity is important process to trigger subsequent processes for sensing.

The *Arabidopsis sgr9* (*shoot gravitropism 9*) mutant exhibited weak shoot gravitropism. The *SGR9* encoding a RING finger protein was mainly expressed in shoot endodermis. Complementation analysis by using endodermis specific promoter *SCR* revealed that *SGR9* has function within endodermis. Interestingly, *SGR9* is localized to amyloplast, and exhibited ubiquitin E3 ligase activity *in vitro*, suggesting that *SGR9* function as ubiquitin E3 ligase on amyloplast within endodermal cell in gravitropism.

Our previous report has shown that some amyloplasts in wild type dynamically move in F-actin dependent manner and some amyloplasts sediment toward the direction of gravity. Amyloplast sedimentation occurs in F-actin independent manner. In *sgr9*, most amyloplasts dynamically move around the cell and did not sediment to the bottom of the cell. Interestingly, this cytological phenotype as well as gravitropic phenotype of *sgr9* was restored by the disruption of F-actin. Live cell imaging of amyloplasts and F-actin showed that the interaction between amyloplasts and F-actin is aberrant in *sgr9*. Clusters of amyloplasts entangled in F-actin was observed only in *sgr9*. These results suggest that *SGR9* modulates the interaction between amyloplasts and F-actin, probably by promoting release of amyloplast from F-actin. Amyloplasts released from F-actin by *SGR9* function may have ability to sediment toward the direction of gravity, leading to subsequent sensing processes. Degradation of unknown substrates by *SGR9* E3 ligase activity might be required for the release of amyloplasts from F-actin.

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H⁺-PYROPHOSPHATASE IS LOCALIZED IN THE MEMBRANE OF VACUOLE AND THE BULB-LIKE STRUCTURE

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H⁺-pyrophosphatase (H⁺-PPase) is a proton pump that generates a H⁺ gradient across membranes using high energy of a phosphoanhydride bond of pyrophosphate (PPI). In higher plants, especially young tissues, a relatively large amount of H⁺-PPase exists in vacuolar membranes, removes the cytoplasmic PPI and maintains vacuolar acidic pH together with V-ATPase. However, it has been reported that H⁺-PPase is localized in not only the vacuolar membrane but also the plasma membrane and is involved in auxin transport indirectly (Li et al., 2005, *Science*).

We focused our attention to the type I H⁺-PPase of *Arabidopsis thaliana* (*AtVHP1/AVP1*) and tried to prepare VHP1-sGFP in order to investigate the cell specificity and actual intracellular localization of the enzyme. In this study, we inserted sGFP into the cytosolic loop of VHP1 and transformed it into the wild type or *vhp1* plant under the control of VHP1 own promoter. First, we confirmed that VHP1-sGFP in the membrane of the transgenic plants had approximately 50% of PPI hydrolysis activity of the wild-type enzyme and was recovered in the same density fractions as that of endogenous VHP1 by sucrose density gradient centrifugation. Therefore, we concluded that this chimera VHP1 is functional and reflects the actual intracellular localization.

VHP1-sGFP was clearly detected in vacuolar membranes as expected. Furthermore, strong fluorescence was observed in membranes of mobile, spherical structures in vacuoles, which are called 'bulbs' (Saito et al. *Plant J.*, 2002). Histological analysis demonstrated that *VHP1* was expressed in almost all tissues, especially in root tips, pericycle cells, and mature pollens. Furthermore, young cells have many bulbs. In conjunction with phenotype of *vhp1* plants, we will discuss physiological role of H⁺-PPase.

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NEW MEMBER OF RAN GTPASE FAMILY OF PROTEINS SPRY DOMAIN-CONTAINING PROTEIN HAS A ROLE IN NUCLEOCYTOPLASMIC TRANSPORT AND IN CELL DIVISION

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The Ran GTPases (Ras-related nuclear proteins) are members of Ras superfamily that is highly conserved in eucaryotes. Ran GTPases ubiquitously regulate the GTP/GDP cycles, control the directionality of nucleoplasmic protein and RNA transport and mediate mitotic spindle assembly. We aimed to investigate yet uncharacterized member of Ran GTPase family proteins SPRY domain-containing protein (At RanSPRY). In Arabidopsis plants At RanSPRY is expressed in roots and aerial part with increased expression in dividing tissues. Analysis of GFP, RFP versions of At RanSPRY in cultured cells and plants of Arabidopsis revealed that protein is localized in cell cycle specific manner in nuclei and in cytoplasm and accumulate in the vicinity of nuclear envelope. In dividing cultured cells smaller portion of protein was localized with microtubules of mitotic spindle, with phragmoplasts, and in telophase in vicinity of reforming nuclei. To understand the role of At RanSPRY protein in relation to Ran GTPase signaling pathway we studied At RanSPRY immunolocalization together with Ran GTPase, RanBP1 and RCC1. To get better insight into At RanSPRY protein interactions, immunopurification and MS/MS analysis are under progress. In T-DNA insertion mutants we found aberrant development of root meristem, cell division defects and clustered stomata. Altogether our data suggested that At RanSPRY protein has function in nucleocytoplasmic transport and in cell division.

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PLASTID TARGETING OF NPP GLYCOPROTEIN FROM THE GOLGI APPARATUS THROUGH THE SECRETORY PATHWAY IN RICE CELL.

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Nucleotide pyrophosphatase/phosphodiesterase (NPP) is a new enzyme family that belongs to a large group of structurally related and functionally divergent nucleotide hydrolases.

We identified six (I)NPP(/I) genes in rice genome and cloned their cDNAs. NPP1, 2 and 6 enzyme proteins were purified and characterized from transgenic rice cells with overexpression of each (I)NPP(/I) gene. These three enzymes exhibited different substrate specificities; NPP1 enzyme effectively hydrolyzes ADP-glucose and ADP-ribose. NPP6 also hydrolyzes ADP-glucose, but being ADP the most favorable substrates. In contrast, NPP2 hardly reacts on nucleotide-sugars and favorably hydrolyzes ADP. All NPP isozymes were conjugated with N-linked oligosaccharide chains.

Confocal-fluorescence microscopy of cells expressing NPP fused with GFP indicated that all NPP-GFPs occur in the plastidial compartment. Plastid targeting of NPP-GFPs were strongly prevented by dominant negative mutants of AFR1 which inhibits the ER-to-Golgi traffic, indicating that NPP glycoproteins are transported from the Golgi apparatus through the secretory pathway to the plastids.

The N-glycan chains released from NPP1, 2, 6 and plastidial NPP1 were captured by glycol blotting method and subjected to the MALDI-TOF-MS/MS with labeling reagents. Compositional annotation for the detected N-glycans revealed that there exist at least 85 different glycan chains conjugated to NPP proteins, and approximately 75% of the glycan chains of plastidial NPP1 isolated from rice chloroplasts were the complex type with the fucose and xylose residues. Furthermore, differences in N-glycan complexities among NPP1, 2 and 6 glycoproteins appeared to show that the oligosaccharide processing may depend upon the protein structures relating to the enzyme functions.

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WOOD CELL-WALL STRUCTURE REQUIRES LOCAL 2D-MICROTUBULE DISASSEMBLY BY A NOVEL PLASMA MEMBRANE-ANCHORED MICROTUBULE-ASSOCIATED PROTEIN

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Proper microtubule distribution is essential to the cellular development of eukaryotes. Plant cells have evolved cortical microtubules in a 2D space beneath the plasma membrane that regulate the movement of the cellulose synthase complex on the plasma membrane. Although recent studies on transverse cortical microtubules in growing plant cells have identified several microtubule-associated proteins (MAPs) that facilitate organization of the cortical microtubules, these MAPs can not fully explain the diverse patterns of cortical microtubules during cell differentiation. Using our newly established *in vitro* wood cell differentiation system, we found that a novel microtubule end-tracking protein, MIDD1 (Microtubule Depletion Domain 1), was anchored to distinct plasma membrane domains and promoted local microtubule disassembly, resulting in pits on wood cell walls. The introduction of RNAi for MIDD1 resulted in failure of local microtubule depletion and the formation of secondary walls without pits. Conversely, the overexpression of MIDD1 suppressed rescue events and reduced microtubule density. MIDD1 has two coiled-coil domains; the first domain associates with microtubules and the second domain is required for the anchorage of MIDD1 to distinct plasma membrane domains. Combination of the two coils caused end-tracking during shrinkage and microtubule disassembly at the specific area. Our results indicate that plants use a distinctive protein that integrates spatial information in the plasma membrane with cortical microtubule dynamics, for determining wood cell wall pattern.

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THE BiP-MEDIATED POLAR NUCLEI FUSION IS ESSENTIAL FOR THE REGULATION OF ENDOSPERM NUCLEI PROLIFERATION IN ARABIDOPSIS THALIANA

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Nuclear fusion is an essential process in the sexual reproduction of animals and plants. In flowering plants, nuclear fusion occurs three times: once during female gametogenesis when two polar nuclei fuse to produce the diploid central cell nucleus, and twice during double fertilization. The yeast immunoglobulin binding protein (BiP) is a molecular chaperone Hsp70 in the endoplasmic reticulum that regulates nuclear membrane fusion during mating. We found that in *Arabidopsis thaliana*, BiP is required for the fusion of polar nuclei during female gametophyte development; the BiP-deficient female gametophytes contained two unfused polar nuclei in close contact. The defect was specific to the fusion of polar nuclei. When fertilized with wild-type pollen, the BiP-deficient female gametophytes were not defective in the fertilization process including fusions of sperm nuclei with the egg and central cell nuclei. However, we found that the endosperm nuclei in the mutant seeds underwent aberrant proliferation, which eventually lead to seed abortion. Analyses of endosperm nuclei division in early seed development showed significant delay in the endosperm nuclei division in the mutant seeds. These results indicate importance of polar nuclei fusion in the proliferation of endosperm nuclei after fertilization.

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IN VITRO PHOSPHORYLATION AND DEPHOSPHORYLATION OF THE PLASMA MEMBRANE H⁺-ATPASE

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Plant plasma membrane H⁺-ATPase creates an electrochemical gradient of H⁺ across the plasma membrane. Recent studies have demonstrated that the H⁺-ATPase is activated via phosphorylation on a penultimate threonine residue in the C-terminus and subsequent binding of 14-3-3 protein to the phosphorylated C-terminus. However, biochemical properties of protein kinase and protein phosphatase, which regulate phosphorylation level of the H⁺-ATPase, are largely unknown. In this study, we investigated *in vitro* phosphorylation and dephosphorylation of the H⁺-ATPase. Both phosphorylation and dephosphorylation of the H⁺-ATPase were detected in the plasma membrane from etiolated seedlings of *Arabidopsis*. Phosphorylation of the H⁺-ATPase was insensitive to K-252a, a potent inhibitor of protein kinase. Furthermore, the dephosphorylation was specifically inhibited by EDTA, a chelating agent for divalent cations, but not by calyculin A, an inhibitor of type 1 and type 2A protein phosphatases. These results indicate that a protein kinase-phosphatase pair co-localizes with the H⁺-ATPase in plasma membrane and regulates phosphorylation status of a penultimate Thr of the H⁺-ATPase. We will report analysis of the H⁺-ATPase complex.

11067

IDENTIFICATION AND FUNCTIONS OF PSV(PROTEIN STORAGE VACUOLES)-TYPE COMPARTMENT IN ARABIDOPSIS MATURE LEAVES

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Plant have multiple vacuoles, some of which function as a storage place or a hydrolytic place. But it has been in a debate that plant cells possess functionally different types of vacuoles in the same cell. Secretory proteins destined for particular vacuoles must possess a specific vacuolar sorting determinant (VSD). α` subunit of β-conglycinin (CON:GFP) has a ctVSD which is responsible for targeting to PSV in *Arabidopsis* seed cells.

However we observed that CON:GFP containing the ctVSD of conglycinin is targeted to LVs and disc compartments in leaf protoplasts.

Therefore we generated transgenic plants to examine here whether disc-like compartment in leaf protoplasts is identical to the PSV in the seeds. In addition we generated transgenic plants with various PSV proteins.

Using here transgenic plants, first we examined localization of various storage proteins such as β-conglycinin (soybean), phaseolin (mung bean), chitinase(soybean) that were transiently expressed in *Arabidopsis* leaf protoplasts and stably in transgenic plants. They all showed at a disc pattern and colocalized at the disc pattern.

Now we characterize PSV-type compartment in plant mature stages.

We also confirmed that these disc patterns were not Protein aggregates by biochemical data.

11068

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF SEQUENCE MOTIFS IN ARABIDOPSIS MITOCHONDRIAL PRESEQUENCES

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Most of mitochondrial proteins are synthesized in cytoplasm and transported to mitochondria via outer and inner membrane translocator complexes. Presequence, the signal peptide located in the N-terminus of nuclear-encoded mitochondrial proteins, is essential for protein targeting and translocation from cytoplasm into mitochondria. The translocation complex of plant mitochondria is distinct from that of yeast or animal, but little is known about the general features of presequence or translocation mechanism of plant mitochondria proteins. To investigate the relationship between presequence and mitochondrial protein translocation, three representative presequences were selected; F1-ATPase-γ, LPD2 (Lipoamide dehydrogenase 2) and IVD (Isovaleryl CoA-dehydrogenase). We generated several deletion or Ala substitution mutants of each presequence to specify the region containing sequence motifs. These mutant presequences were fused with GFP for examining the mitochondrial targeting efficiency and the subcellular localization in *Arabidopsis* leaf protoplasts. As a result, the targeting efficiency of P[31-35] or P[45-50] region-substituted F1-ATPase-γ mutant presequences was significantly decreased than that of original presequence. Ala substitution of P[11-15] in LPD2 presequence also affected to the reporter protein targeting. Interestingly, most of Ala substitution mutants of IVD presequence showed severe inhibitory effect of mitochondrial protein targeting. Fractionation assay indicated that the precursors of the mutant presequence-GFP fusion protein were mainly located in membrane fraction. This study demonstrates that multiple sequence motifs are in each presequence, and the C-terminus of the presequence is important for mitochondria protein translocation, especially in membrane penetration. These results also show the possibility that plant mitochondrial presequences can be divided with multiple groups according to their properties.

11069

ANALYSIS OF SIGNAL-ANCHORED (SA) PROTEINS' TARGETING SEQUENCES AND SCREENING OF PUTATIVE CHLOROPLAST SA PROTEINS THROUGH BIOINFORMATIC TOOLS

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Signal-anchored (SA) proteins have a transmembrane domain (TMD) at their N-terminus working as membrane-anchoring and targeting signal. SA proteins of plastids and mitochondria have various biological roles in plants such as organelle division, translocation receptors, chloroplast positioning and so on. However, small number of SA proteins has been identified compared with soluble proteins of plastids and mitochondria. Also, features of their targeting signals have remained ambiguously. Here, using several bioinformatic tools, we supplemented and improved original targeting rules of chloroplast SA proteins (moderate hydrophobic TMD and the C-terminal positive region). First, through SPECTOPUS (<http://octopus.cbr.su.se/>), we separated transmembrane domain and ER signal peptide which are located at the N-terminus of proteins, and then putative SA proteins were selected. Among them, putative chloroplast SA proteins, which have the C-terminal positive region, were sorted out and tagged with sGFP to define their location in plant cells. We concluded that hydrophobicity calculation with Wimley-White (WW) scale is most proper to divide SA proteins of ER, chloroplast and mitochondria. And above 3 basic amino acids out of 8 amino acids are sufficient to function as the C-terminal positive region. Additionally, based on more detailed targeting rules, we could screen out several undefined SA proteins which are located in chloroplast or mitochondria.

11070

SELECTION AND CHARACTERIZATION OF HIGH TEMPERATURE SENSITIVE GERMINATION MUTANTS OF ARABIDOPSIS

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Temperature is a main environmental factor that defines germination season of winter and summer annual seeds. Germination inhibition of winter annual seeds by high temperature in summer enables the seeds to germinate and to establish seedling growth in autumn. To understand the mechanism of germination regulation by temperature, we have selected high temperature sensitive germination mutants from T-DNA insertion lines of *Arabidopsis*. The seeds of one of the mutants, *nekojita1* (*nkj1*), showed enhanced dormancy and enhanced sensitivity to abscisic acid (ABA). ABA contents in *nkj1* seeds were normal, and decreased to low levels by imbibition even at the restrictive temperature, 28 °C, as in germinating wild type seeds at 22 °C. Interestingly, the germination of *nkj1* seeds was recovered by ABA biosynthesis inhibitor, fluridone, and germination of the seeds of known ABA hypersensitive mutants, *era1-2* and *era3-1*, was inhibited at 28 °C as *nkj1*. These results suggest that ABA hypersensitivity of *nkj1* seeds is the main cause of germination inhibition at 28 °C. Genetic analysis indicated that *nkj1* has single locus recessive mutation, so that *NKJ1* may be a negative regulator of ABA signaling and has a promotive role on germination at permissive temperature conditions. *nkj1* was mapped on the bottom arm of chromosome 4, and the gene was identified by sequencing of the T-DNA border and by genetic complementation tests. The deduced amino acid sequence was homologous to Sec23/Sec24 subunit of COPII coat protein which is involved in vesicular transport from ER to golgi. We are now analyzing germination phenotypes of other COPII component mutant seeds, and will discuss their role on seed development and germination.

11071

ARABIDOPSIS HOMOLOG OF PRENYLATED RAB ACCEPTOR CONTROLS EXIT OF VACUOLAR AND SECRETED PROTEINS FROM THE ER THROUGH THE REGULATION OF COPII VESICLE FORMATION AT THE ER.

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Prenylated rab acceptor (PRA) binds to prenylated Rab proteins, and possibly aids in targeting of Rabs to their respective compartments. In *Arabidopsis*, 19 isoforms have been identified and localized to the ER, Golgi apparatus and endosomes depending on isoforms. However, their biological role has not been addressed. Here, we investigated the biological role of AtPRA1.B5, one of *Arabidopsis* prenylated rab acceptor homologues (AtPRAs). RT-PCR analysis showed that the AtPRA1.B5 was expressed ubiquitously in all the tissues we examined. Localization revealed that AtPRA1.B5 localized primarily to the Golgi complex with a minor portion to the ER when examined with transiently expressed HA-tagged AtPRA1.B5 in leaf protoplasts. In the presence of overexpressed AtPRA1.5, trafficking of vacuolar and secreted proteins, but not the Golgi proteins, was severely inhibited at the ER. AtPRA1.B5 increased the membrane-associated pool of Sar1 and AtSec23 and the amount of AtSec23 interacting with Sar1, but inhibited interaction between AtSec23 and AtSec24. These results suggest that AtPRA1.B5 localized to both the Golgi complex and the ER may control anterograde trafficking from the ER by inhibiting formation of COPII vesicles.

12001

DYNAMIC TAP-TAGGING IDENTIFIES NINJA AND TOPLESS AS NEGATIVE REGULATORS OF JASMONATE SIGNALLING

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Plants respond to biotic attack by activating defence programs that kill or deter the attackers. Such defence responses are costly as they require energy and resources otherwise used for plant growth and reproduction. Hence, a strict control of defence gene activity is pivotal. Jasmonates (JAs), hormones that are instantly produced under these stress conditions, are crucial in this process. In *Arabidopsis*, bioactive JAs are perceived by the SCFCO11 complex which targets the JAZ repressor proteins for degradation. This event causes release of the transcription factor MYC2, thereby activating the first wave of JA-dependent gene expression.

In our research we have applied tandem affinity purification (TAP) to isolate the core JA signalling module and thereby discover new JAZ interactors and their mode of action. For the simultaneous visualization of the dynamics of JAZ complex assembly, TAP was performed with *Arabidopsis* cells elicited or not with JA. Using this proteomics technology we could demonstrate that the JAZ proteins recruit the Groucho/Tup1-type co-repressor TOPLESS (TPL) and TPL-related proteins (TPRs) through a previously uncharacterized adaptor protein, designated Novel Interactor of JAZ (NINJA). NINJA acts as a transcriptional repressor whose activity is mediated by a functional TPL-binding EAR repression motif. Accordingly, both NINJA and TPL proteins function as negative regulators of JA responses and represent a molecular mechanism by which the JAZ proteins repress JA-dependent gene expression. Furthermore, our results point to TPL proteins as general co-repressors that affect multiple (hormonal) signalling pathways through the interaction with specific adaptor proteins.

12002

THE PP2C-SNRK2 COMPLEX: A CENTRAL REGULATORY MODULE OF PROTEIN PHOSPHORYLATION NETWORKS IN ABCISIC ACID SIGNALING

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Abscisic acid (ABA) is a critical phytohormone for maintenance or enhancement of abiotic stress responses in plants, as well as for regulating various developmental processes. Recent identification of the soluble ABA receptors, PYR/PYL/RCARs, triggered emergence of a core ABA signaling pathway. The ABA receptor inhibits protein phosphatase 2C (PP2C), a major negative regulator of ABA response, in an ABA-dependent manner. On the other hand, our group had studied in planta or molecular functions of PP2C or the SNF1-related protein kinase 2 (SnRK2), and we demonstrated 1) SnRK2 functions as a central hub in ABA signaling network, 2) SnRK2 and PP2C interact in various combinations, 3) PP2C inactivates SnRK2 by direct dephosphorylation of some specific residues, 4) abi1-1-type mutation of PP2C constitutively inactivates SnRK2. Furthermore, we reconstituted the simple ABA signaling complex of PYR1, PP2C and SnRK2 and showed this complex is functional for switching ON/OFF of ABA signaling in vitro. These results clearly uncovered the main frame of ABA signaling pathway in plants. Further studies are in progress to provide an overview of protein phosphorylation networks regulated by the PP2C-SnRK2 complex.

12003

CHARACTERIZATION OF VIP1, A NOVEL INTERACTING PARTNER OF HETEROTRIMERIC G PROTEIN B SUBUNIT IN ARABIDOPSIS

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Heterotrimeric G proteins ($G\alpha$, $G\beta$, $G\gamma$) are signaling molecules found in a variety of eukaryotic organisms. In plants, the heterotrimeric G proteins play an important role in signal transduction of phytohormones such as auxin, brassinosteroid, and abscisic acid (ABA). In animal systems, the G proteins transmit signals by regulating activities of effector proteins. In plants, however, there are few reports on such effectors. To gain insight into the G protein-mediated signaling in plants, we performed a yeast two-hybrid (Y2H) screen using *Arabidopsis* $G\beta$ as bait, and identified a bZIP protein, VIP1, as a novel AGB1-interacting partner. A bimolecular fluorescence complementation assay indicated that AGB1 and VIP1 interacted in the nucleus and Golgi apparatus *in planta*. Using the Y2H system, we found that C-terminal half of VIP1 alone interacted with AGB1, and the C-terminal half also interacted with one of *Arabidopsis* $G\gamma$, AGG1. To study the biological role of VIP1 in plants, we generated transgenic *Arabidopsis* plants over-expressing VIP1. They were more sensitive than wild-type plants to ABA during seed germination and early seedling development, supporting the idea that VIP1 is involved in the G protein-mediated ABA signaling as the downstream effector. A yeast one-hybrid assay suggested that VIP1 had a transcriptional activation domain, thus VIP1 is likely to serve as a transcriptional activator in plants. Amino acid sequence of VIP1 is homologous to that of tobacco RSG (Repression of shoot growth), which plays a key role in feedback regulation of gibberellin biosynthesis. VIP1 bound to double-stranded DNA containing rbe (RSG-binding element) *in vitro* in a gel shift assay, suggesting that target genes of VIP1 are similar to those of RSG. Further studies on the $G\beta\gamma$ -VIP1 complex might provide a model mechanism for the G protein-mediated signaling in plants.

12004

CHARACTERIZATION OF A NEW AUXIN BIOSYNTHETIC MUTANT IN ARABIDOPSIS

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Phytohormone auxin is an essential regulator of nearly all aspects of plant growth and development, yet molecular mechanisms of auxin biosynthesis and its regulation are still poorly understood. To uncover novel players of auxin production in plants, we undertook a forward genetic approach. We performed a screen for the suppressors of an auxin-overproducing mutant *sur2*. *SUR2* encodes a CYP83B1 that recruits an auxin precursor indole-3-acetaldoxime (IAOx) into indole-glucosinolate pathway, converting IAOx into IAOx N-oxide. In the *sur2* mutant [where this step is impaired] all of IAOx becomes available for the production of the prevalent form of auxin, IAA; hence the high-auxin defects.

We identified a number of *sur2* suppressors. *rus1*, represented by four independent alleles, nearly completely suppresses phenotypic as well as molecular defects (high DR5:GUS activity) of *sur2*. Importantly, exogenous addition of the IAA precursors tryptophan (TRP) or anthranilate to the *sur2 rus1* double mutant fully reverts *rus1*-mediated suppression of *sur2*, suggesting that the suppression of *sur2* defects by *rus1* is a consequence of reduced levels of the auxin precursors in the *rus1* mutant cells. Consistent with that conclusion is the finding that some of the phenotypes of *rus1* single mutants (such as early embryonic defects) are enhanced by the mutations in the TRP biosynthetic genes *WEI2* and *WEI7*. Interestingly, both *wei2* and *wei7* can also suppress *sur2*.

RUS1 encodes a novel transmembrane protein localized in the inner envelope membrane of the chloroplast. Chloroplasts are the site of TRP biosynthesis in the plant cell, consistent with the genetic interaction between *RUS1* and *WEI2/WEI7*. We hypothesize that *RUS1* is required for recruiting one of the precursors of TRP (chorismate or a shikimate pathway intermediate) into TRP production.

12005

CELL-SPECIFIC AUXIN RESPONSES MEDIATING PHOSPHATE STARVATION ROOT GROWTH ARREST IN ARABIDOPSIS THALIANA

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Plant phosphate starvation growth responses are controlled by quantitative trait loci (QTLs) derived from natural variations in many species. Previous study has shown that root tips contact with low-phosphate mediates reprogramming of plant root architecture. However, there is little known about the molecular mechanism of the plants sense P availability and step-by-step reprogram roots growth. Here we showed that coordinated regulation of phosphate starvation *Arabidopsis* primary root growth arrest and cell-type specific auxin responses at the early stage. We found that reduced PIN1: GFP and PIN7: GUS protein in EDZ region, indicating auxin redistribution plays an important role in regulation of phosphate starvation root architecture responses. We demonstrated that phosphate starvation promoted ethylene biosynthesis and accumulation of EIN3 protein, which in turn enhanced auxin biosynthesis. Furthermore, we generated several NIL lines carrying DR5: GUS from an *Arabidopsis* recombinant inbred line population derived from the Ler and Col-0, we carried on QTL-based cloning and found that phosphate starvation induced ethylene synthesis and cell-specific auxin responses in primary root depended on LOW PHOSPHATE ROOT1 (LPR1) locus. These data indicated that ethylene/auxin signaling might act as important downstream component of LPR1 signaling. Thus, root tips sense exogenous phosphate starvation signal and promote ethylene biosynthesis in LPR1 dependent manners, and further induce local auxin synthesis, which in turn modulate primary root reprogram.

12006

TISSUE-REUNION PROCESS OF THE CUT FLOWERING STEM REQUIRES THE PHYTOHORMONE SIGNALING ACCOMPANIED BY THE UP-REGULATION OF TRANSCRIPTION FACTORS IN ARABIDOPSIS.

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In higher plant, when wounding or grafting interrupted the original organ or tissue connection, de-differentiate and regeneration of cells were induced, these cells acquire embryonic potential and tissue-reunion occurs to restore physiological connections. *Arabidopsis* cut flowering stems were analyzed to elucidate its molecular mechanism. The cell division was initially observed at 3 days after the cut and the tissue-reunion almost completed in 10 days. Microarray analysis demonstrated differential gene expression between cut and non-cut flowering stems and their changes in expression during reunion process. Genes determined to be up-regulated in the reunion process include those involved in cell division, cell wall modification, phytohormone-related gene and transcription factors (TF). To understand

how phytohormone involves in the tissue-reunion process, we focused on the phytohormone related genes and putative downstream TFs. Tissue-reunion was inhibited by decapitation or application of TIBA, and pin1 mutant, in which auxin transport was suppressed, showed lower efficiency in the reunion process. Gene expression analysis showed that AP2- and NAC-type transcription factors (TFs) were up-regulated at 1 and 3 days after cutting and the expression was depend on jasmonic acid (JA) and auxin, respectively. The gene-suppressing transformants for AP2-TF or NAC-TF using CRES-T method did not show normal reunion process. Ethylene-signaling deficient mutant also showed lower efficiency in tissue-reunion and gene expression analysis showed that AP2-TF and NAC-TF were also ethylene-regulated. These results suggest that JA/ auxin /ethylene signaling pathways and putative downstream TFs have an important role in the regulation of gene expression during tissue-reunion of *Arabidopsis* cut flowering stems.

12007

ESTABLISHING THE LINK BETWEEN JASMONATES AND GROWTH CONTROL

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Plant development and stress responses are regulated by complex signalling networks that mediate specific and dynamic plant responses upon activation by various types of exogenous and endogenous signals. The role of Jasmonates (JAs) has been traditionally described in the context of stress mediated responses in plants. However, latest works on JAs signalling have identified new regulatory nodes in the transcriptional network that regulates a number of diverse plant responses to developmental and environmental cues (e. g. senescence regulation) and suggest a common mechanism of JAs action via distinct groups of transcription factors.

Currently, we are interested in dissecting the cellular machinery linking plant stress responses to growth processes. Indeed, recent works revealed that JAs block cell cycle progression by inhibiting G1/S and G2/M transitions in tobacco cells and suppress cell proliferation in human cancer cell lines. While the molecular mechanisms and downstream responses have not been clarified yet, we are excited by the likelihood that jasmonate could act as a distress signal, a physiological role of which is to block cell cycle, slowing vegetative growth during defence responses. A summary of the results obtained so far will be presented.

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12008

THE PLANT RESPONSE TO CELL WALL DAMAGE IS REGULATED THROUGH THE INTERACTION OF ROS AND JA MEDIATED PROCESSES

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The plant cell wall changes its composition and structure in order to fulfil its different biological functions during cell morphogenesis or plant pathogen interaction and to maintain its functional integrity. Evidence has accumulated that a so-called cell wall integrity mechanism exists which monitors wall integrity, initiates changes in wall structure and composition while also activating established jasmonic or salicylic acid dependent stress response networks.

Previously we have shown that *ATRBOHD* (an NADPHoxidase) and *JAR1* are involved in maintaining cell wall integrity and that cell wall damage (generated by cellulose biosynthesis inhibition, CBI) induces jasmonic acid (JA) and lignin production after 6 hours. Here we show that mutations in *THESEUS*, *OXI KINASE1* and *MID1-COMPLEMENTING-ACTIVITY* and calcium as well as ROS signaling inhibitors inhibit CBI induced lignin deposition. Intriguingly, the same mutants exhibit increased JA levels while Ca signaling inhibitors inhibit JA production. In contrast mutants that are deficient in JA perception or production like *coronatine insensitive 1*, *aos* and *jasmonic acid resistant1* exhibit an increase in lignin production. These observations suggest that CBI activates a calcium dependent signaling mechanism that activates ROS and JA mediated signaling processes. Subsequently the dynamic interaction between ROS and JA determines the extent of lignin production in response to cell wall damage. This mechanism seems to enable the plant to respond to an initial input in a quantitatively appropriate way.

12009

MECHANISMS OF ABA PERCEPTION AND EARLY ABA SIGNALING CASCADE

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Abscisic acid (ABA) regulates physiologically important stress and developmental responses. *ABI1* encodes a protein phosphatase 2C that functions early in the ABA signaling cascade. To address the mechanism of *ABI1*-mediated ABA signaling, we generated tagged *ABI1* *Arabidopsis* expression lines and performed affinity column purification of *ABI1*-associated proteins. Interestingly, the most robust in planta *ABI1*-interacting proteins in all LC-MS/MS experiments were nine START domain proteins (Nishimura et al., *Plant J* 2010). The major *in vivo* *ABI1* interactors correspond to PYR/PYL/RCAR proteins, which were recently reported as ABA-binding signal transduction proteins (Park et al., *Science*, 2009, Ma et al., *Science*, 2009), demonstrating *in vivo* PYR/PYL/RCAR interactions with *ABI1* in *Arabidopsis* (Nishimura et al., *Plant J* 2010). To investigate the biological relevance of the PYR/PYLS, we analyzed *pyr1pyl1pyl2pyl4* quadruple mutant plants and found strong insensitivities in ABA-induced stomatal closure and ABA-inhibition of stomatal opening (Nishimura et al., *Plant J* 2010).

We investigated the structural mechanisms by which PYR/PYL/RCAR proteins mediate signaling. PYR1 crystallographic structures reveal an α/β helix-grip fold and homodimeric assembly. The homodimeric assembly was verified in planta by co-immunoprecipitation. ABA binding within a large internal cavity switches structural motifs distinguishing ABA-free "open-lid" from ABA-bound "closed-lid" conformations. Site-directed PYR1 mutants designed to disrupt hormone binding lose ABA-triggered interactions with *ABI1* in planta (Nishimura et al., *Science* 2009). Furthermore, data will be presented indicating that ABA signaling proteins form an ABA signalosome complex.

12010**EIN3/EIL1 COOPERATE WITH PIF1 TO MODULATE SEEDLING DE-ETIOLATION IN ARABIDOPSIS**

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The ability to switch from skotomorphogenesis to photomorphogenesis is essential for seedling development and plant survival. Recent studies revealed that COP1 and phytochrome-interacting factors (PIFs) are key regulators of this transition by repressing the photomorphogenic responses and/or maintaining the skotomorphogenic state of etiolated seedlings. Here we report that the plant gaseous hormone ethylene plays a crucial role in the transition from skotomorphogenesis to photomorphogenesis by facilitating greening of etiolated seedlings upon light irradiation. Activation of EIN3/EIL1 (two master transcription factors in the ethylene signaling pathway) is both necessary and sufficient for ethylene-induced enhancement of seedling greening, as well as repression of the accumulation of protochlorophyllide, a phototoxic intermediate of chlorophyll synthesis. EIN3/EIL1 were found to induce gene expression of two key enzymes in the chlorophyll synthesis pathway, protochlorophyllide oxidoreductase A and B (POR/A/B). ChIP and EMSA assays demonstrated that EIN3 directly binds to the specific elements present in the PORA and PORB promoters. Genetic studies revealed that EIN3/EIL1 function in cooperation with PIF1 in preventing photo-oxidative damage and promoting cotyledon greening. Moreover, activation of EIN3 reverses the blockage of greening triggered by cop1 mutation or far-red light irradiation. Consistently, EIN3 acts downstream of COP1 and its protein accumulation is enhanced by COP1 but decreased by light (Zhong et al., 2009, PNAS). Taken together, EIN3/EIL1 represent a new class of transcriptional regulators along with PIF1 to optimize de-etiolation of Arabidopsis seedlings. This study highlights the essential role of ethylene in enhancing seedling development and survival through protecting etiolated seedlings against photo-oxidative damage.

12011**CYTOKININ-FACILITATED DESTABILIZATION OF ARR2 REGULATES SIGNALING OUTPUT IN ARABIDOPSIS TWO-COMPONENT CIRCUITRY**

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Cytokinins propagate their signals via multiple phosphorelays, similarly observed in bacterial two-component system. In *Arabidopsis*, signal outputs are generally determined by activation state of type-B ARR (Arabidopsis response regulators), a subset of transcription factors; however, their regulatory mechanisms are largely unknown. Here, we demonstrate that the protein stability of ARR2, a type-B ARR, modulates cytokinin signaling outputs. ARR2, but not other type-B ARRs such as ARR1, ARR10, and ARR12, is rapidly destabilized by cytokinin treatment. The degradation of ARR2 is mediated by the 26S proteasome pathway and requires cytokinin-induced phosphorylation of Asp80 residue in the receiver domain. Through mutational analyses of amino acid residues in the receiver domain, we found that substitution of Lys 85 to Gly inhibits ARR2 destabilization by t-zeatin. The expression of ARR2^{K85G} in transgenic *Arabidopsis* conferred enhanced cytokinin sensitivity in various developmental processes including primary root elongation, callus induction, leaf senescence, and hypocotyl growth. ARR2^{K85G} prolonged the high levels of expression of type-A ARRs known as primary cytokinin-responsive genes and indicators for signaling output of the two-component circuitry. These results reveal that cytokinin-induced degradation of ARR2 is a desensitization mechanism of cytokinins and required for fine-tuning of output intensities of developmental signals in *Arabidopsis*.

12012**CKR, A BHLH TRANSCRIPTION FACTOR, FUNCTIONS AS A KEY REGULATOR FOR CELL DIVISION AND ORGAN GROWTH IN CYTOKININ RESPONSE**

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Cytokinins are important for various developmental processes of plants including leaf senescence, apical dominance, chloroplast development, anthocyanin production, and cell division. Cytokinin signaling cascade through two-component phosphorelay has been well-known; however, regulators that determine cytokinin signaling output are still elusive. Here we identified a cytokinin-responsive CKR (Cytokinin responsible growth regulator) encoding a bHLH transcription factor. CKR over-expressing plants exhibited bigger and faster organ growth of flowers, embryo, and cotyledons than did ckr knockout or wild-type plants. CKR expression was induced by cytokinins and ARR2, a major regulator of cytokinin signaling. CKR specifically activated the expression of cyclinD, implicating cytokinin action in cell cycle through the direct activation of CKR as well as the two-component system. Taken together, we propose that CKR is a key downstream regulator in cytokinin-mediated organ growth and cell cycle.

12013**MATERNAL SYNTHESIS OF ABA AND TRANSPORT OF ABA INTO THE EMBRYO DURING ARABIDOPSIS SEED DEVELOPMENT**

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Abscisic acid (ABA) is a plant hormone involved in many aspects of seed development such as accumulation of storage compounds, induction of seed dormancy and inhibition of precocious germination. We conducted detailed ABA quantification analysis in *Arabidopsis* developing seeds by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Detection of ABA from single seed in the population of F₂ generated by crossing wild type and an ABA-deficient mutant, *aba2*, demonstrated that ABA was synthesized in maternal tissues during mid-development, whereas it was synthesized in zygotic tissues in later stages. Despite active ABA biosynthesis in maternal tissues in the middle stage, ABA was highly accumulated in the embryos in this stage, suggesting that ABA was translocated into the embryos from other tissues. Physiological roles of ABA synthesized in different tissues will be discussed.

12014**THREE SNRK2 PROTEIN KINASES ARE THE MAIN POSITIVE REGULATORS OF ABA SIGNALING IN SEED DEVELOPMENT AND DORMANCY IN ARABIDOPSIS**

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ABA is an important phytohormone regulating various plant processes, including stress tolerance, seed development and germination. SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3 are redundant ABA-activated SNF1-related protein kinases 2 (SnRK2s) in *Arabidopsis thaliana*. We examined the role of three homologous *Arabidopsis* ABA-activated SnRK2 protein kinases, SRK2D, SRK2E and SRK2I in seed development and germination. These were mainly expressed in the nucleus during seed development and germination. Unlike *srk2d*, *srk2e* and *srk2i* single and double mutants, the triple mutant (*srk2d srk2e srk2i*) was sensitive to desiccation and showed severe growth defects during seed development. It exhibited elevated seed ABA content, a loss of dormancy and vivipary. The triple mutant showed highly enhanced insensitivity to ABA. It also showed insensitivity to sugars and paclobutrazol (PAC), a gibberellin biosynthesis inhibitor. The triple mutant had greatly reduced phosphorylation activity of in-gel kinase experiments using bZIP transcription factors including ABI5. Microarray experiments revealed that 48% and 30% of the down-regulated genes in *abi5* and *abi3* seeds were suppressed in the triple mutant seeds, respectively. Moreover, disruption of the three protein kinases induced global changes in the up-regulation of ABA-repressive gene expression, as well as the down-regulation of ABA-inducible gene expression. These results indicate that these protein kinases are essential for the control of seed development and dormancy through the extensive control of ABA-responsive gene expression.

12015

ANALYSIS OF AUXIN BIOSYNTHESIS BY USING THE IAA BIOSYNTHESIS INHIBITORS

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We were successful to identify the first auxin-biosynthesis inhibitor, aminoethoxyvinylglycine (AVG), which blocks L-Tryptophan(Trp) aminotransferase which catalyze conversion from L-Trp to indole-3-pyruvic acid. Additional screening allowed us to identify several compounds as auxin-biosynthesis inhibitors, all of which inhibited L-Trp aminotransferase in enzyme extracts from wheat and *Arabidopsis*. We used these inhibitors to investigate the conservation and diversity of the auxin biosynthesis pathway in a monocot plant, rice, and dicot plants, tomato and *Arabidopsis* *in vivo*. These inhibitors reduced endogenous indole-3-acetic acid (IAA) levels both in monocot and dicots, indicating that L-Trp aminotransferase constitutes one of the major auxin biosynthesis pathway conserved among higher plants. However, the inhibitors showed different action spectrum among organs and species. The inhibitors inhibited normal root elongation and the gravitropic response in *Arabidopsis* seedlings, which recovered from the inhibition by exogenous applications of IAA and its precursors almost completely to the level of non-inhibited seedlings. These results provide novel insights into auxin biosynthesis and action.

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12016

ROLE FOR TYROSINE PHOSPHORYLATION IN BRASSINOSTEROID SIGNALING

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Plant and animal cells have receptor kinases that span the plasma membrane and function to transfer extracellular signals to the interior of the cell. Binding of the ligand to the receptor's extracellular domain activates the cytoplasmic protein kinase domain of the receptor via auto- and/or trans-phosphorylation, and the dogma is that animal receptor kinases autophosphorylate on Tyr residues while plant receptor kinases autophosphorylate on Ser/Thr residues. Recent results with the *Arabidopsis* brassinosteroid (BR) receptor kinase, BRI1, and its co-receptor, BAK1, indicate that they are actually dual specificity kinases, capable of autophosphorylation on Ser, Thr and Tyr. With BRI1, phosphorylation of Tyr-831 in the juxtamembrane domain affects BR signaling that controls leaf shape and size, biomass accumulation, and flowering time. With BAK1, which functions as a co-receptor in several pathways including BR signaling, autophosphorylation on Tyr-610 in the carboxy-terminal domain was identified as a major site that is essential for BAK1 function *in vivo*. Plants expressing BAK1(Y610F)-Flag (in the *bak1bak1/bkk1bkk1* background) are severely dwarfed and resemble BR-signaling mutants. A subset of BL-regulated genes, including many growth-promoting genes, is down regulated in the Y610F plants suggesting that phosphorylation of Tyr-610 is essential for effective BR signaling. Interestingly, Tyr phosphorylation of BRI1 and BAK1 appears to have opposing effects on BR signaling. The occurrence of Tyr phosphorylation in BRI1, BAK1, and several other receptor kinases opens a new level of potential regulation of important signaling pathways that may ultimately lead to new strategies to control plant growth and development.

12017

STUDY FOR ROLE OF LONELY GUY CYTOKININ-ACTIVATING ENZYMES IN ARABIDOPSIS

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Cytokinins play crucial roles in diverse aspects of plant growth and development. The free base forms, such as *trans*-zeatin and N⁶-(Δ²-isopentenyl)adenine, are thought to be the hormonally active cytokinin. In the biosynthesis pathway, the nucleotide form is initially produced as a cytokinin precursor. To become active, two pathways are possibly involved in producing the free base forms from the nucleotide: two-step activation pathway and direct activation pathway. In the two-step pathway, the nucleotide forms are successively converted to the corresponding nucleoside and the free-base forms by nucleotidase and nucleosidase, respectively, although the responsible genes have not been identified yet. By contrast, the direct pathway mediates production of the free base forms from the nucleotides. LONELY GUY (LOG) was previously identified as a cytokinin-activating enzyme that works in the direct activation pathway in rice [1] and *Arabidopsis*[2]. However, it is still unclear how the direct activation pathway is biologically important in plant growth and development because *log3 log4 log7* triple mutant only showed slight differences in the visible phenotype [2]. To address this question, we have generated a various combinations of multiple mutants of *Arabidopsis* LOG genes (*AtLOG1-5, 7, 8*). When tracer analyses were performed with stable isotope-labeled cytokinin metabolites, the conversion from the labeled nucleotides to the labeled free base forms is significantly reduced in the multiple mutants. In addition, we undertook detailed phenotypic analyses. The multiple mutants showed altered root and shoot morphology which indicates cytokinin-deficiency. Our results strongly suggested that the direct activation pathway mediated by LOG plays a central role in production of free-base forms.

[1] Kurakawa et al. Nature 452: 652-655 (2007)

[2] Kuroha et al. Plant Cell 21:3152-3169 (2009)

12018

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF PUTATIVE PHOSPHORYLATION SITE OF ATPIN3 IN THE ARABIDOPSIS ROOT HAIR

SYSTEM

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PIN-FORMED (PIN) protein members act as auxin efflux transporters which play a significant role in plant growth and development by maintaining local auxin gradients throughout the plant body. The directional auxin transport is determined by asymmetrical distribution of PIN proteins in the plasma membrane (PM), and phosphorylation of PINs has been implicated in this directional intracellular PIN trafficking. We have taken advantage of the *Arabidopsis* (At) root hair system to assay auxin transporting activity of PIN proteins where enhanced activity of auxin efflux transporters reduces root hair growth likely by lowering cellular auxin levels. Here, using this root hair assay system, we analyzed the putative phosphorylation residues of AtPIN3. While root hair-specific expression of wild type AtPIN3 greatly reduced the root hair growth, certain combinatorial mutations in putative phosphorylation residues of the central hydrophilic loop abolished PIN3-mediated inhibition of root hair growth. Microscopic observation of the PIN3-GFP fusion supports the idea that the restoration of root hair growth in the mutant PIN3-expression lines resulted from disruption of PIN3 trafficking to the PM.

These results provide clues for putative phosphorylation residues in the PIN3 central loop which could be targets of the upstream modulatory pathway.

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12019

THE INTERACTION BETWEEN CYTOKININ AND AUXIN CONTROLS STEM CELL SPECIFICATION DURING *IN VITRO* SHOOT INDUCTION IN ARABIDOPSIS

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Shoot regeneration is an important way for plant propagation in vitro. Stem cells are critical for the formation of shoot apical meristem and must be induced within the callus during shoot induction. Little is understood however about the regulation of this process and how the stem-cell fate becomes determined during this process. In our current study, we showed that the fate of stem cells is determined by cytokinin and auxin within the callus after induction. We next visualized the behavior and interaction of cytokinin and auxin during stem-cell formation. In the non-induced callus, either cytokinin or auxin gradients were established in its surrounding edge region. However, the regional distribution of both hormones occurred following induction after which *WUSCHEL* (*WUS*) was found to be induced in the region containing high levels of cytokinin and low levels of auxin. Furthermore, the polarized PIN-FORMED (PIN) proteins were observed in the regions close to the edge of the callus after induction. Upon the inhibition of cytokinin biosynthesis, the auxin levels decreased and a normal distribution pattern for *WUS* induction was not observed. Disruption of cytokinin signaling also resulted in an abnormal distribution pattern for auxin. Following inhibition of auxin transport or inhibition of *PIN1* expression, the regional distribution of auxin gradients was not detected within the callus and notably, the regional distribution of cytokinin gradients was also not observed. Our current results suggest that a positive interaction between cytokinin and auxin controls the stem-cell fate, and we propose a model for stem-cell specification control during *in vitro* shoot induction in *Arabidopsis* by cytokinin and auxin.

12020

A NOVEL METHOD TO REGULATE THE PROTEIN FUNCTION IN NON-PLANT ORGANISMS BY UTILIZING AN AUXIN RESPONSE

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Plants have evolved a unique system in which the plant hormone directly induces rapid degradation of certain proteins by a specific form of the SCF E3 ubiquitin ligase. In plant cells, auxin promotes the interaction between an auxin receptor TIR1 and transcriptional repressors called AUX/IAAs. As TIR1 is also F-box protein that is a target-recognizing subunit of SCF, AUX/IAAs recruited into SCFTIR1 are polyubiquitinated by SCFTIR1 and degraded by 26S proteasome. Non-plant eukaryotes lack the auxin response, but share the SCF dependent degradation pathway. We expected that we could develop a novel system for rapid depletion of the target protein, based on transplanting this auxin-dependent degradation pathway from plants to non-plant organisms. For this purpose, we expressed the TIR1 gene from *Arabidopsis thaliana* under the GAL promoter. In this strain, GFP fused with IAA17 was depleted within 30 minutes after addition of auxin. This result indicated that we could reconstitute of auxin-dependent degradation pathway in budding yeast by inducing TIR1 to deplete the target protein fused with IAA17. So we named this system Auxin Inducible Degron (AID) system. The AID system allowed rapid and reversible degradation of the target proteins in response to auxin and enabled us to generate efficient conditional mutants of essential proteins in yeast as well as cell lines derived from chicken, mouse and human cells. Thus offering a powerful tool to control protein expression and study protein function in non-plant organisms.

1. K. Nishimura, T. Fukagawa, H. Takisawa, T. Kakimoto and M. Kanemaki An auxin-based degron system for the rapid depletion of proteins in non-plant cells. *Nature Methods* 6, 917-922, 2009

12021

INITIAL CHARACTERIZATION OF THE AGRAVITROPIC ARABIDOPSIS MUTANT THAT IS ORTHOLOGOUS TO RICE *LAZY1*

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Shoots of higher plants show negative gravitropism, where inflorescences bend to direction contrary to the gravity vector. Although genetic studies have identified many genes involved in such gravitropic responses, molecular details of gravity perception and later signal transduction are still largely unknown. By screening of EMS-mutagenized *Arabidopsis* seeds that harbored *MSG2/IAA19 promoter::NLS-GFP* and *MSG2 promoter::GUS*, we isolated a novel mutant which had reduced GFP signal intensity and showed reduced gravitropism in shoots. With map-based cloning method, we identified a mutation in the gene At5g14090 that changed Trp4 to the stop codon. At5g14090 is orthologous to *LAZY1* in rice (Yoshihara and Iino, 2007). Complementation test with a T-DNA insertional line of At5g14090 confirmed that this mutation caused the agravitropic phenotype of shoots. The rice mutant *lazy1* also has reduced shoot gravitropism, suggesting that this gene plays a critical role in gravitropism of higher plants. Our mutant has normal amyloplasts and normal positive gravitropic response in root. These phenotypes are similar to those of rice *lazy1*. *Arabidopsis lazy1* exhibited reduced circumnutation of shoots though circumnutation is completely lost in rice *lazy1* coleoptiles. Under continuous white light condition *Arabidopsis lazy1* apparently displayed plagiogravitropism of shoots. Quantitative RT-PCR analyses confirmed reduced *MSG2* expression in *Arabidopsis lazy1*, but its responsiveness to exogenous auxin was kept almost intact. Though rice *LAZY1* is a single gene, *Arabidopsis LAZY1* (*AtLAZY1*) has a

paralogous gene At3g27025. Its amino acid sequence is 22% identical to AtLAZY1 and also has no known motifs or signals. In this report we will also present phenotypic and molecular genetic analyses of a T-DNA insertion line of the *AtLAZY1* paralog.

12022

CRFS FORM PROTEIN-PROTEIN INTERACTIONS AMONG EACH OTHER AND WITH MEMBERS OF THE CYTOKININ-SIGNALING PATHWAY VIA THE CRF DOMAIN

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Cytokinin is a plant hormone essential for growth and development and the elucidation of its signaling pathway as a variant of the bacterial two-component signaling system (TCS) has led to a better understanding of how this hormone is involved in general plant processes. A set of cytokinin-regulated transcription factors known as Cytokinin Response Factors (CRFs) have been described as a potential branch of the TCS, yet little is known of how CRFs actually interact with each other and with members of TCS pathway. Here we describe the interactions of CRF proteins (CRF1-8) using the yeast two-hybrid system and bimolecular fluorescence complementation in planta assays. We found that CRFs are readily able to form both homo- and heterodimers with each other. Additionally, we provide the first analysis of CRF vs. TCS pathway proteins interactions indicating that CRFs (CRF1-8) are able to specifically interact directly with most of the *Arabidopsis* histidine-phosphotransfer proteins (AHP1-5) further solidifying their link to the cytokinin signaling pathway. Furthermore, we mapped the region of CRF proteins involved in these interactions and determined that the clade specific CRF domain alone is sufficient for these interactions. This is the first described function for the CRF domain in plants.

12023

P-BODY AND STRESS GRANULE LOCALIZED ATTZF1 CAN MEDIATE SUGAR, ABA, AND GA RESPONSES

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Processing-bodies (P-bodies, PBs) and Stress Granules (SGs) are cytoplasmic messenger ribonucleoprotein (mRNP) complexes. It has been shown recently that PBs and SGs play important roles in post-transcriptional regulation and epigenetic modulation of gene expression. While PBs are enriched with translation-repressed mRNAs and mRNA silencing and degradation machineries, SGs are essentially aggregation of stalled pre-initiation complexes and translation regulators. We have found that the *Arabidopsis* (ATTZF1) and human (hTTP) Tandem Zinc Finger proteins can traffic between the nucleus and cytoplasmic foci, where they co-localize with PB and SG markers, AGO1, DCP2, PABP, and XRN4 in plant cells. ATTZF1-associated cytoplasmic foci are dynamic and tissue-specific. They are preferentially formed in active growing cells and induced by various stresses, including stress hormones. Reverse genetic analyses indicate that ATTZF1 is a positive regulator for sugar- and ABA-mediated, and a negative regulator for GA-mediated plant growth, development, and stress responses. Gene expression profiling reveals that over-expression of ATTZF1 mimics the effects of +ABA/-GA. Co-expression analyses identify a cell growth cluster centered by a sugar/ABA/GA regulated peptide hormone and a defense response cluster centered by a WRKY transcription factor. Because ATTZF1 affects hormone accumulation only in specific developmental stages, it is likely that ATTZF1 affects sugar/ABA/GA responses mainly by regulating their signal transduction components. Since ATTZF1 can bind both DNA and RNA *in vitro*, it raises a possibility that ATTZF1 is involved in transcriptional regulation in nucleus and RNA regulation in PBs and SGs.

12024

ABSCISIC ACID AND ETHYLENE CROSSTALK REVEALED BY THE ABA2/ETHYLENE DOUBLE MUTANTS IN ARABIDOPSIS

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Although abscisic acid (ABA) and ethylene have antagonistic functions in the control of plant growth and development, including seed germination and early seedling development, it remains unknown whether a convergent point exists between these two signaling pathways or whether they operate in parallel in *Arabidopsis thaliana*. To elucidate this issue, four ethylene mutants, *ctr1*, *ein2*, *ein3*, and *ein6*, were crossed with *aba2* (also known as *gin1-3*) to generate double mutants. Genetic epistasis analysis revealed that all of the resulting double mutants displayed both *aba2* and ethylene mutants phenotypes. Our currently data therefore demonstrate that ABA and ethylene act in parallel, at least in primary signal transduction pathways. Moreover, by microarray analysis we found that an *ACC oxidase* (*ACO*) was significantly upregulated in the *aba2* mutant, whereas the *NCED3* gene in *ein2* was upregulated, and both the *ABI1* and *CYP707A2* genes in *etr1-1* were downregulated. These data further suggest that ABA and ethylene may control the hormonal biosynthesis catabolism or signaling of each other to enhance their antagonistic effects upon seed germination and early seedling growth.

12025

OVER-EXPRESSION OF CIS-ZEATIN-O-GLUCOSYLTRANSFERASE IN RICE

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In higher plants, *cis*-zeatin (cZ) has been considered as adjunct to *trans*-zeatin with low activity, based on traditional bioassays of cytokinin and studies on cytokinin signaling in *Arabidopsis*. However, cZ and cZ derivates are accumulated in some plant species including maize and rice. In contrast to cytokinin receptors of *Arabidopsis*, maize cytokinin receptor ZmHK1 is sensitive to cZ. Thus, further investigation of cZ is required to understand generality and diversity of the cytokinin metabolism and function in higher plants. In rice, cZ-O-glucoside (cZOG) and cZ riboside-O-glucoside (cZR OG) are accumulated in high levels. It has been reported that two UDP-glucosyltransferase (UDP-GT) family proteins of maize (*cis*ZOG1, *cis*ZOG2) showed cZ specific O-glucosylation activity *in vitro*. Blast search of rice genome database showed that homologous UDP-GT proteins to maize *cis*ZOGs are encoded by 6 genes in rice. Among the 6 UDP-GTs of rice, at least 3 UDP-GTs showed cZ-O-glucosylation activity *in vitro* as well as the maize *cis*ZOGs. Moreover, cZ riboside was a better substrate for O-glucosylation by the UDP-GTs than cZ. In transgenic rice plants ectopically over-expressing the each UDP-GT gene, accumulation of cZOG or cZR OG was likely to be up-regulated. The over-expressing rice indicated some visible phenotypes, which are similar to typical phenotype of cytokinin deficient. Together, we conclude that the UDP-GTs can be involved in inactivation of cytokinin as cZ- or cZR-O-glucosyltransferases in rice.

12026**DIRECT SYNTHESIS OF TZ-TYPE CYTOKININ IS IMPORTANT FOR GALL FORMATION**

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Agrobacterium tumefaciens infection induces tumor formation by integrating the T-DNA region of Ti-plasmid into the plant nuclear genome. Tumors are formed because the T-DNA encodes enzymes that synthesize two plant hormones, auxin and cytokinin. We had demonstrated that a cytokinin biosynthesis enzyme isopentenyltransferase (IPT), Tmr, which is encoded by the T-DNA region, is targeted to and functions in plastids of infected plant. The Tmr creates a metabolic bypass for direct synthesis of tz-type cytokinin by using HMBDP. To reveal biological importance of the Tmr function for gall formation, we studied biochemical properties of Tmr with comparing to Tzs, which functions in the bacterial cell. We generated transgenic *Arabidopsis* overexpressing Tmr, Tzs, or transit peptide-fused Tzs (TP-Tzs) under the control of DEX-inducible promoter. DEX-treatment induced the accumulation of tz-type cytokinin in Tmr-overexpressing *Arabidopsis*, whereas it induced that of both tz and ip in Tzs and TP-Tzs-overexpressing plants. Tmr and Tzs show similar substrate specificity *in vitro*. Thus, it is strongly suggest that Tmr has a specialized function to predominantly use HMBDP for tz biosynthesis in the host plant plastids. We are now examining tumor-formation efficiency of the recombinant *Agrobacteria*, whose Tmr gene on the T-DNA is replaced by Tzs or TP-Tzs.

12027**SKP1-LIKE GENES MEDIATE ABCISIC ACID REGULATION OF SEED GERMINATION, STOMATAL OPENING AND ROOT GROWTH IN ARABIDOPSIS**

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Abscisic acid (ABA) regulates many aspects of plant development, including seed dormancy and germination, root growth and stomatal closure. Plant Skp1-like proteins are, or are thought to be, subunits of the SCF complex E3 ligases, which regulate several phytohormone signaling pathways through protein degradation. However, little is known about the relationship between Skp1 proteins and ABA signaling. Here, we show that overexpression of *Triticum aestivum SKP1-Like 1 (TSK1)* in *Arabidopsis* resulted in delayed seed germination and hypersensitivity to ABA. The opening of stomatal guard cells and the transcription of several ABA-responsive genes were affected in transgenic plants. In addition, *Arabidopsis Skp1-like 1 (ask1)/ask1 ASK2/ask2* seedlings exhibited reduced ABA sensitivity. Real-time PCR results showed ASK1 and ASK2 transcripts downregulated in *abi1-1* and *abi5* mutants. These data suggest that *SKP1-like* genes may positively regulate ABA signaling by SCF-mediated protein degradation and participate in ABA responses.

12028**NOVEL ARABIDOPSIS CYTOKININ-RESISTANT MUTANTS ILLUMINATE AN ETHYLENE-INDEPENDENT PATHWAY FOR GROWTH REPRESSION OF CYTOKININS**

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Cytokinins, with its signal transduction pathway well defined, play key roles in the plant growth and development. Previous studies have implied the significant role of ethylene involved in the growth inhibition by cytokinins. Interestingly, ethylene-insensitive mutants show obvious repressed phenotypes on cytokinin treatment. We asked the question how cytokinins restrained plant growth independent of ethylene signaling. A genetic screening was applied with the *ein2-9* mutant background to select cytokinin resistant mutants beyond ethylene signal transduction. Ten mutants were found to be insensitive to cytokinin inhibition in hypocotyl and root elongation. Allelic analysis showed that these ten mutants belonged to a same complementation group, indicating the high specificity of the screening assay. Surprisingly, after backcross with Col-0, the dark-grown mutants without *ein2-9* background showed constitutive triple response, which can be blocked by ethylene synthesis inhibitor AVG. All the adult mutants exhibited dark green rosette leaves and grew very slowly. Late flowering and late senescence phenotype were observed in the mutants compared with wildtype, suggesting cytokinin signaling pathway was activated in mutants. We hypothesize that the unique mutations caused turbulences of cytokinin metabolism or signaling pathway independent of the promotion of ethylene synthesis, and this novel pathway played a vital role in the cytokinin-induced growth repression.

12029**COP9 SIGNALOSOME TURNS ON THE DEGRADATION OF EIN3/EIL1 IN ETHYLENE SIGNALING**

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Ethylene is a gaseous hormone, which regulates plant growth, development and tolerance to environmental stresses. Ethylene Insensitive 3 (EIN3) and its closest homolog EIN3 Like 1 (EIL1) are two major transcription factors in ethylene signaling. Gene expression analysis and phenotypic observation show that *ein3 eil1* double mutants are insensitive to ethylene, which suggesting that EIN3/EIL1 play key roles in transmitting ethylene signal to their downstream molecules. In the absence of ethylene, the protein level of EIN3/EIL1 is quite low, which is caused by their degradation through two F-box proteins (EBF1/2). After the application of ethylene, the function of EBF1/2 is repressed so that EIN3/EIL1 proteins are accumulated.

COP9 signalosome is protein complex comprising eight subunits, which is responsible for the posttranslationally modifying cullin subunit in E3 ligase. Previous reports have shown that COP9 signalosome interacted with F-box proteins: TIR1, COI1 and UFO to regulate hormone signaling and flower development. However, how about the interactions between COP9 signalosome and E3 target proteins are still unclear.

Here, we showed that EIN3 interacted with one subunit (CSN5) of COP9 signalosome in yeast and plant cells. In the COP9 signalosome mutants, the protein abundance of EIN3 was accumulated, which suggested that COP9 signalosome facilitating the degradation of EIN3. Strikingly, we transferred a reporter line of EIN3 (GUS driven by 5 repeated EIN3 Binding Sites) to *cop9* mutant and found that GUS was constitutively expressed, which proved the enhancement of the function of EIN3 in COP9 signalosome mutants. In order to distinguish the accumulation of EIN3 in COP9 mutant is an effect of missing interaction of COP9-EIN3 and/or repression of EBF1/2 functions, we are going to identify the interaction domain of COP9 and do the mutated analysis.

12030**MINING FOR NOVEL STRIGOLACTONE-RELATED BRANCHING GENES**

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Strigolactones function in the upwardly-mobile, graft-transmissible signalling pathway that keeps axillary buds in a repressed state of growth. However, so far only a handful of genes have been implicated in strigolactone biosynthesis or regulation. The lack of strigolactone branching mutants could be due to redundancy and/or weak branching phenotypes. Using a transcriptomic approach and reverse genetics, we are mining for enzymes and master regulators of the pathway. We present the methodology that allows us to focus on a handful of genes and to make progress in isolating new branching mutants in *Arabidopsis*. Two of our candidates, a transcription factor and an enzyme, show moderate, but significantly increased branching phenotypes in mutants. Branching in these mutant shoots is fully rescued by strigolactone application, but not by grafting with wildtype rootstocks. The transcription factor might be required in the shoot for feedback signalling and strigolactone production. The enzyme appears to be necessary for a late biosynthetic step in shoot tissue and opens opportunities to investigate mobile substrates in strigolactone signalling.

12031**SKP1-LIKE GENES MEDIATE ABSCISIC ACID REGULATION OF SEED GERMINATION, STOMATAL OPENING AND ROOT GROWTH IN ARABIDOPSIS**

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Abscisic acid (ABA) regulates many aspects of plant development, including seed dormancy and germination, root growth and stomatal closure. Plant Skp1-like proteins are, or are thought to be, subunits of the SCF complex E3 ligases, which regulate several phytohormone signaling pathways through protein degradation. However, little is known about the relationship between Skp1 proteins and ABA signaling. Here, we show that overexpression of *Triticum aestivum* SKP1-Like 1 (*TSK1*) in *Arabidopsis* resulted in delayed seed germination and hypersensitivity to ABA. The opening of stomatal guard cells and the transcription of several ABA-responsive genes were affected in transgenic plants. In addition, *Arabidopsis* *Skp1-like 1 (ask1)/ask1 ASK2/ask2* seedlings exhibited reduced ABA sensitivity. Real-time PCR results showed *ASK1* and *ASK2* transcripts downregulated in *abi1-1* and *abi5* mutants. These data suggest that *SKP1-like* genes may positively regulate ABA signaling by SCF-mediated protein degradation and participate in ABA responses.

12032**ATLTP3, A F-BOX PROTEIN WHICH MEDIATE ABSCISIC ACID AND STRESS SIGNALING**

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In this research, we study the function of the AtTLP3 gene using reverse genetic methods. Transgenic plants carrying AtTLP3 promoter::GUS construct were used to determine the temporal and spatial expression patterns of the AtTLP3 gene. According to the results, AtTLP3 had obvious expression during seed germination, suggesting its involvement in this process. ABA-insensitive phenotypes were observed for two independent AtTLP3 knockout lines (*attlp3-1* and *attlp3-2*) while transgenic plants overexpressing AtTLP3 were hypersensitive to ABA during seed germination. RTQ-PCR analysis showed that AtTLP3 expression was repressed by ABA during seed germination. These results suggest that AtTLP3 may participate in the ABA signaling pathway during seed germination. Moreover, transgenic plants were treated with glucose and mannitol to monitor their responses to osmotic pressure during early seedling development. As a result, a glucose-insensitive phenotype was observed in *attlp3-1* and *attlp3-2* while overexpressing AtTLP3 was hypersensitive to 5% glucose during early seedling development. No such responses were observed with the same concentration of mannitol in transgenic plants, suggesting the hypersensitivity was glucose specific rather than the result of an osmotic effect. Transient expression of AtTLP3 in *Arabidopsis* mesophyll protoplasts indicated that AtTLP3 is partially localized in the nucleus. These results suggest that AtTLP3 probably acts through targeting one or more regulatory proteins in the nucleus for ubiquitin-mediated proteolysis to regulate ABA and/or glucose signaling pathways.

12033**EXTENDING THE ABA SIGNALING NETWORK: A WNK KINASE ACTS AS A NEGATIVE REGULATOR**

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As a key regulator of plant growth and development, the phytohormone abscisic acid (ABA) influences almost all aspects of a plant's life, ranging from germination and post germination control to mediating stomatal closure up to the regulation of various stress responses.

To coordinate these functions an extensive network of signal transducers is necessary. Protein phosphatases of the PP2C family are central negative regulators of the ABA signaling pathway. They directly interact with the ABA receptor proteins, thereby regulating the activity of downstream kinases. So far, members of the SnRK2 (SNF1-related protein kinase 2) family are the only identified kinases, which act as positive regulators in ABA signaling.

Our work provides evidence that, opposite SnRKs, a kinase of the WNK family is a new component of the ABA signaling pathway. The *Arabidopsis* WNK family consists of 11 serine/threonine protein kinases exhibiting a characteristic replacement of a unique lysine residue in the catalytic domain, hence the name WNK (With No K (Lysine)).

Phenotypic analysis of *wnk8* mutants, exhibiting altered post germination growth in response to ABA, indicate that WNK8 could be involved in ABA signaling. In addition, double mutant analysis revealed that the *wnk8* alleles are able to modify the ABA-hypersensitive phenotype of the *pp2ca-1* mutant. Direct interaction between WNK8 and PP2CA was observed in the Y2H system and verified *in planta* using the BIFC system. Furthermore, biochemical analysis showed that WNK8 is a direct target of the PP2CA phosphatase.

Taken together our results show that WNK8 can be integrated into the ABA signaling network as a novel negative regulator that might counteract SnRK activity.

12034

CELL SPECIFIC VISUALIZATION OF JASMONATES USING JASMONATE-SPECIFIC ANTIBODIES

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Jasmonates are well-characterized signals in plant development and in the response of plants to abiotic and biotic stresses, such as wounding by herbivores. A gap in our knowledge on jasmonate-induced processes is the occurrence of jasmonates at cellular level. Here, we present a novel antibody-based approach to visualize jasmonates in cross sections of plant material. The antibody raised in rabbit against BSA-coupled jasmonic acid is highly specific for bioactive jasmonates, such as jasmonic acid (JA), its methyl ester and isoleucine conjugate, but does not bind to 12-oxophytodienoic acid, 12-hydroxy-JA and coronatine. With this new antibody a powerful tool is available to detect cell and tissue-specifically the occurrence of jasmonates in any jasmonate-dependent stress response or developmental process of plants.

12035ALTERATION OF SUBSTRATE SPECIFICITY: THE VARIABLE N-TERMINAL DOMAIN OF CA²⁺-DEPENDENT PROTEIN KINASE IS IMPORTANT FOR THE SUBSTRATE RECOGNITION

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Protein kinases are major signaling molecules that are involved in a variety of cellular processes. However, molecular mechanisms of how protein kinases discriminate specific substrates are still largely unknown. Ca²⁺-dependent protein kinases (CDPKs) play central roles in Ca²⁺ signaling in plants. Previously, we found that a tobacco (*Nicotiana tabacum*) NtCDPK1 negatively regulates transcription factor REPRESSION OF SHOOT GROWTH (RSG) that is involved in gibberellin (GA) feedback regulation. Here, we found that the variable N-terminal domain of NtCDPK1 is necessary for the recognition of RSG. A mutation (R10A) in the variable N-terminal domain of NtCDPK1 reduced both RSG-binding and RSG phosphorylation while leaving kinase activity intact. Furthermore, R10A mutation suppressed in vivo function of NtCDPK1. The substitution of the variable N-terminal domain of an *Arabidopsis* CDPK, AtCPK9, with that of NtCDPK1 conferred RSG kinase activities. This chimeric CDPK behaved according to the identity of the variable N-terminal domain in transgenic plants. Our results open the possibility of engineering the substrate specificity of CDPK by manipulation of the variable N-terminal domain, enabling a rational rewiring of cellular signaling pathways.

12036

IDENTIFICATION OF A BAK1-BINDING PROTEIN, BAP228, AND INVESTIGATION OF ITS BRASSINOSTEROID SIGNALING-RELATED GENE EXPRESSION PATTERN

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Brassinosteroid(BR) signal transduction is initiated by the binding of the hormone to the surface receptor BRI1 (BRASSINOSTEROID INSENSITIVE 1). BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1) is a BRI1-binding transmembrane protein cross-activating BR-bound BRI1 protein, thus relaying membrane-initiated brassinosteroid signaling to BR-responding gene expression. To get better understanding how BAK1 is involved in BR signaling, we screened BAK1 binding proteins by performing yeast two-hybrid assay using BAK1 kinase domain as a bait, and identified BAP228 as a LRR(Leucine-rich receptor protein)-typed transmembrane protein. BAP228 binds preferentially to the phosphorylated form of BAK1 than to the non-phosphorylated one determined by yeast tri-hybrid test. Semi-quantitative RT-PCR revealed that *BAP228* expression was high in the vascular tissue of the shoot, root, and flower bud. This result is further evidenced by the fact that GFP signal was highly detected in vascular tissues of the shoot, root, flower bud, and the stamen (pollen) of *pBAP228::GFP* transgenic lines. One micromole of epi-BR treatment to the wild-type plant (Col-0) increased the *BAP228* expression. Interestingly, *BAP228* gene expression was higher in the *bak1-3* mutant than that in the wild-type. This implies that the shortage of BAK1-3 protein might be compensated by the increased *BAP228* gene expression to maintain the normal BR signaling activity by its homeostatic feedback regulation. In conclusion, the BAK1 binding protein, BAP228, is expressed high in the vascular tissue and stamen, and BR signal transduction might be mediated by the interaction between BAP228 and BAK1.

12037

THE EFFECT OF ATRANBP1C, A RAN (RAS-RELATED NUCLEAR G-PROTEIN)-BINDING PROTEIN, AND CALCIUM ON ROOT DEVELOPMENT IN ARABIDOPSIS

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Auxin is one of plant hormones, which has pivotal roles on plant development and growth. AtRanBP1c is one of the binding proteins to Ran/TC4 (Ras-related nuclear small GTP-binding protein. The transgenic *Arabidopsis* expressing antisense strand of *AtRanBP1c* showed 1.5~ 2 times more elongation of the primary root than that of wild-type and had less number of lateral roots. The same transgenic line also showed hypersensitivity to auxin. Based on these results, we have proposed that AtRanBP1c might regulate the nuclear transport of negative regulators of auxin action. To understand the effect of AtRanBP1c-mediated regulation of root growth, we crossed the anti-AtRanBP1c transgenic line with *DR5::GUS* or *pPIN1::PIN1-GFP* expressing transgenic plants to examine the auxin distribution in the root and its transport activity mediated by PIN1 protein. Interestingly, the size of the auxin maximum in the root tip was reduced in the *AtRanBP1c X DR5::GUS* plants and the GFP signal in the *anti-AtRanBP1c X pPIN1::PIN1-GFP* was also significantly disappeared from the vascular tissues compared to the *DR5::GUS* transgenic plant or *pPIN1::PIN1-GFP* transgenic plant control lines, respectively. We investigated the effect of calcium in auxin transport directing to the root tip. Treatments of calcium antagonists decreased the size of the auxin maximum. This phenomenon might be explained by the fact that the expression of *pPIN1::PIN1-GFP* in the roots was reduced by the treatment of calcium antagonist. In addition to their effects on maintenance of auxin maximum, treatments of calcium antagonists frequently resulted in abnormal division of the quiescent center cells and differentiation of the middle cortex. We concluded that both Ran/AtRanBP1c- and calcium-involved signal transduction regulate PIN1 expression, and thus control the auxin-regulated root cell type

determination and maintenance.

12038

A PDR-TYPE ABC TRANSPORTER MEDIATES CELLULAR UPTAKE OF THE PHYTOHORMONE ABSCISIC ACID

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Abscisic acid (ABA) is a ubiquitous phytohormone involved in many developmental processes and stress responses of plants. While ABA moves within the plant, and intracellular receptors for ABA have been identified recently, no ABA transporter has been described to date. Here, we report identification of the ABC transporter AtPDR12/ABCG40 as a plasma membrane ABA uptake transporter. Uptake of ABA into yeast and BY2 cells expressing AtABCG40 was increased, while ABA uptake into protoplasts of atabcg40 plants was decreased, as compared to control cells. In response to exogenous ABA, the up-regulation of ABA responsive genes was strongly delayed in atabcg40 plants, indicating that ABCG40 is necessary for timely responses to ABA. Stomata of loss-of-function atabcg40 mutants closed more slowly in response to ABA, resulting in reduced drought tolerance. Our results integrate ABA-dependent signaling and transport processes and open a new avenue for the engineering of drought tolerant plants.

12039

CONSTITUTIVE EXPRESSION OF ATMYB44 SUPPRESSES JASMONATE-RESPONSIVE GENE ACTIVATION

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AtMYB44 belongs to the R2R3 MYB transcription factor subgroup 22 family in *Arabidopsis thaliana*. AtMYB44 gene is rapidly induced by various phytohormones (abscisic acid, jasmonic acid, salicylic acid, ethylene, etc.) and abiotic stresses (dehydration, low temperature, and salinity). Strong expression was observed in root and in guard cell of the leaf epidermis. When compared to wild-type plants, 35S:AtMYB44 plant showed decreased accumulation of anthocyanin. And the level of chlorophyll accumulation was elevated in 35S:AtMYB44 plant than wild-type. Northern blot analysis revealed that expression of well known jasmonate-responsive genes, including *JR2*, *VSP*, *LOXII* and *AOS*, was suppressed in 35S:AtMYB44 plant under methyl jasmonate (MeJA) treatment. Previously we reported that AtMYB44 plays a role in ABA signaling pathway. Expression of the genes encoding a group of serine/threonine protein phosphatases (PP2Cs) which have been described as negative regulators of ABA signaling was reduced in salt treated 35S:AtMYB44 plants. These data suggest that AtMYB44 regulate JA and ABA inducible stress gene expression and support the hypothesis that jasmonate and ABA antagonistically regulated the expression of stress inducible gene in plants.

12040

FUNCTIONAL ANALYSIS OF THE PROMOTER FROM TOMATO *LEACS5* IN TRANSGENIC ARABIDOPSIS

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Plant hormone ethylene regulates many aspects of plant growth and development. The rate-limiting enzyme in ethylene biosynthesis, 1-amino cyclopropane-1-carboxylic acid (ACC) synthase, is encoded by a multi-gene family whose members are differentially regulated by different developmental, hormonal and environmental conditions. In the past 20 years, there has been nine ACC synthase isogenes reported in tomato.

In this study, an auxin-induced ACS gene in tomato, *LE-ACS5* was investigated. Expression analysis demonstrated that the expression of this gene was in general very low, however its expression can be detected by RT-PCR in young shoot, shoot apex and root where auxin levels are usually higher. In the shoot apex, the transcription of this gene was induced by IAA but inhibited by abscisic acid (ABA) and the suppression of *LE-ACS5* by ABA was in a greater extent than that of other *LE-ACSS*.

To further elucidate the response of this gene to exogenous auxin and ABA in transcriptional level, the expression of a series of *LE-ACS5* promoter (-1117 to +1):: β -glucuronidase (*GUS*) fusion genes were examined in transgenic Arabidopsis. According to PLACE database prediction, its promoter sequence contains putative cis-elements required for auxin and ABA responses. In the seedlings of transgenic Arabidopsis, the cloned promoter was sufficient to modulate GUS in response to exogenous auxin and ABA. Auxin but not ABA increased GUS activity in seedlings. Based on the results, we conclude that regulation of *LE-ACS5* in response to exogenous auxin and ABA is mainly under the transcriptional control of the promoter region of the gene.

This study has revealed some aspects of hormonal interactions, i.e. auxin and ABA on ethylene biosynthesis by regulating the transcription of *LE-ACS5* in seedlings; and enabled us to establish some insights on the nature of auxin-induced ethylene in relation to plant physiology.

12041

A PROMOTER REGION NECESSARY FOR THE TISSUE SPECIFIC AND JASMONATE RESPONSIVE GENE EXPRESSION OF *BRASSICA NTR1* GENE ENCODING A JASMONIC ACID METHYLTRANSFERASE

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The *BcNTR1* gene encodes an S-adenosyl-L-methionine:jasmonic acid methyltransferase (JMT), and is specifically expressed in the floral nectaries of *Brassica campestris* L. ssp. *pekinensis*. We have investigated the regulatory mechanism for the activation of *BcNTR1* gene during floral development. Series of 5'-deletion and recombination constructs of *BcNTR1* promoter were prepared and fused in translational frame to the β -glucuronidase (*GUS*) reporter gene. Histochemical GUS staining and Northern blot analysis revealed that the promoter region spanning about -3.8 kb to -3.1 kb from the translation initiation site is essential for the significant jasmonate responsive and tissue specific gene expression. Comparing with the *Arabidopsis AtJMT* promoter, we found a region having highly homologous sequences each other. Then, we performed yeast-one-hybrid screening using these sequences as a bait to search regulators for the promoter activation. We screened three candidates and confirmed the binding activity in yeast of each protein expressed from full length cDNA clone on the promoter region. We currently conduct more detailed experiment to identify the transcription elements important for

the regulation of *JMT* activity during the floral development.

12042

THE ARABIDOPSIS WOUND-RESPONSIVE PROTEIN *WRP1* REPRESSES GENE EXPRESSION OF *ATJMT* AND SYSTEMIC WOUND SIGNALING VIA INTERACTION WITH SPECIFIC ATJMT PROMOTER ELEMENT

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Previously, we define jasmonate-responsive promoter element in *Brassica NTR1* gene, encoding S-adenosyl-L-methionine:jasmonic acid methyltransferase (JMT). We isolated DNA-binding protein (*WRP1*:*Wound Responsive Protein 1*), using the *BcNTR1* JRE (*JA Responsive Element*) as bait in a yeast one hybrid screening with Arabidopsis cDNA library. *WRP1* was induced by methyl-jasmonate and wound treatments. Through the yeast one hybrid method and EMSA assay, we found that *WRP1* is binding to specific element of *BcNTR1* and *AtJMT* promoter. *AtJMT* gene expression level was altered in *WRP1* overexpressed and inducible transgenic plants. Also systemic wound signaling was changed in *WRP1* knock-out and overexpressed plants compared with wild-type plant. Therfore, we suggest that *WRP1* regulates *AtJMT* gene expression in wound response and it play a important role in systemic wound signaling mechanism.

12043

ANALYSIS OF TRANSCRIPTION FACTORS INVOLVED IN JASMONIC ACID SIGNALING PATHWAY

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Jasmonic acid (JA) is a plant hormone related to biotic and abiotic stress responses. COI1, JAZs and MYC2 transcription factor have been identified to be regulators of JA signal transduction pathway. COI1 is an F-box protein and is thought to be a JA receptor. JAZ proteins are negative regulators of JA signaling. The *coi1* loss-of-function mutants and *jaz* gain-of-function mutants exhibit JA-insensitive phenotypes including male-sterility, while *myc2* loss-of-function mutants are not male-sterile. This indicates that MYC2 would be responsible a part of JA responses but not all, and that there should be other transcription factors that could regulate JA signaling including the regulation of fertility. To identify such transcription factors, we applied our chimeric repressor gene silencing technology (CRES-T), in which a transcription factor that is converted into strong repressor by fusion with EAR-motif repression domain (SRDX), induce dominant negative phenotype similar to loss-of-function of the alleles of the gene for transcription factor by suppressing target genes dominantly. We screened CRES-T lines with impaired JA-responsiveness in root growth and found that one of CRES-T lines, HR0729, exhibited reduced fertility in addition to JA-insensitive root growth. Reduced fertility of HR0729 plants was due to delay of anther dehiscence as observed in other JA-insensitive and -biosynthetic mutants. Gene for HR0729 was expressed in cotyledon, roots, and flowers and was induced by wounding and JA-treatment. In addition, accumulation of anthocyanin was suppressed and the expression of several anthocyanin biosynthetic genes was down-regulated in HR0729 CRES-T plants even in the presence of JA. These results suggest that HR0729 protein acts as regulator of JA signaling pathway as MYC2 does but is responsible for JA-mediated male fertility.

12044

GENETIC INTERACTION BETWEEN *AXR1* AND *SMAP1* THAT MEDIATE 2,4-D RESPONSE IN ARABIDOPSIS

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The *Small Acidic Protein 1 (SMAP1)* gene is responsible for the anti-auxin resistant mutation, *aar1*, and encodes a factor that mediates the synthetic auxin 2,4-D response. The previous results suggested that SMAP1 functions upstream of the AUX/IAA degradation step in auxin signaling pathway. To gain an insight into the function of *SMAP1*, the *aar1* mutant was crossed with known auxin-related mutants. The double mutants, *aar1-1 tir1-1*, *aar1-1 ecr1-1*, and *aar1-1 aar3-2* showed more resistant to 2,4-D than the corresponding single mutants in root growth assay. On the other hand, the *axr1 aar1* double mutants showed severe morphological defects with a lack of root meristem formation. Similar phenotype was observed in the offspring generated by crossing *axr1-12* and the *SMAP1* RNAi transgenic lines. The overexpression of SMAP1-GFP fusion gene under 35S promoter relieved pleiotropic morphological phenotypes of *axr1-12* such as dwarf, multiple shoots, altered flower structure, low fertility and reduced auxin response. The results suggested that the function of *SMAP1* is tightly linked to that of *AXR1* in genetic level. The interaction of SMAP1 to COP9 signalosome and RUB modification system will also be discussed.

12045

SMAP1 IS A POSITIVE REGULATOR FOR 2,4-DICHLOROPHENOXYACETIC ACID MEDIATED ACTIN DEGRADATION AND ACTS INDEPENDENT OF KNOWN AUXIN SIGNALING PATHWAY

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2,4-dichlorophenoxyacetic acid (2,4-D), a chemical analogue of plant hormone auxin, Indole-3-acetic acid (IAA), is widely used as a growth regulator and exogenous source of auxin. Traditionally, it is believed that IAA and 2,4-D share a common signaling pathway. However, recent studies have challenged this idea both at physiological and genetic level. The isolation of a 2,4-D specific mutant *aar1* and identification of the protein SMAP1, which confers specific resistance to 2,4-D, provide evidence that IAA and 2,4-D use partially distinct molecular pathways. Similar to upstream events, 2,4-D and IAA also control the downstream physiological responses differentially. 2,4-D but not IAA, degrades the root actin cell cytoskeleton and inhibits the cell division. To provide a molecular explanation of 2,4-D specific action in Arabidopsis, we characterized the functionally unknown protein SMAP1. The molecular and cell biological analyses revealed that, 1) SMAP1 acts as a positive regulator for 2,4-D induced degradation of actin, cell division and cell elongation processes as the loss of SMAP1 nullify the effect of 2,4-D on all these processes, and 2) SMAP1 acts independent of known ubiquitin-proteasome mediated auxin signaling pathway, as the double mutant of SMAP1 and TIR1, a component of E3 ubiquitin ligases (*aar1-tir1*), in root growth assay, shows complete insensitivity to 2,4-D at the concentration that inhibits eighty percent root growth in respective single mutants. Similarly, the loss of SMAP1 in *tir1* background makes the root actin resistant to 2,4-D mediated degradation. Our results, for the first time, identify a novel 2,4-D specific biologically significant pathway in plants, and also provide a molecular explanation of the difference between IAA and 2,4-D.

12046

BIP1, A BZR1-INTERACTING PROTEIN IN THE BRASSINOSTEROID SIGNAL TRANSDUCTION PATHWAY, NEGATIVELY REGULATES PLANT GROWTH IN ARABIDOPSIS

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Brassinosteroids (BRs) are steroidal plant hormones that control a wide range of plant growth and developmental processes, including cell elongation, vascular development, senescence, flowering, seed germination and stress responses. To understand the molecular mechanisms that BRs regulate plant growth and development, previous molecular genetic studies have depicted a BR signaling pathway in the model plant, Arabidopsis. BRs signal through the BR receptor BRI1, protein kinase BSK1, phosphatase BSU1, and a GSK3-like kinase BIN2, to regulate the BZR1/BES1 family of transcription factors, which directly bind to target gene promoters to activate or repress gene expression and mediate BR-regulated growth responses. However, it remains unclear how such a linear signaling pathway mediated by two transcription factors regulates such diverse developmental processes. To explore whether there are other transcription regulators involved in BR signaling, we performed a BZR1 yeast two-hybrid screen and identified a BZR1-interacting protein, BIP1. BZR1 and BIP1 interact in vivo and in vitro. Similar to BZR1, BIP1 localizes in nucleus and cytoplasm and its accumulation is BR-inducible. However, while BZR1 enhances plant growth and cell elongation, BIP1 inhibits these processes when it is overexpressed. The inhibitory effect of BIP1 seems due to its inhibition to BZR1 activity because BIP1 overexpression suppresses BZR1's hypersensitivity to BR and insensitivity to Brassinazole, the BR biosynthetic inhibitor. These results suggest that BIP1 is a negative regulator of BR signaling and plant growth.

12047

PLANT PHOSPHATIDYLCHOLINE-HYDROLYZING PHOSPHOLIPASES C NPC3 AND NPC4 WITH ROLE IN BRASSINOLIDE SIGNALING

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Phosphatidylcholine-hydrolyzing phospholipase C (PC-PLC) catalyzes the hydrolysis of phosphatidylcholine (PC) to generate phosphocholine and diacylglycerol (DAG). PC-PLC has a long tradition in animal signal transduction to generate DAG as a second messenger besides the classical phosphatidylinositol-splitting phospholipase C (PI-PLC). Based on amino acid sequence similarity to bacterial PC-PLC, six putative PC-PLC genes (*NPC1* to *NPC6*) were identified in the *Arabidopsis* genome. RT-PCR analysis revealed overlapping expression pattern of NPC genes in root, stem, leaf, flower and siliques. In brassinolide-treated (BL) *P_{NPC3}:GUS* and *P_{NPC4}:GUS* seedlings, increase of GUS activity was visible in leaves and shoots. Compared to wild-type, T-DNA insertional knockouts *npc3* and *npc4* showed shorter primary roots and lower lateral root density at low BL concentrations but increased lateral root densities in response to exogenous 0.05–1.0 µM BL. BL-induced expression of *TCH4* and *LRX2*, which are involved in cell expansion, was impaired but not impaired in repression of *CPDCPD*, a BL biosynthesis gene, in BL-treated *npc3* and *npc4*. These observations suggest *NPC3* and *NPC4* are important in BL-mediated signaling in root growth. When treated with 0.1 µM BL, DAG accumulation was observed in tobacco BY-2 cell cultures labeled with fluorescent PC as early as 15 min after application. We hypothesize that at least one PC-PLC is a plant signaling enzyme in BL signal transduction.

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12048

RELATIONSHIPS BETWEEN SHOOT BRANCHING INHIBITION AND STRIGOLACTONE PRODUCTION UNDER PHOSPHORUS DEFICIENCY

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Strigolactones (SLs) are communication signals with root parasitic plants such as *Striga* and *Orobanche* species and symbiotic arbuscular mycorrhizal fungi in the rhizosphere. In the host plants, SLs or downstream metabolites are plant hormones for shoot branching inhibition. Previous studies showed that SL production was elevated by phosphate ion (Pi) starvation. To explore the relationships between SLs production and shoot branching inhibition under phosphorus deficiency, we investigated SL levels and the number of outgrowing tiller buds in rice seedlings grown in hydroponic culture media at varying Pi concentration. We found that tiller bud outgrowth in wild type was inhibited under Pi deficiency, which was correlated with a high SL production. However, the suppression of tillering in response to Pi deficiency was not observed in SL-deficient and -insensitive mutants. The sensitivity for SL perception did not significantly change between +Pi and -Pi condition. After transferring wild type seedling from -Pi to +Pi media, tiller bud outgrowth occurred and the expression of SL-biosynthesis genes, *D10*, *D17*, *D27* and some *OsMAX1-Likes*, was drastically reduced. In contrast, *D3* mRNA levels did not change and *D14* mRNA levels increased only slightly. These results indicate that the regulation of tiller bud outgrowth in rice depends on endogenous SL levels rather than SL responsiveness.

12049

BRASSINOSTEROIDS CONTROL ATEXPA5 GENE EXPRESSION IN ARABIDOPSIS THALIANA

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To elucidate the spatial and temporal roles of *EXPANSIN A5* (*AtEXPA5*) in growth and development of *Arabidopsis thaliana*, phenotypic alterations in loss-of-function mutants were observed. Seedlings of the null mutant, *expA5-1* harboring a T-DNA insertion in the second intron of the *AtEXPA5* gene, had shorter roots and hypocotyls than those of wild-type plants under both light and dark conditions. Compared to wild-type plants, the mutants had smaller rosette leaves. *AtEXPA5* was predominantly expressed in the shoots but little expression was detected in the roots of seedlings. In adult plants, strong expression of *AtEXPA5* was observed in aerial parts of *Arabidopsis thaliana*, especially in the inflorescence stems and flowers. Expression of *AtEXPA5* was enhanced by exogenously applied brassinosteroids. *AtEXPA5* expression was reduced in a brassinosteroid-deficient mutant (*det2*) and a signaling mutant (*bri1-301*), while it was increased in *bzr1-1D*, a dominant mutant of a brassinosteroid transcription factor. A double mutant, *bzr1-1DXexpA5-1*, showed reduced growth compared to the *bzr1-1D* mutant. In addition, the brassinazole resistance of *bzr1-1D* was impaired by *expA5-1* mutation. However, no direct binding of BZR1 protein to *AtEXPA5* promoter was detected. These findings indicate that *AtEXPA5* is a growth-regulating gene whose expression

is controlled by brassinosteroid signaling downstream of BZR1 in *Arabidopsis thaliana*.

12050

GLIP1 IS ASSOCIATED WITH ETHYLENE SIGNALING TO REGULATE THE IMMUNE RESPONSE IN ARABIDOPSIS

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Arabidopsis GDSL LIPASE-LIKE 1 (GLIP1) was previously isolated as a secreted protein in our proteomic analysis. A series of functional analysis demonstrate that GLIP1 plays important roles in pathogen resistance, eliciting both local and systemic resistance in plants. *GLIP1*-overexpressing transgenic *Arabidopsis* (*35S:GLIP1*) exhibited enhanced resistance against necrotrophic pathogens including *A. brassicicola* and *Erwinia carotovora*, and hemibiotrophic pathogen *Pseudomonas syringae*. Application of GLIP1 proteins or petiole exudates from *35S:GLIP1* plants, but not from *glip1*, induced *PDF1.2* and *GLIP1* expression in distal leaves, which was accompanied by the activation of systemic resistance against multiple pathogens. These results imply that GLIP1 may mediate the production of a systemic signaling molecule(s). In addition, GLIP1-elicited systemic resistance was impaired in *etr1-1* plants, but not in *NahG* and *jar1-1*, indicating that the ET response is necessary for GLIP1 function. *35S:GLIP1* plants displayed enhanced triple response compared to wild type, when treated with 1-aminocyclopropane-carboxylic acid, ethephon and ethylene gas. Our results suggest that GLIP1 function in pathogen resistance requires ethylene signaling.

12051

ETHYLENE REGULATES ATEXPA5 GENE EXPRESSION IN ARABIDOPSIS THALIANA

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To investigate physiological functions of *EXPANSIN A5* (*AtEXPA5*) in *Arabidopsis thaliana*, tissue specific GUS activities in *pAtEXPA5-GUS* transgenic plants were examined. In seedlings, *pAtEXPA5-GUS* expression was observed in shoot apex, margin of younger true leaf and petioles. Adult plants showed *pAtEXPA5-GUS* expression in receptacle and nodes. These expression patterns were affected by exogenously applied plant hormones, especially by ethylene. In semi-quantitative RT-PCR results, *AtEXPA5* expression level was decreased by exogenously applied ethylene in concentration-dependent manners at early phase. Up-regulation of *AtEXPA5* gene expression by ethylene biosynthesis inhibitors and RT-PCR results using ethylene insensitive mutants and constitutive mutants indicated that not only ethylene biosynthesis but also signaling are involved in *AtEXPA5* regulation. Hypocotyl length of *AtEXPA5* transgenic plants was reduced by ethylene, suggesting that *AtEXPA5* is involved in hypocotyl elongation which is regulated by ethylene in *Arabidopsis thaliana*.

12052

TGG1 AND TGG2 REDUNDANTLY FUNCTION IN ABA AND MEJA SIGNALING IN ARABIDOPSIS GUARD CELLS

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Guard cells are response to various environment stimuli to lead stomatal opening or closure along with productions of second messengers and activation or inactivation of several kinds of ion channels. However, the ABA and MeJA signal cascades in guard cells are not clarified. Recently, it has been shown that thioglucoside glucohydrolase 1 (TGG1) is abundant in guard cells and is involved in inhibition of light-induced stomatal opening. TGG is an enzyme in glucosinolate-myrosinase system to hydrolyze glucosinolates to toxic compounds as thiocyanate, isothiocyanate, and nitrile, which are related to plant defense against insect, fungi and bacteria. Six TGG genes are identified on *Arabidopsis* genome and TGG1 and TGG2 redundantly function in defense to insect herbivory. In this study, we analyzed *tgg1-3* and *tgg2-1* single mutants and *tgg1-3 tgg2-1* double mutants to clarify whether myrosinase function in ABA and MeJA signaling during ABA- and MeJA-induced stomatal closure. Compare to wild-type, myrosinase activity slightly decreased in *tgg1-3* single mutants and significantly decreased in *tgg1-3 tgg2-1* double mutants. ABA, MeJA, and H_2O_2 -induced stomatal closure were observed in wild-type, *tgg1-3* and *tgg2-1*, but failed to induce stomatal closure in *tgg1-3 tgg2-1*. Unlike ABA, MeJA and H_2O_2 , Ca^{2+} induced stomatal closure in all mutants and wild-type. In addition, ABA induced ROS productions in all mutants and wild-type. Interestingly, cytosolic alkalization in guard cells was induced by ABA in all single mutants like wild-type but less induced in *tgg1-3 tgg2-1* compared with wild-type. These results suggest that TGG1 and TGG2 redundantly function downstream of ROS production and upstream of cytosolic Ca^{2+} elevation in ABA and MeJA signaling in guard cells.

12053

THE ROLE OF ARABIDOPSIS CALCIUM-DEPENDENT PROTEIN KINASE, CPK6, IN GUARD CELL METHYL JASMONATE SIGNALING

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Methyl jasmonate (MeJA) induces stomatal closure dependent on change of cytosolic Ca^{2+} concentration in guard cells. However, details of this molecular mechanism remain unclear. Calcium-dependent protein kinases (CDPKs) function as Ca^{2+} signal transducers in various plant physiological processes. It was suggested that *Arabidopsis* four CDPKs, *CPK3*, *CPK6*, *CPK4*, and *CPK11* function as positive regulators in abscisic acid signaling in guard cells. Here, we examined roles of these CDPKs in MeJA signaling in guard cells using each CDPK knockout mutant. In *CPK6* gene disruption mutants, MeJA-induced stomatal closure was impaired whereas in *CPK3*, *CPK4*, and *CPK11* gene disruption mutants, MeJA-induced stomatal closure were not altered. We also evaluated roles of *CPK6* in MeJA regulation of second messenger production and ion channel activity in guard cells. MeJA induced ROS and NO production in *cpk6* mutant guard cells but failed to activate I Ca channels and S-type anion channels. Our results provide genetic evidence that *CPK6* functions as a positive regulator of MeJA signaling in *Arabidopsis* guard cells.

12054

ROLE OF GLUTATHIONE IN ABA-INDUCED STOMATAL CLOSURE IN ARABIDOPSIS THALIANA

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Abscisic acid (ABA) induces stomatal closure, which suppresses water loss, leading to the tolerance of plants to stress conditions. ABA signaling involves redox regulation including ROS production. Glutathione (GSH), one of the most abundant low molecular-weight thiol compounds maintains redox homeostasis under normal and stressful conditions in higher plants. Although it is reported that GSH is involved in many physiological responses and guard cells accumulate more GSH than other epidermal cells do, roles of GSH on ABA-induced stomatal closure in *Arabidopsis thaliana* remain to be elucidated. In this study, we investigated effects of GSH on ABA-induced stomatal closure. ABA induced stomatal closure along with decreasing GSH contents but dark condition induced stomatal closure without changing GSH contents. In order to clarify whether GSH is involved in the redox regulation of ABA-induced stomatal closure, we employed *Arabidopsis thaliana*, two low GSH mutants, *cad2-1*, which is deficient in the key GSH biosynthesis enzyme, γ -glutamylcysteine synthetase (GCS) and *ch1-1*, which is defective in a light-harvesting antenna in photosystem II and a GSH-decreasing chemical, 1-chloro-2,4-dinitrobenzene (CDNB). The *cad2-1* or *ch1-1* mutation, or depletion of GSH by CDNB enhanced stomatal sensitivity to ABA. Meanwhile, in *cad2-1* and *ch1-1* plants, GSH monoethyl ester (GSHmee) increased GSH level and lowered stomatal sensitivity to ABA. Neither *cad2-1* nor *ch1-1* mutation significantly enhanced ABA-induced ROS production in guard cells. In addition, GSH did not affect activation of I_{Ca} currents by ABA via ROS production during ABA-induced stomatal closure. Taken together, these results suggest that GSH could modulate some signaling factors downstream of activation of I_{Ca} currents in ABA signaling to control ABA sensitivity in guard cells.

12055

ANTAGONISTIC CROSSTALK BETWEEN PLANT HORMONE-RELATED SIGNALING PATHWAYS IN ARABIDOPSIS THALIANA

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Plants are exposed to various stresses, such as pathogen attacks and environmental stress. To survive such stressful environment, plants have evolved unique self-protection systems. In plants, salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA) contribute to responses against biotic and abiotic stresses by influencing the complex plant hormone-networks. Recent studies have shown the mutually antagonistic interactions among plant hormone-mediated signaling pathways. For instance, treatment with ABA suppresses the induction of systemic acquired resistance (SAR) by inhibiting the pathway both upstream and downstream of SA, independently of the JA/ethylene-mediated signaling pathway. Conversely, the activation of SAR suppresses the expression of ABA biosynthesis-related and ABA-responsive genes. These results show that antagonistic crosstalk occurs at multiple steps between the SA-mediated signaling of SAR induction and the ABA-mediated signaling of environmental stress responses. Here, we will discuss the complex plant hormone-networks by analyzing plant hormone mediated-gene expression in SA-, JA-, ABA-treated leaf tissues of *A. thaliana*. We suggest that balance change of plant hormone levels is important for regulation of stress responses in leaf tissues.

12056

CAMALEXIN BIOSYNTHESIS BY A GH3 AMIDO SYNTHASE THROUGH INDOLE-3-ACYL-CYSTEINATE

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Camalexin (3-thizole-2'-yl-indole) is the only phytoalexin discovered in *Arabidopsis*, functioning in defending against invasion of fungal pathogens, which consists of an indole ring and a thizole ring. It has been confirmed that the indole ring derives from tryptophan and its derivatives, and the sulphur atom of the thizole ring was thought from cysteine. However, how the cysteine is catalyzed into the thizole ring remains exclusive. On the other hand, camalexin is generated through the IAN pathway, however, there is still ~20% of total camalexin levels in the IAN pathway knockout mutants, indicative of an additional pathway involved in the biosynthesis of camalexin. We found that the level of camalexin was significantly increased (1.5-2 fold) in the GH3.5 activation-tagged mutant gh3.5-1D than the wild-type after infection by pathogen, which encodes an amido synthase. The GH3.5 protein could catalyze the conjugation of indole-3-carboxylic acid and cysteine to form indole-3-acyl-cysteinate in vitro. Inoculation with Psm(avrRpm1), followed by spraying with indole-3-carboxylic acid and cysteine could significantly increase the levels of camalexin. By extensive genetic study, we documented that GH3.5 functions upstream of PAD3 in camalexin biosynthesis, through generating indole-3-acyl-cysteinate, a potential unrecognized precursor of camalexin. Our current study showed that GH3.5 catalyzes the formation of the thizole ring, and fills in the gap of the key step in camalexin biosynthesis.

12057

REDOX REGULATION OF NPR1 REQUIRES NOC PROTEIN IN PLANT IMMUNITY

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The immune coactivator NPR1 is a master regulator of downstream gene expression during salicylic acid (SA)-mediated defense response. In unchallenged cells, NPR1 is present in the cytoplasm as an oligomer through disulfide bonds. Upon pathogen attack, however, SA-mediated redox changes reduce the NPR1 oligomer to a monomer, allowing it to translocate to the nucleus and activate target gene expression. Thus, oxidative thiol modifications play an important role in modulating activity of NPR1 protein. Here we identified a crucial member of NPR1 oligomer complex by coimmunoprecipitation and mass spectrometry analysis. The NOC protein (NPR1 oligomer component) forms a covalent protein complex with NPR1 through disulfide bonds, and its formation is facilitated by nitric oxide in vitro. The mutations in NOC result in the enhanced disease susceptibility. These results strongly suggest that NOC is responsible for the redox-based regulation of NPR1, and thus coordinately translating cellular redox information into gene expression.

13001

HEXOSES AND OSMOTIC PRESSURE MODULATE THE PLANT CELL WALL STRESS RESPONSE IN ARABIDOPSIS THALIANA

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Plant cell walls are complex and highly dynamic structures, responding and adapting to normal processes of growth and development as well as to biotic and abiotic stresses. Maintaining the functional integrity of plant cell walls during different biological processes is crucial and therefore a mechanism that monitors and maintains wall integrity must exist. In order to determine the effects of cell wall stress on *Arabidopsis thaliana* seedlings a time course experiment containing 8 time points was performed using isoxaben, a cellulose biosynthesis inhibitor. Cellulose biosynthesis inhibition (CBI) caused transcriptional and phenotypic changes, that were dependent on the supply of sugar (both metabolizable or non-metabolizable) in the growth medium and osmotic support, suggesting a role for sugar and osmo-signalling in the cell wall stress response (Hamann et al., 2009). Interestingly, transcriptional down regulation of genes involved in photosynthesis and starch metabolism was detected after 8h of CBI, suggesting a regulatory mechanism coordinating cellulose biosynthesis with photosynthetic activity and primary metabolism. Drug treated seedlings accumulated significantly less soluble sugars, while the starch content was increased (compared to wildtype). We could show that supply of polyethylene glycol (PEG) repressed the metabolic and transcriptional effects of isoxaben treatment in a concentration dependent manner, suggesting turgor pressure as a critical signal connecting cell wall stress with sugar metabolism. The well-known sugar and osmo-signalling mutants *gin2_1*, *gin1_1*, *kin10*, *kin11*, *athk1* and *cre1* are currently being analysed regarding their role during CBI and the results will be presented at the conference. Our analysis shows that cell wall stress affects plant sugar metabolism and implicates turgor pressure and sugars as potential signals in cell wall stress perception.

13002

IDENTIFICATION OF NEW GENES INVOLVED IN CELL WALL EXPANSION USING ACTIVATION TAG SCREENING

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Cell wall expansion, in which the cell wall must increase in surface area while retaining its ability to withstand turgor pressure, is essential for plant growth and development. The expected early lethality of null mutations in genes required for cell wall expansion and the potential for redundancy to mask the phenotypes of mutations in gene family members make conventional screening for mutants in this process difficult. To circumvent these issues, we performed a screen to identify genes whose overexpression causes enhanced cell expansion. As an experimental system we chose etiolated *Arabidopsis* seedlings, in which hypocotyl elongation is driven primarily by cell expansion. From a population of ~30,000 lines transformed with an activation tag construct containing four tandem CaMV 35S enhancers, we identified 39 putative single insertion lines with a heritable elongated hypocotyl phenotype. Identification of the insertion sites for these lines has allowed us to generate a list of candidate genes with potential functions in cell wall expansion. Characterization of these genes, many of which are of unknown function, should enhance our understanding of the molecular events of cell wall expansion and has the potential to inform efforts to more efficiently use plant cell walls for the production of biofuels and renewable materials.

13003

ATCSLD3 LOCALIZES TO A TIP-RESTRICTED PLASMA MEMBRANE DOMAIN IN GROWING ROOT HAIR CELLS.

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In root hairs, cell expansion is highly unidirectional and restricted to the growing apex. In diffuse growth, cell expansion is linked cellulose microfibril orientation and occurs along the entire face of one or more sides of the cell. However during tip-growth there is no obvious directionality to cellulose microfibril orientation at the growing apex. We were interested in the mechanisms controlling cellulose deposition in root hairs and whether mechanisms regulating cellulose synthesis were similar or distinct from cells undergoing diffuse growth. We compared the subcellular distributions of AtCESA cellulose synthases and the closely related AtCSLD3 cellulose-synthase-like protein in growing root hairs. A functional YFP-CSLD3 fusion that restored normal root hair growth in atcsld3 mutant plants was transformed into *A. thaliana*. EYFP-AtCSLD3 localized to a polarized plasma membrane domain in the root hair tip. However, EGFP-AtCESA3 displayed no significant plasma membrane localization in growing root hairs. While EYFP-AtCSLD3 displayed plasma membrane localization in growing root hairs, this fusion protein was restricted to Golgi-like organelles contained within the cytoplasm in non-hair forming root epidermal cells. EGFP-AtCESA3 appeared to associate with linear arrays within the plasma membrane in non-hair forming root epidermal cells, and therefore likely co-localizes with AtCESA6 along cortical microtubule arrays in these cells. However, cortical microtubule arrays are not typically observed in the tips of growing root hair cells where EYFP-AtCSLD3 appears to be plasma membrane localized. While tip-restricted expansion in root hairs is highly dependent on an intact F-actin cytoskeleton, depolymerization of microtubules does not affect polarized growth rates in these cells. This raises the highly intriguing possibility that plasma membrane CSLD proteins may represent a distinct cellulose synthase activity in the tips of growing root hair cells.

13004

TOWARDS THE PLANT GOLGI PROTEOME

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The plant cell wall is comprised of complex sugar polymers including cellulose, hemicellulose and pectin. The Golgi apparatus within the plant cell produces a large proportion of these polysaccharides prior to their incorporation into the cell wall. The details of how these complex sugars are synthesized and delivered are currently poorly understood. Our knowledge of the protein constituent that comprise the plant Golgi is relatively poor when compared to other subcellular components within the cell. In order to better understand the role of this organelle in cell wall biosynthesis we are characterizing this compartment using proteomics. We are employing an orthogonal approach which utilizes density centrifugation followed by charge based separation of the organelle on a Free Flow Electrophoresis system. Analysis of Golgi purified fractions from *Arabidopsis* cell culture by mass spectrometry after FFE separation indicates the method is suitable for isolation of this organelle from plants. We have identified 400 to 500 proteins from these fractions and identified over 50 glycosyl transferases (from multiple gene families) whose role are likely involved in matrix polysaccharide biosynthesis. These glycosyl transferases likely represent the core set of enzymes required for the biosynthesis of hemicellulose and pectin. Overall around half of the proteins identified are of known or likely Golgi in origin; while about a third are unknown or are derived from the endosomal system and 10 — 20% appear to be contaminants from other organelles and membrane systems. Fluorescent protein constructs are being designed to confirm localizations of novel and ambiguous proteins. This technique will enable us to commence in-depth comparative cell wall proteomics focusing on protein function and changes in the plant Golgi apparatus.

13005

FUNCTION OF PECTINS ON CELLULOSE MICROFIBRIL DEPOSITION REVEALED FROM COBTORIN TARGET ANALYSIS

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Cellulose and pectin are major components of primary cell walls in plant. It is thought that their mechanical properties are important for cell morphogenesis. It is a major hypothesis that cortical microtubules guide the cellulose microfibril synthase movement parallel to them, however, the mechanism how cellulose microfibrils correctly align is still unclear. Previously, we have identified a novel inhibitor, cobtorin, which perturbed parallel relationship between cortical microtubules and nascent cellulose microfibrils. In this study, we survey a target of cobtorin, and found that overexpression of pectin methylesterase and polygalacturonase rescued the cobtorin-induced cell swelling phenotype. Also, polygalacturonase treatment recovered the parallel deposition of cellulose microfibrils to cortical microtubules, and cobtorin perturbed the pectin distribution and demethylesterification. These data may suggest that control of pectin property is important for cellulose microfibril deposition and/or maintenance parallel to cortical microtubules.

13006

THE ANALYSIS OF ARABIDOPSIS KAONASHI4 GENE INVOLVED IN THE POLLEN EXINE FORMATION

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Exine, the outer structure of pollen walls, is believed to play an important role for successful pollination because of its species-specific architecture. However, the molecular mechanism of exine construction is still mysterious. The *kaonashi4* (*kns4*) mutant, identified by our previous screening with SEM observation, showed extremely thin exine layer, collapse of the whole structure of pollen grains, and low fertility. The reciprocal cross between wild type and *kns4* indicated that male gametophytes were responsible for the low fertility in *kns4*. The pollen viability and germination ability *in vitro* were not so influenced, but the number of pollen grains adhered to stigma was significantly reduced. These results suggested that low fertility in *kns4* was mainly due to a failure to be recognized by stigmas. TEM observation revealed that the exine abnormality begins at tetrad stage as an extremely thin primexine layer, a scaffold of exine structure between callose wall and microspore plasma membrane. Map-based cloning revealed that the *KNS4* gene encodes a protein which resembles animal β -1,3-galactosyltransferase (GalT) involved in complex *N*-glycan biosynthesis. In contrast to the fact that there are 20 genes homologous to the β -1,3-GalT gene in Arabidopsis genome, other enzymes of this pathway are encoded by a single or small number of genes, and knockout mutants of them did not show any exine abnormalities. These results strongly suggest that KNS4 does not work in the pathway of complex *N*-glycan biosynthesis. We also found that the *KNS4* is specifically expressed in the tapetal cells at around tetrad stage and the KNS4 protein is localized in the Golgi apparatus. It was predicted that the KNS4 functions in the Golgi apparatus of tapetal cell at tetrad stage and is involved in biosynthesis of primexine components.

13007

A NOVEL APPROACH TO DISSECT THE ABSCISSION PROCESS IN ARABIDOPSIS: SINGLE CELL ANALYSIS.

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Abscission in plants is a biological process that involves organ detachment from the main body of the plant; this phenomenon gives the organism the opportunity to shed diseased organs, prepare for cold winters, and facilitate seed dispersal.

It occurs at a specialised layer of cells, the abscission zone which undergoes a complex series of events. Classical experiments of abscission have been conducted by exposing explants to ethylene or IAA for defined periods of time.

The abscission zone is difficult to isolate and usually is contaminated with neighbouring cells, making the analysis of molecular changes associated with the process extremely challenging.

To resolve this problem a transgenic line generated by fusing the promoter of a polygalacturonase gene from Arabidopsis (*At2g41850*) to the reporter Green Fluorescent Protein, which is expressed in the floral abscission zones from Arabidopsis flowers (Gonzalez-Carranza, et al. 2002), has been used to tag, separating cells and facilitate their collection using a fluorescence-dissecting microscope. A cDNA library has been generated from these GFP-tagged cells and mRNA isolated from them. Genes from the zone have been identified, and a selection of promoters has been fused to the GUS and GFP reporters. Results will be presented and possible roles of some of these genes discussed.

Gonzalez-Carranza, et al (2002) Plant Phys. 128: 534-543.

13008

"FUNCTIONAL CHARACTERISATION OF FOUR GDSL-LIPASES"

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Several years ago a large family of lipases, with over a hundred members, was discovered that contained an enzymatic active site different to that of other lipases. Instead of the classical GxSxxG-motif in the center of the protein, GDSL-lipases display a highly conserved octapeptide (GxSxxxG) near their N-terminus (Brick et al., 1995; Upton and Buckley, 1995). Another common feature among the members of this protein family is their potential apoplastic localisation, because: all members of the GDSL-lipases described so far reveal either N-terminal leader sequences or signal peptides for apoplastic secretion. In addition, there is growing evidence that plant GDSL-lipases play a functional role in biotic (*AtGLIP1*, *CaGLIP1*) and abiotic (*AtLTL1*) stress responses (Oh et al., 2005; Narajo et al., 2006; Hwang et al., 2008) possibly through lipolytic activity in the cell wall.

Here, we used a molecular approach to characterise four less-characterised GDSL-lipases, CGM1-4 (contains GDSL-motif). Transcript and Promotor::GUS analysis of the CGMs revealed overlapping but distinct expression patterns in *Arabidopsis* that could underscore functional differences. As in GLIP1, we observed a higher susceptibility of *cgm1* loss-of-function (LOF) lines to the necrotrophic pathogen *Alternaria brassicicola*. In addition, these lines showed a weak hypersensitive response to exogenously applied brassinosteroids. The functional relationship of the apoplastic CGMs with the different signal response pathways, to which they contribute, will be discussed.

13009

NOVEL GENETIC ALLELES CODING FOR RESISTANCE TO FLUPOXAM SUGGEST ASSOCIATION WITH THE CELLULOSE SYNTHASES

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The Cellulose synthases (CESAs) are a group of inverting glycosyltransferases which function on the plasma membrane of all cellulose producing organisms, polymerizing the formation of cellulose microfibrils in a β -1-4 linked fashion. The details of this process in plants remains poorly understand. There are many questions pertaining to cellulose synthesis that remain to be elucidated, such as; Are there accessory proteins which assist in cellulose deposition? How are the CESAs regulated, being that they are only active on the membrane? How are these CESAs tied to the underlying microtubule network, and do they traverse with the assistance of "helper" proteins? The use of cellulose biosynthesis inhibitors in combination with genetics analysis provides a potentially useful way to address some of these questions. To this end, we have used an annual broad-leaf cellulose inhibiting herbicide, flupoxam, to identify 15 mutations conferring resistance to concentrations of flupoxam ranging from 100 nm to 10 μ m. Three of these mutations have been mapped to the C-terminal end of CESA3 (AT5G05170). The 12 remaining mutations, however, have yet to be mapped. It is possible that these mutations might identify cellulose synthase interacting proteins.

13010

ISOXABEN RESISTANCE CONFERRED BY NOVEL *iXR* ALLELES.

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Isoxaben is a pre-emergence broadleaf herbicide which inhibits the incorporation of glucose into cellulose in higher plants by an unknown mechanism. Mutations that confer high degrees of resistance to isoxaben (*iXr*) are of interest since they indirectly reveal information about the mechanics of cellulose biosynthesis and cellulose synthase subunits (CESA). Single amino acid substitutions in the C-terminal region of CESA3 and CESA6 have previously been implicated in isoxaben resistance in *Arabidopsis*. It is speculated that isoxaben specifically binds and prevents CESA3-CESA6 association in primary wall rosette assembly. This notion is supported by the dominant nature of the resistance phenotype. A selection of ethyl methyl sulfonate (EMS) generated mutants yielded six novel lines tolerant to high concentrations of isoxaben. Four contain point mutations localized to *AtCESA3* and *AtCESA6* and are novel alleles of *iXr1* and *iXr2* respectively. The two other mutants possess wild-type alleles of *AtCESA1,3* and *6* and are also resistant to high concentrations of the herbicide in a semidominant fashion. The characterization of these mutations offers the potential for further understanding isoxaben resistance and implicit information about cellulose biosynthesis.

13011

THE MAP KINASES, MPK4 AND MPK11, ARE REQUIRED FOR CYTOKINESIS IN ARABIDOPSIS THALIANA

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Cytokinesis is a critical step of cell division. Both in plants and animals, whereas MAP kinases suggested to regulate cytokinesis, involvement of MAP kinases in cytokinesis is controversial. Here, we show that MPK4 and MPK11 MAP kinase regulate plant cytokinesis in *Arabidopsis thaliana*. Our date demonstrate that kinase activity of MPK4 are highly active in organs containing dividing cells. MPK4 localizes to the equatorial plane at cytokinesis. The *mpk4* mutants contain cells with incomplete cell walls and multiple nuclei. We also identified that MPK11 regulates cytokinesis in absence of MPK4. While the *mpk11* mutants show no defects in cytokinesis, levels of *MPK11* transcripts increase in *mpk4*, the mutation of *mpk11* enhances cytokinesis defects of *mpk4*. Besides, the MPK4 is phosphorylated and activated by ANQ MAP kinase kinase in vitro, and the activity of MPK4 proteins are decreased in *anq-2* mutants in vivo, indicating that MPK4 functions downstream of ANQ.

13012

FUNCTIONS OF α -XYLOSIDASE IN XYLOGLUCAN OLIGOSACCHARIDE METABOLISM, CELL WALL LOOSENING, SEED GERMINATION AND FRUIT DEVELOPMENT

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Temperature is a primary environmental cue for seed germination of many weeds and vegetables. Germination inhibition of winter annual seeds by high temperature in summer enables the seeds to germinate and to establish seedling growth in autumn. To understand the mechanism of germination regulation by temperature, we have selected five high temperature resistant germination mutants of *Arabidopsis* (Tamura et al. 2006, Plant & Cell Physiology 47: 1081-1094). Positional cloning of one of the mutant loci identified that *trg1* is a loss of function mutant of *TRG1/XYL1* gene. *Arabidopsis TRG1/XYL1* gene product have shown to have α -xylosidase activity that removes xylose residue from the non-reducing end of xyloglucan oligosaccharides (Sampedro et al. 2001, Plant Physiology 126: 910-920), and it has been suggested to be a rate limiting step of further degradation of the oligosaccharides. HPLC and MALDI/TOF MS analyses revealed the over-accumulation of XXXG in developing fruits and leaves of *trg1/xy1* mutant. In addition to the germination phenotype, *trg1/xy1* had short and fat fruits, and the cells of the fruit wall showed disordered array and shape. The creep-extension analysis indicated that the elongating flower stem of *trg1/xy1* had lower elasticity and viscosity values than wild type. Reporter gene expression in *TRG1/XYL1* promoter::GUS transgenic plants was detected in sub-epidermal cell layer of germinating seed hypocotyl and in epidermis of the elongating roots. These results suggest that xyloglucan oligosaccharides have enhancing role on cell wall loosening, and α -xylosidase plays a key role to keep cell wall loosening state normal during cell elongation.

13013

CLASS IIII PEROXIDASE PER36 IS REQUIRED FOR MUCILAGE EXTRUSION IN ARABIDOPSIS SEED COAT

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Arabidopsis seed coat has a large quantity of polysaccharide "mucilage". When seeds are imbibed, the mucilage is extruded from seed coat, and then forms a gel-matrix surrounding the seed. We found that the knockdown mutants of seed coat specific peroxidase (*PER36*) showed partial extrusion of the mucilage and failed to form the mucilage gel-matrix. *PER36* promoter contains a putative NAC

transcription factor binding sequence. We reported that plant-specific NAC transcription factors, NAC REGULATED SEED MORPHOLOGY1 (NARS1) and NARS2, are involved in accumulation of the mucilage (1). The expression of *PER36* was remarkably down-regulated in developing seeds of *nars1 nars2* double mutant, suggesting that *PER36* was regulated by NARS1 and NARS2.

PER36-GFP fusion protein preferentially localized to the outer cell wall of the mucilage-accumulation cells and rescued the abnormal mucilage extrusion of *per36* mutants. Cell wall loosening treatment such as EDTA and Na₂CO₃ enhanced the mucilage extrusion in *per36* mutants. These results indicate that *PER36* is involved in cell wall degradation and promotes the mucilage extrusion in the mucilage-accumulation cells.

(1) Kunieda et al., *Plant Cell*, 20, 2631-2642 (2008).

13014

VND-INTERACTING2, A NAC DOMAIN TRANSCRIPTION FACTOR, NEGATIVELY REGULATES XYLEM VESSEL FORMATION IN *ARABIDOPSIS*

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The *Arabidopsis thaliana* NAC domain transcription factor VASCULAR-RELATED NAC-DOMAIN7 (VND7) acts as a master regulator of xylem vessel differentiation. To understand the mechanism by which VND7 regulates xylem vessel differentiation, we used a yeast two-hybrid system to screen for proteins that interact with VND7, and identified cDNAs encoding two NAC domain proteins, VND-INTERACTING1 (VNI1) and VNI2. Binding assays demonstrated that VNI2 effectively interacts with VND7 and the VND family proteins, VND1-5, as well as with other NAC domain proteins at lower affinity. VNI2 is expressed in both xylem and phloem cells in roots and inflorescence stems. The expression of VNI2 overlaps with that of VND7 in elongating vessel precursors in roots. VNI2 contains a predicted PEST motif, and a C-terminally truncated VNI2 protein, which lacks part of the PEST motif, is more stable than full-length VNI2. Transient reporter assays showed that VNI2 is a transcriptional repressor and can repress the expression of vessel-specific genes regulated by VND7. Expression of C-terminally truncated VNI2 under the control of the VND7 promoter inhibited the normal development of xylem vessels in roots and aerial organs. These data suggest that VNI2 regulates xylem cell specification as a transcriptional repressor that interacts with VND proteins and possibly also with other NAC domain proteins.

13015

THE ARABIDOPSIS ATGATL5 GENE IS REQUIRED FOR THE PRODUCTION OF SEED COAT MUCILAGE

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The function of a putative galacturonosyltransferase (GalAT) from *Arabidopsis thaliana* (Atg02720; AtGATL5) was studied. AtGATL5 is expressed in all plant tissues, with highest expression levels in 7 DPA siliques. AtGATL5 transcription appears to be regulated by the transcriptional regulators AP2, TTG1, TTG2 and GL2. A T-DNA insertion in the AtGATL5 gene yields a plant whose seed exhibit a defect in mucilage biosynthesis and abnormal cell separation in the seed coat. Transformation of atgatl5 mutants with the native complete AtGATL5 gene results in the complementation of all morphological phenotypes. Chemical and immunological analysis of the mucilage isolated from atgatl5 mutant seed revealed no apparent changes in sugar composition compared with w.t. mucilage. Macromolecular characterization revealed that the molecular weight of the mutant mucilage increased 47% compared with that of w.t. mucilage. Sub-cellular localization experiments showed that the GATL5 protein is localized to both ER and Golgi in comparison with marker proteins resident to these sub-cellular compartments. These studies demonstrate that AtGATL5 is involved in mucilage synthesis in *Arabidopsis* and may function to regulate the size of the polysaccharide. [Supported by the US National Science Foundation (MCB-0646109).]

14001

LEAFY TARGET GENES REVEAL A DIRECT LINK BETWEEN EXTERNAL STIMULUS RESPONSE AND FLOWER DEVELOPMENT

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The transition from vegetative growth to flower formation is critical for the survival of flowering plants. The plant-specific transcription factor LEAFY (LFY) has central, evolutionarily conserved roles in this process, both in the formation of the first flower during the meristem identity (MI) transition and later in flower patterning. Our combined genome-wide binding and expression studies uncover largely distinct functions for direct LFY target genes at these two stages. We show that LFY directly controls the expression of genes regulating the plant's response to external stimuli during the MI transition in *Arabidopsis*, and regulates a larger set of genes during flower development than previously anticipated. Our computational pipeline for de novo cis motif analysis uncovered stage-specific LFY consensus and cofactor motifs that likely contribute to differential LFY activity at both stages. These studies suggest a molecular mechanism for the coordination of developmental and environmental response programs in plants during the reproductive phase. Regulation of multiple survival programs by a single transcription factor may optimize system-wide plant resource allocation, enhancing species fitness.

14002

IDENTIFICATION OF A MAJOR QTL CONTROLLING LOW-TEMPERATURE TOLERANCE AT SEED GERMINATION STAGE IN RICE

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Tolerance to abiotic stress is an important agronomic trait in crops and is controlled by many genes, which are called quantitative trait loci (QTLs). Identification of these QTLs will contribute not only to the understanding of plant biology but also for plant breeding to achieve stable crop production around the world. Previously, we mapped three QTLs controlling low temperature tolerance at the germination stage (called low temperature germinability). To understand the molecular basis of one of these QTLs, qLTG3-1 (quantitative trait locus

for low temperature germinability on chromosome 3), map-based cloning was performed, and this QTL was shown to be encoded by a protein of unknown function. qLTG3-1 is strongly expressed in the embryo during seed germination. Prior to and during seed germination, specific localization of GUS staining in the tissues covering the embryo was observed. Expression of qLTG3-1 was tightly associated with vacuolation of the tissues covering the embryo. This may cause tissue weakening resulting in reduction of the mechanical resistance to the growth potential of the coleoptile. These phenomena are quite similar to the model system of seed germination presented by dicot plants, suggesting that this model may be conserved in both dicot and monocot plants.

14004

CANDIDATES OF CODING GENES ENCODING NOVEL SIGNALING PEPTIDES IN PLANT GENOME (A. THALIANA AND O. SATIVA)

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Small signaling peptides called peptide hormone recently have shown a significant role in various aspects of either plant development or stress response. However, it is believed that such the peptides tend not to be identified because small coding genes tend not to be identified as coding genes due to the conservative nature of gene prediction algorithms. Using our recently published pipeline (Hanada et al., Genome Res. 2007, Hanada et al., Bioinformatics 2010), we identified novel 7,442 and 28,883 sORF small open reading frames (sORFs) with high coding potential in *Arabidopsis thaliana* and *Oryza sativa* genomes. Our aim is to identify small genes encoding novel peptide showing hormone-like functions from these newly identified sORFs as well as annotated small coding genes. Out of 12812 and 67557 small coding genes, our designed microarray identified 6552 and 19068 small coding genes with high expression level under various organs and various stress conditions in *A. thaliana* and *O. sativa*, respectively. There are 7943 and 37797 small coding genes with high homology in 7 plant species (*Arabidopsis lyrata*, *Lotus japonicus*, *Medicago truncatula*, *Populus trichocarpa*, *Vitis vinifera*, *Sorghum bicolor*, *Zea mays*). With developing a new method to identify amino acid compositions specific to peptide hormone by Bayes' approach, we identified 2227 and 13188 small coding genes with high similar hormone-like amino acid compositions in *A. thaliana* and *O. sativa*, respectively. Taken together, we found 892 and 6431 small coding genes with high expression, high homology in other plant genomes and similar hormone-like amino acid compositions. These genes are thought to be candidate genes encoding novel signaling peptides. Interestingly, the candidate genes tend to be specifically expressed in flower, siliques (*Arabidopsis*) or ear (*Oryza*), indicating that there is unknown mechanisms related to morphogenesis of reproductive organs by new peptide hormones.

14005

FUNCTIONAL ANALYSIS OF SHORT OPEN READING FRAMES (SORFS) IN PLANTS

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Open reading frame predicted to encode more than 100 amino acids has been annotated as a gene. Therefore, the initial annotation did not include many short open reading frames (sORFs). However, recent transcriptome studies have discovered that small nonannotated protein-coding genes were expressed in the intergenic region. These peptides encoded by sORFs are reported to be involved in various functions in yeast and *Drosophila*. In the *Arabidopsis* genome, we identified 7,159 short open reading frames (sORFs) ranging from 90 to 300 bp in the intergenic regions. We developed an agilent custom microarray including sORFs, AGI genes and intergenic transcripts in *Arabidopsis* to detect sORF expression. The expression of about 1,500 sORFs could be detected in various tissues (rosette leaves, roots, stems, flowers, siliques). Some sORF expressions were confirmed by RT-PCR analysis to validate microarray results. The sORF expression profile was also analyzed during illumination process to identify light-regulated sORFs. Dark-grown seedlings for 3 days were transferred to continuous illumination. Seedlings were collected after 1, 6, 24 h illumination and extracted RNA was subjected to microarray analysis. We could detect light-dependent sORF expression. This suggests that sORF might have a role in light-regulated development in *Arabidopsis*. Moreover, we generated sORF overexpression *Arabidopsis* plants and observed their phenotypes. Some mutants showed altered flower size, hypocotyl length and root length. We also created rice custom microarray to analyze the expression profile of sORF in rice. About 10,000-12,000 sORFs were also expressed in various tissues (leaves, roots, stems, panicles, seeds). Thus, sORFs might have a role in plant development and the perception of environmental stimuli in higher plants.

14006

DWARF MUTANT CHARACTERIZATION IN HEAVY ION-BEAM MUTATED DIPLOID WHEAT (*TRITICUM MONOCOCCUM*.)

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The short stature and sturdy stalks of the dwarf varieties of wheat rendered them resistant to flattery by wind, rain or high densities and more effective in converting fertilizer input into higher yields. Most of the modern dwarf and semi-dwarf wheat lines having the dwarf gene from the Japanese variety Norin10 among their ancestors. The importance of nitrogen use efficiency and to meet the food demand of growing population, this project was initiated to find the alternative and efficient dwarf genes in wheat. Mutations in plants are powerful tools, not only for classifying physiological mechanisms in plants but also for developing new plant varieties in practical breeding programs. Heavy ion-beam mutagenesis is generally accepted as an effective method for producing mutations, as well as for functional studies of genes. The mutated population can be used to develop germplasm that contains gene knockouts, and fast track the development of non-GM, high value traits. Two diploid wheat strains (DV 92 & KT 3-5) were selected for ion-beam treatment (^{14}N -135 MeV/u) and around 2700 M_2 lines were evaluated. Dwarf and semi-dwarf mutants were identified by phenotypically. Due to the importance of phytohormone in relation to plant height, the gibberellin acid response of selected lines were evaluated through external GA_3 experiments at the seedling stage. Based on phenotype and GA_3 experiment, in total, ten lines were short listed and carried out the high throughput hormone analysis in order to measure the endogenous level of GA, auxin, cytokinin and abscisic acid. For genetic studies, selected lines were back-crossed with parent. Now the molecular characterization is underway to find the deletion mutant for any of the hormone related genes.

14007

TRIFLDB AND TRIMEDB: INTEGRATED INFORMATION RESOURCES FOR TRITICEAE COMPARATIVE GENOMICS

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The Triticeae Full-Length CDS Database (TriFLDB, <http://TriFLDB.psc.riken.jp/>) contains available information regarding full-length coding sequences (CDSs) of the Triticeae crops wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) and includes functional annotations and comparative genomics features. TriFLDB provides a search interface using keywords for gene function and related Gene Ontology terms and a similarity search for DNA and deduced translated amino acid sequences to access annotations of Triticeae full-length CDS (TriFLCDS) entries. Annotations consist of similarity search results against several sequence databases and domain structure predictions. The deduced amino acid sequences in TriFLDB are grouped with the proteome datasets for *Arabidopsis* (*Arabidopsis thaliana*), rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*), providing hierarchical clustering results based on full-length protein sequences. The current TriFLDB contains 15,871 full-length CDSs from barley and wheat and includes putative full-length cDNAs for barley and wheat, which are publicly accessible. Additionally, the Triticeae mapped EST database (TriMEDB, <http://TriMEDB.psc.riken.jp/>) provides information, along with various annotations, regarding mapped cDNA markers that are related to barley and their homologues in wheat. The current version of TriMEDB provides map-location data for barley and wheat ESTs retrieved from three published barley linkage maps and one diploid wheat. To provide the possible genetic locations of full-length CDSs, TriFLCDS entries are also assigned to the genetically mapped cDNA sequences of barley and diploid wheat, which are currently accommodated in TriMEDB. These relational data are searchable from the search interfaces of both databases. This integrated database provides informatics gateway for Triticeae genomics and grass comparative genomics.

14008

LEGUMETFDB: AN INTEGRATIVE DATABASE FOR FUNCTIONAL GENOMICS OF GLYCINE MAX, LOTUS JAPONICUS AND MEDICAGO TRUNCATULA TRANSCRIPTION FACTORS

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Sequence-specific DNA-binding transcription factors (TFs) are often termed as "master regulators" which bind to DNA and either activate or repress gene transcription. We have computationally analyzed the genome sequence data of soybean (*Glycine max*), *Lotus japonicus* and *Medicago truncatula* to identify the complete sets of TFs in these three legume species based on the Hidden Markov Model profiles of DNA-binding domain families. We have then established an integrative database named LegumeTFDB to provide access to all three TF repertoires. LegumeTFDB integrates unique information for each TF gene and family, including sequence features, gene promoters, domain alignments, gene ontology (GO) assignment and sequence comparison data derived from comparative analysis with TFs found within legumes, in *Arabidopsis*, rice and poplar as well as with proteins in NCBI nr and UniProt. With particular interest in abiotic stress signaling, we analyzed the promoter regions for all of the TF encoding genes of the three legume species as a means to identify abiotic stress responsive *cis*-elements as well as all types of *cis*-motifs provided by the PLACE database. Additionally, we supply hyperlinks to available expression data of 1548 soybean TF genes. LegumeTFDB provides an important user-friendly public resource for the elucidation of transcriptional regulation underlying different developmental and physiological processes in agriculturally important legumes. Furthermore, with its unique features LegumeTFDB enables comparative genomics of TF repertoires both within legume species, among legumes, non-legume plants and other organisms.

Availability: <http://legumetfdb.psc.riken.jp/>

14009

DEVELOPMENT OF CASSAVA DATABASE: CASSAVA.PSC.RIKEN.JP

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Cassava (*Manihot esculenta*, Euphorbiaceae) is a starch crop known for its ability to grow in diverse environments, ranging from dry to humid climates and acidic to alkaline soils. Further, this crop can grow in nutrient-poor soil and is found worldwide. Cassava is considered an energy source and is used in industries and as food. In fact, information on cassava, such as published journal articles and sequence registration in public databanks, is increasing, and this increase is associated with high expectations. However, unfortunately, the information is scattered and not organized. Therefore, at present, we are unable to refer to it effectively. Similar to the study of *Arabidopsis*, comprehensive researches such as a cDNA collection and microarray experiments will be performed for cassava. In fact, we have successfully obtained a cassava full-length cDNA collection. Because it is expected that the volume of information will be generated from such researches will be large, organization of the information will be important.

Cassava.psc.riken.jp provides cassava mRNA sequences and ESTs currently available from NCBI (Genbank/EMBL/DDBJ) and their annotations. So far, this database equips search function by gene function, accession number and sequence similarity (BLAST). The annotation of Cassava.psc.riken.jp is based on the similarity search results against several protein databases and the similarity map results in the castor bean (*Ricinus communis*, Euphorbiaceae), poplar (*Populus trichocarpa*, Salicaceae), grape (*Vitis vinifera*, Vitaceae) and *Arabidopsis thaliana* (Brassicaceae) genome sequences. This database is available at <http://cassava.psc.riken.jp/>.

14010

EFFECT OF SILICON ON CELL WALL MATRIX POLYSACCHARIDES IN RICE

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Rice (*Oryza sativa*) is a typical Si-accumulating plant and is able to accumulate Si to >10% of the shoot dry weight. In the shoot, it is known that Si exists as a polymerized form in motor cells or beneath the cuticle layer. On the other hand, it is suggested that there are silicon-containing high molecular weight compounds in cell wall. In addition to that, it was also reported that cell wall became thicker in Si deficient condition. There are some differences in composition of cell wall between dicot and monocot. Pectin content is about 30 - 40% in cell wall of dicot, but pectin content is very little in cell wall of monocot. Hemicellulose is mainly constructed by xyloglucan in dicot, but it is mainly constructed by glucuronoarabinoxylan and (1-3),(1-4) β -D-glucan in monocot. Therefore, our objective is to investigate

the relationship between Si and cell wall matrix polysaccharides, especially the effects of Si on the cell wall matrix polysaccharides composition. Sugar composition of cell wall fractions isolated from rice grown in hydroponics with or without 1.5 mM silicic acid were analyzed by gas-liquid chromatography. As a result, the balance of pectic, hemicellulosic and cellulosic fractions was differed between two Si conditions and the amounts of some sugars were different in the pectic fraction. In the creep test, the cell wall extensibility of the immature leaf was increased in Si-deficient condition. Based on these results, the interaction between Si and cell wall matrix polysaccharides will be discussed.

14011

ANALYSIS OF THE PHENOTYPE OF POLYGALACTURONASE OVER-EXPRESSED RICE

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Liquid fuels derived from cellulosic biomass is one of the candidates of alternative to conventional fossil fuel source. However, cell walls, which consist of cellulose, hemicellulose, pectin and lignin, are locked in complex polymers. In particular, pectin might be a barrier for enzymatic saccharification of cellulosic ethanol, because pectin degradation treatments are effective in ethanol production process. Compared with the pectin-abundant dicotyledious cell wall (40%), the cell wall of rice contains less pectin (10%). Therefore, pectin was thought to be less important for the growth and development in rice. To understand the function of pectin in the development of rice, we analyzed the polygalacturonase over-expressed rice driven by maize ubiquitin promoter. Overexpression of PG caused the reduction of pectin content and alteration of sugar composition. This PG overexpressors show the brittle phenotype in leaves. These results suggest that pectin might be important for the normal development and the property of cell wall in rice.

14012

SNP-BASED GENETIC MAPS IN CULTIVATED TOMATO

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In order to advance genetics and genomics in tomato, an interspecific standard linkage map has been constructed with RFLP, SSR, and SNP markers based on a population derived from a cross between *Solanum lycopersicum* and *S. pennellii*, and a draft sequence of tomato genome has been released by the International Tomato Genome Sequencing Consortium. However, intraspecific dense linkage maps of cultivated tomato have not been developed because of less polymorphism between germplasms. In the present study, we discovered 5,607 candidate SNPs by comparison of 49,972 contigs that derived from 229,086 ESTs registered in public databases. Out of the 5,607 candidate SNP markers, 1,536 were tested for polymorphism using Illumina GoldenGate system for two mapping populations, namely AMF2, consisting of "Ailsa Craig", "Micro-Tom", and its E_2 progeny, and MMF2, consisting of "M82", "Micro-Tom", and its E_2 progeny. As the results, a total of 646 and 639 SNP markers showed polymorphism in the AMF2 and the MMF2 populations, respectively, and were subjected to linkage analysis. A linkage map of the AMF2 consisted of 991 loci, including 646 SNPs and 345 SSRs, which were previously developed from EST and BAC-end sequences, and covered 1,468 cM. In the MMF2 population, a total 637 SNP loci were located onto 12 linkage groups representing 1,126 cM. Meanwhile, we applied the Illumina GoldenGate SNP assay for 27 tomato cultivars to evaluate the availability of the SNPs. Among the 27 cultivars, 919 of 1,536 SNP markers were segregated. The results in this study will contribute to physiology, molecular genetics, genomics, and breeding in tomato.

14013

ENTRAPMENT OF ENDOGENOUS CIRCADIAN RHYTHMS IN ARABIDOPSIS BY GLK MYB TRANSCRIPTION FACTORS.

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GLK1 and GLK2 transcription factors have been studied extensively with respect to their role in chloroplast biogenesis (Waters et al. (2009) *The Plant Cell*, 21:1109-1128). Our previous studies identified that overexpression of GLK1 in *Arabidopsis* led to the reprogramming of gene expression networks to enhance a high constitutive expression of genes encoding disease defense related proteins and conferred resistance to *Fusarium graminearum* (Savitch et al. (2007) *BBRC*, 359: 234-238). Comprehensive transcriptome analyses of *Arabidopsis* GLK1 OE, GLK2 OE, GLK1 KO, GLK2 KO and GLK1&2 KO performed at ZT4 and ZT12 identified that transcription of a large number of genes was similarly controlled in OE and KO plants, in particular genes regulated by circadian clocks. In addition, comparison of transcriptional changes at ZT4 with that of ZT12 identified that large number of genes affected in OE and/or KO plants reversed their expression, such that genes typically induced at ZT4 were induced in OE and KO plants at ZT12 and vice versa. This observation suggests that GLK1 and GLK2 might be involved in regulation of circadian clocks in *Arabidopsis*. In this presentation we report the monitoring of diurnal changes in transcription of major clock related genes performed by qPCR analysis. Our studies identified that GLK1 OE, GLK2 OE, GLK1 KO, GLK2 KO and GLK1&2 KO affected differentially either diurnal amplitude or time of appearance of CCA1, LHY, APRR3, APRR5, APRR7, APRR9, TOC1, GI, PCL1 indicating that both overexpression of GLK1 and GLK2 or knockouts of GLK1 and GLK2 perturb diurnal rhythmicity in *Arabidopsis*. Our results also indicated that GLK1 OE suppresses endogenous GLK2 and GLK2 OE suppresses endogenous GLK1, thus, suggesting that observed changes in diurnal rhythmicity might be combinatorial effect of perturb GLK locus.

15001

DIVERSITY ANALYSIS OF NAN3-INDUCED COMMON BEAN MUTANTS USING AFLP AND MORPHO-AGRONOMICAL CHARACTERISTICS

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Common bean (*Phaseolus vulgaris* L.) recently gains much interest in Taiwan because it is characterized as a healthy food. However, only limited varieties are available for common bean grower. Chemical-induced mutagenesis has been used to obtain genetic diversity and produce new variety in many crop species. In the present study, amplified fragment length polymorphism (AFLP) and morpho-agronomic diversities in a common bean variety Hwachia and its'34 NaN3-induced mutants (M7 generation) were investigated. The results showed that eight primer combinations generated 516 fragments among the tested materials, of which 448 fragments were polymorphic. The calculated Jaccard similarity coefficients based on AFLP data ranged from 0.469 to 0.839. Both clustering and principal coordinate

analyses confirmed the presence of molecular diversities between variety Hwachia and its mutants. Statistical analyses also revealed the presence of morpho-agronomic variations among the tested mutants. The relations among Hwachia and mutants based on AFLP data were correlated with those based on morpho-agronomic characteristics. Thus, NaN3-induced mutagenesis can broaden genetic variation in common bean. Moreover, mutants SA-09 and SA-14 can be released as new common bean varieties; others (SA-07, SA-08, SA-30 and SA-31) are also useful as a source of variation to derive new variety.

15002

AN ARABIDOPSIS TRICHOME SPECIFIC PROMOTER DRIVES REPORTER GENE EXPRESSION AND RETAINS ITS SPECIFICITY IN BRASSICA JUNCEA

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In several cases tissue specific expression of a transgene in plant species is desirable. Trichomes are specialized epidermal cells that produce secretions and that are thought to provide a first line of defence against pests and pathogens. Many trichome-secreted compounds are used commercially as flavourings, medicines, etc. The trichome specific promoter isolated from the upstream sequence of Ethylene Response Factor gene (At5g11190.1). Hypocotyles of Brassica juncea were used to develop an in vitro culture system for plant regeneration and Agrobacterium mediated transformation. Plants were regenerated from hypocotyle derived callus precultured on Murashige and Skoog medium supplemented with 2 mg/L 2,4-dichlorophenoxyacetic acid. Transgenic plants were obtained by inoculation of precultured explant with a disarmed Agrobacterium strain (GV3101) containing the binary vector pBI101-TSP, which carried the genes encoding β -glucuronidase under trichome specific promoter. This promoter is shown to direct the specific expression of the reporter gene, β -glucuronidase (GUS), in trichomes of in vitro regenerated brassica plantlets. The expression of gus gene was predominantly more in trichomes of young leaves, petiole and stem. PCR and RT-PCR analysis revealed the presence and expression of gus gene in transgenic plants respectively. This promoter may provide efficient bioengineering to enhance pest and pathogen resistance, and for molecular farming.

15003

MOLECULAR DETECTION OF NINE RICE VIRUSES BY RT-LAMP

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In the context of rapid and unpredictable epidemic outbreaks of rice virus diseases, a system for quick and accurate identification of the causal viruses is critical for not only epidemiological study but also for monitoring and controlling the outbreaks. Such an assay system, when available, will have to be specific, sensitive and adaptable to detect newly evolved strains. To address these needs, we established the assays for molecular detection of nine major rice viruses that occur in Asia based on Reverse-Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) principles - a cost effective, rapid and sensitive assay for molecular diagnosis in many fields.

RT-LAMP assays were developed and evaluated for the detection of nine viruses from infected rice plants, including Rice black-streaked dwarf virus (RBSDV), Rice dwarf virus (RDV), Rice gall dwarf virus (RGDV), Rice ragged stunt virus (RRSV), Rice transitory yellowing virus (RTYV), Rice stripe virus (RSV), Rice grassy stunt virus (RGSV), Rice tungro bacilliform virus (RTBV) and Rice tungro spherical virus (RTSV). Primers were designed based on sequences coding for structural proteins of each of the viruses. All primer sets, except for RBSDV, detected corresponding target sequences from infected rice at 63°C within 60 minutes. The accuracy of the assays was monitored with the use of external and internal (*OsRAC1*) controls. The sensitivities of the assays were either superior (for RSV, RTBV and RTYV) or similar (for RDV) to that of one-step RT-PCR. Furthermore, the assays worked reliably and reproducibly with RNA extracted by 0.5N NaOH which further reduces the cost of regular diagnosis. For the first time, with these RT-LAMP assays, it was possible to detect the presence of RTBV and RTSV from viruliferous vectors.

The assay system we reported here should facilitate studies on rice disease epidemiology, outbreak surveillance and molecular pathology.

15004

TRANSCRIPTIONAL REGULATION OF FLAVONOL BIOSYNTHESIS IN GRAPEVINE: NOVEL INSIGHTS INTO FLAVONOL DIVERSITY

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Flavonoids represent an important class of plant secondary compounds that play pivotal roles in grape berries and contribute substantially to their taste, quality and nutrition. They exhibit anti-oxidant and anti-proliferative capacities and thereby account for health-promoting effects of the consumption of grapes and many other fruits. Interestingly, extensive modification patterns strongly influence pharmacological activity of the respective flavonoid.

To elucidate regulation of flavonoid synthesis in fruit, the project aimed at the identification and functional characterisation of the MYB transcription factors *VvMYBF1* and *VvMYBF2* from *V. vinifera* L. cv 'Shiraz'. *VvMYBF1* was shown to be a light-inducible flavonol-specific transcription factor by correlation of gene expression patterns, transient reporter systems and complementation of a flavonol-deficient *Arabidopsis* mutant phenotype. These studies have been conducted to *VvMYBF2* to elucidate to which extent *VvMYBF1* and *VvMYBF2* spatiotemporally regulate the accumulation of flavonols during grape berry development.

While the general flavonoid pathway is well characterized in several plant species, little is known about the genes modifying flavonol aglycones and other core compounds to produce the tremendous diversity of flavonoids observed in plants. These complex modification patterns and differences in the general flavonoid pathway between plant species result in more than 6000 flavonoid compounds in plants. In a forward genetic approach, *VvMYBF1* and *VvMYBF2* have been expressed in grapevine hairy root cultures to perform DNA microarray experiments to identify new genes specifically involved in modification and accumulation of flavonols. Co-expression of these target genes with the respective MYB factor in grapevine hairy roots might lead to the synthesis of specifically modified flavonols and the characterization of the enzymes modifying the basic flavonol skeleton.

15005

ENGINEERING JA-REGULATED SECONDARY METABOLISM IN PLANTS

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Plants produce many small molecules useful as pharmaceuticals, insecticides, flavours, and fragrances which derive from secondary metabolism. The ubiquitous plant hormone jasmonic acid induces the biosynthesis of defence proteins and protective secondary metabolites. The lack of genomic data for the majority of medicinal plants limits global analyses such as transcriptomics, proteomics and metabolomics. We propose here to manipulate jasmonate-responsive genes controlling a pathway of potential importance for the production of therapeutic drugs, in the model plant *Arabidopsis thaliana* and in tobacco with the dual aim of developing a greater understanding of the terpenoid-indole-alkaloid pathway, and developing small molecules or precursors for new medicines. Success in manipulation of the targeted metabolic pathway will be analyzed by LC-MS, GC-MS and through a novel functional screening system. The analysis of engineered plant lines will improve in particular the understanding of this key pathway that leads to the production of compounds acting as toxins, antimalarial, or antineoplastic drugs in human medicine. The success of this project will provide an inexpensive way to test the function of economically important compounds in plants and to engineer their production *in planta*.

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15006

A SYNTHETIC-BIOLOGY APPROACH CONFERRING DROUGHT TOLERANCE BY INDUCING γPGA SYNTHETASE GENES IN ARABIDOPSIS

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Environment changes cause desertification all over the world and breadbasket issue following world population explosion have become a serious problem. To contribute these problems, it is important to provide drought tolerance in plants and increase arable fields.

We focused γPGA (gamma-polyglutamic acid), which has high water retention ability. γPGA is a primary component of the sticky paste of natto which is a traditional Japanese food made from fermented soybeans. It is a very safe substance and has no affect on the environment. γPGA is produced with three synthetase genes, pgsA, B, and C. Usually, it is a time-consuming process to induce three genes into a plant if we induce each gene separately and hybridize them. Instead of this process, we applied IRES (Internal Ribosome Entry Site) and connected three synthetase genes into one transcript, like bacterial operon. Additionally, we optimized the codon frequency into *Arabidopsis* and the DNA sequence was synthesized by chemical method. After the induction of the three synthetase genes, we performed drought tolerance assay. When we put the plants under the dry condition, we could not confirm any difference between wild type and γPGA synthetase induced plants. After re-watering to the dried plants, γPGA synthetase induced plants showed revitalization and started to grow. To measure the growth objectively, we manipulated the image files focusing the amount of the leaves and confirmed around 1.7 times difference between wild type and γPGA synthetase induced plants. For further analysis, now we are performing proteome analysis to validate γPGA in the plants.

It was confirmed that γPGA was strong in the re-growth by the re-water supply. This can draw the re-growth when it rains after it dries long in the desert or other dry grounds.

15007

ARABIDOPSIS AS A PLATFORM FOR PLANT SYNTHETIC BIOLOGY

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Synthetic biology is the study and engineering of biological systems for both practical applications and to gain insight into natural biological systems. It is beneficial for plant synthetic biologists to engineer their systems in a characterized and easily transformable model organism such as *Arabidopsis* during the development of system components and proof-of-concept stages. Using this strategy, we have designed and tested a synthetic signaling circuit in *Arabidopsis* that links highly specific and sensitive ligand binding to gene expression, and in this example, alters the plant phenotype. Receptors, computationally re-designed bacterial periplasmic binding proteins, are targeted to the plant's apoplast. When the ligand specific for the receptor is present, the receptor-ligand complex naturally develops high affinity for the extracellular domain of chemotactic proteins. The ligand used in our current system, 2,4,6-trinitrotoluene (TNT), is an environmental pollutant and explosive. We produced chimeric transmembrane molecules that initiate an intracellular histidine kinase (HK) phospho-relay. To reduce complexity typical of eukaryotic signal transduction, we developed one protein, PhoB-VP64, that links the membrane-localized HKs to a signal sensitive transcriptional promoter (PlantPho). In response to the external ligand and the HK initiated phospho-relay, PhoB-VP64 translocates to the nucleus and activates transcription from the synthetic PlantPho promoter. To produce a visible phenotype, we linked the PlantPho promoter to a synthetic de-greening gene circuit that causes a loss of chlorophyll and accumulation of ROS (reactive oxygen species). These results show that it may be possible, with refinement, to produce highly specific detector plants.

15008

SITE-DIRECTED MUTAGENESIS IN HIGHER PLANTS USING ZINC FINGER NUCLEASES

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The induction of double-strand DNA breaks (DSBs) greatly increases rates of homologous recombination-mediated gene targeting or of gene mutation by error-prone non-homologous end joining. Both repair mechanisms would be important basis for molecular breeding to modify the targeted gene on the genome. However, these approaches depend on the capability to create a DSB on the specific genomic sequence of interest. Zinc finger nucleases (ZFNs) are recently developed to introduce a site-specific DSB. ZFNs function as dimers with each monomer composed of a non-specific cleavage domain from the *Fok I* endonuclease fused to a zinc-finger array engineered to bind a target DNA sequence. By modification of zinc finger DNA binding domain, ZFNs can cleave virtually any long stretch of DNA specifically.

Here, we demonstrated a reliable approach for targeted-gene inactivation in *Arabidopsis* using ZFNs. The engineered ZFNs for the stress-responsive regulator, the *ABA-INSENSITIVE4* (*ABI4*) gene cleaved the recognition sequences in *ABI4* specifically *in vitro*. Next, *Arabidopsis* plants were transformed with the ZFNs genes driven by a heat-shock promoter. After heat-shock induction, gene mutations with deletion and substitution in *ABI4* via the ZFNs-mediated cleavage were observed in somatic cells at frequencies as high as 3%. We

further established the homozygote mutant lines, zfn-abi4-1-1 for *ABI4*. zfn-abi4-1-1 exhibited expected mutant phenotypes; ABA and glucose insensitivity. These data provide the evidence that our approach using ZFNs can be used for the efficient production of mutant plants for reverse genetics and molecular breeding. We are also on going to establish the system of site-directed mutagenesis in rice.

15009

THE FAST TECHNIQUE FOR AGROBACTERIUM-MEDIATED TRANSIENT GENE EXPRESSION IN SEEDLINGS OF ARABIDOPSIS AND OTHER PLANT SPECIES

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Genome sequencing has identified a massive number of uncharacterized genes in *Arabidopsis thaliana* and several other plant species. To decipher these unknown gene functions, several transient transformation systems have been developed as quick and convenient alternatives to the lengthy transgenic assay. These transient assays include biotic bombardment, protoplast transfection and Agrobacterium-mediated transient transformation, each having advantages and disadvantages depending on the research purposes. We present a novel transient assay based on cocultivation of young *Arabidopsis thaliana* seedlings with *Agrobacterium tumefaciens* in the presence of the surfactant Silwet L-77. This Fast Agro-mediated Seedling Transformation (FAST) was used successfully to express a wide variety of constructs driven by different promoters in *Arabidopsis* seedling cotyledons (but not roots) in diverse genetic backgrounds. Localizations of three previously uncharacterized proteins were identified by cotransformation with fluorescent organelle markers. The FAST procedure requires minimal handling of seedlings and was also adaptable for use in 96-well plates. The high transformation efficiency of the FAST procedure enabled protein detection from eight transformed seedlings by immunoblotting. Protein-protein interaction, in this case HY5 homodimerization, was readily detected in FAST-treated seedlings with Förster resonance energy transfer and bimolecular fluorescence complementation techniques. Initial tests demonstrated that the FAST procedure can also be applied to other dicot and monocot species, including tobacco, tomato, rice and switchgrass. The FAST technique thus provides a rapid, efficient and economical assay of gene function in intact plants with minimal manual handling and without dedicated device. This method is potentially ideal for future automated high-throughput analysis.

15010

DETECTION OF SALINITY TOLERANCE GENES IN ARABIDOPSIS BY QTL

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Arabidopsis is an important staple food and cash crop of the world. To obtain better yield from saline soils and saline waters, it is imperative that salt tolerant *arabidopsis* varieties should be developed. Soil salinity is one of the greatest problems faced by agriculture today. There are many mechanisms by which plants tolerate the problems associated with salinity all of which are genetically controlled. Salt tolerance is a mixture of different morpho-physiological processes which are controlled by many genes across the *arabidopsis* genome. DNA markers enhance the recovery rate of isogenic recurrent genome after hybridization and facilitate the introgression of multiple genes with additive effects necessary to increase stress tolerance. Molecular markers are used to transfer alleles of interest from wild relatives into cultivated genotypes. SSR primer pairs were used to find DNA marker linked with salinity resistance. A total 200 SSR primer pairs used. Out of which four SSR primers were polymorphic with the parents. These four polymorphic primers applied on F2 population. Development of microsatellite markers provided a new approach to MAS in breeding practice. Based on these criteria microsatellite markers are preferable to RFLP markers. Once developed microsatellite markers are easy to use and less expensive to assay because the procedure for microsatellite analysis virtually involves only amplification and electrophoresis.

15011

EFFICIENT MOLECULAR BREEDING OF TORENIA FLOWERS BY COLLECTIVE INTRODUCTION OF CHIMERIC REPRESSORS FOR ARABIDOPSIS TRANSCRIPTION FACTORS

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Since flowers of horticultural plants are attractive, flowers with new colors and/or shapes have been produced by breeding. Molecular breeding enables us to produce novel floral traits which could not be obtained by traditional breeding. Our recent study using transgenic torenias (*Torenia fournieri*) revealed that utilization of *Arabidopsis* genes is effective for the generation of new floral traits of horticultural plants. Because many transcription factors, such as MADS-box and TCP family proteins, participate in flower development, manipulation of transcription factors can be a powerful tool for modification of floral traits. However, it demands time and effort to find out useful genes by generating transgenic plants one by one. To facilitate this process, we collectively generated many transgenic torenias harboring different transgenes by bulk introduction of around 50 *Arabidopsis* transcription factor genes. In addition, we converted all the transcription factors into chimeric repressors, which act dominantly even in the presence of functionally redundant factors, by fusing the repression domain (SRDX).

Two sets of transcription factor genes were introduced; 42 genes which are highly expressed in *Arabidopsis* flowers and 50 genes which participate in regulation of organ development and/or cell differentiation. In the observations of totally 750 transgenic plants, we found many attractive phenotypes in petal color and/or shape, such as big corollas, serrated or white margined petals and color patterns. These phenotypes seemed more valuable and variegated contrary to our expectations from the putative gene function in *Arabidopsis*. We found that functionally unknown genes also induced flower variation. These results suggest that the transgenic torenias will provide novel insight into the functions of the transcription factors which could not be revealed by previous studies in *Arabidopsis*.

15012

DEVELOPMENT OF SOYBEAN TRANSGENIC PLANTS WITH THREE GENES, ORE7, ATSIZ AND SMV CP-RNAI USING HIGHLY EFFICIENT SOYBEAN TRANSFORMATION SYSTEM

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Korean soybean variety Kwangan was transformed with three genes using highly efficient soybean transformation system. The genes

are leaf senescence delaying gene (*Ore7*), salt stress resistance gene (*AtSIZ*), and soybean mosaic virus coat protein. These genes were transformed into Kwangan and kept growing to harvest seeds. Most transgenic soybean lines produced T₂ seeds. Their T₂ plants are investigated for gene introduction and their expression using PCR, RT-PCR, and Southern blot. Medium resistance of soybean mosaic virus was confirmed from some of the transgenic lines overexpressing RNAi form of viral coat protein gene. For the two other genes, *Ore7* and *AtSIZ*, we currently analyzing a set of transgenic lines to investigate target trait expression such as leaf senescence delay and salt stress resistance.

15013

MOLECULAR CHARACTERIZATION OF OSPAP1: TRANSGENIC EXPRESSION OF A PURPLE ACID PHOSPHATASE UP-REGULATED IN PHOSPHATE-DEPRIVED RICE SUSPENSION CELLS

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Phosphate starvation-induced purple acid phosphatase cDNA was cloned from rice, *Oryza sativa*. The cDNA encoding *O. sativa* purple acid phosphatase (*OsPAP1*) has 1195 bp with an open reading frame of 355 amino acid residues. The amino acid sequence of *OsPAP1* cDNA shows identities of 50-51% with other plant purple acid phosphatases and appears to have five conserved motifs containing the residues involved in metal binding. *OsPAP1* was expressed in the rice plant and in cell cultures in the absence of phosphate (Pi). The induced expression of *OsPAP1* is a specific response to Pi starvation, and is not affected by the deprivation of other nutrients. The expression was organ specific, with strongest expression in Pi-deprived roots. The *OsPAP1* cDNA was expressed as a 37 kDa polypeptide in *Escherichia coli* BL21 (DE3). In addition, the *OsPAP1* gene was introduced into *Arabidopsis* via an *Agrobacterium*-mediated transformation. Functional expression of the *OsPAP1* gene in the transgenic *Arabidopsis* line was confirmed by northern and western blot analysis. *OsPAP1* overexpression lines revealed a higher phosphatase activity compared with the wild-type. These results suggest that the *OsPAP1* gene can be used to develop new transgenic dicotyledonous plants that are able to adapt to Pi-deficient conditions.

15014

ESTABLISHMENT OF CELL LINES OF SUSPENSION CULTURES AND TISSUE CULTURE SYSTEMS FOR INDUCING SOMATIC EMBRYOGENESIS IN ARABIDOPSIS THALIANA

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Plant cell, tissue and organ cultures are important biological resources used extensively for a broad range of studies. Undifferentiated cell cultures are used as valuable tools for investigating cellular processes. Furthermore, tissue culture systems to induce redifferentiation are useful not only for researches of totipotency and developmental processes, but also for agricultural applications such as clonal propagation of plants and regeneration of transgenic plants.

The ability of plant cells to undergo somatic embryogenesis is experimental evidence of their totipotency. We have established a simple culture system for inducing somatic embryogenesis in *Arabidopsis thaliana*. Light-green somatic embryos developed from germinating embryos cultured in the presence of 2,4-dichlorophenoxyacetic acid. Using this method, we examined the capability of 352 natural accessions to form somatic embryos. This simple culture system for inducing somatic embryogenesis from germinating embryos will be a valuable tool for investigating molecular mechanisms of somatic embryogenesis.

We have established *Arabidopsis thaliana* cell-suspension cultures derived from leaves of 3 accessions (Col, Ws, and Ler). These cell lines maintained different physiological properties. We have also developed simple protocols for cryopreservation of the cell lines. A standardized method for evaluating characteristics of cultured cells may be required for quantitative monitoring and quality control of the cell cultures.

15015

EVALUATION OF ARABIDOPSIS METHIONINE SULFOXIDE REDUCTASE B GENES AS EFFECTIVE NOVEL NON-ANTIBIOTIC SELECTABLE MARKERS FOR PLANT TRANSFORMATION

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Plant transformation is an important tool for basic research and crop improvement. In most cases, selection is based on antibiotic or herbicide resistance to identify putative transformed plantlets. If plant gene(s) can not only tolerant to abiotic- or biotic-stress but also be used as the selection marker, it will provide more benefit for crop improvement. Here, a new selection system based on the enhanced expression of specific methionine sulfoxide reductase B (*MsrB*) genes and the use of methyl viologen (MV) as selection agent was developed. To clarify the potential use of the cytosolic *MsrB* genes, transgenic plants overexpressing individual *MsrB* genes were created and their MV-tolerance capability determined. *MsrB7*, *MsrB8* and *MsrB9* transgenic plants, but not *MsrB5* and *MsrB6* transgenic plants, were germinated successfully and were later efficiently selected on MV (300 nM)-supplemented MS medium. Southern blot analysis and RT-PCR of transgenic plants selected by MV revealed 35S::*MsrB7/8/9* integration and increased *MsrB7/8/9* mRNA transcripts. MV treatment caused significant accumulation of H₂O₂ in the wild-type plants compared to *MsrB7*, *MsrB8* and *MsrB9* overexpressing plants. In addition, these transgenic plants exhibited higher activity of ROS-scavenging enzymes such as peroxidase and thioredoxin reductase under MV treatment. *MsrB7/8/9* overexpressing plants not only displayed tolerance to MV and H₂O₂ treatments on culture plates but also exhibited resistance to MV-spreading compared to wild-type plants when planted in soil. The *MsrB*/MV system may provide a new selection system distinct from conventional antibiotic selection systems.

15016

HIGHER EXPRESSION OF VITAMIN C BIOSYNTHESIS GENES LEADS TO HIGHER VITAMIN C CONTENTS IN ACEROLA (*MALPIGHIA GLabra*) COMPARED TO ARABIDOPSIS THALIANA

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Micronutrient deficiency is rife among over 2 billion people living on earth. Human is unable to synthesize vitamin C and so depend majorly on fruits and vegetables in his diet for the supply of this essential vitamin. Acerola (*Malpighia glabra*) is a tropical fruit with an abundant vitamin C content that is more than 20-fold that found in Strawberry and Oranges. Although vitamin C has a long history dating from the 18th century, the pathway to its synthesis in plant was unknown. Over a decade ago, a breakthrough came as a functional biosynthetic pathway was proposed using the model plant *Arabidopsis thaliana*. All effort has since then been jolted towards understanding and utilizing the genes in this pathway to improve the quality of plants. We have focused on three important genes in the

proposed pathway namely; phosphomannomutase (PMM), GDP-D-mannose pyrophosphorylase (GMP) and GDP-L-galactose phosphorylase (GGP). The activity of the precursor to PMM, phosphomannose isomerase, has been questionable in plants. Although reported recently in *A. thaliana*, we could not find the activity in acerola. Correlations were observed between PMM activities and vitamin C contents in *A. thaliana*, tomato and acerola. In *A. thaliana*, mutation in GMP and GGP genes led to the vtc1 and vtc2 mutants respectively with reduced vitamin C contents compared to the wild type plant. Acerola GGP gene has the highest expression level among the genes analyzed and it was found to be about 700-fold greater than the expression in wild type *A. thaliana*. In acerola, there was a correlation between GMP gene expression and vitamin C content in the fruits during ripening. Overexpression of each of these genes led to 2- to 3-fold increase in the vitamin C contents of tobacco. The combined overexpression of these acerola genes in other economically important crops may lead to tremendous elevation of vitamin C.

15017

AGROBACTERIUM-MEDIATED TRANSFORMATION OF THE PARASITIC PLANT *PHTHEIROSPERMUM JAPONICUM*

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Parasitic plants in the Orobanchaceae invade roots of neighboring plants to take them of water and nutrients. Despite the fact that some parasitic plants are the world's most damaging agricultural pests, the molecular mechanisms involved in the parasite and host interactions are largely unknown. To exploit reverse genetic approaches for functional characterization of genes involved in parasitism, we developed transient and stable transformation systems for the facultative parasitic plant *Phtheirospermum japonicum* using the hairy-root-inducing bacteria, *Agrobacterium rhizogenes*. An efficient protocol was established by combination of sonication, vacuum and acetosyringone treatment using young seedlings as starting materials. Using this method, transient transformation was visible in cotyledons, and stable transgenic hairy roots of *P. japonicum* had visibly emerged from cotyledons 2 weeks after *A. rhizogenes* inoculation. Longer sonication increased the efficiency of transient transformation, whereas in stable root transformation these effects were not evident. Without vacuum treatment, higher percentages of seedlings showed successful transient transformation. In contrast, the vacuum-treated plants showed a significant increase in the stable transformation efficiency. We also verified that the addition of acetosyringone is essential for stable root transformation. The presence of a transgene in *P. japonicum* was verified by genomic PCR using GFP-specific primers

15018

INVOLVEMENT OF MAP3K δ 4 IN GROWTH REGULATION AND STRESS SIGNALING IN *ARABIDOPSIS THALIANA*

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MAP3K δ 4 (At4g23050) is a member of RAF-related MAPKKKs (mitogen activated protein kinase kinase kinases), classified as subgroup B2 in *Arabidopsis* MAPKKKs. It consists of 736 amino acids and contains two domains, that is, a PAS domain located in the N-terminus and a kinase domain in the C-terminus. It has been reported that many PAS domains bind cofactors or ligands to detect sensory signals and that many PAS-containing proteins are involved in the sensing of environmental stimuli, such as oxygen, redox or light. Although MAP3K δ 4 having PAS domain may play a role in signaling and environmental response, its function has not been reported. It is also unclear whether MAP3K δ 4 functions as MAPKKK. In order to clarify the function of MAP3K δ 4, we investigated expression patterns of the gene in different tissues and in response to stress conditions. In addition, we characterized transgenic *Arabidopsis* plants over-expressing MAP3K δ 4.

RT-PCR analysis in mature plants indicated that MAP3K δ 4 was expressed mainly in vegetative tissues such as rosette leaves, cauline leaves, stems, and in roots at higher level. On the other hand, its expression level was much lower in inflorescence and hardly detectable in siliques. It is also shown that the expression level in leaves fluctuated in response to salt stress. Transgenic *Arabidopsis* plants constitutively expressing MAP3K δ 4 exhibited earlier bolting and more vigorous growth in stems. In addition, the transgenic plants were more tolerant to salt stress. These results suggest that MAP3K δ 4 is involved in the growth regulation and/or the stress signaling.

15019

MONITORING OF IRES-MEDIATED TRANSLATION EFFICIENCY BY DUAL-COLOR LUCIFERASE REPORTER SYSTEM IN HIGHER PLANTS

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Internal Ribosome Entry Site (IRES) was first identified as a sequence in viral genomes that allow cap-independent translation initiation. Recent studies on plant viral gene expression revealed the presence of IRES-mediated translation mechanisms among plant viruses. It has been shown that the 5'UTR sequence from several plant viruses can mediate cap-independent translation in plant cells. In order to conduct an assessment of various IRES-dependent translation efficiencies in plant cells, we have developed a dual-color bioluminescence reporter system using luciferase genes derived from a luminous click beetle, *Pyrophorus plagiophthalmus*. Two luciferase genes, designated CBR and CBG, are separated by an IRES element and expressed under the control of the CaMV 35S promoter. Although they share 99% identical amino acid sequences, the CBR emits red light while the CBG emits green light. These luciferases catalyze D-luciferin that is also catalyzed by firefly luciferase and the red and the green lights can be separated by the optical filter. Using an appropriate detection system, we are able to monitor the IRES activity as a red/green ratio. Unlike the conventional dual-luciferase assay, which requires cell disruption and protein extraction, dual-color assay enables us to observe the IRES activity in planta. We are currently using this technology for identification and characterization of IRESes and for the isolation of mutants exhibiting altered IRES activity.

15020

GABI-TILL: EXPANSION AND NOVEL USE OF THE GABI-TILLING PLATFORM FOR GENE FUNCTION ANALYSIS IN ARABIDOPSIS

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As part of the GABI-TILL consortium, that aims at the use and further development of the TILLING-technology for the analysis of gene functions in crop species of major importance in Germany and Europe, this subproject explores means of technical improvements, it utilises and further expands *Arabidopsis* TILLING populations, and demonstrates novel TILLING applications in addition to supporting

the establishment of TILLING platforms for other species and conductance of mutant searches in close co-operation with other GABI-TILL partners. Thus, pre-existing Arabidopsis TILLING populations (Col-0) are expanded and deepened (aiming at increased mutation frequency), and new populations (C24, Cvi) are created. The TILLING methodology is further advanced through testing novel pooling strategies and mutation detection through sequencing. To demonstrate novel TILLING applications, splice site mutations have been identified in the alternatively spliced Arabidopsis genes RSp31, RSp31a, RSZ32, and RSZ33 that encode splicing factors. Furthermore, mutations have been detected in the Arabidopsis miRNA824 precursor gene that plays an important function in the regulation of the AGL16 gene, which is required for proper development of stomatal complexes.

15021

DEVELOPMENT OF A HIGH THROUGHPUT SCREENING SYSTEM TO MONITOR PATHOGEN-RESPONSIVE GENE EXPRESSION IN ARABIDOPSIS THALIANA (GROWTH STAGE 1.0 SEEDLING)

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To develop a bioluminescence monitoring of plant defense gene expression, we exploited firefly luciferase (Fluc) reporter system and tested several defense-related gene promoters using transgenic Arabidopsis. Results of *in vivo* bioluminescence assay indicated that the promoters are induced by treatment with chemicals or pathogen inoculation and the luminescence levels are in parallel with the endogenous mRNA levels. In order to adapt to the high throughput screening (HTS) system in 96-multiwell format, we further selected promoters that are functional in growth stage 1.0 seedlings (cotyledon fully opened), and found that the *Pathogenesis Related protein 1a* (*PR-1a*) from tobacco BY-2 and the *Vegetative Storage Protein 1* (*VSP1*) promoter from *Arabidopsis thaliana* showed clear Fluc activity induction in response to treatment with chemicals in stage 1.0 seedlings. To improve the *in planta* HTS system, we are currently introducing the dual-color luciferase assay system using click beetle luciferase reporter genes.

16001

IN SILICO APPROACHES TO UNDERSTANDING REGULATION OF ARABIDOPSIS CLOCK GENES

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Comparative genomics is a powerful method to elucidate which bases in DNA sequences are important for transcription factor binding. The rationale behind these approaches is that random mutations are unlikely to be maintained during evolution if they affect functional regions of a genome; therefore bases that are conserved across species are likely to correspond to functionally important regions. We have developed a novel alignment-based comparative genomics tool called EARS (Evolutionary Analysis of Regulatory Sequences) that is specifically tuned for the analysis of plant genomes. This method compares two sequences by breaking them into windows of a fixed length, and computes the optimal alignment score between all possible window pairs. Therefore, to align two 2kb sequences, approximately four million individual window alignments are performed. As all alignments are optimal global alignments, rather than heuristic, a loss-free comparison of the sequences is achieved. A conservation profile is generated showing the p-value of the maximum alignment score for each given window to its best matching window in the other sequence. Peaks above the significance threshold identify putative conserved sequences. The conservation profile allows a rapid assessment of whether or not two promoters may exhibit regions of sequence similarity.

We demonstrate the power of this method by discovering evolutionarily conserved sequences in the promoters of *Arabidopsis* circadian clock genes, including TOC1, LHY, CCA1 and LUX (PCL1). Within these sequences we demonstrate conservation of known transcription factor binding sites, and uncover putative novel elements. To test whether the elements detected are involved in the rhythmic transcription of these genes, we have developed software that performs a statistical test for over-representation of motifs in groups of genes with similar temporal patterns of expression. EARS is available online at <http://wsbc.warwick.ac.uk/ears/main.php>.

16002

THE CIS-ACTING ELEMENTS IN COLD AND DEHYDRATION INDUCIBLE PROMOTERS OF ARABIDOPSIS AND RICE

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Abstract: Low temperature and dehydration are adverse environmental conditions that affect plant growth and productivity. Many genes have been described that respond to both stresses at the transcriptional level, and their gene products are thought to function in stress tolerance and response even though these stresses are quite different. The *cis*-acting elements that function in stress-responsive gene expression have been analyzed to elucidate the molecular mechanisms of gene expression in response to these stresses. We have reported that DREB1A/CBF3 and AREB family specifically interact with the dehydration-responsive element (DRE) and ABA-responsive element (ABRE), respectively. DRE and ABRE are found in many cold- and dehydration-inducible promoters. However, very few attempts have been made to clarify general tendency of promoter sequences in cold- or dehydration-inducible genes. In our current study, we investigated all ($4^6=4096$) hexamer sequences in promoters of *A. thaliana* and *O. sativa* and demonstrate general tendencies of conserved sequences in cold- and/or dehydration-inducible promoters. The DRE and Evening Element are the highest and the second highest conserved sequences in cold-inducible promoters of *A. thaliana*, respectively. The ABRE and G box are the highest and the second highest conserved sequences in dehydration-inducible promoters of *A. thaliana*, respectively. In addition to these elements, we found several other conserved sequences in cold- or dehydration-inducible promoters of *A. thaliana* and *O. sativa*. Moreover, representative conserved sequences in promoters of downstream genes of DREB1A/CBF3 and AREB family are similar to those in cold- and dehydration-inducible promoters, respectively. Our promoter analyses suggest that DREB1A/CBF3 and AREB families play major roles in cold- and dehydration-inducible genes expression, respectively.

16003

RANK OF CORRELATION COEFFICIENT AS A COMPARABLE MEASURE FOR BIOLOGICAL SIGNIFICANCE OF GENE COEXPRESSION

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Information regarding gene coexpression is useful to predict gene function. Several databases have been constructed for gene coexpression in model organisms based on a large amount of publicly available gene expression data measured by GeneChip platforms. In

these databases, Pearson's correlation coefficients (PCCs) of gene expression patterns are widely used as a measure of gene coexpression. Although the coexpression measure or GeneChip summarization method affects the performance of the gene coexpression database, previous studies for these calculation procedures were tested with only a small number of samples and a particular species. To evaluate the effectiveness of coexpression measures, assessments with large-scale microarray data are required. We first examined characteristics of PCC and found that the optimal PCC threshold to retrieve functionally related genes was affected by the method of gene expression database construction and the target gene function. In addition, we found that this problem could be overcome when we used correlation ranks instead of correlation values. This observation was evaluated by large-scale gene expression data for four species: *Arabidopsis*, human, mouse and rat. The rank-based coexpression data is available in our coexpression databases, ATTED-II (<http://atted.jp>) for *Arabidopsis* and rice, COXPRESdb (<http://coxpresdb.jp>) for 7 animal species.

16004

PLANT SNP ANNOTATION ANALYSIS USING A WEB-BASED PIPELINE TOOL FOR NEXT-GENERATION SEQUENCING DATA

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High throughput sequencing is an increasingly important tool in biomedical research, due in part to rapidity, precision and cost-effectiveness of next-generation sequencer (NGS). Tools for automated or semi-automated analysis of sequencing reads would increase the utility of NGS for molecular biologists. In June 2009, the DNA Data Bank of Japan (DDBJ) released DDBJ Read Archive (DRA), an archive database for NGS data. DRA collaboratively exchanges data with the Sequence Read Archive (SRA) at NCBI and the European Read Archive (ERA) and EBI. To annotate raw sequence data stored in DRA, we developed the DDBJ Read Annotation Pipeline, a semi-automated analytical tool. The proposed pipeline consists of two processes: basic analysis for genome mapping and de novo assembly, and high-level analysis for structural and functional annotations such as single nucleotide polymorphism (SNP) detection and expression tag counts.

The pipeline has three distinct features. First, analytical results may be easily submitted to DDBJ databases using a streamlined process, whereby map outputs are converted to DRA formats, and similarly the results of assembly/annotations are converted to DDBJ-based International Nucleotide Sequence Database (INSD) formats (<http://www.insdc.org/>). Second, a web-based graphical user interface enables biologists without high-level bioinformatics expertise to analyse large amounts of raw sequencing data. Third, the use of cluster computing systems and computers with large memory in DDBJ infrastructure allows for high throughput. To accomplish basic analysis functions, we installed popular mapping and assembly tools including bowtie, velvet and others. For high-level analysis, analytical tools for SNP detection have been implemented in the current pipeline system. Other annotation tools will be implemented in the future version. In this report, we demonstrate pipeline analysis of reference mapping and genomic variation from plant short reads.

16005

MATHEMATICAL BIO-INFORMATION SYNTHESIZING FROM NEXT GENERATION SEQUENCING AND TILLING ARRAY DATA OF ARABIDOPSIS

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An observed transcript's information has increased explosively by the emergence of next-generation sequencer. The technology enables users to try a new complicated analysis apart from like simple counting of RNA tags. Now, tiling array has also plenty tags and can cover the transcriptions of whole genome by one experiment. In addition, we discovered that the correlations matrix made with tiling array probes of many experiments shows transcription units clearly. By effectively using these characteristics, we developed a mathematical method called "ARTADE2.0" for genome-wide identification of transcription units of *Arabidopsis thaliana* from tiling arrays of many experiments. We used Affymetrix genome tiling array results from eighteen conditions, including plants under various stresses or several organs. Moreover, it is considered that the more stack observations of other aspects the clearer gene structure or system would be seen. We therefore enhanced the method by embedding new kind of transcription data. Here, we implemented 3 millions of CAGE (Cap Analysis Gene Expression) tags from plants under non-treated condition, drought or hormone-treated conditions to the method. The new method can predict more than 18000 TU with marking high precision which was higher than 91.5% for known TUs compared to 84.6% from the result of one experiment version (ARTADE1.2.2.1). In the result, we found more than a thousands novel gene structures which were not predicted previously. Then, CAGE bursts 5' predictions power from the comparison with result of non CAGE use. The availability for implementation of RNA-sequence data will be also described. We show techniques for the synthesizing of multi kinds' transcript information through mathematical and statistical approach.

16006

DEVELOPMENT OF AN *IN SILICO* CIS-ELEMENT PREDICTION PROGRAM, MAMA, AND APPLICATION TO *ORYZA SATIVA*, *ARABIDOPSIS THALIANA*, AND *HOMO SAPIENS*

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Cis-elements are indispensable factors for controlling gene expressions under various conditions. *In silico* methods to predict *cis*-elements greatly help the understanding of the controlling mechanism of genes. Among *cis*-elements, transcription factor binding sites are essential genomic sequences for the regulation of gene expression. We found that some *cis*-element sequences, together with some adjacent bases, are overrepresented especially within 500 bp upstream from transcription start site of their putative regulated genes. This tendency was clearly correlated with gene expression ratios in microarray data. Based on this finding, we developed a novel *in silico* method to predict *cis*-element sequences using microarray data. All 7-10 base-long sequences within 500 bases upstream from transcription start site of regulated genes were searched in the upstream sequences of all genes on microarray data as motif candidates. When a similar sequence was found on upstream sequence of a gene, the number and length of sequences matched to the candidate sequence were associated with expression ratio of the gene on microarray data to evaluate accumulation of similar sequences in regulated genes. This *in silico* method, Microarray Associated Motif Analysis method (MAMA), was applied to microarray data of *Oryza sativa*, *Arabidopsis thaliana* and *Homo sapiens*, and several candidates of novel *cis*-acting elements were extracted together with significant overlap to known *cis*-acting elements. Furthermore, many predicted motifs were co-localized in the same upstream sequences of regulated genes. This suggested the cooperative regulation of gene expression by several *cis*-elements. These results demonstrated that MAMA is useful for

finding novel functional *cis*-elements.

16007

MASCP GATOR: AN AGGREGATING PORTAL FOR PROTEOMIC DATA

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The plant research community has widely adopted and integrated proteomics as part of their research agenda. There are currently a number of online *Arabidopsis* proteomics resources that house both primary data and integrated studies. Unfortunately these resources exist independently, and lack a level of integration that makes for easy analysis. To address this lack of data integration, a portal application has been developed to integrate these disparate resources - presenting proteomic experimental data in a summarized single location. The portal operates through the input of a single AGI, with the application then retrieving mass spectrometric, protein modifications, and sub-cellular localisation data from a variety of external sources on demand. This data is collated and summarised, taking advantage of the contextual amino acid sequence to present the data in a way that assists with easy comprehension by the user. Researchers within the *Arabidopsis* proteomics community have co-ordinated their online resources through standardizations to develop the new portal. This collaborative effort involved modifying existing resources to enable programmatic access to the data, and agreeing upon common protocols for the exchange of data. As well as developing the standalone portal, the resources used to create this portal are all freely available as components for others to integrate into their websites to make their own proteomic data mash-ups.

16008

EXTENDED FLOWERING PATHWAYS IN ARABIDOPSIS BASED ON INTEGRATIVE LINKS BETWEEN PROTEIN-PROTEIN AND MIRNA-TARGET INTERACTIONS

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Flowering is a complex biological system involving several pathways such as circadian, photoperiodic, and vernalization. MicroRNA (miRNA) is a class of non-coding small RNAs that regulate gene expression in post-transcriptional and translational levels. Here, we investigated miRNAs that might affect protein-protein interactions in flowering. Specifically, we computationally identified the integrative links between reported protein-protein interactions (PPIs) obtained from TAIR9 (<http://www.arabidopsis.org>) and our miRNA-target interactions (MTIs), predicted from secondary structures of miRNA-target duplexes between *Arabidopsis* miRNAs from miRBase and TAIR9 cDNAs. Flowering-related genes were categorized based on GO terms into five datasets: "circadian," "flower," "gibberellin," "vernalization," and "photoperiod," and searched against the known PPIs and predicted MTIs. The resulted network consists of 91 MTIs of 34 miRNA families and 51 PPIs of 60 proteins. Fifteen genes/proteins (e.g., CKB3, LKP2, FUS1, TFL2, SWN, DCL1) are identified as links between PPIs and MTIs. CKB3, for instance, a potential target of miR397, interacts with core clock components CCA1 and LHY1. LKP2 in circadian pathway interacts with COL1 which is potentially targeted by miR847. GI, PHYA, and PHYB proteins in photoperiodic pathway interact with FUS1, whose mRNA is potentially targeted by miR869. In vernalization, TFL2 and SWN are potentially targeted by miR414. To discover additional links, neighbors of neighbors of known PPIs were also used to search against the MTIs. This resulted in 12 PPIs of 14 proteins that are conserved across the datasets and 4 out of 14 proteins are identified as potential targets of miR173, miR847, and miR414. This larger network brings common interactions that might affect flowering over the boundary of individual pathways. These extended networks highlight a more comprehensive view of possibly involving mechanisms in pathways of flowering.

16009

EXPLORING PREDICTED TARGETS AND BINDING SPECIFICITY OF CONSERVED MIRNAS ACROSS PLANT SPECIES

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MiRNA target prediction in plants was straightforward with the scanning for nearly perfect binding sequences between a miRNA and its targets. This concept was then devised in following work with empirical parameters from target binding sites of some *Arabidopsis* miRNA families. To expand the understanding of miRNA-target recognition in plants, here, we computationally explored the conservation of miRNA targets and binding site specificities across plant species and miRNA families. We obtained miRNAs of 25 plants from miRBase and identified 14 miRNA families: miR156, miR159, miR160, miR164, miR166, miR167, miR168, miR169, miR171, miR172, miR319, miR395, miR396, and miR397 that are mostly common across 9 plants (6 eudicots and 3 monocots) and *Arabidopsis*. The secondary structures of duplexes between these common miRNAs and EST sequences of corresponding plants compiled from PlantGDB were predicted for potential targets. Functions of all predicted targets (e.g., miR156: SBP-box gene family, miR167: auxin response factor, and miR169: CCAAT-binding factor) across plant species were conserved with validated targets of *Arabidopsis*. Binding sites of these predicted targets were then analyzed for their specificity. We found that patterns of binding sites differ across miRNA families but are conserved across most plant species within a family. For instances, most target binding sites of miR172 uniquely contain GU pair at position 7 while binding sites of miR167 mostly have mismatches at positions 1, 2, and 14. Binding positions 1-19 are mostly complementary matches in miR396. While these specificities are identified, the disallowed mismatch at positions 10-11 from previous empirical work are still preserved. These discovered patterns enable the refinement of computational methods in plant miRNA-target prediction and target family classification. Also, they provide a good starting point for explaining the site accessibility between a miRNA and its target in a 3D structural model.

17001

MITOCHONDRIAL GENE EXPRESSION IN HETEROPALSMIC INDIVIDUALS

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The regulation of gene expression in plant mitochondria is still underinvestigated. Differences in the mitochondrial (mt) DNA and

nuclear-encoded factors were found to underly mt RNA variation among accessions of *Arabidopsis thaliana* (Forner et al. 2008). However, the extent of mt DNA polymorphism in *A. thaliana* is rather limited, confined to non-coding regions. Additional plant models are necessary to better understand the complex processes associated with large mt genomes of flowering plants. Whereas most plant species have highly conserved mt protein coding genes, there are some taxa with elevated substitution rates. *Silene vulgaris* (Caryophyllaceae) shows high variation in mt DNA caused by both DNA rearrangements and nucleotide substitutions. Occasional co-existence of several different copies of mt genome in the same individual (heteroplasmy) caused by rare paternal inheritance of mt DNA was found in this species. It makes possible to study the impact of heteroplasmy on gene expression and the interaction of two or more different "alleles" of mt genes. We found that re-arrangements of DNA led to the changes of transcription pattern of several mt genes in *S. vulgaris* (Elansary et al. 2010). Multiple transcription start sites and co-transcription with adjacent genes were responsible for this mRNA polymorphism. Here, we demonstrate that the transcript levels do not correspond to respective *atp1* copy numbers in heteroplasmic individuals of *S. vulgaris*. We also used sequence similarity with known *A. thaliana* genes to search for *S. vulgaris* homologs, which could be involved in mt DNA recombination and gene transcription.

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17002

TRANSLATIONAL CONTROL DURING THE G2/M TRANSITION OF THE CELL CYCLE

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Plant cell division is a highly coordinated process. G1-to-S and G2-to-M transitions are governed by cyclins and cyclin-dependent kinases and a significant amount of the genome is transcribed in a cell cycle phase-dependent manner. Although this indicates the importance of transcriptional activation for cell cycle progression, mRNA stability or decay and translational control mechanisms are largely responsible for shaping the cell cycle phase specific proteome. To gain insight in translational control aspects during mitosis in plants we performed a genome-wide expression analysis of the G2-to-M transition in synchronised tobacco BY-2 cells. A comparative analysis between total and ribosome-associated mRNA samples indicated that only 20% of the identified transcripts was loaded on the ribosomes. At least 160 transcripts were specifically present in mitotic ribosomes, while showing no differential expression characteristics in total RNA samples. These transcripts are hence subject to a translational control mechanism during the onset of mitosis, demonstrating the impact of translational control on cell cycle progression in plants. In addition, by making use of a protoplast bioassay and bi-cistronic gene constructs that contained the 5'-untranslated region from *Arabidopsis* homologues we demonstrate the potential involvement of internal ribosome entry site (IRES) elements in translational control mechanisms of the plant cell cycle.

17003

ARABIDOPSIS PSEUDO-RESPONSE REGULATORS 9, 7 AND 5 ARE TRANSCRIPTIONAL REPRESSORS IN THE CIRCADIAN CLOCK

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An interlocking transcriptional-translational feedback loop of clock-associated genes is thought to be the clock's 'central oscillator' in *Arabidopsis thaliana*. Recent genetic studies have suggested that *PSEUDO-RESPONSE REGULATOR 9* (*PRR9*), *PRR7*, and *PRR5* act within or close to the loop, however their molecular functions remain unknown. In this presentation, we demonstrate that *PRR9*, *PRR7*, and *PRR5* protein act as transcriptional repressors of *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*). Each *PRR9*, *PRR7*, and *PRR5* suppresses *CCA1* and *LHY* promoter activities, and confers transcriptional repressor activity to a heterologous DNA binding protein in a transient reporter assay. Using a glucocorticoid-induced *PRR5-GR* (glucocorticoid receptor) construct, we found that *PRR5* directly down-regulates *CCA1* and *LHY* expression. Furthermore, *PRR9*, *PRR7*, and *PRR5* associate with the *CCA1* and *LHY* promoters *in vivo* from morning to midnight, coinciding with the time these genes are repressed. These results suggest that the repressor activities of *PRR9*, *PRR7* and *PRR5* on the *CCA1* and *LHY* promoter regions constitute the molecular mechanism that accounts for the role of three PRR proteins in the feedback loop of the circadian clock.

17004

FUNCTIONAL ANALYSIS OF PLANT GLUTAMATE RECEPTORS (GLRs)

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Amino acids have long been suggested in N signaling, but the mechanisms for amino acid perception are far from being understood. Searches for nitrogen sensors resulted in the identification of 20 genes in *Arabidopsis* with sequence and structural homology to mammalian ionotropic glutamate receptors (iGluRs), known as glutamate-like receptors (GLRs)(1). Mammalian ionotropic GluRs are known to sense extracellular glutamate and regulate membrane potential of synapses through conduction of cations(2). While evidence is emerging that plant GLRs also function as non-selective cation channels, the function of plant glutamate receptors *in vivo* is not well understood, particularly with regard to ligand specificity(3).

Plant GLRs are homologous to NMDA receptors that require the formation of tetramers or pentamers for channel functionality(4). This research aims to investigate whether these proteins also require the formation of oligomers to form a functional channel. Interaction between different GLR subunits have been examined using a modified yeast-2-hybrid technique known as mating-based split ubiquitin system. Additionally, Foerster Resonance Energy Transfer (FRET) will be utilized to examine intra- and inter-molecular interactions of GLR genes. Preliminary results suggest that AtGLR3.3 and 3.4, mutations in which were shown to attenuate glutamate responses (5), may interact with each other. These constructs, along with other interacting GLRs, will be used to analyze conformational changes induced by potential ligands of these proteins.

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17005

COMPARISON OF CONSENSUS BINDING-SITE SEQUENCES OF SEVEN AP2-TYPE TRANSCRIPTION FACTORS OF ARABIDOPSIS THALIANA WITH TWO AP2 DNA-BINDING DOMAINS

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Arabidopsis thaliana contains 14 APETALA2 (AP2)-type transcription factors (TFs) with two AP2 DNA-binding domains, which include important regulators of meristem function, organ development, and seed oil accumulation. We used a simplified random-sequence oligonucleotide selection to compare consensus binding-site sequences (CBSSs) of them. The CBSSs of seven AP2-type TFs fell into three groups that correspond to three protein subfamilies based on structural similarities of the dual AP2 domain, and they were highly similar among TFs within the same subfamily. The CBSS common to two WRINKLED1 (WRI1) subfamily TFs, 5'-[CnTnG]_n[CCGCC]-3', where n represents any nucleotides, was similar to that shared by three AINTEGUMENTA (ANT) subfamily TFs, 5'-[CnTnG]_n[GTGCC]-3'. DNA-binding experiments with mutant forms of WRI1, in which the R1 and R2 AP2 domains were deleted or swapped with the respective AP2 domains of ANT, suggested that the presence of both AP2 domains is required for DNA binding and that the R1 domain plays central role in the recognition of CCGCC or GTGCC core motif. Unlike TFs in the WRI1 and ANT subfamilies, the CBSSs of two AP2 subfamily TFs, AP2 and TARGET OF EAT3 (TOE3), both consisted of eight consecutive bases, 5'-ACGTTGTT-3', and the DNA-binding activity of AP2 was not abolished by deletion of the R2 AP2 domain. These results suggest that AP2 subfamily TFs actually function as single AP2 domain TFs.

17006

STRUCTURE AND FUNCTION STUDIES OF SIZ1, A PIAS-TYPE SUMO E3 LIGASE FROM ARABIDOPSIS

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Arabidopsis SIZ1, a SUMO E3 ligase, is an ortholog of mammalian PIAS (Protein Inhibitor of Activated STAT) and yeast SIZ (SAP/Miz) proteins. SIZ1 plays a pivotal role in controlling SUMOylation, and disruption of its function has been reported to affect stress responses, growth, and development. We performed a structural and functional analysis of SIZ1 by determining phenotypes of *siz1* knockout mutants transformed with SIZ1 alleles carrying point mutations in predicted SIZ1 domains. SIZ1 contains all canonical domains of PIAS/SIZ-type proteins, such as the SAP, PINIT, SP-RING, and plant-specific PHD domains. Our study revealed that an intact zinc finger motif in the SP-RING domain as a major component of SUMOylation activity, and reliable nuclear localization of SIZ1. The SP-RING domain appears also as a feature that is involved in gauging salicylic acid levels. Mutations of the SP-RING domain of SIZ1 resulted in diminished plant size, enhanced plant defense capacity, ABA inhibition of cotyledon greening, and reduced thermotolerance. In contrast, alleles with mutationally altered PHD and PINIT domains of SIZ1 conferred a dominant long-hypocotyl phenotype that is dependent on sugar such as glucose and sucrose and light. Finally, the SXS domain was involved in SA-mediated ABA signaling during early growth after germination. The results indicate that the diverse phenotypes that are characteristic of SIZ1 protein activity are associated with specific domains that can be functionally separated.

17007

THE GENETIC BASIS OF HETEROSES IN A HIGHLY HETEROSES RICE HYBRID

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We have been investigating genetic and molecular bases of yield and heterosis in rice using a combination of approaches, with the ultimate goal to achieve a comprehensive understanding of the biology of yield and heterosis. We focused the effort in yield traits of Shanyou 63, a cross between Zhenshan 97 and Minghui 63, which is the mostly widely cultivated rice hybrid in China for more than two decades. Several genetic populations were developed from this cross, including F2, F2:3, recombinant inbred line (RIL) and "immortalized F2" populations. Hundreds of QTLs and digenic interactions for yield traits and heterosis have been detected from analyses of these populations and several QTLs have been cloned providing data for evaluating contributions of individual loci. For complete dissection of the genetic effects for yield heterosis in this cross, we constructed a bin map composed of ultra-high density SNPs based on low-depth sequences of RILs using next generation sequencing. Analysis of the immortalized F2, specifically designed for dissecting the genetic basis of heterosis, revealed dominant effects in the majority of the bins. In most cases, heterozygotes performed better than the means of the two homozygotes, although the magnitudes were variable and mostly small. Thus, accumulation of small advantages of the heterozygotes over the mid-parents at large numbers of loci accounts for the genetic basis of heterosis in the F1 hybrid. Several lines of follow-up work have been undertaken to characterize the molecular basis underlying the genetic components of heterosis, including: construction of chromosomal segmental substitution lines to evaluate loci for yield and heterotic effects under near isogenic backgrounds; transcriptomes of the parents, the hybrid and RILs to characterize the regulatory network and to associate the genotypes with phenotypes, and; molecular cloning and characterizing major loci contributing to yield and heterosis.

17008

INTERACTION ANALYSES OF ARABIDOPSIS TUBBY-LIKE PROTEINS WITH ASK PROTEINS

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In this study, we have investigated protein-protein interactions between 10 *Arabidopsis* TUBBY-like proteins (AtTLPs) and various *Arabidopsis* Skp1-like proteins (ASKs) using the yeast two-hybrid system. The results indicate that six AtTLPs (AtTLP1, 2, 3, 9, 10, 11) are able to specifically interact with ASK1. AtTLP6 is able to interact with ASK1, ASK2, and ASK11, while AtTLP5, AtTLP7, and AtTLP8 did not show any significant interaction with any ASK protein. Serial deletion analyses of AtTLP2 have demonstrated that both F-box domain and tubby domain are required for AtTLP2-ASK1 interaction. Domain swapping suggests that variations at the N-terminus of AtTLP7 and C-terminus of AtTLP5 result in the inability to interact with ASK1. Taking these observations together, we propose that AtTLP1, 2, 3, 9, 10,

and 11 can specifically interact with ASK1 and may have an overlapping function. Since AtLPS5, 7, and 8 cannot interact with any ASK, it is suggested that they may have lost their original function(s) and/or acquired new function(s) during evolution.

17009

COMPONENTS OF THE SAME LIGAND-RECEPTOR SIGNALING SYSTEM CONTROL CELL SEPARATION BOTH DURING FLORAL ORGAN ABSCISSION AND LATERAL ROOT EMERGENCE

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Small peptides can act as signaling molecules that coordinate development, growth and differentiation. *INFLORESCENCE DEFICIENT IN ABSCISSION (IDA)* encodes a putative peptide ligand necessary for the regulation of floral organ abscission in *Arabidopsis*. IDA is expressed specifically in the abscission zone at the base of the organ to be shed, and it is dependent on the two receptor-like kinases (RLK) HESA (HAE) and HESA-LIKE 2 (HSL2) to exert its function (Butenko et al., *Plant Cell*, 2003; Stenvik et al., *Plant Cell*, 2008; Cho et al., *PNAS*, 2008). The *ida* and *hae hsl2* mutations block the final cell separation step. Based on the expression pattern of members of a small family of *IDA-LIKE (IDL)* genes and other HSL genes, we have suggested that similar ligand-receptor pairs are involved in other cell separation processes (Butenko et al., *Trends Plant Sci*, 2009). The active part of the IDA/IDL ligands resides in a short C-terminal conserved region (EPIP), and synthetic EPIP peptides can trigger signaling. Ethylene is known to promote abscission, but IDA-HAE-HSL2 represents an ethylene-independent signaling pathway. Recently we found, however, that *IDA* is auxin-inducible in the root. During lateral root (LR) development *IDA* is expressed in cortex and epidermal cells overlying lateral root primordia. These cells have to separate to allow LR emergence. Mutation in *IDA* not only leads to total deficiency in floral organ abscission, but also to a delay in LR emergence, suggesting that *IDA* plays a similar role during these two cell separation events. Consistent with this, mutation in a putative *IDA* receptor results in a similar defect in LR emergence. Thus, a similar signaling system seems to be used for cell separation in two different developmental processes.

17010

ROLE OF WHIRLY PROTEINS IN ARABIDOPSIS ORGANELLE GENOME REPAIR

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DNA double-strand breaks are highly detrimental to all organisms and need to be quickly and accurately repaired. Although several proteins are known to maintain chloroplast and mitochondria genome stability in plants, little is known about the mechanisms of DNA repair in these organelles. Recently, the proteins AtWhy1 and AtWhy3 were shown to maintain chloroplast genome stability. Indeed, in the absence of these proteins, microhomology-mediated DNA rearrangements accumulate in the chloroplast genome. AtWhy1 and AtWhy3 belong to the Whirly family of proteins whose members are mainly found in the plant kingdom. Whirly proteins bind single-stranded DNA and perform multiple tasks in DNA metabolism ranging from transcription regulation to telomere homeostasis. In *Arabidopsis*, AtWhy1 and AtWhy3 are targeted to the chloroplasts whereas AtWhy2 is targeted to the mitochondria. To verify if Whirly proteins play a role in plant organelle DNA repair, we monitored the effects of DNA-damaging agents in *Arabidopsis* plants lacking either mitochondria- or chloroplast-targeted Whirly proteins. Our experiments suggest that Whirly proteins are involved in the accurate repair of double-stranded DNA breaks. To gain molecular insights on the role of Whirly proteins in this process, we also solved the crystal structure of a Whirly protein in complex with single-stranded DNA.

17011

POLYPLOIDY AND HYBRID VIGOR

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Hybrid plants such as maize or sorghum grow bigger and stronger than their parents. This is also true for allopolyploids such as wheat or cotton that contain two or more sets of chromosomes from different species. The phenomenon, known as hybrid vigor or heterosis, was systematically characterized by Charles Darwin in 1876. Although genetic models for heterosis have been rigorously tested, the molecular bases remain elusive. Using *Arabidopsis* as a model system, we have shown the roles of nonadditive gene expression, small RNAs, and epigenetic regulation, including circadian-mediated metabolic pathways, in hybrid vigor, which could lead to better use and exploitation of the increased biomass and yield in hybrids and allopolyploids for food, feed, and fuels.

17012

HYBRID INCOMPATIBILITY IN ARABIDOPSIS THALIANA

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Epistasis—the nonadditive interaction between genes—critically determines the performance of hybrid genotypes. Hybrid necrosis is an example of a deleterious epistatic interaction observed in many intra- and interspecific plant crosses. We have developed *A. thaliana* for the study of this recurrent phenomenon in plant evolution. About two percent of intraspecific crosses yield F1 progeny that express hybrid necrosis. Several independent cases result from epistatic interactions that trigger autoimmune-like responses. I will discuss our progress in identifying the causal genes, both for several crosses with hybrid necrosis and for two non-immune hybrid weakness cases.

17013

ABCD, ANALYSIS BASED ON COPY NUMBER OF GENOMIC DNA, METHOD REVEALS THE NUMBER OF TRANSCRIPTS, PROTEINS AND METABOLITES IN A CELL OF ARABIDOPSIS

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Cellular levels of transcripts, proteins, and metabolites are usually quantified relative to the value for a known, reliable, cellular component. When the factor used to normalize these values remains constant between samples, quantification of transcripts, proteins, or metabolites is straightforward. However, normalization factors often vary among samples from distinct or novel mutants and thus

must be developed for each new analytical method. Here we demonstrate quantification transcript, protein, and metabolite levels in Arabidopsis based on genomic DNA copy number, which is comparatively constant between cells. We extracted total nucleic acid from Arabidopsis rosette leaves, and quantified the number of transcripts by qRT-PCR using the genomic DNA copy number for normalization. Assuming that each cell was diploid, each cell of 3-week-old Arabidopsis rosette leaf was calculated to have 3.0×10^3 transcripts of the large subunit of Rubisco gene (RBC-L), 3.9×10^3 transcript of RBC-S and 5.6×10^5 18S rRNA. We similarly quantified the numbers of RBC-L protein and chlorophyll (i.e., metabolite) in extracts of the wild-type plant, and DNA copy number was determined by direct qPCR analysis of the extract using a DNA polymerase with tolerance to a wide range of common PCR inhibitors. Assuming that each cell was diploid, we determined that each cell in a 3-week-old Arabidopsis rosette leaf had 1.21×10^8 RBC-L molecules and 8.48×10^8 chlorophyll molecules; these values had relatively low coefficients of variation, underscoring the reliability of our method. Thus, normalization to genomic DNA copy number provides an easy and accurate method for determining the number of transcripts, proteins, and metabolites in a cell-potentially from any species. We designated the quantification method as ABCD (Analysis Based on the Copy number of genomic DNA) method.

17014

HOW IS THE FLOWERING REGULATED IN DAY-NEUTRAL PLANT, TOMATO?

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The Flowering Locus T (FT) gene of Arabidopsis integrates long-day flowering signals and promotes flowering induction. Hd3a, a rice homologue of FT, also functions as a central flowering promoter in rice, a short-day plant. For both, it is now believed that the pathway from the day-length signal to FT regulation is broadly conserved in plant species. In day-neutral plants, however, the function and regulation of FT homologues are not well characterized.

In tomato, three FT homologues - SP3D, SP5G and SP6A - have been cloned. Since the late-flowering mutant, single flower truss (sft), has a mutation in the SP3D gene, it is considered to be an FT orthologue in tomato. However, the functions of SP5G and SP6A in tomato flowering remain unclear. In Arabidopsis, FT expression shows diurnal pattern; it is low at dawn, increases through the day and peaks at dusk. To investigate the relationship between day-length and expression of FT homologues in tomato, we examined the expression of SP3D, SP5G, and SP6A under long- and short-day conditions. In contrast to the domesticated tomato cultivar, some of tomato wild relatives show short-day flowering habit. We also examined the expression pattern of SP genes as well as tomato CONSTANS and GIGANTEA homologue genes in these wild relatives.

We made over-expression and knockdown transgenic plants of SP3D, SP5G, and SP6A to observe flowering time and other phenotypes and speculate their functions. We will present these results and discuss about the flowering pathway of day-neutral tomato: how FT homologous genes contribute it.

17015

DEFECTS IN PLASTID DIVISION AND PLANT GROSS MORPHOLOGY OF CRUMPLED LEAF MUTANT OF ARABIDOPSIS THALIANA IS ENHANCED BY FTSZ1-1

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A mutation in *CRUMPLED LEAF* (*CRL*) of *Arabidopsis thaliana* causes a severe defect in plastid division and generates cells without detectable plastids. In addition, the mutation causes distortion of cell division planes and aberrant cell differentiation. *FtsZ1-1* of *A. thaliana* is another gene that is involved in plastid division. Mutations in *FtsZ1-1* also cause a severe defect in chloroplast division. To investigate the genetic interaction between *CRL* and *FtsZ1-1*, we generated and analyzed a line that segregates *crl ftsZ1-1* double mutants, using null alleles of these genes. The number of chloroplast in cells was reduced in the double mutant in comparison to either *crl* or *ftsZ1-1*. This result indicated that *CRL* is involved in a plastid division pathway that is different from the *FtsZ*-dependent one. Besides this, our whole mount *in situ* immunostaining of *FtsZ* revealed that most chloroplasts had multiple *FtsZ* rings in *crl*. Thus *CRL* is also involved in the suppression of the *FtsZ* ring formation except for the plastid constriction site. Morphological analysis of the double mutant revealed that *ftsZ1-1* mutation enhances morphological abnormalities of the *crl* plants. Namely, most of *crl ftsZ1-1* embryos had more than three cotyledons. Size of shoot apical meristem of *crl ftsZ1-1* embryos was enlarged. Some embryos of the double mutants had more than two shoot apical meristems. The seedlings of the double mutants showed aberrant gross morphology of plants: small, pale, and with narrow and asymmetric leaves. Considering that *FtsZ1-1* functions exclusively in plastid division, our results suggested that the suppression of plastid division is one of the causes of the aberrant gross morphology of the *crl* plants.

17016

CIRCADIAN RHYTHM OF ARABIDOPSIS PETAL MOVEMENT

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Some flowers open in the morning. And some ones do in the evening. Linne proposed the idea of the floral clock which was made up of various kinds of flowers opening at different time each other. Recently, variation of the timing among closely related plant species in the field has been studied on aspect of the differentiation of reproductive timing. Here, we present chronobiological features of flower opening in *Arabidopsis thaliana*. The plant opens flower in the morning, but the detail is unknown. Then, we examined it under diurnal light/dark cycle. The petal began the expansion when lamp started illumination. Then, it opened completely for 3 hours in the light. We examined whether the rhythm continued in light without the daily dark periods. Observation of four days in light showed the rhythm with 23 hours period. The period was not changed in low or high temperature. In addition, the rhythm was entrained by external light/dark cycles. These properties are essential to circadian rhythm. Thus, *Arabidopsis* petal movement is under circadian clock. The chronobiological properties will allow us to know signal transduction of the movement under circadian clock.

17017

NOVEL SELF-COMPATIBLE LINES OF BRASSICA RAPA L. ISOLATED FROM TRADITIONAL AND COMMERCIAL POPULATIONS

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Self-incompatibility (SI) in Brassicaceae is controlled by the SRK and SP11/SCR genes situated at the S locus. Although a few of following downstream factors in SP11-SRK signaling cascade have been identified, a comprehensive understanding of the SI mechanism still remains in Brassicaceae. Analysis of self-compatible (SC) mutants is significant for understanding of the molecular mechanism on SI reaction, thus we screened SC lines from a variety of Japanese bulk-populations of *B. rapa* vegetables. Two lines, TSC4 and 28, seem to have disruptions in the SI signaling cascade, while other line, TSC2, seem to have a deficiency in a female S determinant, SRK. In TSC4 and 28, known SI-related factors, i.e. SRK, SP11, MLPK, THL, and ARK1, were expressed normally and their expression levels were comparable with those in SI line. On a *B. rapa* genetic linkage map, potential SC genes in TSC4 and 28 were mapped on linkage group A3 and A1, respectively, whereas MLPK, ARK1, and THL were mapped on A3, A4, and A6, respectively. Although potential SC gene of TSC4 and MLPK were on a same linkage group, their positions were apparently discriminated. These results indicate that the SC genes of TSC4 and 28 are independent from the S-locus or known SI-related genes. Thus, the SC lines selected here have mutations in novel factors of the SI signaling cascade and they will contribute to fill pieces in a signal transduction pathway of the SI system in Brassicaceae.

17018

THE INTERNATIONAL ORYZA MAP ALIGNMENT PROJECT (I-OMAP): A GOLDEN PATH TO UNLOCK THE GENETIC POTENTIAL OF THE WILD RELATIVES OF RICE

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The availability of close comparative systems provides one with the ability to address basic and applied questions within a robust phylogenetic framework. After completion of the rice genome in 2004, our consortium set out to create a closed experimental system for the genus *Oryza* which contains 23 species (2 cultivated species; 10 distinct genome types; 6 diploid & 4 polyploid species; 3.6 genome size variation; wide distribution & habits) under the rubric – the International *Oryza* Map Alignment Project (I-OMAP: www omap.org). The wild relatives of rice contain a virtually untapped reservoir of agriculturally important genes that could be used to improve the world's most important food crop. The core I-OMAP resource is composed of 17 deep-coverage BAC libraries, 13 BAC fingerprint/end sequenced physical maps aligned to the rice reference genome, and 9 chromosome 3 short arm sequences. These physical maps serve as entry points to virtually any region of the collective *Oryza* genome for interrogation (e.g. *Adh1*, *Moc1*, *Hd1*, *Cen8*), as well as scaffolds to guide reference genome assembly. In November 2009 the I-OMAP consortia developed a plan to generate high-quality reference genome sequences for representatives of all 14 diploid *Oryza* species, and the outgroup *Leersia perrieri*, by mid 2011. Our goal is to provide the highest quality reference genome sequences as possible, which can then be used as resequencing templates to capture allelic diversity, and structural variation for functional and evolutionary studies.

Recent analyses of the I-OMAP system will be presented, along with an update of the communities' efforts to generate RefSeqs for all diploid *Oryza*: including the newly completed *Oryza glaberrima* (West African cultivated rice) RefSeq.

17019

THE BASIS OF RAPID GENOME SIZE CHANGE IN ARABIDOPSIS

*Detlef Weigel (Max Planck Institute for Developmental Biology, Germany)

In 2005, the Joint Genome Institutes of the US Department of Energy approved a Community Sequencing Proposal to produce high-quality reference genomes for the two *Arabidopsis thaliana* relatives *A. lyrata* and *Capsella rubella*. I will present our analysis of the *A. lyrata* genome, based on a collaboration between the groups of Mikhail Nasrallah (Cornell), Joy Bergelson (Univ. of Chicago), James C. Carrington (Oregon State Univ.), Brandon S. Gaut (UC Irvine), Jeremy Schmutz (Hudson Alpha), Klaus F. X. Mayer (MPI), Yves Van de Peer (VIB), Igor V. Grigoriev (JGI), Magnus Nordborg (USC/GMI) and my lab. The main folks in our labs responsible for this analysis were Tina T. Hu (UCS), Pedro Pattyn (VIB) and Ya-Long Guo (MPI).

Despite little variation in the types of genes they encode, the nuclear DNA content of angiosperms varies dramatically, from the tiny 64 Mb genome of the corkscrew plant *Genlisea* to the enormous 124 Gb genome of *Fritillaria* lilies. Several factors, in particular polyploidization and transposable elements (TEs), are known to be involved in causing these differences, but the responsible processes are poorly understood because genomes sufficiently closely related for accurate inference of evolutionary events have not been available. We have compared the genome sequence of *A. lyrata*, a self-incompatible perennial, with that of its close relative, the model plant *A. thaliana*, a self-compatible annual. The two species diverged about 10 million years ago (Mya), but at 125 Mb, the *A. thaliana* genome is much smaller than that of *A. lyrata*, which is more than 200 Mb. Although there is no closely related outgroup with which one can polarize differences between the two genomes, it is generally assumed that the small genome of *A. thaliana* constitutes the derived state for the family. Apparent shrinkage in genome size can be partially attributed to large-scale rearrangements involving the loss of three centromeres, but the main cause lies in the vast number—hundreds of thousands—of small deletions found throughout the genome, primarily in non-coding DNA and TEs, but also involving multi-gene families coding for proteins. Analysis of deletions segregating in *A. thaliana* indicates that the process is still ongoing, and suggests that it is due to pervasive selection for a smaller genome.

17020

COMPARATIVE ANALYSIS OF POLYPLOIDY, GENE LOSS AND CHROMOSOMAL REARRANGEMENTS IN THE SISTER-FAMILIES BRASSICACEAE AND CLEOMACEAE

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Ancient whole genome duplication (WGD) and the associated processes of chromosomal repatterning, duplicate gene divergence and gene loss have greatly influenced the complexity of life on earth. Genomic data show most angiosperm lineages have at least one WGD in their history. Identifying evolutionary outcomes of WGDs is integral to understanding plant diversification and function. Comparative analyses of diverse species (i.e. *Arabidopsis* and grape) or within one family (i.e. grasses) have revealed significant patterns of genomic change after polyploidization. What is lacking are strategic basal-lineage and sister-family comparative analyses allowing for much more

precise ancestral genome reconstruction. Inferred ancestral genomes can serve as Rosetta stones to interpret processes of gene retention and loss, origin of novel genes and chromosomal rearrangements. The Brassicaceae is an ideal group for comparative genomics of WGD and ancestral genome reconstruction. First, it contains the premier model *Arabidopsis* and *Brassica* crops. Second, there are twelve-plus genome projects underway. Third, there is significant synteny across species. Fourth, phylogenetic results support a crown-group radiation, the genus *Aethionema* as basal and the Cleomaceae as its sister-group. Finally, the crown-group shares the alpha (α) WGD and Cleomaceae has an independent (Cs-α) WGD. I will discuss the beginning phases of a “crown-base-sister” comparative genomic analysis based on data from the crown Brassicaceae, the basal *Aethionema arabicum* and the sister-lineage *Cleome spinosa*. Analyses of WGD associated with a plant family radiation allows use to address fundamental questions about polyploidy, gene retention and chromosomal rearrangements and to translate findings to other lineages.

18001

FOCUSING ON PHOSPHORYLATION

Alex Jones (The Sainsbury Laboratory, UK)

The study of phosphorylation in *Arabidopsis* has benefited in recent years from several large-scale studies which have identified many thousands of phosphopeptides. However, some low abundance proteins, such as receptor like kinases, still need to be analyzed individually through targeted approaches such as in vitro expression and co- immunoprecipitation. I will discuss the online resources available to mine published information, before moving to experimental methods to maximize coverage and identification of phosphopeptides for selected proteins. Finally quantification of phosphorylation remains challenging and I will discuss some developments in this area.

18002

PHOSPHOPROTEOMICS IN PLANTS

Hirofumi Nakagami (RIKEN PSC, Japan)

Recent technological breakthroughs enabled large-scale *in vivo* phosphorylation site mapping, so-called shotgun phosphoproteomics. However, shotgun phosphoproteomics is not yet a mature technology, and progress is still rapid. The scope and limitations of current methods, and some key technological issues in this field will be discussed.

18003

AN ONLINE ARABIDOPSIS PROTEOMICS DATA AGGREGATOR

Hiren Joshi (Lawrence Berkeley National Laboratory, USA)

There is currently a number of useful *Arabidopsis* proteomic resources available online. While useful, these resources all exist independently. Thus obtaining a proteomic overview of a given protein requires visiting multiple sources. To address this lack of integration, a portal application has been developed to integrate the disparate resources - presenting proteomic experimental data in a single location. The MASC Proteomics community was tapped to develop the portal, and a new bioinformatic resource for the experimenter has been developed. Through the supply of a single AGI, the portal application retrieves mass spectrometric, phosphorylation, and sub-cellular localization data. This data is collated and summarized, taking advantage of the contextual sequence to present the data in a way that assists with comprehension.

18004

APPROACHES TO ACCURATE TRACKING AND ANALYSIS OF DATA FROM A LARGE FUNCTIONAL GENOMICS PROJECT

Robert L. Last (Michigan State Univ. USA)

The Chloroplast 2010 project (www.plastid.msu.edu) is a large reverse genetics functional genomics project that has queried >4,000 mutants for 85 traits. During the course of this project we have handled hundreds of thousands of samples and collected phenotypic data from a diverse set of assays ranging from morphology through LC-MS/MS metabolite data. Approaches for accurate tracking of plants and will be discussed, as will approaches to analyze data from single grow-outs or across four years of assays.

18005

QUANTIFICATION OF SPATIO-TEMPORAL GENE EXPRESSION OF ARABIDOPSIS LUCTAG LINE USING DIGITAL IMAGE SEQUENCE ANALYSIS

Eli Kaminuma (National Institute of Genetics, Center for Information Biology and DNA Data Bank of Japan, Japan)

To find the function of a gene, systematic analysis of phenotypes is vital. We have proposed a new methodology of mutant phenotypic analysis based on precise three-dimensional (3D) measurement by a laser range finder and micro X-ray computed tomography.

However, their measurement systems cannot acquire gene expression data. In this report, we introduce a method to quantify gene expression and location in time series of images. Concretely, we collected two-dimensional image sequence of gene expression using Luciferase tag (LucTag) lines developed in the RIKEN PSC by the ARGUS-50 system. In preliminary data, we found gene expression in the digital image data at shoot apical meristem, root apical meristem, lateral root, and so on. In special, difference of quantified expression values at the cotyledons and true leaves from the digital image data is discussed.

18006

ARABIDOPSIS PHENOTYPING AT THE AUSTRALIAN PLANT PHENOMICS FACILITY

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The Australian Plant Phenomics Facility is a \$50M investment in high throughput and high resolution phenotyping national facilities across two nodes; the High Resolution Plant Phenomics Centre in Canberra and the Plant Accelerator in Adelaide. The Canberra node was commissioned in August 2009 and the Adelaide facility in January 2010. The Canberra node is comprised of 4 modules, one specifically targeted toward model plants such as *Arabidopsis*. Plants are grown in growth cabinets in trays of 20 individual small pots. These trays are imaged from the top using chlorophyll fluorescence imaging, FIR imaging or RGB imaging to extract photosynthetic, stomatal and growth responses to abiotic and biotic stresses in high throughput (1500 plants per day). A variety of conveyor-belt based and in-cabinet imaging tools are used for these purposes. Individual pots can also be removed and imaged with side views to capture 3-D growth and visualise floral architecture. A bioinformatics capacity based on a Phenomics Ontology Driven Database (PODD) is under development

to support the facility. The technical infrastructure is reviewed in this presentation with some examples of the calculated data which has been extracted from non-invasive imaging.

18007

HIGH-THROUGHPUT PHENOTYPING OF ARABIDOPSIS SHOOT GROWTH UNDER DIFFERENT WATER DEFICIT TREATMENTS.

Olivier Loudet (INRA, National Institute for Agricultural Research Versailles, France)

To screen among either accessions, RILs or mutants, we have developed our own high-throughput phenotyping platform (*Phenoscope*) that can handle and treat hundreds of plants simultaneously in controlled conditions. We now have 2 robots like the one shown below, capable of moving ~1,500 individual plants throughout the growth chamber following a 4-hours cycle, while automatically adjusting soil water content (according to different established treatment programs) and taking pictures of the plants for further automatic leaf area analysis.

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Bomer, Moritz (15005)*	Caesar, Katharina (11041)*	Chen, Jui-Hung (03109)*
Bond, Charles (10003)	Caiyun, Yang (07008)	Chen, Li-mei (03039)
Bones, Atle M. (C504)	Caldana, Camila (03042)	Chen, Li-Qing (02021)
Bonetta, Dario (13009)	Calhoun, Christopher (C802)	Chen, Li-Teh (C404)
Bonetta, Dario (13010)	Campilho, Ana (08122)	Chen, Li-Yu (08066)*
Bonham-Smith, Peta (08075)	Campilho, Ana (08171)	Chen, Long-Fang O. (08110)
Bonnet, Eric (07003)*	Cao, Xiaofeng (04015)*	Chen, Meng (08014)*
Bonnot, Clemence (08036)	Cao, Xiaofeng (04029)	Chen, Mingsheng (C203)
Borges, Filipe (C405)*	Cappadocia, Laurent (17010)*	Chen, Mingsheng (02024)
Borges, Filipe (09018)	Cardarelli, Maura (08024)	Chen, Mingsheng (17018)
Boudolf, Veronique (11027)	Carlsbecker, Annelie (08122)	Chen, Po-Hong (17008)
Bourguignon, Jacques (03010)	Carmody, Melanie (03031)	Chen, Po-Hsuan (10011)

Chen, Po-Hsuan (12032)	Choi, Hong-Kyu (03048)	Clemence, Bonnot (C304)
Chen, Po-Hsuan (17008)	Choi, Hong-Kyu (03049)	Clement, Mathilde (C304)
CHEN, QINFANG (03004)	Choi, Hye-Kyung (02037)	Clement, Mathilde (08036)
Chen, Qin-Fang (06002)	Choi, Hyunmo (08078)	Clouse, Steven (12016)
Chen, Xiangbin (12005)	Choi, Jaemyung (02034)	Codreanu, Mirela-Corina (11004)
Chen, Xuemei (04016)	Choi, Seung Hee (08160)	COLOT, Vincent (C402)*
Chen, Ying (12056)	Choi, Seung-won (11043)*	Colot, Vincent (04037)
Chen, Yuling (17015)	Choi, Sunhwa (12012)	Colot, Vincent (08060)
Chen, Yutao (12028)	Choi, Sun-Mee (04039)*	Conn, Vanessa (03105)
Chen, Z. Jeffrey (04016)*	Choi, Wonkyun (09005)	Conrad, Udo (12034)
Chen, Z. Jeffrey (17011)*	Choi, Yang Do (03089)	Cook, Douglas R. (02029)
Cheng, Wan-Hsing (12024)*	Choi, Yang Do (12039)	Cook, Douglas R. (02031)
Cheng, Zhi Juan (12019)	Choi, Yang Do (12041)	Cordon, Nicole (15004)
Cheon, Choong-Ill (04023)	Choi, Yang Do (12042)	Cortijo, Sandra (04037)*
Cheon, Choong-Ill (08071)	Choi, Yeonhee (04032)	Coruzzi, Gloria M. (03078)
Cheong, Hyeonsook (08115)	Choi, Yeonhee (04043)	Costa de Oliveira, Antonio (17018)
Cheong, Jong-Joo (03069)	Choi, Yeonhee (08134)	Costantino, Paolo (08024)
Cheong, Jong-Joo (12039)	Choi, Yeonhee (08138)	Coupland, George (04020)
Cheong, Jong-Joo (12041)	Choi, Yeonhee (08154)	Coupland, George (08097)
Cheong, Jong-Joo (12042)	Choi, Yeonhee (12012)	Coutuer, Silvie (11031)
Cheong, Mi Sun (09005)	Chong, Kang (01004)	Covington, Michael (03014)
Cheong, Mi Sun (17006)*	Chong, Kang (12027)	Creff, Audrey (C304)
Chetty, Raymond (C803)	Chong, Kang (12031)	Creff, Audrey (08036)
Chhun, Tory (03045)	Chory, Joanne (08014)	Cremer, Julia (12030)
Chiang, Ming-Hau (12024)	Chory, Joanne (12009)	Crisp, Peter A. (03032)
Chiarenza, Serge (C304)	Chou, Po-Hsuan (08161)*	Crist, Deborah (C802)
Chiba, Yukako (02038)	Chow, Brenda (PL301)	Crist, Deborah (09007)
Chien, Chih-Cheng (02009)	Christendat, Dinesh (07013)	Crist, Deborah (09010)
Chien, Ching-Te (03109)	Christmann, Alexander (C302)	Cruz-Ramirez, Alfredo (08021)*
Cho, Chang Woo (02029)	Christov, Nikolai (03128)	Cuellar Perez, Amparo (12001)
Cho, Chang Woo (02031)	Christova, Petya (03128)	Cui, Haitao (C203)
Cho, Chang-Woo (03047)*	Chu, Jinfang (02024)	Cui, Haitao (02024)*
Cho, Chang-Woo (03048)	Chua, Nam-Hai (03066)	Cui, Xia (04029)
Cho, Chang-Woo (03049)	Chua, Nam-Hai (17003)	Curtis, Wilkerson (C603)
Cho, Chang-Woo (03050)*	Chung, Eui-Hwan (02011)*	Cutcliffe, James (12022)
Cho, Chul-Oh (03040)	Chung, Eun Sook (02029)	Cutler, Sean (09011)
Cho, Daeshik (11017)	Chung, Eun Sook (02031)	Cutler, Sean (12009)
Cho, Hong Joo (08131)*	Chung, Eunsook (03047)	Cvjetko, Petra (03090)*
Cho, Hui Kyong (11036)*	Chung, Eunsook (03048)*	Czemmel, Stefan (15004)*
Cho, Hyun Jung (08140)	Chung, Eunsook (03049)	da-Cunha, Luis (02011)
Cho, Hyung-Taeg (08086)	Chung, Eunsook (03050)	Dainobu, Tomoko (11044)
Cho, Hyung-Taeg (11026)*	Chung, Joo Hee (12050)	D'Angelo, Cecilia (08026)
Cho, Hyung-Taeg (12018)	Chung, Woo Sik (02055)	Dangl, Jeffery (02011)
Cho, Jung-Nam (04027)	Chung, Woo Sik (08106)	Daudi, Arsalan (09017)
Cho, Kiu-Hyung (08109)	Chung, Woo Sik (11056)	David-Assael, Ora (04008)
Cho, Myeon Haeng (03106)	Chung, Woo Sik (11059)	Davies, Brendan (08119)
Cho, Myeon Haeng (08143)	Chung, Young Soo (02028)	Davis, Seth J (08011)
Cho, Myeon Haeng (08144)	Chung, Young Soo (02029)	Dawe, R. Kelly (11002)
Cho, Seok Keun (03082)	Chung, Young Soo (02031)	De Clercq, Rebecca (09024)
Cho, Seok Keun (03083)	Chung, Young Soo (15012)	de Folter, Stefan (08076)
Cho, Seok Keun (03086)	Chung, Young-Soo (03050)	de Folter, Stefan (08077)*
Cho, Young-hyun (12011)	CHYE, MEELEN (03004)	De Groeve, Steven (04012)
Choi, Hong Kyu (02028)	Chye, Mee-Len (06002)	De Jaeger, Geert (C804)*
Choi, Hong Kyu (02029)	Cierlik, Izabela (08035)	De Jaeger, Geert (04012)
Choi, Hong Kyu (02031)	Cierlik, Izabela (08159)*	De Jaeger, Geert (07001)
Choi, Hong-Kyu (03047)	Ciesielski, Arkadiusz (03076)	De Jaeger, Geert (08084)

De Jaeger, Geert (12001)	Doonan, John H. (11019)	Eulgem, Thomas (02006)
de Meaux, Juliette (K001)	Dreher, Kate (PL602)	Eulgem, Thomas (02012)*
De Meyer, Bjorn (09024)	Dreher, Kate (C803)	Eun, Changho (C401)
De Veylder, Lieven (08084)	Dreyer, Ingo (08009)	Ezaki, Bunichi (03001)
De Veylder, Lieven (11027)	DU, ZHIYAN (03004)*	Ezhova, Tatiana (10019)
De Veylder, Lieven (17002)	Duan, Ying (C301)	Falasca, Giuseppina (01002)
DeFraia, Christopher (02023)*	Dubouzet, Joseph Gogo (02044)	Falasca, Giuseppina (08022)
Defries, Andrew (09011)*	Duettmann, Verena (06023)	Fan, Rong (12040)*
Delannoy, Etienne (08036)	Dun, Elizabeth (12030)	Fanata, Wahyu Indra (03074)
Deleris, Angelique (04048)*	Dunatunga, Damayanthi (08085)	Fanata, Wahyu Indra (03075)
Della Rovere, Federica (01002)	Durrant, Wendy (C201)	Farhi, Ronit (04008)
Della Rovere, Federica (08022)	Dutilleul, Christelle (03010)	Farmer, Lisa M. (11049)*
DellaPenna, Dean (C603)	Ebert, Berit (10012)	Fattorini, Laura (01002)
Demura, Taku (04014)	Ebert, Berit (10024)	Fattorini, Laura (08022)
Demura, Taku (08054)	Ebine, Kazuo (04025)	Febrer, Melanie (PL503)
Demura, Taku (11019)	Ebine, Kazuo (11042)	Fedorenko, Olga (10019)
Demura, Taku (13005)	Ebine, Kazuo (11044)*	Feechan, Angela (02016)
Demura, Taku (13014)	Ebine, Kazuo (11052)	Feijo, Jose A. (09018)
Deng, Xian (04015)	Echeverria, Manuel (08151)	Felippes, Felipe (04004)*
Denness, Lucy (12008)	Ecker, Joseph (04046)	Felix, Georg (C504)
Dennis, Elizabeth (04001)	Ecker, Joseph R. (PL403)*	Feng, Dongru (03071)
Dennis, Liz (04010)	Eckert, Christian (03012)	Feng, Xiaoqi (08067)*
Deokar, Amit (15002)	Edger, Patrick (17020)	Feng, Yue (03069)
Desaki, Yoshitake (13012)	Eeckhout, Dominique (07001)	Feraru, Elena (11016)*
Desnos, Thierry (C304)	Effgen, Sigi (10013)	Feraru, Mugurel (11016)
Desnos, Thierry (08036)*	Efroni, Idan (08112)	Ferjani, Ali (08001)*
Desveaux, Darrell (PL101)	Egami, Mai (04033)	Fernandez Barbero, Gemma (12001)
Dettmer, Jan (C706)	Ehrhardt, David W. (11058)	Fernie, Alisdair (06019)
Dettmer, Jan (08122)	Eklund, D Magnus (08159)	Fernie, Alisdair (07017)
Devanathan, Sriram (06030)*	Eklund, Magnus (08035)	Fernie, Alisdair (13001)
Devoto, Alessandra (12007)	Elazar, Meirav (04008)	Fernie, Alisdair R. (06018)
Devoto, Alessandra (15005)	Elgass, Kirstin (11040)	Fiehn, Oliver (PL602)
Di Biase, David (07013)	Elgass, Kirstin (11041)	Figueiredo, Duarte (03127)*
Diaz, Celine (03084)*	Elharrar, Einat (04008)	Filardo, Fiona (12030)
Diaz-Trivino, Sara (08021)	Eliaz, Dror (04008)	Finkenstadt, Barbel (07005)
Dickerson, Julie (PL602)	Ellis, Brian (08059)	Finnegan, Jean (04010)
Dickinson, Hugh (08067)	Elo, Annakaisa (C706)	Fischer, Robert (08154)
Diehl, Patrik (08005)	Elo, Annakaisa (08165)	Fitzgerald, Heather (08125)
Dinesh-Kumar, Savithramma (07015)	El-Showk, Sedeer (C706)	Fleming, Andrew (08070)
Ding, Hong (03024)	El-Showk, Sedeer (08171)	Fleury, Delphine (04012)
Ding, Zhiwen (08006)	Endo, Makoto (03118)	Floyd, Sandra (08112)
Dinwiddie, Brendan (04018)	Endo, Makoto (04009)	Forde, Brian G (08162)
Dissmeyer, Nico (11051)	Endo, Takaho (04002)	Forner, Susanne (12034)
Dixon, Laura (07006)*	Endo, Takaho (04006)	Forrest, Alan (10018)
Dixon, Phillip (PL602)	Endo, Toshiya (11065)	Franke, Rochus (08005)
Dobra, Jana (03005)	Enju, Akiko (03105)*	Frankel, Neta (04008)
Dobrowolska, Grazyna (03076)	Erbs, Gitte (C205)	Fricker, Mark (03094)
Doherty, Colleen (PL301)	Eriksson, Maria E. (03038)*	Frickey, Tancred (12030)
Dohra, Hideo (11012)	Esaka, Muneharu (15016)	Friml, Jiri (08045)
Doi, Koji (09006)*	Escobar Guzman, Rocio (08077)	Friml, Jiri (08157)
Dolja, Valerian V. (11024)	Eshed, Yuval (08051)	Friml, Jiri (11016)
Domeki, Yukie (08050)	Eshed, Yuval (08112)	Fristedt, Rikard (11009)
Domeki, Yukie (08091)	Estavillo, G. (18006)	Frommer, Wolf B (C604)*
Dong, Xinnian (C201)*	Estavillo, Gonzalo M. (03032)	Frommer, Wolf B (02021)
Dong, Xinnian (12057)	Etchells, Peter (C702)	Frommer, Wolf B. (11058)
Donohue, Kathleen (K001)	Etcheverry, Mathilde (04037)	Fu, Jihong (02024)

Fu, Shih-Feng (08120)	Fukatsu, Kazue (11012)	Ghorbel, Abdelwahed (08113)
Fu, Xiangdong (12005)*	Fukazawa, Jutarou (12035)	Ghosh, Sibdas (07002)
Fucile, Geoff (07013)	Fukazawa, Mitsue (13013)	Gierth, Markus (03106)
Fujii, Nobuharu (08137)	Fukuda, Hiroo (C502)*	Gilroy, Simon (11011)
Fujii, Nobuharu (08147)	Fukuda, Hiroo (05005)	Glasner, Shira (06007)
Fujii, Sota (10003)*	Fukuda, Hiroo (08124)	Glazebrook, Jane (C204)*
Fujiki, Yuki (08046)*	Fukuda, Hiroo (08157)	Glazebrook, Jane (C605)
Fujiki, Yuki (11030)	Fukuda, Hiroo (08167)	Glazebrook, Jane (02003)
Fujikura, Ushio (08054)*	Fukuda, Hiroo (08172)	Glazebrook, Jane (02010)
Fujimoto, Masaru (11015)	Fukuda, Hiroo (08173)	Go, Jae Pil (08106)
Fujimoto, Masaru (11020)*	Fukuda, Hiroo (11064)	Goch, Grazyna (03076)
Fujimoto, Masaru (11021)	Fukuda, Hiroo (13014)	Goda, Hideki (07009)
Fujimoto, Masaru (11042)	Fukudome, Akihito (04033)*	Goebel, Ulrike (08097)
Fujimoto, Masaru (11044)	Fukuhara, Ryuta (08132)*	Goerlich, Esther (12033)*
Fujimoto, Ryo (04001)*	Fukuhara, Toshiyuki (04033)	Goertzen, Leslie (12022)
Fujino, Kenji (14002)*	Fukuhara, Toshiyuki (04045)	Goh, Chang-Sook (12037)
Fujioka, Shozo (07009)	Fukui, Kiichi (11022)	Goh, Hoe Han (08070)*
Fujioka, Shozo (12015)	Fukuoka, Hiroyuki (14012)	Goh, Tatsuaki (08080)*
Fujioka, Shozo (12023)	Fukuoka, Midori (03126)	Goh, Tatsuaki (11044)
Fujioka, Tomoaki (03118)	Fukusaki, Eiichiro (06027)	Goldshmidt, Alexander (08112)
Fujita, Masahiro (04046)	Fukushima, Atsushi (07016)*	GOMEZ-MINGUET, Eugenio (08025)
Fujita, Miki (03045)	Fukushima, Atsushi (07017)	Gondwe, Martha Y (08006)
Fujita, Miki (03117)*	Fukushima, Ery (06034)*	Gong, Zhizhong (C301)*
Fujita, Tomomichi (10020)	Furbank, R.T. (18006)	González Besteiro, Marina A (C202)
Fujita, Yasunari (03017)	Furukawa, Jun (14010)	Gonzalez-Carranza, Zinnia H. (08027)*
Fujita, Yasunari (03018)	Furukawa, Jun (14011)	Gonzalez-Carranza, Zinnia Haydee (13007)*
Fujita, Yasunari (03019)	Furutani, Ayako (02004)	Goodrich, Justin (04013)
Fujita, Yasunari (03033)	Furutani, Ayako (02005)	Goossens, Alain (12001)*
Fujita, Yasunari (03045)*	Furutani, Masahiko (08095)	Gordon, Matthew (03031)*
Fujita, Yasunari (03096)	Furutani, Masahiko (08126)*	Gordon, Sean (C102)
Fujita, Yasunari (03117)	Furutani, Masahiko (08133)	Gorte, Maartje (08037)*
Fujita, Yasunari (11023)	Furuya, Tomoyuki (03125)*	Goto, Derek (08103)
Fujita, Yasunari (12014)	Futamura, Norihiro (08166)	Goto, Fumiyuki (06029)
Fujiwara, Makoto (11010)*	G.Hatcher, Patrick (06027)	Goto, Hiroto (07017)
Fujiwara, Masayuki (08050)	Gaash, Rachel (04008)	Goto, Koji (08116)
Fujiwara, Masayuki (08167)	Gadeyne, Astrid (C804)	Goto, Koji (17014)*
Fujiwara, Masayuki (11012)	Gafni, Chen (06007)	Goto, Nobuharu (06040)
Fujiwara, Masayuki (11045)	Gajdanowicz, Pawel (08009)*	Gottlieb, Dror (04008)
Fujiwara, Sumire (08031)	Galarneau, Erin (11054)	Gottowik, M. (15020)
Fujiwara, Toru (02047)	Galvao, Rafaelo (08014)	Grabowski, Stephanie (05001)
Fujiwara, Toru (02051)	Gan, Eng-Seng (C705)	Graf, Alexander (02042)
Fujiwara, Toru (03103)	Ganguly, Anindya (11026)	Gras, Diana Ester (03122)*
Fujiwara, Toru (03124)	Ganguly, Anindya (12018)*	Gray, Gordon (08075)
Fujiwara, Toru (06001)*	Gao, Xin Qi (12019)	Greco, Raffaella (08076)
Fujiwara, Toru (06045)	Gao, Yawei (08005)	Green, Pamela J. (03078)
Fujiwara, Toru (11015)	Gao, Yongshun (03054)*	Grefen, Christopher (13008)
Fukagawa, Tatsuo (12020)	Gardner, Rui (C405)	Grill, Erwin (C302)*
Fukai, Eigo (04009)*	Gardner, Rui (09018)	Grini, Paul (08060)
Fukaki, Hidehiro (06027)	Garnett, Philip (07014)*	Gritskikh, Marina (10019)
FUKAKI, Hidehiro (06036)	Garnett, Trevor P. (03105)	Grossman, Arthur (06046)
Fukaki, Hidehiro (08080)	Gasch, Philipp (11001)	Grossmann, Guido (11058)*
Fukaki, Hidehiro (08094)	Geerinck, Jan (C804)	Grotewold, Erich (C704)
Fukao, Yoichiro (08050)	Geerinck, Jan (12001)	Grotewold, Erich (C802)*
Fukao, Yoichiro (08167)	Gelova, Zuzana (11062)	Grotewold, Erich (07007)
Fukao, Yoichiro (11045)	Gendron, Joshua (PL301)	Grotewold, Erich (08004)
Fukao, Youichirou (11012)	Getzoff, Elizabeth (12009)	Grotewold, Erich (09007)

Grotewold, Erich (09010)	Haney, Cara H. (11058)	Hayashi, Makoto (10016)
Gruissem, Wilhelm (04022)	Haque, Md. Ashraful (02015)	Hayashi, Nagao (02044)
Gruissem, Wilhelm (08034)	Hara, Hiroko (08111)	Hayashi, Rika (08151)
Gu, Lianfeng (04015)	Harada, Erimi (16005)	Hayashi, Shimpei (12002)
Guenot, Bernadette (PL803)	Harada, Kazuo (06027)	Hayashi, Yasuko (11018)
Guo, Hongwei (12010)*	Hara-Nishimura, Ikuko (PL202)*	Hayashi, Yoriko (09015)*
Guo, Hongwei (12028)	Hara-Nishimura, Ikuko (C505)	Hayashi, Yoriko (09019)
Guo, Hongwei (12029)	Hara-Nishimura, Ikuko (04005)	Hayashi, Yoshikazu (11020)
Guo, Woei-Jiun (02021)	Hara-Nishimura, Ikuko (09004)	Hayashi, Yuki (11066)*
Gust, Andrea A (C205)	Hara-Nishimura, Ikuko (11018)	Hayashi, Yuko (09014)
Gutierrez, Laurent (01002)	Hara-Nishimura, Ikuko (11024)	Hayashi, Yuko (09016)
Gutierrez, Laurent (08023)	Hara-Nishimura, Ikuko (11025)	He, Junna (C301)
Gutierrez, Rodrigo (03095)	Hara-Nishimura, Ikuko (13013)	He, Junxian (12046)*
Gutierrez, Rodrigo A. (03078)	Harashima, Hirofumi (11051)*	He, Yuke (08008)*
Gutierrez, Rodrigo Antonio (03122)	Harkness, Troy (08075)	He, Zemiao (C705)
Gyula, Peter (03038)	Harmer, Stacey (03014)	He, Zuhua (12056)*
Ha, Misook (04016)	Harmoko, Rikno (03074)	Heazlewood, Joshua (07020)
Ha, Tracy (03013)	Harmoko, Rikno (03075)*	Heazlewood, Joshua (13004)*
Hah, Cyrus (12023)	Harmsen, Eef (17020)	Heazlewood, Joshua L. (16007)
Hahn, Michael G. (13015)*	Harris, Nilangani N (15004)	Heese, Maren (08060)
Hajslova, Jana (02002)	Harter, Klaus (08026)	Hegie, Alicia (03002)
Hakozaki, Hirokazu (11046)	Harter, Klaus (11040)	Heidstra, Renze (08028)
Halada, Petr (11062)	Harter, Klaus (11041)	Heidstra, Renze (08037)
Hall, Anthony (03038)	Harter, Klaus (13008)	Heidstra, Renze (08153)
Hall, Hardy (08059)*	Hase, Takeshi (14004)	Helariutta, Ykä (C706)*
Hamada, Jumpei (02054)	Hase, Yoshihiro (08113)	Helariutta, Yka (08122)
Hamada, Kazuki (03118)	Hasebe, Mitsuyasu (C103)*	Helariutta, Yka (08165)
Hamann, Thorsten (12008)*	Hasebe, Mitsuyasu (01001)	Helariutta, Yka (08171)
Hamann, Thorsten (13001)	Hasebe, Mitsuyasu (01005)	Helper, Anne (PL301)
Hamasaki, Hidefumi (08149)*	Hasebe, Mitsuyasu (01007)	Helliwell, Chris (04010)
HAMES, Cecile (08025)	Hasegawa, Koji (06040)	Hellmann, Eva (12022)
Hamilton, Elizabeth (PL301)	Hasegawa, Paul M. (03003)	Help, Hanna (C706)
Hammann, Philippe (11051)	Hasezawa, Seiichiro (11024)	Hennig, Lars (04022)
Han, Bin (17018)	Hasezawa, Seiichiro (13005)	Henriques, Rossana (03066)
Han, Brian (03079)	Hashida, Shin-nosuke (06029)*	Henriques, Rossana (17003)
Han, Hay Ju (02055)	Hashimoto, Haruki (06011)	Henry, Robert (17018)
Han, Hay Ju (08106)*	Hashimoto, Kenji (03012)*	Heo, Jee-Eun (03047)
Han, Hay Ju (11056)	Hashimoto, Kenji (08026)*	Heo, Jung-Ok (08114)*
Han, Jeong A (11035)*	Hashimoto, Mika (06009)	Hepworth, Jo (10008)*
Han, Jeong-A (11032)	Hashimoto, Takashi (08052)	Herrero, Eva (08011)*
Han, Jeong-A (11036)	Hashimoto, Takashi (11031)	Heyl, Alexander (12022)
Han, Muho (04039)	Hashinokuchi, Hiromi (06042)	Hibara, Ken-ichiro (PL201)
Han, Soon-Ki (04039)	Hatsugai, Noriyuki (PL202)	Hibberd, Julian (17020)
Hanada, Atsushi (03035)	Hattori, Kazuki (02018)	Higaki, Takumi (13005)
Hanada, Atsushi (06013)	Hattori, Sayoko (11048)	Higashi, Katsumi (15014)
Hanada, Atsushi (12048)	Hattori, Tsukaho (03029)	Higashide, Makiko (02014)
Hanada, Kousuke (02032)	Hause, Bettina (11034)	Higashitani, Atsushi (03020)*
Hanada, Kousuke (04014)	Hause, Bettina (12034)	Higashitani, Nahoko (03020)
Hanada, Kousuke (09006)	Hauser, Felix (03013)	Higashiyama, Tetsuya (C503)*
Hanada, Kousuke (14004)*	Hauser, Marie-Theres (11019)	Higashiyama, Tetsuya (08043)
Hanada, Kousuke (14005)	Hauser, Marie-Theres (11028)	Higuchi, Mieko (C801)*
Hanada, Kousuke (16005)	Hayakawa, Toshihiko (08105)	Higuchi, Mieko (02032)
Hanamata, Shigeru (02054)	Hayashi, Hideki (03065)	Higuchi, Mieko (03057)
Hanano, Keiko (08102)	Hayashi, Keiko (10009)*	Higuchi, Mieko (08149)
Hanano, Shigeru (08116)*	Hayashi, Ken-ichiro (06038)	Higuchi, Mieko (14004)
Hanba, Yuko (03059)	Hayashi, Makoto (04009)	Higuchi, Mieko (14005)*

Hiki, Takeshi (08052)	Holomuzki, Nicholas (C802)	Huber, Stefan (02039)
Hikosaka, Kouki (10015)*	Hong, Hyewon (17006)	Huber, Steven (12016)*
Hilson, Pierre (09023)*	Hong, Jeum Kyu (11026)	Hudson, Andrew (10001)
Hilson, Pierre (09024)*	Hong, Jong-Pil (03061)*	Huep, Gunnar (08062)
Hindle, Matthew (C703)	Hong, Lan (03052)	Huettel, Bruno (08097)
Hirabayashi, Takayuki (06029)	Hong, Suk-Whan (03113)	Hugouvieux, Veronique (03010)*
Hiraguri, Akihiro (04033)	Hong, Suk-Whan (03115)	Huijser, Peter (08064)
Hiraguri, Akihiro (04045)	Hong, Suk-Whan Hong (03110)	Hunt, Arthur (04017)
Hirai, Akiko (06004)	Hong, Sung Myun (08140)	Huppenberger, Peter (11041)
Hirai, Masami (C601)	Hong, Sung Myun (09012)*	Huppenberger, Peter (13008)*
Hirai-Yokota, Masami (06026)	Hong, Zonglie (08101)	Hur, Yoon-Sun (08071)
Hirakawa, Yuki (C502)	Hong-Hermesdorf, Anne (12033)	Hwang, Hau-Hsuan (02022)*
Hirakawa, Yuki (08173)*	Hongo, Hiroaki (08111)	Hwang, Ihhwan (11071)
Hirano, Hiroyuki (08088)	Hongo, Hiroaki (08148)	Hwang, Ildoo (12011)*
Hirano, Hiro-Yuki (08079)	Hony, David (08121)	Hwang, Ildoo (12012)
Hirano, Hiro-yuki (08090)	Horiguchi, Gorou (08001)	Hwang, Indeok (08115)
Hirano, Tomonari (09014)	Horiguchi, Gorou (08015)	Hwang, Inhwang (PL203)*
Hirano, Tomonari (09015)	Horiguchi, Gorou (08054)	Hwang, Inhwang (11067)*
Hirano, Tomonari (09016)*	Horii, Yoko (02044)	Hwang, Inhwang (11068)
Hirano, Tomonari (09019)	Horii, Yoko (08148)	Hwang, Inhwang (11069)
Hirano, Tomonari (09021)	Horii, Yoko (14005)	Hwang, Jae-Ung (12038)*
Hiraoka, Yasuko (06032)*	Horikawa, Akihiko (09020)	Hwang, Jung Eun (03026)
Hiratsu, Keiichiro (C104)	Horvath, Beatrix (08021)	HWANG, JUNG-YUN (06035)
Hiratsuka, Kazuyuki (15019)	Hosaka, Aeni (09020)	Hwang, Ildoo (02034)
Hiratsuka, Kazuyuki (15021)	Hoshino, Kyoko (01001)	Hwang, San-Gwang (12024)
Hirayama, Takashi (12002)	Hoshino, Tamotsu (03128)	Hwang, Seong Cheol (03114)*
Hirochika, Hirohiko (02044)	Hossain, Khaled (07002)	Hwang, Sung Min (03097)
Hirochika, Hirohiko (02054)	Hossain, Md. Aktar (03113)	Hwang, Sung Min (08123)*
Hirochika, Hirohiko (03029)	Hou, Xingliang (08065)*	Hyun, Youbong (04032)
Hirochika, Hirohiko (03057)	Houben, Andreas (04012)	Hyun, Youbong (04043)
Hirochika, Hirohiko (04009)	Hronkova, Marie (03005)	Iandolino, Alberto (02031)
Hirochika, Hirohiko (08061)	Hsieh, En-Jung (03088)	Iba, Koh (PL303)*
Hirochika, Hirohiko (10006)	Hsieh, En-Juun (03109)	Ichikawa, Hiroaki (09020)
Hirochika, Hirohiko (10022)	Hsieh, Hsu-Liang (03109)	Ichikawa, Takanari (C801)
Hirota, Tomo (06024)*	Hsing, Yue-ie (17018)	Ichikawa, Takanari (02044)
Hirschberg, Joseph (06007)	Hsiung, Yu-chuan (08049)	Ichikawa, Takanari (03057)
Hiruma, Kei (02026)*	Hsu, Fu-Chiun (03006)	Ichikawa, Takanari (08002)
Hitomi, Chiharu (12009)	Hsu, Fu-Chiun (03021)*	Ichikawa, Takanari (08017)
Hitomi, Kenichi (12009)	Hu, Bo (03052)	Ichiki, Tamaki (15003)
Hiwatashi, Yuji (01001)	Hu, Fengyi (17018)	Ichimura, Kazuya (02042)*
Hiwatashi, Yuji (01005)	Hu, Gaosheng (02029)	Ichitani, Katsuyuki (10006)
Hiwatashi, Yuji (01007)	Hu, Gaosheng (02031)	Ichitani, Katsuyuki (10022)
hninsi, Moe (06040)	Hu, Heng-Cheng (11017)	Ide, Yoko (06045)*
HO, William Wing-Ho (08083)*	Hu, Honghong (11014)*	Igarashi, Hisako (05006)
Hoang, My Hanh Thi (08106)	Hua, Deping (C301)	Igarashi, Hisako (08163)*
Hoang, My Hanh Thi (11059)	Huala, Eva (C803)*	Igarashi, Kazuei (08130)
Hobbs, Jamie (08070)	Huang, Hao-Jen (08049)	Iasaki, Tomohiro (08166)
Hobo, Tokunori (03029)	Huang, Hao-Jen (08120)	Iida, Hidetoshi (11047)
Hoeberichts, Frank (07003)	Huang, Jen-Pan (10011)	Iida, Kazuko (11047)
Hoefgen, Rainer (03041)	Huang, Jia-Yuan (08110)	Iida, Kei (16005)
Hoefgen, Rainer (03042)	Huang, Nien-Chen (08072)	Iida-Okada, Keiko (09020)
Hoehmann, Susanne (08064)	Huang, Xueqing (10013)*	Iijima, Yoko (03011)
Holdorf, Meghan M. (06030)	Huang, Yiding (08085)	Iima, Makoto (07019)
Holland, John-Todd (06006)	Hubbard, Katharine (03013)	Iizuka, Kazuya (02050)
Hollunder, Jens (07001)	Hubberten, Hans-Michael (03041)	Ikawa, Yuki (06020)
Holmstrom, Kjell-Ove (07002)	Hubberten, Hans-Michael (03042)*	Ikeda, Akiro (03107)

Ikeda, Miho (03065)	Ishida, Takashi (08044)	Ito, Yusuke (03034)
Ikeda, Miho (08135)*	Ishida, Tetsuya (03043)*	Ito, Yusuke (03037)
Ikeda, Torazo (11022)	Ishiguri, Futoshi (02050)	Itoh, Ryuichi (11010)
Ikeda, Yoko (04031)	Ishiguro, Sumie (02018)*	Iuchi, Satoshi (03107)
Ikeda, Yoko (04038)*	Ishiguro, Sumie (13006)	Iuchi, Satoshi (09022)*
Ikeda, Yoko (04042)	Ishiguro, Sumie (17005)	Iwabuchi, Masaki (03057)
Ikeda, Yuriko (04038)	Ishihama, Yasushi (09017)	Iwai, Hiroaki (14010)
Ikeuchi, Masahiko (06026)	Ishihama, Yasushi (12002)	Iwai, Hiroaki (14011)
Ikeyama, Yoshifumi (08094)*	Ishii, Tadashi (14010)	Iwakawa, Hidekazu (08096)
Ikezaki, Masaya (08051)	Ishii, Tadashi (14011)	Iwakawa, Hidekazu Iwakawa (08051)
Ilk, Nadine (10007)*	Ishii, Takahiro (12015)	Iwama, Katsuhisa (03044)
Il-Ryong, Choi (15003)	Ishikawa, Haruki (03121)	Iwamoto, Akitoshi (07018)*
Im, Chak Han (03097)	Ishikawa, Kazuya (02027)*	Iwasa, Yoh (04041)
Imai, Akihiro (01007)*	Ishikawa, Kazuya (06017)	Iwasaki, Akira (08146)*
Imai, Aya (11053)	Ishikawa, Kazuya (06028)	Iwasaki, Mayumi (08051)
Imai, Hiroyuki (03120)*	Ishikawa, Masaki (01005)*	Iwasaki, Mayumi (08174)
Imai, Ryozo (03123)	Ishikawa, Masaki (01007)	Iwase, Akira (C104)*
Imai, Ryozo (03128)*	Ishikawa, Shouta (11048)	Iwata, Mineko (01001)
Imai, Tomoya (08174)	Ishikawa, Takahiro (03044)*	Iwata, Yuji (02046)
Imai, Yu (C505)	Ishikawa, Takahiro (03054)	Iyer-Pascuzzi, Anjali (PL802)
Imaizumi, Takato (03066)*	Ishikawa, Takahiro (03063)	Izui, Katsura (03039)
Imamura, Kenro (01006)*	Ishikawa, Takahiro (03087)	Izumi, Masanori (11057)*
Immanen, Juha (C706)	Ishikawa, Takahiro (03116)	Jürgens, Gerd (C701)*
Imre, Katherine (C603)	Ishikawa, Tomoko (03119)	Jackson, David (08063)*
Imura, Yoshiyuki (03018)	Ishimizu, Takeshi (11046)	Jackson, Scott (17018)
Imura, Yuko (08148)	Ishimizu, Takeshi (13005)	Jahan, Md. Sarwar (12054)
Imura, Yuko (08149)	Ishitani, Manabu (14009)	Jahan, Md.Sarwar (12052)
Inaba, Takehito (11003)*	Ishiura, Masahiro (06009)	Jahns, Oliver (06023)*
Inagaki, Soichi (04026)*	Ishiyama, Kanako (12014)	Jaimie, Van Norman (PL802)
Ingram, Gwyneth (05004)	Ishizuka, Toru (09020)	Jain, Pradeep Kumar (15002)
Ingsriswang, Supawadee (16008)	Isobe, Sachiko (14012)	Jan, Asad (03034)*
Ingsriswang, Supawadee (16009)	Isogai, Akira (04046)	Jang, Jyan-Chyun (12023)*
Innes, Roger (C203)	Isogai, Akira (17017)	Jang, Mihue (11067)
Inoue, Eri (06010)	Isokawa, Sachiyo (10002)	Jang, Seonghoe (08097)
Inoue, Takahiro (03116)	Isokawa, Sachiyo (17017)	Jang, Yun Hee (04035)*
Inoue, Yasunori (C104)	Isono, Erika (11028)*	Jansweijer, Vera (08037)
Inzé, Dirk (08084)	Israelsson-Nordstrom, Maria (11014)	Jarillo, Jose A. (08098)*
Inzé, Dirk (PL601)*	Isram, Mohammad Mahbub (12052)	Jarillo, Jose A. (08100)
Inze, Dirk (C804)	Itai, Reiko N (16006)	JAUVION, Vincent (04003)*
Inze, Dirk (07001)	Ito, Daisuke (06028)*	Je, Ji Hyun (03026)
Inze, Dirk (11027)	Ito, Emi (11044)	Jensen, Shane T. (14001)
Inze, Dirk (11031)	Ito, Jason (07020)*	Jeon, Byeongwook (11017)
Inze, Dirk (17002)	Ito, Jun (08133)*	Jeon, Eun Hee (02028)
Ioki, Motohide (09013)*	Ito, Jun (11045)	Jeon, Eun Hee (15012)
Isawa, Tsuyoshi (12055)	Ito, Jun (11052)	Jeon, Hyesung (03069)
Isemer, Rena (08033)*	Ito, Masaki (11019)*	Jeon, Jae Ok (08169)*
Isemer, Rena (11005)	Ito, Motomi (10016)	Jeon, Jin (03040)*
Ishibashi, Nanako (08081)*	Ito, Shogo (03066)	Jeon, Jong-Seong (04039)
Ishida, Hiroyuki (11057)	Ito, Takeshi (12035)*	Jeon, Young (11035)
Ishida, Juliane K. (15017)*	Ito, Takuya (06010)	Jeong, Mi-Jeong (02036)
Ishida, Junko (03023)	Ito, Takuya (13005)	Jeong, Soon Chun (15012)
Ishida, Junko (04002)	Ito, Toshiro (C705)*	Jeong, So-Yoon (12051)
Ishida, Junko (04006)	Ito, Toshiro (08012)	Jeong, SuYeong (08078)*
Ishida, Junko (04014)	Ito, Toshiro (08158)	Jeong, Yeon Sim (02029)
Ishida, Sarahmi (12035)	Ito, Yusuke (03019)	Jeong, Yeon Sim (02030)*
Ishida, Takashi (08031)*	Ito, Yusuke (03027)	Jeong, Yeon Sim (02031)

Jeong, Yeon Sim (15013)*	Kakutani, Tetsuji (04001)	Kanno, Satomi (C304)
Jeong, Young-Min (04027)	Kakutani, Tetsuji (04026)	Kanno, Yuri (12013)
Jeung, Soon-Jae (02040)	Kakutani, Tetsuji (04028)	Karimi, Mansour (09024)
Jiang, Caifu (12005)	Kakutani, Tetsuji (04038)	Kariya, Ayano (08102)
Jiang, Han-Wei (03109)	Kalousova, Marcela (02002)	Karrenberg, Sophie (10021)
Jiang, Ning (08007)	Kamada, Hiroshi (08117)	Kasahara, Hiroyuki (01007)
Jiao, Yuling (01003)	Kamada, Hiroshi (08118)	Kasahara, Hiroyuki (06013)
Jie, Xu (08047)	Kamada, Hiroshi (08142)	Kasahara, Hiroyuki (06038)*
Jikumaru, Yusuke (03102)	Kamada, Hiroshi (12006)	Kasahara, Hiroyuki (12026)
Jikumaru, Yusuke (06013)*	Kamada-Nobusada, Tomoe (09003)	Kasai, Koji (02047)*
Jikumaru, Yusuke (06038)	Kamide, Yukiko (06004)	Kasajima, Ichiro (06045)
Jikumaru, Yusuke (10017)	Kamide, Yukiko (06047)	Kasschau, Kristin (04016)
Jikumaru, Yusuke (11070)	Kaminaka, Hironori (02014)*	Katagiri, Fumiaki (C204)
Jikumaru, Yusuke (12013)	Kaminaka, Hironori (03123)	Katagiri, Fumiaki (C605)*
Jikumaru, Yusuke (12023)	Kaminuma, Eli (16004)*	Katagiri, Fumiaki (02003)
Jin, Jing Bo (17006)	Kaminuma, Eli (18005)*	Katagiri, Fumiaki (02010)
Jin, Yong-Mei (03069)	Kamiya, Asako (C305)	Katagiri, Takeshi (12014)
Jisaka, Mitsuo (11048)	Kamiya, Asako (08148)	Katahata, Shin-Ichiro (08166)*
Jo, Eun Jeong (02037)	Kamiya, Yoko (06042)	Kato, Mariko (11038)
Johannes, Frank (04037)	Kamiya, Yuji (03102)	Kato, Nobuo (03039)
Johansson, Mikael (03038)	Kamiya, Yuji (06013)	Kato, Tomoaki (02026)
Johnston, Amal (08034)*	Kamiya, Yuji (06038)	Kato, Tomoaki (02035)
Jones, Alex (18001)*	Kamiya, Yuji (10017)	Katori, Taku (03107)*
Jones, Alexandra (02008)*	Kamiya, Yuji (11070)	Katsiarimpa, Anthi (11028)
Jones, Matt (03014)*	Kamiya, Yuji (12006)	Katsunuma, Norio (10006)
JOO, SE-HWAN (06035)*	Kamiya, Yuji (12013)	Katsunuma, Norio (10022)
Joshi, Hiren (18003)*	Kamiya, Yuji (12023)	Katsura, Hitomi (03126)
Joshi, Hiren J (16007)*	Kamiya, Yuji (12048)	Katsura, Shogo (03015)
Jourdain, Agnes (03010)	Kamiya, Yuji (14006)	Kawabe, Akira (04028)
Ju, Geonho (08134)	Kamoun, Sophien (02008)	Kawabe, Akira (10018)*
Juenger, Thomas E. (03072)	Kanai, Tomoyuki (08103)	Kawade, Kensuke (08015)*
Jun, Sang Eun (08109)	Kanamori, Hiroyuki (10006)	Kawagishi-Kobayashi, Makiko (03118)
Jung, Ho Won (02028)	Kanamori, Norihito (12014)	Kawaguchi, Masayoshi (05002)
Jung, Ho Won (02029)	Kanaoka, Masahiro (08101)*	Kawaguchi, Shuji (16005)*
Jung, Ho Won (02031)	Kanaya, Akihiro (04033)	Kawai, Tsutae (06009)*
Jung, Hye Seung (09012)	Kanehara, Kazue (02013)	Kawai, Tsutae (17005)
Jung, Hyun Ju (03056)	Kanehara, Kazue (08012)	Kawai-Yamada, Maki (06029)
Jung, In Jung (03074)	Kaneko, Fumi (03118)	Kawakami, Jessica (04018)
Jung, In Jung (03075)	Kaneko, Kensuke (11012)	Kawakami, Jessica (04047)
Jung, Ji Hyun (03111)	Kaneko, Kentarou (11063)*	Kawakami, Naoto (11070)
Jung, Jinwook (03069)	Kanemaki, Masato (12020)	Kawakami, Naoto (13012)
Jung, Mi Soon (02055)*	Kang, Chang Ho (03111)	Kawamura, Ayako (08002)
Jung, Mi soon (11056)	Kang, Hong-Gu (02003)*	Kawamura, Ayako (08017)
Jung, Min Hee (03104)*	Kang, Hunseung (02025)	Kawamura, Ayako (08018)
Jung, Young Jun (03114)	Kang, Hunseung (03056)	Kawamura, Ayako (08044)
Jusoh, Malinna (03094)*	Kang, In Soon (03093)	Kawamura, Setsuko (09022)
Jutt, Dominic (03051)	Kang, Jae-Sook (03069)	Kawano, Eri (08136)
K. Watahiki, Masaaki (03102)	Kang, Jee-Sook (03048)	Kawano, Shigeyuki (09021)
Kachroo, Pradeep (02003)	Kang, Jeong-Gu (02016)	Kawarazaki, Tomoko (11053)*
Kaiser, Brent N. (03105)	Kang, Joohyun (12038)	Kawasaki, Tsutomu (02005)
Kakehi, Junichi (08130)	Kang, Min-Jeong (04043)	Kawashima, Mika (07017)
Kakehi, Jun-ichi (08136)*	Kang, Na Young (03040)	Kawashima, Mika (08148)
Kakei, Yusuke (16006)*	Kang, Shin Gene (12023)	Kawashima, Mika (09006)
Kakimoto, Tatsuo (12020)	Kang, Sun Bin (02048)*	Kawashima, Mika (14005)
Kakizaki, Tomohiro (11003)	Kang, Sun Bin (03112)	Kawaura, Kanako (04044)*
Kakutani, Tetsuji (PL402)*	Kang, Yeon Hee (08093)	Kawaura, Kanako (06042)

Kawaura, Kanako (14006)	Kim, Ji Yeon (02025)*	Kim, Sunghan (08071)*
Kay, Steve (03066)	KIM, Ji Yun (04040)	Kim, Sun-Ho (08089)
Kay, Steve A. (PL301)*	Kim, Jinho (11069)	Kim, Sunmi (03040)
Kaya, Hidetaka (11053)	Kim, Jong-Myong (04002)*	Kim, Tae-Houn (03013)*
Kaya, Hidetaka (11055)	Kim, Jong-Myong (04006)	Kim, Wan-hui (08125)
Kayabe, Taketo (04046)	Kim, Jun_Hyeok (03110)*	Kim, Wan-hui (08139)*
Kazama, Ayumi (06020)	Kim, Jungmook (03040)	Kim, Woe Yeon (02048)
Kazama, Yusuke (09014)*	Kim, Jungmook (08053)*	Kim, Woe Yeon (02049)
Kazama, Yusuke (09015)	Kim, Ju-Won (12036)	Kim, Woe Yeon (03111)
Kazama, Yusuke (09016)	Kim, Kangmin (12011)	Kim, Woe Yeon (03112)
Kazama, Yusuke (09019)	Kim, Kyoung-Mee (03047)	Kim, Woe Yeon (03114)
Kazama, Yusuke (09021)	Kim, Kyoung-Mee (03048)	Kim, Woe-Yeon (09005)
Kazama, Yusuke (11010)	Kim, Kyoung-Mee (03049)	Kim, Woo Taek (03082)
Kelley, Lawrence (07013)	Kim, Kyoung-Mee (03050)	Kim, Woo Taek (03083)
Kemmerling, Birgit (13008)	Kim, Kyoung-Sook (03047)	Kim, Woo Taek (03086)
Kerstetter, Randall (PL201)	Kim, Kyoung-Sook (03048)	KIM, Woo Taek (03092)
Keta, Sumie (08155)*	Kim, Kyoung-Sook (03049)	Kim, Yoon (12037)
Khorobrykh, Sergey (03011)	Kim, Kyoung-Sook (03050)	Kim, Young-hye (03113)*
Kiba, Takatoshi (03066)	Kim, Kyung Eun (02055)	Kim, Yujung (03016)
Kiba, Takatoshi (06015)*	Kim, Kyung Eun (08106)	Kim, Yun-Kyung (04023)*
Kiba, Takatoshi (17003)	Kim, Kyung Eun (08140)	Kim, Yu-Young (12038)
Kidokoro, Sataoshi (03045)	Kim, Kyung Eun (11056)*	Kimizu, Mayumi (08061)
Kidokoro, Satoshi (03017)	Kim, Kyung Eun (11059)	Kim-To, Taiko (04002)
Kidokoro, Satoshi (03018)*	Kim, Mi Jin (02028)	Kimura, Hiroshi (04002)
Kidokoro, Satoshi (03019)	Kim, Mi Jin (02029)	Kimura, Makoto (02035)
Kidokoro, Satoshi (12014)	Kim, Mi Jin (02031)*	Kimura, Sachie (03058)
Kidokoro, Satoshi (16002)	Kim, Mi Jin (15012)*	Kimura, Sachie (11055)*
Kikuchi, Jun (03121)	Kim, Mi Ri (02049)*	King, John (C703)
Kikuchi, Shoshi (09020)	Kim, Mi Ri (03112)	Kinoshita, Atsuko (08152)
Kikuchi, Shunsuke (03118)	Kim, Min Chul (02020)	Kinoshita, Atsuko (08167)
Kikuta, Rina (17017)	KIM, MIN KYUN (06035)	Kinoshita, Atsuko (08172)*
Kim, Anna (08143)	Kim, Min Kyung (03056)	Kinoshita, Kengo (16003)
Kim, Anna (08144)	Kim, Minhee (08154)	Kinoshita, Tetsu (04031)
Kim, Beg Hab (03062)	Kim, Min-Hee (12036)	Kinoshita, Tetsu (04038)
Kim, Byung-Hoon (09002)	Kim, Minkyun (03069)*	Kinoshita, Tetsu (04042)
Kim, Dae Heon (PL203)	Kim, Myung-Hee (03123)*	Kinoshita, Toshinori (11038)
Kim, Dae Won (08123)	Kim, Nan Young (03040)	Kinoshita, Toshinori (11066)
Kim, Do Hoon (02028)	Kim, Nan Young (08053)	Kinoshita, Yuki (04031)
Kim, Do Hoon (02029)	Kim, Ohkmae (12050)	Kinoshita, Yuki (04038)
Kim, Do Hoon (02031)	Kim, Sang-Tae (10014)*	Kinoshita, Yuki (04042)
Kim, Doh Hoon (02030)	Kim, Sangwook (11069)	Kiriwa, Yoshikazu (11012)
Kim, Doh Hoon (15013)	Kim, Seo Hyun (08138)*	Kitagawa, Youichiro (02054)
Kim, Gyung-Tae (08109)*	KIM, SEONG-KI (06035)	Kitajima-Koga, Aya (11063)
Kim, Hae Jin (11026)	Kim, Seong-Ki (12049)	Kitakura, Saeko (08045)
Kim, Hee Kyu (02036)	Kim, Seong-Ki (12051)	Kitamura, Satoshi (06039)*
Kim, Ho Soo (02055)	Kim, Soo Jin (03083)*	Kiyoduka, Masahiro (02054)
Kim, Hye Gi (12050)	Kim, Soo Jin (11026)	Kiyota, Eri (04045)*
Kim, In A (08114)	Kim, Soo youn (11071)	Klein, Ilse (09001)*
Kim, Jae Joon (08140)	Kim, Soo-Hwan (12036)*	Klein, Ilse (11001)
Kim, Je Hein (03074)*	Kim, Soo-Hwan (12037)*	Klessig, Daniel F. (02003)
Kim, Je Hein (03075)	Kim, Soon-Kap (04035)	Klie, Sebastian (03042)
Kim, Jeong Hoe (08168)	Kim, Sun Ho (08106)	Kloepper, Joseph W. (02033)
Kim, Jeong Hoe (08169)	Kim, Sun Ho (11056)	Knee, Emma (C802)
Kim, Jeong Hoe (08170)	Kim, Sun Young (02049)	Knee, Emma (09007)
Kim, Jeong-Kook (04035)	Kim, Sun Young (03062)*	Knee, Emma (09010)
Kim, Jeongsik (C805)	Kim, Sunghan (04023)	Knierim, Bernhard (13004)

Knox, Kirsten (03022)*	Kondawar, Vishwajith (15002)	Kubo, Minoru (13014)
Ko, Jae Pil (11059)*	Kondo, Eri (07018)	Kubo, Shigeru (03039)*
Ko, Jong-Hyun (04032)	Kondo, Maki (PL202)	Kubo, Takahiro (03009)
Ko, Ki Seong (03074)	Kondo, Maki (06011)	Kuboyama, Tsutomu (10006)*
Ko, Ki Seong (03075)	Kondo, Maki (08150)	Kuboyama, Tsutomu (10022)
Ko, Sukmin (08117)	Kondo, Maki (11018)	Kuchitsu, Kazuyuki (02054)*
Koabayashi, Akie (04026)	Kondo, Maki (13013)	Kuchitsu, Kazuyuki (03058)
Kobayashi, Akie (04028)	Kondo, Yoichi (09009)	Kuchitsu, Kazuyuki (11053)
Kobayashi, Akie (08137)	Kondo, Yuki (C502)	Kuchitsu, Kazuyuki (11055)
Kobayashi, Akie (08147)	Kondo, Yuki (08173)	Kudla, Joerg (03012)
Kobayashi, Keiko (06044)	Kondo-Osanai, Kumiko (03080)*	Kudla, Joerg (08026)
Kobayashi, Koichi (06003)	Kondou, Youichi (C801)	Kudo, Toru (12025)*
Kobayashi, Koichi (06011)*	Kondou, Youichi (01007)	Kudoh, Hiroshi (04001)
Kobayashi, Kosuke (11019)	Kondou, Youichi (02044)	Kufel, Joanna (04021)
Kobayashi, Makoto (06047)	Kondou, Youichi (03057)	Kuhlemeier, Cris (PL803)*
Kobayashi, Makoto (07016)	Kondou, Youichi (07017)	Kuhlemeier, Cris (08020)
Kobayashi, Makoto (07017)	Kondou, Youichi (08002)	Kuhlemeier, Cris (08055)
Kobayashi, Masatomo (C801)*	Kondou, Youichi (08017)	Kuhlmann, Markus (04007)
Kobayashi, Masatomo (03107)	Kondou, Youichi (08148)	Kuijt, Suzanne J.H. (08076)
Kobayashi, Masatomo (03117)	Kondou, Youichi (09006)	Kulik, Anna (03076)
Kobayashi, Masatomo (09022)	Kondou, Youichi (15006)	Kumar, Shailesh (08111)*
Kobayashi, Masatomo (12014)	Kong, Dongdong (11017)	Kumar, Vajinder (15002)
Kobayashi, Masatomo (15014)	Kong, Hyun Gi (02037)	Kumlein, Jochen (04007)
Kobayashi, Norio (04006)	Kong, Yingzhen (13015)	Kumpf, Robert (08056)
Kobayashi, Norio (09006)	Konishi, Mineko (04025)	Kumpf, Robert (17009)
Kobayashi, Shouko (17016)	Konishi, Mineko (06005)*	Kumura, Hiromi (03011)
Kobayashi, Takanori (16006)	Konishi, Mineko (08152)	Kun, He (PL602)
Kobayashi, Toshihiro (15014)*	Koo, Ja Choon (11007)*	Kunieda, Tadashi (13013)*
Kodaira, Kensuke (03015)	Koo, Sung Cheol (09005)	Kuo, Yi-Chun (02009)
Kodaira, Kensuke (03028)	Koo, Yoon Duck (09005)	Kupriyanova, Evgeniya (10019)*
Kodaira, Ken-Suke (03033)*	Koohi, Mehrana (06043)	Kurata, Nori (04046)
Kodama, Yuichi (16004)	Koornneef, Maarten (K001)*	Kurata, Nori (17018)
Koga, Hironori (02035)	Koornneef, Maarten (10007)	Kurata, Tetsuya (01001)
Koiwa, Hisashi (03069)	Koornneef, Maarten (10010)	Kurihara, Daisuke (11022)
Koizumi, Koji (08157)	Koornneef, Maarten (10013)	Kuriyama, Tomoko (08148)
Koizumi, Nozomu (02046)*	Kopriva, Stanislav (C602)*	Kuroda, Hirofumi (02044)
Koizumi, Ryota (08012)	Kouchi, Hiroshi (04009)	Kuroha, Takeshi (PL801)
Kojima, Mikiko (C104)	Kouno, Takafumi (03001)*	Kuroha, Takeshi (09003)
Kojima, Mikiko (03029)	Kousetu, Ken (13011)*	Kuromori, Takashi (C305)*
Kojima, Mikiko (09003)*	Kousyu, Tsutomu (11063)	Kuromori, Takashi (06010)
Kojima, Mikiko (12017)	Kowalczyk, Mariusz (08023)	Kuromori, Takashi (08141)
Kojima, Mikiko (12025)	Koyama, Tomotsugu (C104)	Kuromori, Takashi (08148)
Kojima, Mikiko (12026)	Koyama, Tomotsugu (08040)*	Kuromori, Takashi (08149)
Kojima, Shoko (08051)	Koyama, Tomotsugu (08145)	Kuromori, Takashi (08167)
Kojima, Shoko (08151)	Kozaki, Akiko (06020)*	Kuromori, Takashi (09006)
Kojima, Shoko (08156)	Krause, Kirsten (08033)	Kurusu, Takamitsu (02054)
Kojima, Shoko (08174)*	Krupinska, Karin (08033)	Kusajima, Miyuki (02056)*
Kojima, Soichi (08105)	Krupinska, Karin (11005)	Kusama, Masahiro (15021)*
Kojima, Yusuke (03060)	Krusche, Peter (16001)	Kusano, Hiroaki (11008)*
Kojoma, Mareshige (06032)	Krzyszton, Michal (04021)	Kusano, Miyako (06047)
Kolmos, Elsebeth (PL301)	Krzywinska, Ewa (03076)	Kusano, Miyako (07016)
Kolmos, Elsebeth (08011)	Ku, Su-Jin (03040)	Kusano, Miyako (07017)*
Komaki, Shinichiro (11031)*	Kubo, Akihiro (03098)	Kusterer, Barbara (10012)
Komatsu, Satoshi (17017)	Kubo, Minoru (01001)	Kutsuna, Natsumaro (11024)
Komeda, Yoshibumi (08132)	Kubo, Minoru (01005)	Kutsuna, Natsumaro (13005)
Komeda, Yoshibumi (08141)	Kubo, Minoru (01007)	Kutsuna, Shinsuke (17016)

Kuwabara, Chikako (03128)	Lee, Chang-Lung (17008)	Lee, Kyunghee (02036)
Kuwahara, Ayuko (06004)	Lee, Cynthia (C803)	Lee, kyunghee (11059)
Kuwasiro, Yoshitaka (08130)	Lee, Deok Ho (03114)	Lee, Mi-Hyun (02046)
Kwack, Yeon-Joo (03047)	Lee, Dong Hee (15012)	Lee, Mi-Hyun (08114)
Kwack, Yeon-Joo (03048)	Lee, Dong Ju (03040)	Lee, Mi-Hyun (08127)*
Kwack, Yeon-Joo (03049)	Lee, Dong Wook (11068)	Lee, Miyoung (12038)
Kwack, Yeon-Joo (03050)	Lee, Dong-Wook (PL203)	Lee, Myeong Min (08093)
Kwak, June M (11017)*	Lee, Eun-Jung (03040)	Lee, Myeong Min (08114)
KWAK, June M. (03092)	Lee, EunKyoung (C704)	Lee, Myoung Hui (11071)*
Kwak, Kyung Jin (03056)*	Lee, EunKyoung (08004)	Lee, Ok chan (08154)
Kwon, Eunjung (02016)	Lee, Hak-Soo (12037)	Lee, Ok Ran (11026)
Kwon, Kwang-Chul (08144)	Lee, Han Woo (08053)	Lee, Ruey-Hua (08049)*
Kwon, So Hyun (08168)*	Lee, Han Yong (03089)*	LEE, SANG CHEUL (06035)
Kwon, Soon Il (08131)	Lee, Han Yong (12039)	Lee, Sang Ho (11026)
Kwon, Sun Jae (12050)*	Lee, Hanna (08125)*	Lee, Sang Ho (12018)
Kwon, Tackmin (02040)	Lee, Hanna (08139)	Lee, Sang Joon (03089)
Kwon, Yerim (03113)	Lee, Hojoung (03110)	Lee, Sang Joon (12041)
Kwon, Yerim (03115)*	Lee, Hojoung (03113)	Lee, Sang Joon (12042)
Kwon, Young Sang (02036)	Lee, Hojoung (03115)	Lee, Sang Min (11059)
Kyotani, Toshinobu (02052)	Lee, Horim (08099)*	Lee, Sang Yeol (02048)
Lahner, Brett (07011)	Lee, Ho-Seok (11032)	Lee, Sang Yeol (02049)
Lai, Chia-Ping (10011)*	Lee, Hwa Jung (02025)	Lee, Sang Yeol (03069)
Lai, Chia-Ping (12032)*	Lee, Hye Bin (03114)	Lee, Sang Yeol (03074)
Lai, Chia-Ping (17008)*	Lee, Hyoung Ju (02037)	Lee, Sang Yeol (03075)
Lai, Erh-Min (02022)	Lee, Il-Hwan (08160)*	Lee, Sang Yeol (03111)
Lai, Hui-Chuan (03088)	Lee, In Chul (08160)	Lee, Sang Yeol (03112)
Lai, Kwok Wai (06022)*	Lee, Insuk (PL602)	Lee, Sang Yeol (03114)
Lajunen, Heini (C205)	Lee, Jai Heon (02028)	Lee, Sangmee (11017)
Lalonde, Sylvie (C604)	Lee, Jai Heon (02029)	Lee, Se Jeong (08144)*
Lalonde, Sylvie (02021)	Lee, Jai Heon (02031)	Lee, Seonghee (02001)*
Lamesch, Philippe (C803)	Lee, Jai-Heon (03047)	Lee, Seon-Woo (02037)
Landberg, Katarina (08164)*	Lee, Jai-Heon (03048)	Lee, Shin Ae (08114)
Lange, B. Markus (PL602)	Lee, Jai-Heon (03049)	Lee, Shin Ae (08115)*
Lanz, Christa (10005)	Lee, Jai-Heon (03050)	Lee, Shu-Hong (15015)*
Larrieu, Antoine (08056)	Lee, Jee Woong (08154)	Lee, Soohyun (02036)
Larrieu, Antoine (17009)	Lee, Jent-turn (03091)	Lee, Soohyun (12050)
Larsson, Dennis (07002)	Lee, Jeong Hee (02043)	Lee, SookJin (PL203)
Last, Robert L. (C603)*	Lee, Jeong Hwan (08125)	Lee, Stephen (03013)
Last, Robert L. (18004)*	Lee, Jeong Hwan (08140)*	Lee, Stephen (12009)
Laurie, Rebecca (08041)	Lee, Jin Suk (PL801)	Lee, Sumin (PL203)
Laux, Thomas (08043)	Lee, Jinwon (04018)	Lee, Sumin (11068)*
Law, Ching Wen (08012)	Lee, Jinwon (04047)*	Lee, Sun Yong (03111)
Law, Robert (13009)*	Lee, Jiyoung (17006)	Lee, Sun-Young (08089)
Law, Robert (13010)	Lee, Ji-Young (08122)	Lee, Yew (12037)
Law, Rona (04048)	Lee, Ji-Young (08165)	Lee, Ying-Ling (02022)
Lazaro, Ana (08098)	Lee, Jong Seob (08134)	Lee, Yong-Hwan (04039)
Lazarowitz, Sondra (02019)	Lee, Jong Seob (08140)	Lee, Yongjik (PL203)
Lazarowitz, Sondra (08058)*	Lee, Junho (PL203)	Lee, Young Mee (03114)
Lazzaro, Michelle (11017)	Lee, Junho (11069)*	Lee, Youngsook (11030)
Le, Dinh Huan (03093)	Lee, Keh Chien (04035)	Lee, Youngsook (12038)
Le, Dung (15003)*	Lee, Kyun Oh (02048)	Lehesranta, Satu (C706)
Leaflight, Ren (C802)	Lee, Kyun Oh (02049)	Lehesranta, Satu (08122)
Leaver, Christopher J. (11021)	Lee, Kyun Oh (03074)	Lehmann, Martin (06019)
Lee, Byung Ha (08168)	Lee, Kyun Oh (03075)	Lepage, Etienne (17010)
Lee, Byung Ha (08169)	Lee, Kyun Oh (03111)	Lewandowska-Gnatowska, Elzbieta (03077)
Lee, Byung Ha (08170)*	Lee, Kyun Oh (03114)	Leyser, Ottoline (07014)

Leyser, Ottoline (10008)	Lin, Tsan-Piao (03088)	Luijten, Marijn (08037)
Li, Chia-Wen (03067)*	Lin, Tsan-Piao (03109)	Lumba, Shelley (PL101)
Li, Chia-Wen (03068)	Lin, Wan-Chen (08120)	Lundh, Dan (07002)
Li, Chia-Wen (15015)	Lin, Wen Yong (03125)	Luo, Lilan (08096)*
Li, Chijun (12027)	Lin, Yann-Rong (17018)	Luo, Xue (08007)
Li, Chijun (12031)	Lindgren, Ove (08122)	Luo, Yonghai (10021)*
Li, Donghui (C803)	Lindsay, Donna (08075)*	Lyons, Rebecca (02032)*
Li, Dongmei (C704)	Lingard, Matthew J. (11049)	Lyu, Aram (09012)
Li, Dongmei (08004)	Lipka, Volker (02008)	Ma, Hong (08007)
Li, Haihang (03052)	Lisec, Jan (06043)	Ma, Hong (08010)
Li, Hongju (11029)*	Lisec, Jan (10012)	Ma, Hong (12027)
Li, Hongzhe (14001)	Lisec, Jan (10024)	Ma, Hong (12031)
Li, Hui (08005)	Liu, Bing (03071)	Ma, Jian Feng (14010)
Li, Jia (01004)	Liu, Chang (08128)*	Ma, Jinyu (06002)
Li, Jian-Feng (15009)*	Liu, Chunyan (04015)	Ma, Shisong (07015)*
Li, Jianming (11017)	Liu, Chunyan (04029)	Machida, Chiyoko (08051)*
Li, Jing (03024)	Liu, Jie (08066)	Machida, Chiyoko (08081)
Li, Lei (08010)	Liu, Jie (08068)	Machida, Chiyoko (08096)
Li, Lina (08109)	Liu, Jie (08069)	Machida, Chiyoko (08151)
Li, Ling (03052)	Liu, Jun (03071)	Machida, Chiyoko (08155)
Li, Lixin (11018)	Liu, Man (04017)	Machida, Chiyoko (08156)
Li, Meina (08014)	Liu, Mao-Sen (08110)*	Machida, Chiyoko (08174)
Li, Ning (03112)	Liu, Shenkui (12003)	Machida, Chiyoko (11027)
Li, Q. Quinn (04017)*	Liu, Tie (PL201)	Machida, Yasunori (08051)
Li, Qianfeng (12046)	Liu, Xiao Min (08106)	Machida, Yasunori (08081)
Li, Qun (12056)	Liu, Xiao Min (11056)	Machida, Yasunori (08096)
Li, Wen-Xue (03024)*	Liu, Xu (03052)*	Machida, Yasunori (08151)
Li, Wenyang (12028)	Liu, Yang (09014)	Machida, Yasunori (08155)
Li, Xing Guo (12019)	Liu, Yang (09016)	Machida, Yasunori (08156)
Li, Xinran (08068)*	Liu, Yang (13007)	Machida, Yasunori (08174)
Li, Yi-Hang (C404)	Liu, Yue (C301)	Machida, Yasunori (11027)
Li, Yi-Ho (02022)	Liu, Yu-Shan (08072)	Machida, Yasunori (13011)
Lian, Heng (08008)	Liu, Zhongchi (08042)*	Machida, Yasunori (17015)
Liang, Wanqi (08005)	Liu, Zuojun (12027)	Mackey, David (02011)
Liang, Wanqi (08006)	Liu, Zuojun (12031)	Macknight, Richard (08041)
Liang, Wanqi (08007)	Loake, Gary (02016)	Madhou, Priya (12008)
Liao, Hui (C301)	Long, David E. (06030)	Maeda, Satoru (02044)*
Liao, Lili (12005)	Long, Sharon R. (11058)	Maeda, Yutaka (03058)
Liao, Shih-Yi (08161)	Long, Terri (07011)*	Maehashi, Kenji (03107)
Liao, Yu-Chen (02022)	Lopes, Telma (09018)	Maekawa, Shugo (08050)
Lichtenberger, Raffael (C706)	Lopez-Gonzalez, Leticia (08100)	Maekawa, Shugo (08091)*
Lim, Chae Oh (03016)*	Losse, R. (15020)	Maekawa, Shugo (08092)
Lim, Chae Oh (03025)*	Loudet, Olivier (PL701)*	Maeo, Kenichiro (06009)
Lim, Chae Oh (03026)*	Loudet, Olivier (18007)*	Maeo, Kenichiro (17005)*
Lim, Chae Oh (03062)	Lu, Chaofu (06014)*	Maes, Lies (08036)
Lim, Chan Ju (03062)	Lu, Cheng (03078)	Maeshima, Masayoshi (03059)
Lim, Hyun Ju (02029)	Lu, Chung-An (08072)	Maeshima, Masayoshi (03121)
Lim, Jun (08114)	Lu, Falong (04015)	Maeshima, Masayoshi (08001)
Lim, Jun (08115)	Lu, Falong (04029)*	Maeshima, Masayoshi (11038)
Lim, Jun (08127)	Lu, Jie (04016)	Maeshima, Masayoshi (11061)
Lim, Poay N. (10001)*	Lu, Kuan-Ju (08072)*	Magnani, Enrico (PL201)
Lin, Changfa (08086)*	Lu, Sheen (03066)	Magome, Hiroshi (12048)
Lin, Chentao (PL302)*	Lu, Xunli (02013)	Mahadeo, Nawkar Ganesh (02049)
Lin, Chun-Hung (08110)	Lu, Yan (C603)	Maibam, Punyakishore (02048)
Lin, Pei-Chi (12023)	Lucas, Jessica (C704)	Makaroff, Dr.Christopher A. (06030)
Lin, Pei-Chi (12024)	Lucas, Jessica (08004)	Maki, Yuko (08103)*

Makino, Amane (11057)	Masuda, Choji (12052)	Matsuzaki, Jun (03102)*
Makino, Sachi (11061)	Masuda, Shinji (03108)	Matsuzawa, Atsushi (08142)
Makita, Nobue (09003)	Masuda, Tatsuru (06003)*	Matsuzawa, Atsushi (09008)
Makita, Nobue (12025)	Masuda, Tatsuru (06011)	Matthes, Annemarie (06019)
Makita, Yuko (15006)*	Masuko, Hiromi (11046)	Matthewman, Colette (C602)
Malenica, Nenad (03090)	Masuya, Hiroshi (09006)	Matzke, Antonius (C401)
Mamedov, Fikret (11009)	Matallana, Lilian (03030)	Matzke, Marjori (C401)*
Manabe, Katsushi (17016)	Mathieu, Olivier (04028)	McCourt, Peter (PL101)*
Manavella, Pablo (04020)*	Matser, Vera (08119)*	McKenna, Joe (12008)
Mandal, Abul (07002)*	Matsuda, Fumio (06010)	McKenzie, Neil (PL503)
Mandel, Therese (PL803)	Matsuda, Fumio (06012)	McWatters, Harriet (03094)
Manickavelu, Alagu (14006)*	Matsuda, Fumio (06039)	McWatters, Harriet G. (03038)
Mann, James (C802)	Matsuda, Fumio (07017)	Meagher, Richard (04030)
Mann, Varda (06007)*	Matsuda, Yasuyuki (14002)	Meier, Iris (11050)
Mano, Jun'ichi (03011)*	Matsui, Akihiro (04002)	Meijer, Annemarie H. (08076)
Mano, Jun'ichi (06024)	Matsui, Akihiro (04006)*	Meixner, Alfred A. (11041)
Mansfield, John (12008)	Matsui, Akihiro (04014)	Melchinger, Albrecht (10012)
Manuel Chico, Jose (12001)	Matsui, Akihiro (16005)	Mellor, Nathan (C703)
Marcotte, Edward (PL602)	Matsui, Keiko (14005)	Menke, Frank (08057)*
Marechal, Alexandre (17010)	Matsui, Kyoko (04005)*	Mette, Michael Florian (04007)*
Marin, Esther (08038)	Matsui, Kyoko (09020)	Meyer, Etienne (07020)
Marquardt, Katrin (03051)	Matsui, Minami (C801)*	Meyer, Rhonda (06043)
Marsch Martinez, Nayelli (08077)	Matsui, Minami (01007)	Meyer, Rhonda (10012)
Marsch-Martinez, Nayelli (08076)*	Matsui, Minami (02032)	Meyer, Rhonda C. (10024)
Marshall, Alex (07008)	Matsui, Minami (02044)	Meyer, Tom (C803)
Marshall, Alex (08047)	Matsui, Minami (03057)	Meyerowitz, Elliot (01003)
Martienssen, Robert A (C405)	Matsui, Minami (03061)	Meyerowitz, Elliot (08030)
Martin, Gregory B. (02003)	Matsui, Minami (07017)	Meyerowitz, Elliot M. (K002)*
Martinec, Jan (12047)*	Matsui, Minami (08002)	Meyerowitz, Elliot M. (C102)*
Martinoia, Enrico (12038)	Matsui, Minami (08017)	Meyers, Emma (12030)
Maruta, Takanori (03044)	Matsui, Minami (08084)	Mhuanthong, Wuttichai (16008)
Maruta, Takanori (03053)	Matsui, Minami (08111)	Mhuanthong, Wuttichai (16009)
Maruta, Takanori (03060)	Matsui, Minami (08148)	Miao, Ying (08033)
Maruta, Takanori (03063)	Matsui, Minami (08149)	Miao, Ying (11005)*
Maruta, Takanori (03087)	Matsui, Minami (09006)	Michikawa, Masataka (11053)
Maruta, Takanori (03116)	Matsui, Minami (09009)	Middleton, Alistair (C703)
Maruta, Takanori (06016)	Matsui, Minami (13005)	Mielke, Kati (12034)*
Maruta, Takanori (06017)	Matsui, Minami (14004)	Mikamori, Hiroki (02014)
Maruta, Takanori (06037)*	Matsui, Minami (14005)	Millar, A. Harvey (16007)
Maruyama, Daisuke (11065)	Matsui, Minami (15006)	Millar, Andrew (07006)
Maruyama, Kyonoshin (03017)	Matsukura, Satoko (03027)	Millar, Andrew J (03022)
Maruyama, Kyonoshin (03018)	Matsukura, Satoko (03037)*	Millar, Andrew J (08011)
Maruyama, Kyonoshin (03019)	Matsumaru, Masamichi (02015)	Millar, Andrew J. (03038)
Maruyama, Kyonoshin (03033)	Matsumoto, Shogo (06044)	Millar, Harvey (07020)
Maruyama, Kyonoshin (03037)	Matsumoto, Takashi (10006)	Miller, Gad (03002)
Maruyama, Kyonoshin (03045)	Matsumoto, Takayuki (06009)	Miller, Julie (C802)
Maruyama, Kyonoshin (03055)	Matsumura, Hideo (11003)	Mimura, Tetsuro (C601)*
Maruyama, Kyonoshin (12014)	Matsumura, Yoko (08151)*	Mimura, Tetsuro (06027)
Maruyama, Kyonoshin (16002)*	Matsumura, Yoko (08174)	Mimura, Tetsuro (06031)
Maruyama, Shinichiro (10023)	Matsunaga, Sachihiro (11022)*	MIMURA, Tetsuro (06036)
Maruyama, Yosuke (02038)	Matsuoka, Naoko (15019)	Mimura, Tetsuro (08080)
Maruyama-Nakashita, Akiko (03099)*	Matsuoka, Daisuke (03125)	Minami, Anzu (03023)
Masaoka, Kanari (02018)	MATSUOKA, Daisuke (15018)	Minamisawa, Kazunori (08031)
Mashiguchi, Kiyoshi (06013)	Matsuoka, Makoto (PL103)*	Mira-Rodado, Virtudes (08026)
Mashiguchi, Kiyoshi (06038)	Matsuoka, Makoto (09003)	Mira-Rodado, Virtudes (11040)
Mason, Mike (12030)*	Matsushima, Ryo (11018)	Mishra, Girish (06002)

Mithoe, Sharon (08057)	Mochida, Keiichi (09017)	Mugford, Sarah G. (C602)
Mitina, Irina (04032)	Mochida, Keiichi (14007)*	Muller, Karel (17001)
MITINA, Irina (04040)	Mochida, Keiichi (14008)*	Muller, Robert (C803)
Mitsuda, Nobutaka (C104)	Mochizuki, Takako (16004)	Mullin, Nic (08070)
Mitsuda, Nobutaka (03098)	Mochizuki, Yoshiki (04006)	Munemasa, Shintaro (12053)
Mitsuda, Nobutaka (08135)	Mohnen, Debra (13015)	Munemasa, Shintaro (12054)
Mitsuda, Nobutaka (08145)	Momma, Takayuki (06025)	Munnik, Teun (11008)
Mitsuda, Nobutaka (09009)*	Moon, Dae-Sung (03104)	Munoz-Bertomeu, Jesus (06018)
Mitsuda, Nobutaka (09020)	Moon, Stephanie (PL602)	Muranaka, Toshiya (06032)
Mitsuda, Nobutaka (12006)	Moon, Yong-Hwan (03073)	Muranaka, Toshiya (06033)
Mitsuda, Nobutaka (12043)	Moon, Yong-Hwan (03093)	Muranaka, Toshiya (06034)
Mitsuda, Nobutaka (13014)	Moon, Yong-Hwan (08089)	Muranaka, Toshiya (06042)
Mitsuda, Nobutaka (15011)	Moore, Michelle (17004)*	Muranaka, Toshiya (06044)
Mitsui, Toshiaki (11063)	Moreno, Nuno (09018)	Murata, Ariaki (06021)
Mittler, Ron (03002)	Moreno-Risueno, Miguel (PL802)	Murata, Satoko (02018)
Miura, Asuka (04026)	Moreno-Risueno, Miguel (08013)*	Murata, Yoshiyuki (12052)
Miura, Asuka (04028)	Moreno-Risueno, Miguel A. (08016)	Murata, Yoshiyuki (12053)*
Miura, Kenji (03003)*	Moreno-Risueno, Miguel A. (08122)	Murata, Yoshiyuki (12054)
Miura, Kenji (06040)	Mori, Izumi (12053)	Muroya, Mitsuhiro (17016)*
Miura, Kenji (08031)	Mori, Izumi C. (12052)	Murray, James AH (C704)
Miura, Shinya (03020)	Mori, Izumi C. (12054)	Murray, James AH (08004)
Miwa, Kyoko (02047)	Mori, Masaki (02044)	Mussar, Kristofer (04030)*
Miwa, Kyoko (03103)*	Mori, Masashi (C505)	Muszynska, Grazyna (03077)
Miwa, Kyoko (06001)	Mori, Tatsuya (03053)	Muthappa, Senthil-Kumar (02001)
Miyaji, Takaaki (C305)	Morimoto, Kyoko (03028)	Muthuvel, Bhuma (C802)
Miyamoto, Mayu (05003)	Morinaga, Shin-Ichi (10016)*	Muto, Shu (08002)
Miyamoto, Mayu (08162)	Morishita, Teruyuki (03060)	Muto, Yukari (08001)
Miyao, AKio (03029)	Morita, Haruka (03029)	Myouga, Fumiyoshi (03080)
Miyao, Akio (08061)	Morita-Terao, Miyo (11052)	Myouga, Fumiyoshi (12002)
Miyao, Akio (09020)	Mori-Ueda, Nanae (12026)*	Myrenas, Mattias (08035)
Miyao, Akio (10006)	Moriwaki, Teppei (08137)	Mysore, Kirankumar (02001)
Miyao, Akio (10022)	Moriwaki, Teppei (08147)*	Mysore, Kirankumar (08041)
Miyashima, Shunsuke (C706)	Moriyama, Hiromitsu (04033)	Nagai, Kazuo (08156)
Miyata, Kana (08087)*	Moriyama, Hiromitsu (04045)	Nagamura, Yoshiaki (03118)
Miyata, Kana (08113)	Moriyama, Yoshinori (C305)	Nagamura, Yoshiaki (09020)
Miyawaki, Kaori (01001)*	Morohashi, Kengo (C704)	Nagano, Atsushi J (11025)
Miyawaki, Tatsuya (06025)	Morohashi, Kengo (07007)*	Nagano, Atsushi J. (10016)
Miyazawa, Yutaka (03020)	Morohashi, Kengo (08004)	Nagano, Kuniaki (03118)
Miyazawa, Yutaka (08137)*	Morosawa, Taeko (03023)	Nagano, Mutsumi (06004)
Miyazawa, Yutaka (08147)	Morosawa, Taeko (04002)	Nagano, Mutsumi (06012)
Mizoguchi, Masahide (03096)*	Morosawa, Taeko (04006)	Nagasaki, Atsushi (03121)
Mizoguchi, Masahide (12002)	Morosawa, Taeko (04014)	Nagase, Yuta (03007)
Mizoguchi, Tsuyoshi (02042)	Morsa, Stijn (03002)	Nagata, Chisako (11038)*
Mizoguchi, Tsuyoshi (08087)	Moses, Alan (PL101)	Nagata, Maki (12055)*
Mizoguchi, Tsuyoshi (08113)	Motohashi, Reiko (11012)*	Nagata, Noriko (11012)
Mizoi, Junya (03017)	Motose, Hiroyasu (08104)	Nagata, Toshifumi (09020)
Mizoi, Junya (03018)	Motose, Hiroyasu (08108)	Nagato, Yasuo (08061)
Mizoi, Junya (03028)	Motose, Hiroyasu (08130)*	Nagatoshi, Yukari (03098)*
Mizoi, Junya (03036)*	Motose, Hiroyasu (08136)	Nagaya, Tsutomu (11048)
Mizoi, Junya (03037)	Mou, Zhonglin (02023)	Nagayama, Youichi (15014)
Mizoi, Junya (03045)	Mouchel, Celine (10008)	Nagel, Dawn (PL301)
Mizoi, Junya (11023)	MOYROUD, Edwige (08025)	Nagumo, Miho (03119)
Mizoi, Junya (16002)	Mueller, Isabel (11028)	Nagy, Ferenc (08011)
Mizuno, Takeshi (17003)	Mueller, Margarete (03130)*	Nahal, Hardeep (07013)
Mizutani, Masaharu (06033)	Mueller, Ralf (04013)*	Nahar, Most. (08129)*
Mizutani, Megumi (03029)	Mueller-Roeber, Bernd (03030)	Nahar, Noor (07002)

Naito, Satoshi (04034)	Nakashima, Kazuo (03019)	Nieminen, Kaisa (08165)
Naito, Satoshi (07016)	Nakashima, Kazuo (03027)	Niibori, Hitomi (11053)
Naito, Satoshi (11015)	Nakashima, Kazuo (03034)	Niihama, Mitsuru (08074)
Nakabayashi, Kazumi (08082)*	Nakashima, Kazuo (03045)	Niikura, Satoshi (17017)
Nakabayashi, Ryo (06010)	Nakashima, Kazuo (03096)	Niinuma, Kanae (08018)*
Nakagami, Hirofumi (03023)	Nakashima, Kazuo (11023)	Niinuma, Kanae (08087)
Nakagami, Hirofumi (09017)*	Nakashima, Kazuo (12014)*	Niitsu, Masaru (08108)
Nakagami, Hirofumi (18002)*	Nakashita, Hideo (02053)	Nikolau, Basil J. (PL602)
Nakagawa, Ayami (06041)	Nakashita, Hideo (02056)	Nishida, Ikuo (08046)
Nakagawa, Ayami (08155)	Nakashita, Hideo (12055)	Nishida, Ikuo (11030)
Nakagawa, Ayami (08156)*	Nakasone, Akari (12044)	Nishihara, Kiyoshi (09016)
Nakagawa, Mayu (08113)	Nakasone, Shoko (03045)	Nishihara, Kiyoshi (09019)
Nakagawa, Tsuyoshi (03054)	Nakata, Masaru (12035)	Nishihara, Kiyoshi (09021)*
Nakagawa, Tsuyoshi (11039)	Nakata, Masaru (12043)*	Nishikawa, Shuh-ichi (11065)*
Nakagawa, Tsuyoshi (11048)	Nakayama, Katsuhiro (11003)	Nishimura, Kohei (12020)*
Nakahira, Yoichi (02045)	Nakayama, Naomi (PL803)	Nishimura, Kohji (11048)*
Nakajima Munekage, Yuri (03081)*	Nakayama, Naomi (08020)*	Nishimura, Mikio (PL202)
Nakajima, Keiji (08052)*	Nakayama, Yasumune (06027)	Nishimura, Mikio (06011)
Nakajima, Masaki (11021)	Nakazawa, Yukihiro (04033)	Nishimura, Mikio (08150)
Nakajima, Masami (02056)	Nakazono, Mikio (05002)	Nishimura, Mikio (10016)
Nakajima, Nobuyoshi (09013)	Nam, Hong Gil (08078)	Nishimura, Mikio (11018)
Nakajyo, Haruyuki (06040)*	Nam, Hong Gil (08160)	Nishimura, Mikio (11025)
Nakamichi, Norihito (17003)*	Nam, Hong-Gil (11017)	Nishimura, Mikio (13013)
Nakaminami, Kentaro (03023)*	Nam, Jaesung (02040)*	Nishimura, Noriyuki (03013)
Nakamori, Chihiro (05006)	Nam, Kyoung Hee (03062)	Nishimura, Noriyuki (12009)*
Nakamura, Atsuko (14010)	Nam, Myung Hee (12050)	Nishimura, Shin-Ichrou (11063)
Nakamura, Atsuko (14011)	Nam, Youn Jeong (03064)*	Nishina, Momoko (11025)
Nakamura, Kenzo (02018)	Namba, Shigetou (15017)	Nishitani, Aiko (11044)
Nakamura, Kenzo (06009)	Nambara, Eiji (12006)	Nishitani, Kazuhiko (14010)
Nakamura, Kenzo (08155)	Namigoshi, Keita (10006)	Nishitani, Kazuhiko (14011)
Nakamura, Kenzo (13006)	Nanmori, Takashi (03125)	Nishiuchi, Takumi (02026)
Nakamura, Kenzo (17005)	NANMORI, Takashi (15018)	Nishiuchi, Takumi (02035)
Nakamura, Miyuki (04026)	Naramoto, Satoshi (08157)*	Nishiura, Yasumasa (07019)
Nakamura, Miyuki (04042)*	Narisawa, Tomoko (06047)	Nishiyama, Rie (03064)
Nakamura, Moritaka (11060)*	Narumi, Issay (06039)	Nishiyama, Rie (03070)*
Nakamura, Suguru (11066)	Narumi, Issay (12044)	Nishiyama, Tomoaki (01001)
Nakamura, Yasukazu (16004)	Narumi, Takako (15011)	Nishiyama, Tomoaki (11044)
Nakamura, Yoshimasa (12052)	Nath, Suvadeep (09011)	Nishizawa, Naoko K (16006)
Nakamura, Yoshimasa (12053)	Natsui, Yu (08113)	Nishizawa-Yokoi, Ayako (03060)
Nakamura, Yoshimasa (12054)	Naumann, Ulf (C401)	Nishizawa-Yokoi, Ayako (03065)*
Nakamuura, Yuki (08012)*	Navarova, Hana (02002)	Nito, Kazumasa (12009)
Nakanishi, Hiromi (16006)	Navarova, Hana (02039)*	Niu, Lifang (04015)
Nakanishi, Tomoko (C304)	Nebenfuehr, Andreas (15009)	Nobusawa, Takashi (08073)*
Nakano, Akihiko (08157)	Nefissi, Rim (08113)*	Noguchi, Ko (03085)
Nakano, Akihiko (11020)	Neirynck, Sandy (07001)	Noh, Bosl (04027)
Nakano, Akihiko (11042)	Nelissen, Hilde (04012)	Noh, Bosl (04032)
Nakano, Akihiko (11043)	Nelson, Jeffrey (PL301)	Noh, Bosl (04039)
Nakano, Akihiko (11044)	Neubert, Christoph (11037)*	NOH, Bosl (04040)*
Nakano, Akihiko (11045)	Neuhaus, Gunther (03051)	Noh, Bosl (04043)
Nakano, Akihiko (11052)	Newell, Nicole (PL201)	Noh, Hana (03110)
Nakano, Masataka (11047)*	Newman, Mari-Anne (C205)	Noh, Hana (03113)
Nakano, Rie (11027)	Neyt, Pia (04012)	Noh, Yoo-Sun (04027)*
Nakano, Ryohei Thomas (11018)*	Nguyen, Long (11004)	Noh, Yoo-Sun (04032)*
Nakano, Takeshi (10020)	Nguyen, Xuan Canh (08106)	Noh, Yoo-Sun (04039)
Nakano, Yasukazu (08126)	Nguyen, Xuan Canh (11059)	NOH, Yoo-Sun (04040)
Nakashima, Kazuo (03018)	Nielsen, Erik (13003)*	Noh, Yoo-Sun (04043)

Noir, Sandra (12007)*	Ohashi-Ito, Kyoko (08124)*	Oka, Atsuhiro (03129)
Nokajima, Hiroshi (02054)	Ohbayashi, Iwai (04025)*	Oka, Atsuhiro (11008)
Noma, Satoko (11065)	Ohbayashi, Iwai (08151)	OKA, Mariko (15018)
Nomura, Hironari (02045)*	Ohbu, Sumie (09014)	Oka, Nodoka (12057)*
Nomura, Takahito (10020)*	Ohbu, Sumie (09015)	Okada, Kazunori (02054)
Nosaka, Misuzu (04036)*	Ohbu, Sumie (09016)	Okada, Kiyotaka (05006)
Nosaka, Ryota (03065)	Ohbu, Sumie (09021)	Okada, Kiyotaka (08101)
Noshi, Masahiro (03063)*	Ohlrogge, John (C603)	Okada, Kiyotaka (08146)
Nou, Ill-Sup (11046)	Ohme-Takagi, Masaru (C104)	Okada, Kiyotaka (08150)
Noudomi, Keiko (08118)*	Ohme-Takagi, Masaru (03027)	Okada, Kiyotaka (08163)
NOUTOSHI, Yoshiteru (02041)	Ohme-Takagi, Masaru (03065)	Okada, Kiyotaka (12006)
Noutoshi, Yoshiteru (02052)*	Ohme-Takagi, Masaru (03098)	Okada, Ryo (04045)
Novak, Ondrej (03040)	Ohme-Takagi, Masaru (04005)	Okada, Toshihiro (03046)
Novotna, Zuzana (02002)	Ohme-Takagi, Masaru (08040)	Okai, Masahiko (11055)
Nozaki, Hisayoshi (10023)	Ohme-Takagi, Masaru (08135)	Okajima, Koji (03126)*
Ntoukakis, Varids (02008)	Ohme-Takagi, Masaru (08145)	Okamoto, Haruko (03094)
Nuernberger, Thorsten (C205)*	Ohme-Takagi, Masaru (09009)	Okamoto, Masahiro (14004)
Numark, Somrak (16008)	Ohme-Takagi, Masaru (09020)	Okamoto, Masanori (16005)
Nunes-Nesi, Adriano (06018)	Ohme-Takagi, Masaru (12006)	Okamoto, Satoru (05002)*
Nunes-Nesi, Adriano (13001)	Ohme-Takagi, Masaru (12043)	Okano, Yuko (11070)
Nusinow, Dmitri (PL301)	Ohme-Takagi, Masaru (13014)	Okatani, Yusuke (11044)
Nussaume, Laurent (C304)*	Ohme-Takagi, Masaru (15011)	Okazaki, Masateru (02052)
Nussaume, Laurent (08036)	Ohmori, Hiroyuki (06001)	Okazaki, Yozo (06047)*
Nyunoya, Hiroshi (02015)*	Ohmori, Shinnosuke (08061)	Okiyama, Yuuya (10006)
Nyunoya, Hiroshi (11047)	Ohmori, Yoshihiro (08088)*	Okiyama, Yuuya (10022)*
Obara, Mari (01001)	Ohmura, Tomohiro (11022)	Okubo, Kousaku (16004)
Obata, Toshihiro (06019)*	Ohneda, Masako (08090)	Okuma, Eiji (12054)*
Obayashi, Takeshi (16003)*	Ohnishi, Erika (05002)	Okumoto, Sakiko (06006)*
Obayashi, Takeshi (17013)	Ohnishi, Miwa (06027)*	Okumoto, Sakiko (17004)
Ochiai, Hirokazu (02004)	Ohnishi, Miwa (06031)	Okuno, Tetsuro (02026)
Ochiai, Hirokazu (02005)	OHNISHI, Miwa (06036)	Okushima, Yoko (08109)
Oda, Atsushi (08113)	Ohnishi, Toshiyuki (06033)	Oliveira, M. Margarida (03127)
Oda, Kenji (02044)	Ohno, Akiko (02018)	Omura, Toshihiro (15003)
Oda, Kenji (03057)*	Ohno, Carolyn (08030)	Onai, Kiyoshi (06009)
Oda, Susumu (03118)*	Ohno, Ryoko (03058)	O'Neill, Malcolm (13015)
Oda, Yoshihisa (08124)	Ohr, Hyonhwa (08154)	Ono, Hirokazu (03119)
Oda, Yoshihisa (11064)*	Ohshima, Masumi (01001)	Ono, Kazuko (14002)
Odaira, Shota (02015)	Ohsumi, Yoshinori (08046)	Ono, Yutaka (12045)
Ogata, Shin-ichi (15021)	Ohsumi, Yoshinori (11033)	Onouchi, Hitoshi (04034)
Ogata, Yoshiyuki (03055)	Ohta, Hiroyuki (03108)	Onouchi, Hitoshi (07016)
Ogawa, Daisuke (03029)*	Ohta, Hiroyuki (06011)	Onoue, Noriyuki (04034)
Ogawa, Eriko (08104)*	Ohta, Hiroyuki (08012)	Ooga, Atsushi (07019)
Ogawa, Ken-ich (12054)	Ohta, Masaru (03003)	Oono, Yutaka (12044)*
Ogawa, Takahisa (02027)	Ohtake, Miki (02044)	Orita, Izumi (03039)
Ogichi, Riichi (10015)	Ohtani, Misato (01006)	Osada, Hiroyuki (13005)
Ogihara, Yasunari (04044)	Ohtani, Misato (04014)*	Osaka, Masaaki (17017)*
Ogihara, Yasunari (06042)	Ohtani, Misato (13014)	Osakabe, Keishi (15008)*
Ogihara, Yasunari (14006)	Ohtsubo, Norihiro (08145)	Osakabe, Yuriko (03015)*
Ogo, Yuko (16006)	Ohtsubo, Norihiro (15011)	Osakabe, Yuriko (03018)
Ogura, Rieko (15019)*	Ohtsuki, Hitomi (07016)	Osakabe, Yuriko (11023)
Ogura, Takehiko (12015)	Ohyama, Kiyoshi (06033)	Osanai, Takashi (06026)*
Oguri, Yasuko (01005)	Ohyama, Kiyoshi (06034)	Oshima, Haruka (17016)
Oguri, Yasuko (01007)	Ohyama, Kiyoshi (06042)	Oshima, Yoshimi (08145)*
Oh, Chang-Sik (02003)	Oikawa, Akira (06026)	Oshima, Yoshimi (15011)
Oh, Jee Eun (03113)	Oikawa, Akira (06027)	Oshino, Takeshi (03020)
Oh, Man-Ho (12016)	Oiwa, Yuki (08105)	Osnato, Michela (08038)*

Osteryoung, Katherine (C603)	Park, Ju-Young (04039)	Poirier, Yves (C304)
Ostler, Sandra (02039)	Park, Kyung Hyuk (08138)	Pomeranz, Marcelo (12023)
Otori, Kumi (06016)	Park, Kyunghyuk (08134)	Popescu, Sorina (07015)
Otori, Kumi (06037)	Park, Ohkmae K. (08131)	Pornsiriwong, Wannarat (03032)
Otsuka, Kurataka (08152)*	Park, Sang-Youl (12009)	Porto, Matthew (07015)
Otsuki, Hitomi (06047)	Park, Su Jin (08160)	Postnikoff, Spike (08075)
Otsuki, Hitomi (07017)	Park, Sungjin (13003)	Pridmore, Tony (C703)
Ott, Felix (04004)	Park, Young Ju (02025)	Provart, Nicholas (07013)*
Ott, Sascha (07005)	Parker, Jane E. (03013)	Prunedá-Paz, Jose (PL301)
Ott, Sascha (16001)	Parry, Geraint (03078)	Prunedá-Paz, Jose (03066)
Otto, Markus (11034)*	Parsons, Harriet (13004)	Putterill, Joanna (08041)*
Ouwerkerk, Pieter B.F. (08076)	Patel, Kanu (C404)	Pyo, Young Jae (03106)*
Pacurar, Daniel (08023)	Patel, Shalaka (11050)	Pyo, Young Jae (08143)*
Pacurar, Monica (08023)	Patil, Gunvant (15002)*	Pyo, Young Jae (08144)
Padilla, Francisco (C802)	Pauwels, Laurens (12001)	Qi, Wenqing (06002)
Paeng, Seul Ki (03111)*	Pavlidis, Pavlos (10002)	Qin, Feng (03028)*
Page, Carla (07015)	Paz-Ares, Javier (C304)	Qin, Feng (03033)
Pai, Hyun-Sook (11032)	Peaucelle, Alexis (08070)	Qin, Feng (03036)
Pai, Hyun-Sook (11035)	Peck, Scott C. (C202)*	Qin, Yuan (08085)
Pai, Hyun-Sook (11036)	Pederson, Eric (08164)	Quail, Peter (03127)
Pajerowska-Mukhtar, Karolina (C201)	Pei, Zhen-Ming (11017)	Quan, Li (08175)*
Pak, Jung Hun (02028)*	Peine, Nora (03013)	Queval, Guillaume (09023)
Pak, Jung Hun (02029)*	Pejchar, Premysl (12047)	Quodt, Vanessa (08064)
Pak, Jung Hun (02031)	Pekker, Irena (08051)	Rahman, Abidur (03008)*
Pak, Jung Hun (15012)	Pelaz, Soraya (08038)	Rahman, Abidur (12045)
Palanivelu, Ravishankar (08085)	Pencik, Ales (11016)	Rahman, Mohammad A. (04011)
Pallas, Jacqueline (02016)	Peng, Hsiao-Ping (03021)	Ramachandran, Vanitharani (04016)
Pan, I-Chun (03068)*	Pereira, Andy (08076)	Rambo, Robert (12009)
Pan, Xiao-Ying (03024)	Pereira, Andy (08077)	Rand, David (07005)
PARCY, Francois (08025)*	Peret, Benjamin (C703)	Rashotte, Aaron (12022)*
Parent, Jean-Sebastien (17010)	Perez-Torres Jr, Rodolfo (06030)	Ratet, Pascal (08041)
Parizot, Boris (07003)	Persiau, Geert (07001)	Rathjen, John (02008)
Park, Chan Ho (12049)*	Peters, Janny L. (08027)	Rausch, Thomas (15004)
Park, Dong-Jin (02048)	Peterson, Kylee (PL801)	Ravanat, Jean-Luc (03002)
Park, Eun Jin (02037)	Petrocelli, Valentina (08024)	Reback, Maxwell A. (14001)
Park, Eunsook (15009)	Petrovska, Beata (11062)	Redestig, Henning (07016)
Park, Geuntae (12012)	Petutschnig, Elena (02008)	Reinhart, Brenda (PL201)
Park, Geun-Tae (08134)*	Petzold, Chris (07020)	Renak, David (08121)*
Park, Geun-Tae (08154)	Petzold, Christopher (13004)	Reyes Olalde, Irepan (08077)
Park, Hee Jin (09005)*	Phan Tran, Lam-Son (15003)	Reymond, Matthieu (K001)
Park, Heesung (02040)	Picot, Emma (16001)*	Reymond, Matthieu (10007)
Park, Hee-Yeon (03073)	Pignocchi, Cristina (11019)	Reymond, Matthieu (10010)
Park, Hee-Yeon (03093)	Pineiro, Manuel (08100)*	Rhee, Seung Yon (PL602)*
Park, Hee-Yeon (08089)	Pineiro, Manuel A. (08098)	Rhee, Seung Yon (PL602)*
Park, Hyeong Cheol (02020)*	Ping, Lan (07004)	Rhee, Sue (C604)
Park, Hyeong Cheol (09005)	Pinon, Violaine (C702)	Rho, Jin-Su (03104)
Park, Hyeong Cheol (17006)	Pinot, Franck (08005)	Ries, Amber (11014)
Park, Hyo Bee (02034)	Pires, J. Chris (17020)	Riewe, David (06043)*
Park, Hyo-Young (04035)	Pirrung, Michael (09011)	Riewe, David (10012)
Park, Jeong Mee (02043)*	Pitaksaringkarn, Weerasak (12006)	Riewe, David (10024)
Park, Jin Ho (02048)	Ploetz, Larry (C803)	RIVARD, Maud (04003)
Park, Jin-Sup (08154)*	Plute, Thomas (C802)	Riveras, Eleodoro (03095)
Park, Jong-In (03118)	Pochylova, Zaneta (11062)	Rivero, Luz (C802)
Park, Jong-In (11046)	Pogson, B. (18006)*	Rivero, Luz (09007)
Park, Joonghyuk (12012)*	Pogson, Barry (03031)	Rivero, Luz (09010)*
Park, Jung Hoon (02020)	Pogson, Barry J. (03032)*	Robert, Stephanie (08045)

Robert, Stephanie (11016)	Saito, Kazuki (06026)	Sasayama, Daisuke (03125)
Roberts, Christina J (08122)*	Saito, Kazuki (06033)	SASAYAMA, Daisuke (15018)*
Roberts, Jeremy A (13007)	Saito, Kazuki (06034)	Sasidharan, Rajkumar (C803)
Roberts, Jeremy A. (08027)	Saito, Kazuki (06039)	SASKA, Ivana (02041)*
Roberts, Nadia (03013)	Saito, Kazuki (06047)	Satake, Akiko (04041)*
Robertson, Masumi (04010)*	Saito, Kazuki (07016)	Satake, Akiko (07019)
Robinson, Simon P (15004)	Saito, Kazuki (07017)	Sato, Atsuko (12021)
Robviewx, Florent (08141)*	Saito, Tamio (13005)	Sato, Junya (14011)*
Rodriguez-Franco, Marta (03051)	Saji, Hikaru (03098)	Sato, Kazuhito (09020)
Roeder, Adrienne (08030)*	Sakai, Tatsuya (03035)*	Sato, Masa H. (11044)
Roesler, Jutta (11001)	Sakai, Yasuyoshi (03039)	Sato, Masanao (C204)
Rogers, Jane (PL503)	Sakakibara, Hitoshi (C104)	Sato, Masanao (C605)
Rolcik, Jakub (11016)	Sakakibara, Hitoshi (03029)	Sato, Masanao (02003)
Ros, Roc (06018)*	Sakakibara, Hitoshi (03039)	Sato, Masanao (02010)
Ross, Annegret (02013)	Sakakibara, Hitoshi (06015)	Sato, Muneharu (10006)
Rossignol, Pascale (C702)	Sakakibara, Hitoshi (09003)	Sato, Naoki (06011)
Rosso, Mario G. (15020)*	Sakakibara, Hitoshi (12017)	Sato, Nobuo (08156)
Roth, Mary (PL602)	Sakakibara, Hitoshi (12025)	Sato, Shigeru (06008)*
Rothan, Christophe (14012)	Sakakibara, Hitoshi (12026)	Sato, Shusei (04009)
Roudier, Francois (04037)	Sakakibara, Hitoshi (14006)	Sato, Shusei (05002)
Roudier, Francois (08060)	Sakakibara, Hitoshi (17003)	Sato, Shusei (14012)
Rounslley, Steve (17018)	Sakamoto, Atsushi (06041)	Sato, Tadashi (09015)
Roy, Bijoyita (09002)	Sakamoto, Atsushi (17013)	Sato, Takeo (08050)*
Russinova, Eugenia (11004)	Sakamoto, Hideki (03033)	Sato, Takeo (08091)
Russinova, Eugenia (11031)	Sakamoto, Norihito (08095)*	Sato, Takeo (08092)
Ruzicka, Kamil (C706)	Sakamoto, Takuya (03124)*	Sato, Yoshikatsu (01001)
Ruzicka, Kamil (08171)*	Sakata, Tadashi (03020)	Sato, Yoshikatsu (01007)
Rychel, Amanda (PL801)	Sakata, Yoichi (03107)	Sato, Yutaka (04036)
Ryu, Choong-Min (02033)*	Sakata, Yoichi (03119)	Satoh, Shinobu (12006)
Ryu, Choong-Min (02034)*	Sako, Kaori (08103)	Satoh, Shinobu (14010)
Ryu, Choong-Min (02036)*	Sakurai, Naoki (13012)	Satoh, Shinobu (14011)
Ryu, Choong-Min (12050)	Sakurai, Nozomu (03011)	Sato-Nara, Kumi (03121)*
Ryu, Hak-Sung (04039)	Sakurai, Nozomu (06031)	Satoshi, Iuchi (03117)
Ryu, Hojin (12011)	Sakurai, Tetsuya (09006)	Saul, Helen (04008)
Ryu, Jee-Youn (04027)	Sakurai, Tetsuya (14007)	Sauveplane, Vincent (08005)
Ryu, Jong Sang (08078)	Sakurai, Tetsuya (14008)	Savage, Linda (C603)
Ryu, Kook Hui (08093)	Sakurai, Tetsuya (14009)*	Savage, Natasha (07006)
Ryu, Moon Young (03082)*	Salt, David (07011)	Savitch, Leonid (14013)*
Ryu, Stephen Beungtae (11026)	Sappl, Pia (08112)*	Sawa, Mariko (PL301)
Ryusui, Rie (03080)	Sarkeshik, Ali (12009)	Sawa, Shinichiro (05005)
Sabatini, Sabrina (12011)	Sarojam, Rajani (08112)	Sawa, Shinichiro (08167)
Sack, Fred (C704)*	Saruhashi, Satoshi (16004)	Sawa, Shinichiro (08172)
Sack, Fred (08004)*	Sasabe, Michiko (08096)	Sawa, Yoshihiro (03044)
Saez-Vasquez, Julio (08151)	Sasabe, Michiko (11027)*	Sawa, Yoshihiro (03054)
Sagasser, Martin (08062)	Sasabe, Michiko (13011)	Sawada, Yuji (06004)
Saibo, Nelson (03127)	Sasaki, Eriko (07009)	Sawada, Yuji (06012)*
Saidi, Noor (02016)	Sasaki, Kanako (06025)	Sawai, Satoru (06033)
Saiga, Shunsuke (C105)	Sasaki, Katsutomo (15011)	Sawai, Satsuki (03007)
Saijo, Yusuke (02013)*	Sasaki, Kentaro (03128)	Sawaki, Naoya (03100)*
Saito, Chieko (11052)*	Sasaki, Kentro (03123)	Sayama, Hiroko (03017)
Saito, Kazuki (PL603)*	Sasaki, Nobumitsu (02015)	Saze, Hidetoshi (04026)
Saito, Kazuki (03041)	Sasaki, Ryosuke (06027)	Scacchi, Emanuele (12011)
Saito, Kazuki (03099)	Sasaki, Ryosuke (06031)	Scherer, Guenther F.E. (12047)
Saito, Kazuki (06004)	Sasaki, Ryousuke (02035)	Scheres, Ben (08021)
Saito, Kazuki (06010)	Sasaki, Shu (12021)*	Scheres, Ben (08028)
Saito, Kazuki (06012)	Sasaya, Takahide (15003)	Scheres, Ben (08037)

Scheres, Ben (08057)	Seo, Mitsunori (12013)*	Shimada, Hiroaki (08148)
Scheres, Ben (08153)	Seok, Hye-Yeon (03073)*	Shimada, Hiroaki (08149)
Scheres, Ben (17005)	Seok, Hye-Yeon (08089)*	Shimada, Hiroaki (11008)
Schiefelbein, John (08093)	Sezaki, Noriko (08104)	Shimada, Hiroshi (06041)
Schmidt, Renate (04007)	Shah, Bhavank (03079)	Shimada, Hiroshi (17013)*
Schmidt, Romy (10012)	Shahollari, Bationa (C202)	Shimada, Takashi L. (09004)*
Schmidt, Romy (10024)	Sharma, Pooja (15002)	Shimada, Tomoo (C505)*
Schmidt, Wolfgang (07004)*	Shaul, Orit (04008)*	Shimada, Tomoo (09004)
Schneider, Katja (08017)*	Shaw, Jei-Fu (10011)	Shimada, Tomoo (11018)
Schneider, Katja (08031)	Shaw, Jei-Fu (12032)	Shimada, Tomoo (11024)
Schnittger, Arp (08060)	Shaw, Jei-Fu (17008)	Shimada, Yukihisa (06021)
Schnittger, Arp (11051)	Sheen, Jen (08099)	Shimada, Yukihisa (07009)*
Schoen, Daniel (17020)	Shen, Mingzhe (02020)	Shimada, Yukihisa (12015)
Scholl, Randy (C802)	Shi, Chun-Lin (C504)	Shimada-Beltran, Harumi (02019)
Scholl, Randy (09007)*	Shi, Chun-Lin (08056)	Shimada-Beltran, Harumi (08058)
Scholl, Randy (09010)	Shi, Chun-Lin (17009)	Shimamoto, Ko (02005)
Schrantz, M. Eric (17020)*	Shi, Dongqiao (08068)	Shimamoto, Ko (08019)
Schreiber, Lukas (08005)	Shi, Dong-Qiao (08066)	Shimizu, Hidetada (C305)
Schroeder, Julian (03106)	Shi, Hui (12010)	Shimizu, Kentaro (08101)
Schroeder, Julian (11014)	Shiau, Jeng-Yuan (07004)	Shimizu, Kentaro K. (10002)*
Schroeder, Julian (12009)	Shiba, Hiroshi (04046)*	Shimizu, Satoko (08102)
Schroeder, Julian I. (03013)	Shiba, Hiroshi (17017)	Shimizu-Inatsugi, Rie (10002)
Schroeder, Mercedes (02012)	Shibagaki, Nakako (06046)*	Shimmen, Teruo (11024)
Schubert, Daniel (04013)	Shibasaki, Kyohei (03008)	Shimotohno, Akie (08153)*
Schulze-Lefert, Paul (02013)	Shibata, Daisuke (03011)	Shimotohno, Akie (17005)
Schumacher, Karin (11037)	Shibata, Daisuke (03055)	Shin, Junhye (04018)
Schumacher, Karin (12033)	Shibata, Kyomi (10020)	Shin, Mi Rim (02049)
Schwager, Nicole (11028)	Shibuya, Naoto (13012)	Shin, Ryoung (03061)
Schwechheimer, Claus (11028)	Shichijo, Chizuko (06027)	Shin, Ryoung (03064)
Sebastian, Jose (08122)	SHICHIJO, Chizuko (06036)	Shin, Ryoung (03084)
Sedbrook, John C. (02014)	Shigemori, Hideyuki (06040)	Shin, Sang Hyun (02028)
Segami, Shoji (11061)*	Shigeoka, Shigeru (02027)	Shin, Yun-Jeong (04023)
Segawa, Yuta (08108)	Shigeoka, Shigeru (03044)	Shinano, Takuro (03101)
Segura, Juan (06018)	Shigeoka, Shigeru (03053)	Shinohara, Kenji (08166)
Seidel, Claus, AM (05001)	Shigeoka, Shigeru (03054)	Shinozaki, Kazuo (C303)
Seki, Hikaru (06032)	Shigeoka, Shigeru (03060)	Shinozaki, Kazuo (C305)
Seki, Hikaru (06033)*	Shigeoka, Shigeru (03063)	Shinozaki, Kazuo (02042)
Seki, Hikaru (06034)	Shigeoka, Shigeru (03065)	Shinozaki, Kazuo (03015)
Seki, Motoaki (C104)	Shigeoka, Shigeru (03087)	Shinozaki, Kazuo (03017)
Seki, Motoaki (03023)	Shigeoka, Shigeru (03116)*	Shinozaki, Kazuo (03018)
Seki, Motoaki (04002)	Shigeoka, Shigeru (06016)	Shinozaki, Kazuo (03019)
Seki, Motoaki (04006)	Shigeoka, Shigeru (06017)	Shinozaki, Kazuo (03023)
Seki, Motoaki (04014)	Shigeoka, Shigeru (06028)	Shinozaki, Kazuo (03027)
Seki, Motoaki (08040)	Shigeoka, Shigeru (06037)	Shinozaki, Kazuo (03028)
Seki, Motoaki (14004)	Shigeyama, Takuma (13012)*	Shinozaki, Kazuo (03033)
Seki, Motoaki (14009)	Shigyo, Mikao (03100)	Shinozaki, Kazuo (03034)
Sekiguchi, Hiroshi (14002)	Shih, Ming-Che (03006)	Shinozaki, Kazuo (03036)
Selbig, Joachim (10012)	Shih, Ming-Che (03021)	Shinozaki, Kazuo (03037)
Sena, Giovanni (C101)	Shiina, Takashi (02045)	Shinozaki, Kazuo (03045)
SEO, Dong Hye (03092)*	Shikata, Masahito (08145)	Shinozaki, Kazuo (03055)
Seo, Jun Sung (12041)*	Shikata, Masahito (15011)*	Shinozaki, Kazuo (03080)
Seo, Jun Sung (12042)*	Shim, Isaac (13009)	Shinozaki, Kazuo (03096)
Seo, Ju-Seok (03089)	Shim, Isaac (13010)*	Shinozaki, Kazuo (03107)
Seo, Mitsunori (06013)	Shim, Jae Sung (03089)	Shinozaki, Kazuo (03117)
Seo, Mitsunori (10017)	Shim, Jae Sung (12039)	Shinozaki, Kazuo (03119)
Seo, Mitsunori (11070)	Shimada, Hiroaki (08111)	Shinozaki, Kazuo (04002)

Shinozaki, Kazuo (04006)	Sinozaki, Satoshi (12055)	Stadler, Thomas (10002)
Shinozaki, Kazuo (04014)	Sirault, X. (18006)	Stahl, Yvonne (05001)*
Shinozaki, Kazuo (06010)	Slotkin, R. Keith (C405)	Stahl, Yvonne (05004)
Shinozaki, Kazuo (08040)	Small, Ian (10003)	Staldal, Veronika (08035)*
Shinozaki, Kazuo (08141)	Smirnoff, Nicholas (03054)	Staldal, Veronika (08159)
Shinozaki, Kazuo (08167)	Smith, Harley (08074)	Stals, Hilde (07001)
Shinozaki, Kazuo (09006)	Smith, Richard (PL803)	Steinfath, Matthias (10012)
Shinozaki, Kazuo (11023)	Snyder, Michael (07015)	Stenvik, Grethe-Elisabeth (C504)
Shinozaki, Kazuo (12002)	So, Hyun A (02029)	Stepanova, Anna (12004)*
Shinozaki, Kazuo (12014)	So, Hyun A (02031)	Stepney, Susan (07014)
Shinozaki, Kazuo (14004)	So, Hyun-A (03047)	Stich, Benjamin (10010)
Shinozaki, Kazuo (14006)	So, Hyun-A (03048)	Stierhof, York-Dieter (11028)
Shinozaki, Kazuo (14007)	So, Hyun-A (03049)*	Stitt, Mark (10010)
Shinozaki, Kazuo (14008)	So, Hyun-A (03050)	Stoddard, Thomas (02010)
Shinozaki, Kazuo (14009)	Soeno, Kazuo (07009)	Storchova, Helena (03005)
Shinozaki, Kazuo (16002)	Soeno, Kazuo (12015)*	Storchova, Helena (17001)*
Shinozaki, Kazuo (16005)	Soga, Keiko (01001)	Stougaard, Jens (04009)
Shinozaki, Satoshi (02053)	Solano, Roberto (12001)	Stracke, Ralf (06023)
Shiota, Hajime (08117)*	Solymosi, Katalin (11009)	Stracke, Ralf (15004)
Shiota, Hajime (08118)	Somers, David (C805)*	Straight, Alexander (03066)
Shiota, Hajime (08142)	Somers, David E. (03038)	Strnad, Miroslav (03040)
Shiota, Hajime (09008)	Somerville, Chris (13002)	Stronberg, Verlyn (06006)
Shiraiwa, Yoshihiro (09013)	Somerville, Shauna (02007)	Stroud, Hume (04048)
Shirasawa, Akira (17017)	Somerville, Shauna (02017)	Styring, Stenbjorn (11009)
Shirasawa, Kenta (14012)*	Son, Bo Hwa (03074)	Su, Jianbin (03071)
Shirasu, Ken (01007)	Son, Bo Hwa (03075)	Su, Liangchen (03052)
Shirasu, Ken (02032)	Son, Daeyoung (03104)	Su, Ying Hua (12019)
SHIRASU, Ken (02041)	Son, Keum-Joo (03104)	Suda, Kunihiro (06031)
Shirasu, Ken (02042)	Son, Seung-Hyun (12051)*	Sudo, Hiroshi (06033)
Shirasu, Ken (02052)	Son, Young Sim (03097)*	SUDO, Keisuke (11046)*
Shirasu, Ken (03023)	Song, Chen (08035)	Sueyoshi, Kuni (08050)
Shirasu, Ken (08131)	Song, Chi Eun (03025)	Sugano, Shigeo S. (C505)
Shirasu, Ken (09017)	Song, Hae-Ryong (04039)	Sugano, Shoji (02044)
Shirasu, Ken (10023)	Song, Jae Hyo (08093)*	Sugawara, Hideaki (16004)
Shirasu, Ken (11033)	Song, Jie (07008)	Sugawara, Satoko (06010)
Shirasu, Ken (15017)	Song, Ji-Hye (12036)	Sugawara, Satoko (06038)
Shiu, Shinhan (C603)	Song, Junqi (C201)	Sugimoto, Eriko (C305)
Shoda, Keiko (11042)	Song, Sang-Kee (08093)	Sugimoto, Kaoru (C102)
Shoji, Kazuhiro (06029)	Song, Sang-Kee (08114)	Sugimoto, Kaoru (01003)*
Shu, Huan (04022)*	Song, Young Hun (03066)	Sugimoto, Keiko (C104)
Shualev, Vladimir (03002)	Sono, Takako (08133)	Sugimoto, Keiko (08002)
Shui, Guanghou (08012)	Sonoda, Yutaka (03123)	Sugimoto, Keiko (08017)
Shulaev, Vladimir (PL602)	Sonoike, Kintake (06011)	Sugimoto, Keiko (08018)
Shulze, Waltraud X (16007)	Soppe, Wim (08082)	Sugimoto, Keiko (08031)
Shumizu, Takumi (15003)	Souk, Seo (03015)	Sugimoto, Keiko (08044)*
Siegel-Gaskins, Dan (07007)	Soyano, Takashi (13011)	Sugimoto, Nagisa (01001)
Sijacic, Paja (08042)	Sozzani, Rosangela (PL802)	Sugino, Ryosuke (03081)
Silva, Francisco Goes da (02029)	Sozzani, Rosangela (08013)	Sugita, Maiko (08061)
Silva, Francisco Goes da (02031)	Sparks, J. Alan (08175)	Sugiura, Masahiro (03046)
Simon, Rüdiger (C501)*	Spetea, Cornelia (11009)*	Sugiyama, Haruna (08087)
Simon, Ruediger (05001)	Spoel, Steven (02016)	Sugiyama, Maki (06013)
Simon, Ruediger (05004)	Spoel, Steven (12057)	Sugiyama, Munetaka (C105)*
Singh, Jas (14013)	Sprott, David (14013)	Sugiyama, Munetaka (01006)
Singh, Prashant (02009)	Srinivasan, Ramamurthy (15002)	Sugiyama, Munetaka (04014)
Singh, Shanker (C803)	Stacey, Gary (11011)	Sugiyama, Munetaka (04025)
Sinha, Neelima (08107)	Stacey, Nicola (08031)	Sugiyama, Munetaka (07018)

Sugiyama, Munetaka (08151)	Tabata, Satoshi (05002)	Takasaki, Hironori (16002)
Sugiyama, Munetaka (08152)	Tabata, Satoshi (14012)	Takase, Tomoyuki (03121)
Sugiyama, Naoyuki (09017)	Tabuchi, Tomoki (06037)	Takashima, Yuya (02050)
Sugiyama, Naoyuki (12002)	Tachibana, Chika (08019)	Takatsujii, Hiroshi (02044)
Sugiyama, Yuko (06027)	Tachikawa, Tomoe (12015)	Takatsuka, Michiyuki (08117)
Sugiyama, Yuko (06031)	Tada, Yasuomi (12057)	Takayama, Seiji (04046)
Suh, Ji yeon (03086)*	Tada, Yuichi (03007)*	Takayama, Seiji (10002)
Sulpice, Ronan (10010)	Tadege, Million (08041)	Takayama, Seiji (17017)
Sumner, Lloyd (PL602)	Tainaka, Hitoshi (03065)	Takeda, Seiji (08102)*
Sun, Bo (C705)	Taji, Teruaki (03107)	Takeda, Seiji (08146)
Sundberg, Eva (08035)	Taji, Teruaki (03119)*	Takeda, Shin (03029)
Sundberg, Eva (08159)	Takabayashi, Atsushi (02038)	Takeda-Kamiya, Noriko (12048)
Sundberg, Eva (08164)	Takada, Yoshinobu (17017)	Takiguchi, Yuko (09009)
Sung, Jih Min (15001)*	Takagi, Kazuteru (06021)	Takiguchi, Yuko (09020)
Sung, Nu Ri (03111)	Takagi, Toshihisa (16004)	Takisawa, Haruhiko (12020)
Sung, Z. Renee (08089)	Takahara, Kentaro (06029)	Tamada, Yosuke (04032)
Sung, Zinmay Renee (08110)	Takahashi, Akira (02054)	Tamai, Atsushi (C505)
Suwabe, Keita (03118)	Takahashi, Fuminori (02042)	Tamaki, Hiroaki (C105)
Suwabe, Keita (10002)	Takahashi, Hideki (02003)	Tamaki, Shojiro (08019)
Suwabe, Keita (11046)	Takahashi, Hideki (03099)	Tamaki, Takayuki (11043)
Suwabe, Keita (17017)	Takahashi, Hideki (05003)	Tamaru, Hisashi (04024)*
Suzaki, Takuya (08079)	Takahashi, Hideki (08162)	Tamayo, Karem (03095)
Suzaki, Takuya (08090)	Takahashi, Hideki (08167)	Tameshige, Toshiaki (08150)*
Suzuki, Akinori (05003)	Takahashi, Hideyuki (03020)	Tamoi, Masahiro (03063)
Suzuki, Akinori (08162)*	Takahashi, Hideyuki (08137)	Tamoi, Masahiro (06016)*
Suzuki, Akinori (08167)	Takahashi, Hideyuki (08147)	Tamoi, Masahiro (06037)
Suzuki, Go (03118)	Takahashi, Hiro (08051)	Tamura, Kentaro (PL202)
Suzuki, Go (10002)	Takahashi, Hiro (08156)	Tamura, Kentaro (04005)
Suzuki, Go (11046)	Takahashi, Hirokazu (05002)	Tamura, Kentaro (11018)
Suzuki, Go (17017)	Takahashi, Kazuma (02038)	Tamura, Kentaro (11024)
Suzuki, Hideyuki (03055)	Takahashi, Maho (12045)*	Tanabe, Noriaki (03053)
Suzuki, Hiromu (02050)	Takahashi, Naoki (03061)	Tanaka, Ayumu (02038)
Suzuki, Hitoshi (03121)	Takahashi, Naoki (08084)*	Tanaka, Hidenori (03015)
Suzuki, Iwane (09013)	Takahashi, Naoki (08111)	Tanaka, Hirokazu (08045)*
Suzuki, Katsunori (12026)	Takahashi, Naoki (08149)	Tanaka, Ichiro (08117)
Suzuki, Makoto (06012)	Takahashi, Sachiko (11012)	Tanaka, Ichiro (08118)
Suzuki, Masashi (06042)	Takahashi, Shinya (03058)*	Tanaka, Ichiro (08142)
Suzuki, Masashi (06044)*	Takahashi, Taku (08104)	Tanaka, Ichiro (09008)
Suzuki, Miho (11012)	Takahashi, Taku (08108)	Tanaka, Kan (06026)
Suzuki, Nobuhiro (03002)	Takahashi, Taku (08130)	Tanaka, Keita (06013)
Suzuki, Shunji (08087)	Takahashi, Taku (08136)	Tanaka, Kiwamu (11011)*
Suzuki, Toshiya (13006)*	Takahashi, Yohsuke (12035)	Tanaka, Maho (03023)
Suzuki, Tsukasa (10006)	Takahashi, Yuji (13011)	Tanaka, Maho (04002)
Suzuki, Yutaka (04046)	Takahata, Yoshihito (03009)	Tanaka, Maho (04014)
Swanson, Sarah (11011)	Takahata, Yoshihito (08032)	Tanaka, Mayuki (02051)*
Swarbreck, David (C803)	Takaku, Mami (04044)	Tanaka, Mayuki (11015)
Swarup, Ranjan (C703)	Takamune, Makiko (03099)	Tanaka, Ryoichi (02038)
Sygusch, Jurgen (17010)	Takanaga, Hitomi (02021)	Tanaka, Shigeo (03107)
Szakonyi, Dora (C702)	Takanashi, Hideki (11020)	Tanaka, Shigeo (03119)
Szczegielniak, Jadwiga (03077)*	Takano, Junpei (02047)	Tanaka, Tsuneyuki (15021)
Szumlanski, Amy (13003)	Takano, Junpei (11015)*	Tanaka, Wakana (08090)*
Szurmak, Blanka (03077)	Takano, Sho (08074)	Tanaka, Yuji (11039)*
Szymona, Lidia (03077)	Takano, Tetsuo (12003)	Tanaka-Matsuoka, Kaya (08105)
T. Yamamoto, Kotaro (03102)	Takano, Yoshitaka (02026)	Tandstad, Nora M. (08056)
T.Morita, Miyo (11060)	Takasaki, Hironori (03019)*	Tang, Jianwei (06042)*
Tabata, Satoshi (PL501)*	Takasaki, Hironori (03096)	Tang, Jianwei (06044)

Tang, Yuhong (08175)	Tomabechi, Mari (03020)	Tsukaya, Hirokazu (08039)
Tani, Chiharu (12052)	Tominaga, Motoki (11052)	Tsukaya, Hirokazu (08054)
Taniguchi, Kakeru (11070)	Tominga-Wada, Rumi (08002)	Tsukaya, Hirokazu (08079)
Taniguchi, Masatoshi (03129)	Tomita, Masaru (09017)	Tsukikawa, Mai (08029)
Tanokura, Masaru (11055)	Tong, Wurina (08108)*	Tsunaga, Yuta (03020)
Tanouchi, Aoi (03063)	Toriba, Taiyo (08079)*	Tsurumaru, Yusuke (06025)*
Tasaka, Masao (08074)	Torii, Keiko U. (PL801)*	Tsurumi, Seiji (03008)
Tasaka, Masao (08094)	Toth, Reka (04020)	Tsutsumi, Nobuhiro (11020)
Tasaka, Masao (08095)	Toyoda, Atsushi (02047)	Tsutsumi, Nobuhiro (11021)
Tasaka, Masao (08102)	Toyoda, Testuro (04002)	Tsutsumi, Nobuhiro (11044)
Tasaka, Masao (08126)	Toyoda, Tetsuro (04006)	Turck, Franziska (06023)
Tasaka, Masao (08129)	Toyoda, Tetsuro (04014)	Twigg, Richard (PL802)
Tasaka, Masao (08133)	Toyoda, Tetsuro (09006)	Tyczewska, Agata (C401)
Tasaka, Masao (11052)	Toyoda, Tetsuro (09017)	Tzeng, Yun-Huei (10011)
Tasaka, Masao (11060)	Toyoda, Tetsuro (14004)	Uchida, Eiji (08061)
Tasaka, Masao (12006)	Toyoda, Tetsuro (15006)	Uchida, Mayumi (08137)
Tatematsu, Kiyoshi (08146)	Toyoda, Tetsuro (16005)	Uchida, Mayumi (08147)
Tatsuo, Sato (02056)	Toyokura, Koichi (08150)	Uchimiya, Hirofumi (06029)
Taura, Satoru (10006)	Tran, Lam-Son (14008)	Uchimiya, Hirofumi (12044)
Teo, Zhi Wei (08012)	Tran, Lam-Son Phan (03033)	Uchiyama, Asako (02019)*
Tepperman, James (03127)	Tran, Lam-Son Phan (03064)	Uchiyama, Asako (08058)
Teramoto, Takashi (07019)*	Tran, Lam-Son Phan (03070)	Uchiyama, Susumu (11022)
Teraoka, Yuuki (02014)	Trang, Duong Huyen (08109)	Ueda, Haruko (11018)
Terao-Morita, Miyo (12006)	Triantaphylides, Christian (03002)	Ueda, Haruko (11024)*
Terashima, Ichiro (03085)	Troffer-charlier, Nathalie (11051)	Ueda, Minako (08043)*
Terauchi, Ryohei (11003)	Tsai, Chia-Hong (02009)	Ueda, Takashi (08157)
Terpstra, Inez (08037)	Tsai, Yun-Long (02022)	Ueda, Takashi (11015)
Tester, Mark A. (03105)	Tsuchida, Yuhei (05006)	Ueda, Takashi (11020)
Testerink, Christa (11008)	Tsuchida, Yuhei (08163)	Ueda, Takashi (11042)
Thelander, Mattias (08164)	Tsuchida-Mayama, Tomoko (03035)	Ueda, Takashi (11043)
THEVENON, Emmanuel (08025)	Tsuchida-Mayama, Tomoko (09020)*	Ueda, Takashi (11044)
Thibaud, Marie-Christine (C304)	Tsuchihira, Ayako (03059)*	Ueda, Takashi (11045)
Thitamadee, Siripong (08122)	Tsuchihira, Ayako (03121)	Ueda, Takashi (11052)
Thordal-Christensen, Hans (11044)	Tsuchimatsu, Takashi (10002)	Ueguchi-Tanaka, Miyako (09003)
Thorstensen, Tage (04011)	Tsuchiya, Tokuji (02006)*	Uehara, Ai (03128)
Thu, May Phyo (04035)	Tsuchiya, Tokuji (02012)	Uehara, Yukiko (03035)
Tian, Lu (04016)	Tsuda, Kenichi (C204)	Uemura, Matsuo (03008)
Tintor, Nico (02013)	Tsuda, Kenichi (C605)	Uemura, Matsuo (03023)
Tiskin, Alexander (16001)	Tsuda, Kenichi (02010)*	Uemura, Shuhei (02045)
Tobin, Elaine (03066)	Tsugama, Daisuke (12003)*	Uemura, Tomohiro (11042)*
Toda, Yosuke (03029)	Tsuge, Seiji (02004)	Uemura, Tomohiro (11043)
Todaka, Daisuke (03027)*	Tsuge, Seiji (02005)	Uemura, Tomohiro (11044)
Todaka, Daisuke (03034)	Tsuge, Tomohiko (03129)	Uemura, Tomohiro (11045)
Todaka, Daisuke (03037)	Tsuge, Tomohiko (11008)	Uemura, Tomohiro (11052)
Toh, Shegeo (PL101)	Tsugeki, Ryuji (08146)	Uenishi, Yumi (03121)
Toh, Shigeo (11070)	Tsugeki, Ryuji (08150)	Ueno, Yoshihisa (08051)
Toh, Shigeo (13012)	Tsuji, Hiroyuki (08019)*	Ueno, Yoshihisa (08081)
Tohge, Takayuki (06039)	Tsujimoto, Ryoma (03100)	Ueno, Yoshihisa (08096)
Tohge, Takayuki (07017)	Tsukagoshi, Hironaka (PL802)	Ueno, Yoshihisa (08174)
Tokairin, Hideo (09015)	Tsukagoshi, Hironaka (07011)	Ulm, Roman (C202)
Toki, Seiichi (15008)	Tsukagoshi, Hironaka (08003)*	Um, Hae Young (02037)
Tokuchi, Konatsu (11070)*	Tsukahara, Sayuri (04028)*	Umeda, Masaaki (03058)
Tokuchi, Konatu (13012)	Tsukamoto, Tatsuya (08085)*	Umeda, Masaaki (08031)
Tokuda, Tsuyoshi (17005)	Tsukaya, Hirokazu (04012)	Umeda, Masaaki (08073)
Tokunaga, Hiroki (12017)*	Tsukaya, Hirokazu (08001)	Umeda, Masaaki (08109)
Tokutomi, Satoru (03126)	Tsukaya, Hirokazu (08015)	Umeshara, Mikihisa (12048)*

Umeshara, Yosuke (04009)	Vidan, Nikolina (03090)	Watanabe, Masao (03118)
Umemoto, Naoyuki (06025)	Vie, Ane Kjersti (C504)	Watanabe, Masao (10002)
Umezawa, Taishi (03096)*	Villamor, Joji Grace C. (03072)	Watanabe, Masao (11046)
Umezawa, Taishi (12002)*	Virata, Rebecca (PL801)	Watanabe, Masao (17017)
Umezawa, Taishi (12014)	Voigt, Christian (02007)*	Watanabe, Mutsumi (03041)*
Umezawa, Yukiko (11063)	Volc, Jindrich (11062)	Watanabe, Naoharu (06021)
Underwood, William (02017)*	von Arnim, Albrecht (09002)*	Watanabe, Nobuyoshi (10006)
Uraji, Misugi (12052)*	von Wirén, Nicolaus (08105)	Watanabe, Nobuyoshi (10022)
Urano, Kaoru (03055)*	W. Innes, Roger (02024)	Watanabe, Ryohei (08052)
Urata, Nobuaki (15021)	Waadt, Rainer (03012)	Watanabe, Shunsuke (06041)*
Ursache, Robertas (C706)	Wachsmann, Guy (08028)*	Watanabe, Yasuko (03070)
Urushiji, Risa (06016)	Wachsmann, Guy (08037)	Watanabe, Yuichiro (C605)
Ushifusa, Chika (04006)	Wada, Takuji (08002)	Watanabe, Yuichiro (08148)
Vaculik, Lukas (02002)	Wagner, Doris (08029)	Watanabe-Sugimoto, Megumi (12052)
Valentova, Olga (02002)*	Wagner, Doris (14001)	Watanabe-Sugimoto, Megumi (12054)
Van Breusegem, Frank (03002)*	Waite, Darren (PL503)	Watkins, Kenneth P. (11054)
Van Breusegem, Frank (07003)	Waites, Richard (08119)	Weber, Andreas (C603)
Van Breusegem, Frank (17002)	Waki, Takamitsu (08052)	Weckwerth, Wolfram (09001)
Van Damme, Daniel (C804)	Walker, Amanda R (15004)	Weckwerth, Wolfram (16007)
van Damme, Mireille (02008)	Wang, Angela (12009)	Wehrstedt, G. (15020)
van de Cotte, Brigitte (03002)	Wang, Chunming (12046)	Weigel, Detlef (PL702)*
Van de Peer, Yves (07003)	Wang, Hongbin (03071)*	Weigel, Detlef (04004)
Van De Slijke, Eveline (07001)	Wang, Hsiao-Lin (04018)	Weigel, Detlef (04020)
Van Der Kelen, Katrien (17002)*	Wang, Hsiao-Lin (04047)	Weigel, Detlef (08083)
Van Der Straeten, Dominique (04012)	Wang, Jinfa (03071)	Weigel, Detlef (10005)
van der Winden, Johannes (C401)	Wang, Jing (08063)	Weigel, Detlef (10014)
van Dijken, Anja (08037)	Wang, Li (C301)	Weigel, Detlef (17012)*
Van Isterdael, Gert (07001)	Wang, Lin (C204)	Weigel, Detlef (17019)*
Van Leene, Jelle (C804)	Wang, Lin (C605)	Weigelt, Kathleen (10012)
Van Leene, Jelle (07001)*	Wang, Min (01004)	Weigelt, Kathleen (10024)*
Van Lijsebettens, Mieke (04012)*	Wang, Mingfu (06002)	Weiller, Georg (12030)
Van Norman, Jaimie M. (08016)*	Wang, Ming-Hsuan (02022)	Weisshaar, Bernd (06023)
van Wijk, Klaas (16007)	Wang, Muyang (12056)	Weisshaar, Bernd (08062)
van Wijk, Klaas J. (11054)	Wang, Ning-Ning (03006)	Weisshaar, Bernd (15004)
Vanden Bossche, Robin (12001)	Wang, Peng (03071)	Weller, Jim (08041)
Vandenbussche, Phillip (04012)	Wang, Ruozhong (12027)	Wells, Darren (C703)
Vanderauwera, Sandy (03002)	Wang, Ruozhong (12031)	Welti, Ruth (PL602)
Vanderhaeghen, Rudy (09023)	Wang, Shui (C201)	Wen, Tuan-Nan (07004)
Vanderhaeghen, Rudy (09024)	Wang, Wen (17018)	Wenk, Markus R. (08012)
Vanderhaeghen, Rudy (17002)	Wang, Xiaofeng (12016)	Wenkel, Stephan (PL201)
Vankova, Radomira (03005)*	Wang, Xiaomin (01004)	Werck-Reichhart, Danièle (08005)
Vaten, Anne (C706)	Wang, Xiu-Jie (04016)	Western, Tamara (13015)
Vaten, Anne (08122)	Wang, Xuelu (08010)*	Whelan, James (03032)
VAUCHERET, Herve (04003)	Wang, Yao (03079)	Wibowo, Juliarni (05003)
Vaughn, Justin (09002)	Wang, Yin (03085)*	Wichadakul, Duangdao (16008)*
Veflingstad, Siren (07005)	Wang, Yujing (C203)	Wichadakul, Duangdao (16009)*
Veiseth, Silje V. (04011)*	Wang, Yujing (02024)	Widmer, Alex (10021)
Vener, Alexander (11009)	Wang, Zhi-Yong (12046)	Wienkoop, Stefanie (09001)
Venkatakrishnan, Sowmya (11050)*	Wangwattana, Bunyapa (06010)	Wilks, Chris (C803)
Verboom, Robert (07020)	Wasaki, Jun (03101)*	Williams-Carrier, Rosalind E. (11054)
Verkest, Aurine (C804)	Wasternack, Claus (11034)	Willmann, Roland (C205)
Verkest, Aurine (07001)	Watahiki, Masaaki (12021)	Willmitzer, Lothar (03042)
Vermeer, Joop E.M. (11008)	Watanabe, Asuka (13012)	Willmitzer, Lothar (06043)
Verslues, Paul E. (03072)*	Watanabe, Keiro (08150)	Willmitzer, Lothar (10012)
Vichas, Athea (11054)	Watanabe, Makoto M. (09013)	Willmitzer, Lothar (10024)
Vidal, Elena A. (03078)*	Watanabe, Masao (03020)	Wilson, Pip B. (03032)

WILSON, Zoe (08047)*	Yabuta, Yukinori (03087)	Yamamoto, Yuka (02014)
Wilson, Zoe A (08006)	Yabuta, Yukinori (03116)	Yamane, Hisakazu (02054)
Wimalasekera, Rinukshi (12047)	Yabuuchi, Hikaru (C305)	Yamaoka, Shohei (11021)*
Wing, Rod A. (17018)*	Yadav, Shri Ram (C706)	Yamaoka, Yasuyo (11030)*
Wink, Rene H. (05004)*	Yajima, Masamiki (08081)	Yamashita, Yui (04034)*
Winter, Cara M. (14001)*	Yamada, Hiroki (03087)*	Yamaya, Tomoyuki (08105)
Wirtz, Markus (03032)	Yamada, Keiji (09008)	Yamazaki, Mami (06010)
Witters, Erwin (C804)	Yamada, Kenji (11025)*	Yamazaki, Mami (06039)
Witters, Erwin (04012)	Yamada, Kohji (03015)	Yamazaki, Takashi (12006)
Witters, Erwin (07001)	Yamada, Kohji (11023)*	Yan, Cunyu (02024)
Witthoeft, Janika (11040)*	Yamada, Kosumi (06040)	Yan, Huili (03051)*
Won, So Youn (03069)	Yamada, Masashi (05005)*	Yan, Shun-ping (C201)
Woo, Dong-Hyuk (03093)*	Yamada, Masashi (08172)	Yanada, Ken-ichi (09008)*
Woo, Je-Tae (08156)	Yamada, Yutaka (06012)	Yanagida, Ai (11063)
Wormit, Alexandra (13001)*	Yamaguchi, Ayako (08029)*	Yanagisawa, Shuichi (C601)
Wright, Jon (PL503)	Yamaguchi, Ayako (14001)	Yanagisawa, Shuichi (03043)
Wright, Stephen (17020)	Yamaguchi, Hiroyasu (15011)	Yanagisawa, Shuichi (03100)
Wu, Ai-Jiuan (02011)	Yamaguchi, Junji (02038)*	Yanagisawa, Shuichi (06005)
Wu, Anhui (03030)	Yamaguchi, Junji (08050)	Yanagisawa, Shuichi (06008)
Wu, Jianzhong (10006)	Yamaguchi, Junji (08091)	Yang, Caiyun (08006)
Wu, Juan (03046)*	Yamaguchi, Junji (08092)	Yang, Caiyun (08047)
Wu, Miin-Feng (14001)	Yamaguchi, Junji (08103)	Yang, Chih-Fu (12032)
Wu, Shu-Hsing (C404)	Yamaguchi, Koji (02005)*	Yang, Chin-Ying (03006)*
Wurtzel, Eleanore (06007)	Yamaguchi, Masatoshi (13014)*	Yang, Fong-Jhieh (02022)
Xiao, Jun (12031)*	Yamaguchi, Shinjiro (PL102)*	Yang, Thomas (07004)
Xiao, Langtao (12027)	Yamaguchi, Shinjiro (03035)	Yang, Weicai (08068)
Xiao, Langtao (12031)	Yamaguchi, Shinjiro (06013)	Yang, Wei-Cai (08066)
XIAO, SHI (03004)	Yamaguchi, Shinjiro (12006)	Yang, Wei-cai (08069)
Xiao, Shi (06002)*	Yamaguchi, Shinjiro (12048)	Yang, Xijia (08007)
Xie, Bo (08101)	Yamaguchi, Takahiro (04012)	Yano, Kentaro (03118)
Xie, Weibo (17007)	Yamaguchi, Takahiro (08039)*	Yano, Kentaro (04046)
Xie, Zidian (C704)	Yamaguchi, Takahiro (08079)	Yano, Masahiro (14002)
Xie, Zidian (08004)	Yamaguchi-Shinozaki, Kazuko (C303)*	Yano, Ryoichi (10017)*
Xing, Lijing (01004)	Yamaguchi-Shinozaki, Kazuko (03015)	Yao, Qinfang (12005)
Xing, Shuping (08064)*	Yamaguchi-Shinozaki, Kazuko (03017)	Yao, Yao (03052)
Xiong, Yongzhong (17007)	Yamaguchi-Shinozaki, Kazuko (03018)	Yap, Kyoko (04011)
Xu, Caiguo (17007)	Yamaguchi-Shinozaki, Kazuko (03019)	Yasuda, Michiko (02053)*
Xu, Morgan (08063)	Yamaguchi-Shinozaki, Kazuko (03027)	Yasuda, Michiko (02056)
Xu, Ruqiang (04017)	Yamaguchi-Shinozaki, Kazuko (03028)	Yasuda, Michiko (12055)
Xu, Yifeng (C705)	Yamaguchi-Shinozaki, Kazuko (03033)	Yasuda, Shigetaka (08050)
Xu, Yifeng (08158)*	Yamaguchi-Shinozaki, Kazuko (03034)	Yasuda, Shigetaka (08091)
Xu, Yunyuan (01004)*	Yamaguchi-Shinozaki, Kazuko (03036)	Yasuda, Shigetaka (08092)*
Xu, Yunyuan (12027)*	Yamaguchi-Shinozaki, Kazuko (03037)	Yasumoro, Mariko (04044)
Xu, Yunyuan (12031)	Yamaguchi-Shinozaki, Kazuko (03045)	Yates, John (12009)
Xue, Gang-Ping (03030)	Yamaguchi-Shinozaki, Kazuko (03055)	Yazaki, Junshi (04046)
Xue, Jie (08006)	Yamaguchi-Shinozaki, Kazuko (03096)	Yazaki, Kazufumi (06025)
Xue, Li (C203)	Yamaguchi-Shinozaki, Kazuko (03117)	Ye, Qianqian (08010)
Xue, Li (02024)	Yamaguchi-Shinozaki, Kazuko (11023)	Yeu, Song Yion (12039)
Xue, Shaowu (03013)	Yamaguchi-Shinozaki, Kazuko (12002)	Yeu, Song Yion (12041)
Xue, Shaowu (11014)	Yamaguchi-Shinozaki, Kazuko (12014)	Yi, Hwi-su (02033)
Y.Taniguchi, Yumiki (03129)	Yamaguchi-Shinozaki, Kazuko (14008)	Yi, Young Byung (08109)
Yabe, Kimihiko (05006)*	Yamaguchi-Shinozaki, Kazuko (16002)	Yi, Young-Byung (02040)
Yabuta, Yukinori (03044)	Yamakawa, Takashi (02056)	Yilmaz, Alper (07007)
Yabuta, Yukinori (03060)*	Yamamoto, Hiroko (02038)	Yin, Haibo (C301)
Yabuta, Yukinori (03063)	Yamamoto, Kotaro (12021)	Yin, Lan (11009)
Yabuta, Yukinori (03065)	Yamamoto, Tsuyoshi (14010)*	Yin, Minghui (02016)

Yin, Yanhai (08010)	Yoshimura, Kazuya (03053)*	Zhang, Jing (08165)*
Yip, Wing Kin (06022)	Yoshimura, Kazuya (03063)	Zhang, Jingyu (04019)*
Yip, Wing Kin (12040)	Yoshimura, Kazuya (03116)	Zhang, Lulu (08041)
Yokoi, Shuji (03009)*	Yoshimura, Kazuya (06017)	Zhang, Ping (08005)
Yokoi, Shuji (08032)	Yoshimura, Kazuya (06028)	Zhang, Qifa (PL502)*
Yokota Hirai, Masami (06004)	Yoshimura, Mika (08031)	Zhang, Qifa (17007)*
Yokota Hirai, Masami (06012)	Yoshimura, Mika (08044)	Zhang, Shanshan (08010)
Yokota Hirai, Masami (06045)	Yoshinaga, Akiko (06001)	Zhang, Shuaibin (04029)
Yokota Hirai, Masami (06047)	Yoshioka, Hirofumi (02045)	Zhang, Wen-Juan (08066)
Yokota Hirai, Masami (07016)	Yoshioka, Yasushi (17015)*	Zhang, Xian Sheng (12019)*
Yokota, Akiho (03081)	Yoshizawa, Nobuo (02050)	Zhang, Xiaoyu (04030)
Yokota, Etsuo (11024)	Yoshizumi, Takeshi (08111)	Zhang, Xinyan (12028)*
Yokota, Kazushige (11048)	Yoshizumi, Takeshi (08148)*	Zhang, Xudong (02023)
Yokota, Shinso (02050)*	Yoshizumi, Takeshi (08149)	Zhang, Xuebin (08027)
Yokota, Takao (10020)	Yoshizumi, Takeshi (09009)	Zhang, Yan (08064)
Yokotani, Naoki (03057)	Yoshizumi, Takeshi (14005)	Zhang, Yang (03071)
Yokoyama, Kunihiro (03053)	Yoshizumi, Takeshi (15006)	Zhao, Meng (03024)
Yokoyama, Ryusuke (14010)	You, Wanhai (C401)	Zhao, Rongmin (03079)*
Yokoyama, Ryusuke (14011)	Youn, Ji Hyun (12049)	Zhong, Shangwei (12010)
Yoneda, Arata (08111)	Yu, Hai Yang (03047)	Zhou, Gang (17007)
Yoneda, Arata (13005)*	Yu, Hai Yang (03048)	Zhou, Gongke (13015)
Yoneda, Yuki (03081)	Yu, Hai Yang (03049)	Zhou, Jian-Min (C203)*
Yonekura-Sakakibara, Keiko (06010)*	Yu, Hai Yang (03050)	Zhou, Jian-Min (02024)
Yonekura-Sakakibara, Keiko (06039)	Yu, Hao (08065)	Zhou, Jing (08122)
Yoo, Jae Yong (03074)	Yu, Hao (08128)	Zhu, Shan-shan (08069)*
Yoo, Jae Yong (03075)	Yu, Nan-Ie (08127)	Zhu, Wenjiao (08010)
Yoo, Seong Jeon (08125)	Yu, Tien-Shin (08072)	Zhu, Ziqiang (12029)*
Yoo, Seung Kwan (08125)	Yuan, Shuai (C101)	Zilberman, Daniel (C403)*
Yoshida, Hitoshi (08061)*	Yuan, Zheng (08006)	Zimmerli, Laurent (02009)*
Yoshida, Hitoshi (10009)	Yukawa, Yasushi (03046)	Zoe A, Wilson (07008)*
Yoshida, Keigo (08105)*	Yun, Byung-Wook (02016)*	Zuniga-Mayo, Victor M. (08076)
Yoshida, Riichiro (08087)	Yun, Dae-Jin (02020)	
Yoshida, Saiko (PL803)	Yun, Dae-Jin (03112)	
Yoshida, Saiko (08055)*	Yun, Dae-Jin (09005)	
Yoshida, Satoko (10023)*	Yun, Dae-Jin (17006)	
Yoshida, Satoko (15017)	Yun, Hye In (04043)*	
Yoshida, Shigeo (06021)	Yun, Jeonga (12004)	
Yoshida, Shigeo (07009)	Yurimoto, Hiroya (03039)	
Yoshida, Shigeo (12015)	Zaki, Haitham (08032)*	
Yoshida, Shuhei (08126)	Zakrzewska-Placzek, Monika (04021)*	
Yoshida, Takuhiro (14007)	Zamioudis, Christos (08021)	
Yoshida, Takuhiro (14008)	Zamir, Dani (06007)	
Yoshida, Takuhiro (14009)	Zdunek, Jeffrey (15007)*	
Yoshida, Takumi (03037)	Zeidler, Mathias (09001)	
Yoshida, Takuya (03017)*	Zeidler, Mathias (11001)*	
Yoshida, Takuya (03045)	Zeier, Juergen (02039)	
Yoshida, Takuya (12014)	Zeier, Tatiana E. (02039)	
Yoshida, Takuya (16002)	Zhang, Biyu (03052)	
Yoshida, Yuko (09017)	Zhang, Dabing (08005)*	
Yoshihara, Toshihiro (06029)	Zhang, Dabing (08006)	
Yoshimoto, Kohki (08131)	Zhang, Dabing (08007)*	
Yoshimoto, Kohki (11033)*	Zhang, Dabing (08047)	
Yoshimoto, Kohki (11052)	Zhang, Dasheng (08006)*	
Yoshimoto, Tadashi (06017)*	Zhang, Fu-Suo (03024)	
Yoshimura, Kazuya (02027)	Zhang, Hui (08007)	
Yoshimura, Kazuya (03044)	Zhang, Jing (C706)	

GENERAL MEETING INFORMATION

Registration

The registration desk will be opened on the following schedule at the 2nd floor of Conference Center.

June 6 (Day 1) 14:00 – 20:00

June 7 (Day 2) 8:30 – 18:30

June 8 (Day 3) 8:30 – 18:30

June 9 (Day 4) 8:30 – 18:30

June 10 (Day 5) 8:30 – 12:30

Welcome Drink

Organizing Committee will welcome all participants.

Welcome Drink will be served on June 6 (Day 1) from 18:00 to 20:00 at the 3rd floor of Conference Center.

Oral Sessions

Oral sessions will be held at the Main Hall and the 3rd floor of Conference Center.

For Oral Presentation Speakers

Oral speakers are requested to bring their presentation file (MS Powerpoint) or their own PCs to the PC desk at least 30 min before their session (Not their talk) starts.

Speakers who will present at main hall:

Please bring your PC or presentation file to room "024" at B1F of Conference Center.

Speakers who will present at 3rd floor:

Please bring your PC or presentation file to room "316" at 3F of Conference Center.

Exhibits

Exhibits will be opened in the Exhibition Hall A from June 7 (Day 2) to June 9 (Day 4).

Temporary Childcare Service

"Kid's Square"

Kid's Square (in Pan Pacific Yokohama Bay Hotel) is the nearest childcare service from Pacifico Yokohama.

Please contact directly.

TEL/FAX: 045-640-6008

Website: http://www.alpha-co.com/english/ks01_yokohama.html

Banks

Around the conference area, there are post offices and convenience stores which allow international ATM withdrawals.

Banks

1. Bank of Yokohama Head Office: Near the Sakuragicho Station, 15min walking distance from the conference venue

Teller window: 9:00-15:00 (Monday to Friday)

Tel: 045-225-1111

2. Citibank Yokohama Branch: Yokohama Station West Exit

Teller window: 9:00-15:00 (Monday to Friday)

Tel: 045-314-0716

ATMs

1. Japan Post Bank (JP Bank): Queen's Square Yokohama 1F
9:00-19:00 (Monday to Friday)
2. Bank of Yokohama Head Office: Near the Sakuragicho Station, 15min walking distance from the conference venue
7:45-21:00 (Monday to Friday)
9:00-21:00 (Saturday and Sunday)
3. Citibank Yokohama Branch: Yokohama Station West Exit
24 hours a day, 365 days a year
4. Daily Yamazaki (Convenience Store): Exhibition Hall 1st Floor
7:00-23:00, 365 days a year (Japanese Bank/ Credit Card only)

Banquet

Conference Banquet will be held on June 9 (Day 4) from 19:00 to 21:00 at the Ballroom, 3rd floor of Intercontinental Yokohama Grand Hotel.

Banquet Ticket Sales

The delegates who have already registered will receive the Banquet tickets at the time of registration. You can purchase Banquet tickets onsite anytime the registration desk is open. Banquet ticket is 8,000 Japanese yen. Please bring your ticket to the Banquet Venue and give it to the staffs.

Onsite T-shirt Sales

You can purchase T-shirts at the 2nd floor of Conference Center anytime between June 7 at 8:30 and June 10 at 12:30. Please check opening times of the registration desk on specific days. We accept only Japanese cash.

Internet

Internet is freely available at 3rd floor of Conference Center. (Except for rooms 301, 302, 303 and 304. Please check the floor map)

NSF 2010 and Beyond Session

The session will be held on June 8 (Day 3), 13:00 – 15:00, at room 301+302+303+304. Served with lunch.

Yokohama Citizen Open Seminar

The seminar "Seek the origin of foods –wheat and rice–" will be held for public on June 8 (Day 3), 15:30 – 17:00, at Conference Center 3rd floor, room 315. (Language: Japanese)

Smoking Policy

Smoking is only allowed at smoking areas: Conference Center 1st and 3rd floors and Exhibition Hall 1st floor. Yokohama city legally prohibits smoking on the streets and restaurants to prevent passive smoking.

Meals and Refreshments

Lunch will be served to attendees on day 2 (June 7), day 3 (June 8) and day 4 (June 9). Coffee and other beverages will be served during coffee breaks.

Cameras/Video/Audio Recording

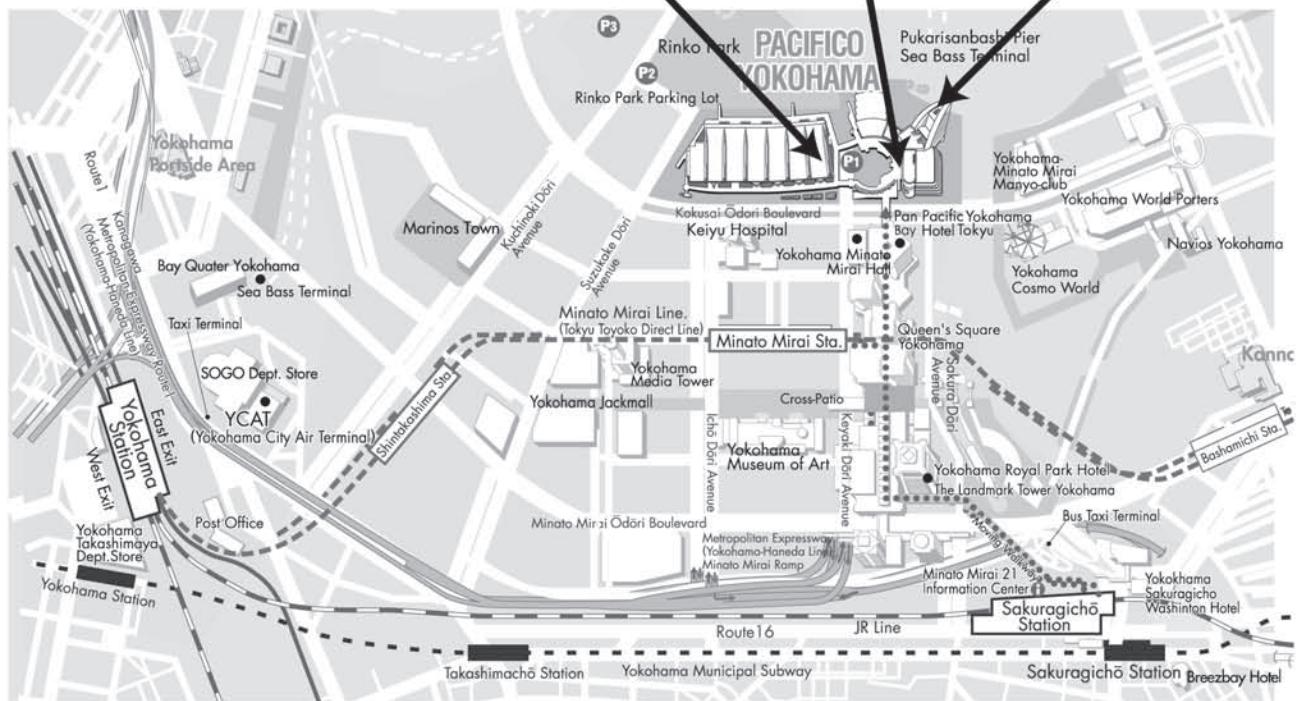
Taking photos, videos, or audio recording of any kind of the posters and sessions will be PROHIBITED.

PACIFICO YOKOHAMA FLOOR PLAN



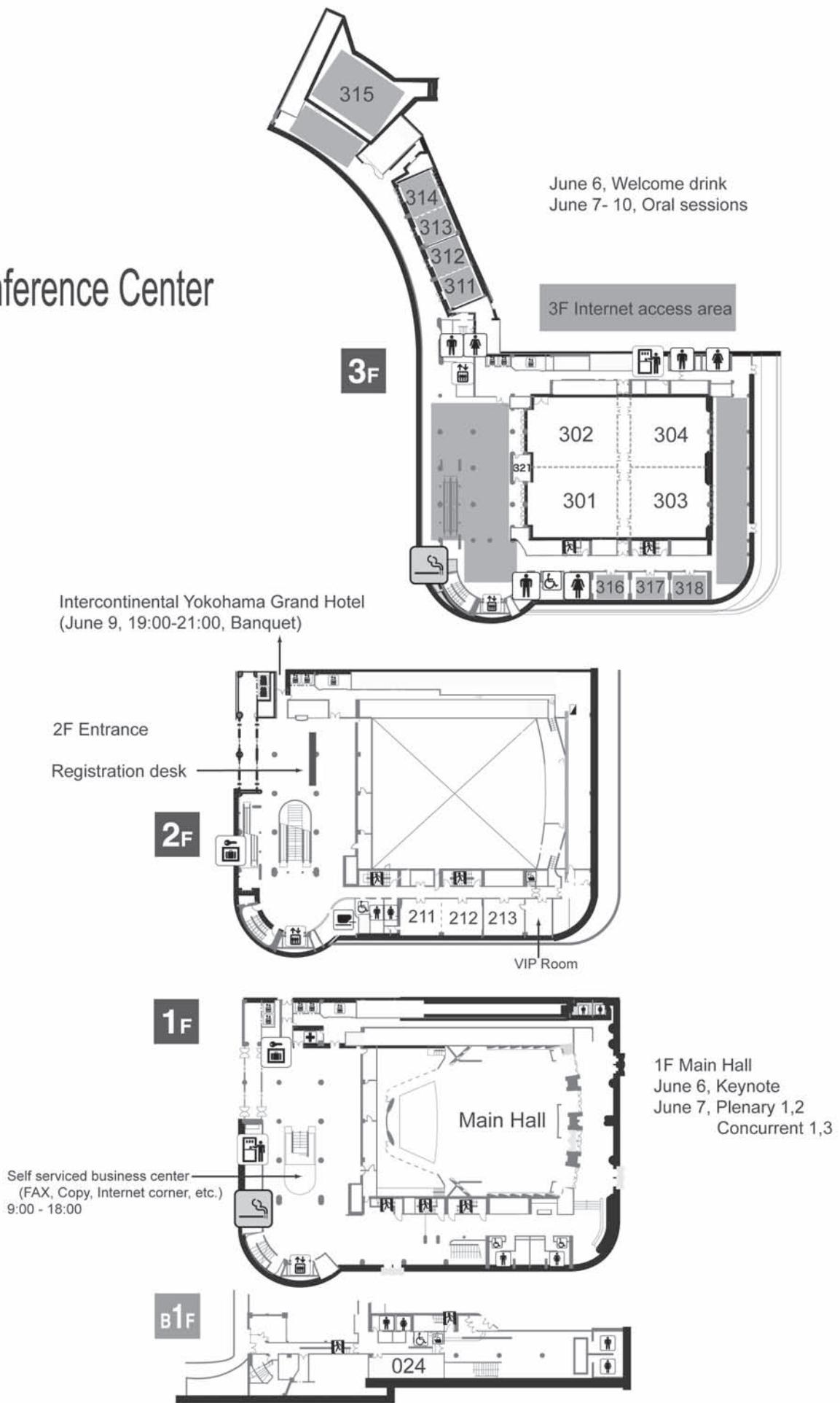
Exhibition Hall A
(Posters & Exhibits)

Conference Center
(Registration desk 2F)
(Oral sessions)



..... On foot

Conference Center



MEMO

MEMO

International Rational-Genome-Design Contest with Arabidopsis

GENOCON for Synthetic Biology

We-based contest aimed at supporting a future generation of scientists – including a category for high-school students.

A challenge for green innovation: rational genome design of a plant with an environmental detoxification function.

Acumulation and sharing of genome-design theories and programs from researchers around the world.



GenoCon contestants design DNA sequences in a web-browser-based programming environment. RIKEN evaluates their functions experimentally.

<http://genocon.org>

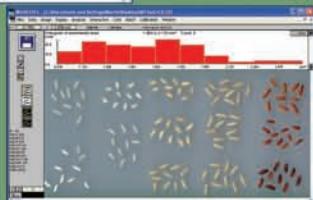
Image Analysis Systems for Plant Scientists

Based on High Resolution Scanners and Digital Cameras • For PC Computers with Windows Operating Systems Including Windows 7



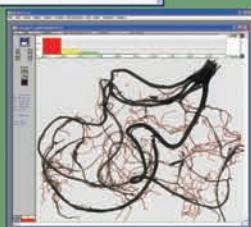
WinFOLIA™

Analyses leaf morphology and leaf shape (Fractals)
Measures healthy and diseased leaf area
Quantifies pest damage,...



WinSEEDLE™

Analyses seed and needle morphology
Counts and classifies seeds
Measures healthy and diseased area,...



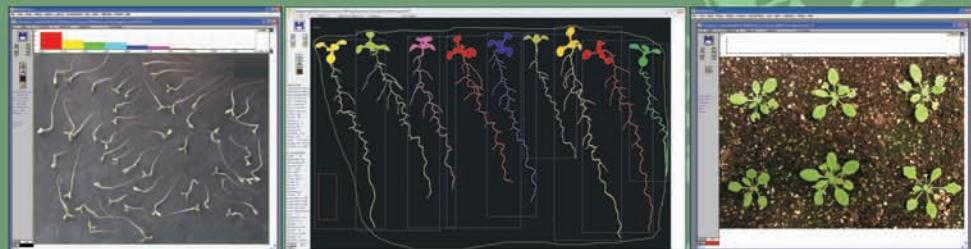
WinRHIZO™

Analyses washed root morphology, link, topology and architecture
Measures healthy and diseased area, thin lateral vs. main roots,...

WinRHIZO™ Arabidopsis

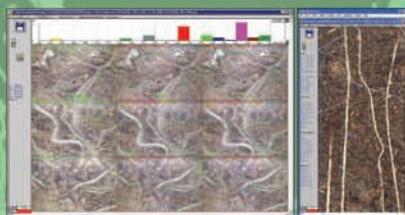
Includes WinRHIZO™ measurements plus:

- Seed germination analysis
- Seedling count
- Individual seedling area
- Plant height and width (side view)
- Leaf length and width (top view)....



WinRHIZO™ Tron

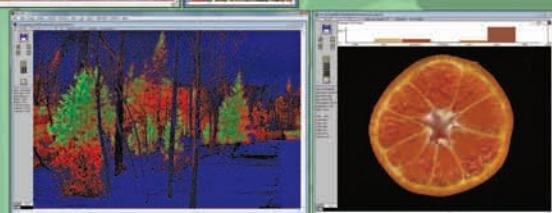
Analyses morphology, architecture and topology of roots in minirhizotron and soil
Compatible with most imaging acquisition devices for root growth monitoring in soil



WinCAM NDVI™

Measures areas in function of color and morphology of objects selected by using color levels

New in 2010: Color indexes and NDVI analysis (Vegetation health status)



More details at www.regentinstruments.com

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