10th International Conference on Arabidopsis Research

The University of Melbourne, Australia

4-8 July 1999

==========

PROGRAM AND ABSTRACTS

==========

Major Sponsors

CSIRO Division of Plant Industry
National Science Foundation (USA)
Novartis Agribusiness
Victorian Department of State Development

Other Sponsors

AgrEvo
Agriculture Victoria
Blackwell Science
Elsevier
Florigene
Lehle Seeds
Perkin Elmer
Sigma/Aldrich

Web-site provided by EnCompass Bioinformatics Pty Ltd
http://arabidopsis.en-bio.com.au

==========

International Conference Advisory Committee

Jiyiang Li, Chinese Academy of Sciences, China
Hong Gil Nam, Pohang University of Science and Technology, South Korea
Kiyotaka Okada, Kyoto University, Japan
Joanna Putterill, University of Auckland, New Zealand
Venkatesan Sundaresan, Institute of Molecular Agrobiology, Singapore

==========

==========

Australian Program Committee

Chris Cobbett, University of Melbourne, Melbourne
Liz Dennis, CSIRO Division of Plant Industry, Canberra
Ed Newbigin, University of Melbourne, Melbourne
Jim Peacock, CSIRO Division of Plant Industry, Canberra
David Smyth, Monash University, Melbourne (Convenor)
Richard Williamson, Asutralian National University, Canberra

==========

==========

Local Organizing Committee

Chris Cobbett, University of Melbourne (Sponsorships)
Ed Newbigin, University of Melbourne (Treasurer)
Roger Parish, La Trobe University
Carolyn Schultz, University of Melbourne (Secretary)
David Smyth, Monash University (Convenor)

==========

PROGRAM

Sunday 4 July

Noon - 5:00 PM

Registration, Foyer, Copland Theatre (Economics and Commerce Building)

Poster set-up, Wilson Hall

5:00 PM - 6:00 PM

Keynote Address
Copland Theatre

Chair: **David Smyth**, Monash University, Australia

Elliot Meyerowitz, California Insitute of Technology, USA

Arabidopsis past and future

6:00 PM - Midnight

Welcome Mixer (sponsored by Novartis Agribusiness)
Wilson Hall

Drinks, Savories

Poster set-up and viewing

Registration

Monday 5 July

8:30 AM - 10:30 AM

SESSION 1 **Environmental Perception and Abiotic Stress**
(with support from AgrEvo)

Copland Theatre

Chair: **Chris Cobbett**, University of Melbourne, Australia

1-1 **Carol R. Andersson**, The Scripps Research Institute, USA
The Reveille (RVE) family of DNA binding proteins and the circadian clock

1-2 **Kazuo Shinozaki**, The Institute of Physical and Chemical Research (RIKEN), Japan
Molecular responses to drought stress in Arabidopsis

1-3 **Suk-Bong Ha**, University of Melbourne, Australia
Phytochelatin synthase genes from fungal, plant and animal species

1-4 **Hye Ryun Woo**, Pohang University of Science and Technology, South Korea
Analysis of Arabidopsis mutants that confer delayed senescence symptoms or prolonged longevity

1-5 **Tatsuya Sakai**, Kyoto University, Japan
Analysis of root phototropism mutant, rpt2, of Arabidopsis

1-6 **Peter Hunt**, CSIRO Division of Plant Industry, Australia
Evolution of non-symbiotic hemoglobins and expression of two classes of non-symbiotic hemoglobins in Arabidopsis thaliana

10:30 AM - 11:00 AM

Tea and Coffee
Marquee

Monday 5 July

11:00 AM - 12:30 PM

SESSION 2 **Silencing, Epigenetics and Gene Expression**
Copland Theatre

Chair: **Liz Dennis**, CSIRO Division of Plant Industry, Australia

2-1 **Hervé Vaucheret**, INRA, France (with support from CSIRO)
Gene silencing in Arabidopsis

2-2 **Mark A. Johnson**, Michigan State University, USA
Genetic determinants of mRNA degradation in Arabidopsis

2-3 **Colin MacDougall**, University of Edinburgh, UK
Protein-Protein interactions between Polycomb-group members in Arabidopsis

2-4 **Feng Zheng**, Novartis Agribusiness Biotechnology Research Inc., USA
Comparison of global gene expression profiles of herbicide-treated and antisense "knock-out" plants

12:30 PM - 2:00 PM

Lunch
Union House

Monday 5 July

2:00 PM - 3:30 PM

SESSION 3 **Structural Genomics**
(with support from Perkin Elmer)
Copland Theatre

Chair: **Ed Newbigin**, University of Melbourne, Australia

3-1 **Steve Rounsley**, Cereon Genomics, USA
Whole Genome Shotgun Sequencing of Arabidopsis - analysis and insight

3-2 **Satoshi Tabata**, Kazusa DNA Research Institute, Japan
Structural analysis of the Arabidopsis thaliana genome toward functional genomics

3-3 **Robin Buell**, The Institute for Genomic Research, USA
The Arabidopsis genome annotation database: a resource for plant biologists

3-4 **Larry Parnell**, Cold Spring Harbor Laboratory, USA
The nature of the EST: Distributation across chromosome IV

3:30 PM - 4:00 PM

Tea and Coffee
Marquee

Monday 5 July

4:00 PM - 5:30 PM

SESSION 4 **Functional Genomics**
(with support from Perkin Elmer)
Copland Theatre

Chair: **Venkatesan Sundaresan**, Institute of Molecular Agrobiology, Singpore

4-1 **David Bouchez**, INRA, France (with support from CSIRO)
T-DNA insertional mutagenesis for reverse genetics in Arabidopsis

4-2 **Iain Wilson**, Carnegie Institution of Washington, USA (with support from AgVic)
Characterization of the powdery mildew disease resistance locus, RPW10, using DNA microarrays

4-3 **Serguei Parinov**, Institute of Molecular Agrobiology, Singapore
Analysis of flanking sequences from 1000 independent Ds-transposon insertion lines in Arabidopsis thaliana

4-4 **Nina Fedoroff**, Penn State University, USA
Global analysis of environmental stress and developmentally regulated genes in Arabidopsis

5:30 PM - 10:30 PM

Posters, Odd numbers (sponsored by Victorian Department of State Development)
Wilson Hall

Drinks available, cash bar

Dinner, own arrangements

Tuesday 6 July

8:30 AM - 10:30 AM

SESSION 5 Metabolism, Transport and Trafficking
Copland Theatre

Chair: **Jiyiang Li**, Chinese Academy of Sciences, China

5-1 **Natasha Raikhel**, Michigan State University, USA (with support from Florigene)
Various mechanisms of vesicular trafficking to the plant vacuole

5-2 **Mary Lou Guerinot**, Dartmouth College, USA (with support from CSIRO)
Understanding the complexities of metal uptake: a true test of one's mettle

5-3 **Whitney Robertson**, University of Wisconsin, USA
Analysis of T-DNA-disrupted K^+ channel genes in Arabidopsis thaliana

5-4 **Anthony Millar**, University of British Columbia, Canada
Molecular genetic manipulation of very-long-chain fatty acid biosynthetic pathways in Arabidopsis

5-5 **Michael Santos**, Virginia Polytechnic Institute and State University, USA
Expression of phage-derived antibody genes to modulate flavonoid biosynthesis in transgenic Arabidopsis

5-6 **N Peeters**, INRA, France
Aminoacyl-tRNA synthatases, a set of essential proteins in translation distributed between cytosol, mitochondria and plastids

10:30 AM - 11:00 AM

Tea and Coffee
Marquee

Tuesday 6 July

11:00 AM - 12:30 PM

SESSION 6 **Signal Transduction**
Copland Theatre

Chair: **Hong Gil Nam**, Pohung University of Science and Technology, South Korea

6-1 J**oseph Ecker**, University of Pennsylvania, USA
Perception, signaling and responses to a simple hydrocarbon

6-2 **Tai-Ping Sun**, Duke University, USA (with support from CSIRO)
Gibberellin signal transduction in Arabidopsis

6-3 **Robert Pruitt**, Harvard University, USA
Regulating cellular interactions by modifying extracellular properties

6-4 **June Myung Kwak**, Pohang University of Science and Technology, South Korea, and University of California San Diego, USA
A plant homolog of mammalian ionotropic glutamate receptors controls light- and auxin-induced opening of stomatal aperture

12:30 PM - 2:00 PM

Lunch
Union House

Tuesday 6 July

2:00 PM - 3:30 PM

SESSION 7 **Plant Pathogen Interactions**
Copland Theatre

Chair: **John Manners**, University of Queensland, Australia

7-1 **Xinnian Dong**, Duke University, USA
Systemic acquired resistance is controlled by both positive and negative regulators

7-2 **Jane Parker**, Sainsbury Laboratory, UK (with support from CSIRO)
Molecular genetic dissection of R gene-mediated disease resistance pathways

7-3 **Stephen Howell**, Boyce Thompson Institute, USA
Identification of Arabidopsis proteins that interact with the movement protein of cauliflower mosaic virus (CaMV)

7-4 **Uwe Köhler**, University of Cambridge, UK
Trans-splicing ribozymes for potential virus resistance in plants and for use as "RNA sensors”

3:30 PM - 4:00 PM

Tea and Coffee
Marquee

Tuesday 6 July

4:00 PM- 5:30 PM

SESSION 8 Vegetative Development and Differentiation
Copland Theatre

Chair: **Bruce Veit**, Massey University, New Zealand

8.1 **Martin Hülskamp**, University of Tübingen, Germany

8-2 **Kiyotaka Okada**, Kyoto University, Japan
Role of intercellular signaling in the cell fate specification

8-3 **Takashi Hashimoto**, Nara Institute of Science and Technology, Japan
Arabidopsis *SPIRAL1* gene is required for directional control of cell elongation

8-4 **John Bowman**, University of California Davis, USA
Members of the *YABBY* gene family specify abaxial cell fate in Arabidopsis

5:30 PM - 10:30 PM

Posters, Even numbers (sponsored by Victorian Department of State Development)
Wilson Hall

Drinks available, cash bar

Dinner, own arrangements

Wednesday 7 July

EXCURSIONS

Great Ocean Road (Twelve Apostles)

Philip Island Penguin Parade and Koala Conservation Park

Healseville Sanctuary and Fergusson's Winery, Yarra Valley

Details to be advised

8:30 AM- 5:00 PM

Posters
Wilson Hall

Thursday 8 July

**** POSTERS TO BE REMOVED FROM WILSON HALL BY 2:00 PM TODAY ****

8:30 AM - 10:30 AM

SESSION 9 Floral Induction
Copland Theatre

Chair: **Joanna Putterill**, University of Auckland, New Zealand

9-1 **Wim Soppe**, Wageningen University, The Netherlands
The gain of function epi-mutant *fwa* causes late flowering

9-2 **Tony Gendall**, John Innes Centre, UK
Vernalization in Arabidopsis - a molecular genetic approach

9-3 **Candice Sheldon**, CSIRO Plant Industry, Australia
FLF/FLC is a repressor of flowering in Arabidopsis

9-4 **Joanna Putterill**, University of Auckland, New Zealand
Characterising *GIGANTEA*: A circadian-regulated gene which regulates photoperiodic flowering in Arabidopsis

9-5 **Yasushi Kobayashi**, Kyoto University, Japan
The flowering-time gene *FT* acts downstream of *CONSTANS* and antagonistically with its homolog, *TERMINAL FLOWER1*

9-6 **Doris Wagner**, California Institute of Technology, USA
Switching on flowers

10:30 AM - 11:00 AM

Tea and Coffee
Marquee

Thursday 8 July

11:00 AM - 12:30 PM

SESSION 10 Flower and Ovule Development
Copland Theatre

Chair: **John Bowman**, University of California Davis, USA (formerly Monash University, Australia)

10-1 **Martin Yanofsky**, University of California San Diego, USA
FRUITFULL and *SHATTERPROOF* MADS-box genes interact during fruit development in Arabidopsis

10-2 **Charles Gasser**, University of California, Davis, USA
Building an ovule: genetic control of patterning, polarity, identity, and directional expansion

10-3 **Yuval Eshed**, University of California Davis, USA
Distinct mechanisms promote polarity establishment in the carpels of Arabidopsis thaliana

10-4 **Koji Goto**, Kyoto University, Japan
Non-cell-autonomous function of the floral homeotic gene *PISTILLATA* and*APETALA3* is exerted by their downstream molecule

12:30 PM - 2:00 PM

Lunch
Union House

**** POSTERS TO BE REMOVED FROM WILSON HALL BY 2:00 PM ****

Thursday 8 July

2:00 PM - 3:30 PM

SESSION 11 **Fertilization and Embryogenesis**
Copland Theatre

Chair: **Jim Peacock**, CSIRO Plant Industry, Australia

11-1 **Jean-Philippe Vielle-Calzada**, Cold Spring Harbor Laboratory, USA
(with support from CSIRO)
Genomic imprinting during seed development in Arabidopsis

11-2 **Robert Fischer**, University of California, Berkeley, USA
Initiation of endosperm development in Arabidopsis

11-3 **Abdul Chaudhury**, CSIRO Plant Industry, Australia
Fertilization independent seed development: a pathway to understanding apomixis

11-4 **Sacco de Vries**, Wageningen University, The Netherlands
The Arabidopsis somatic embryogenesis receptor-like kinase (*AtSERK*) gene

3:30 PM - 4:00 PM

Tea and Coffee
Marquee

Thursday 8 July

4:00 PM - 5:30 PM

SESSION 12 **Meristems: Roots and Shoots**
Copland Theatre

Chair: **Kiyotaka Okada**, Kyoto University, Japan

12-1 **Thomas Laux**, University of Tübingen, Germany
Regulation of cell identity in the shoot meristem

12-2 **Rüdiger Simon**, University of Köln, Germany
CLV3 acts via *CLV1* and *CLV2* to repress *WUSCHEL*

12-3 **Philip Benfey**, New York University, USA
Radial pattern formation

12-4 **Dimitris Beis**, Utrecht University, The Netherlands
Pattern and polarity in Arabidopsis root development

7:30 PM - Midnight

CLOSING DINNER (entry by ticket only)
The Regent Theatre's Plaza Ballroom
191 Collins Street, Melbourne

==========

ABSTRACTS

For

TALKS and POSTERS

==========

PRESENTING AUTHORS ARE IN BOLD

Keynote Address

Arabidopsis Past and Future

Elliot M. Meyerowitz

Division of Biology, California Institute of Technology, Pasadena, CA, 91125, USA

A brief introduction on the origins of genetical research using Arabidopsis, and of the international Arabidopsis meetings, will be given. This will be followed by a survey of some of the surprises and conclusions that come from our present knowledge of the genomic sequence of Arabidopsis. Among the conclusions are that plants and animals have used their common patrimony of eukaryotic genes rather differently, with the differences most apparent in cell-cell and cell-environment signal transduction mechanisms, and in the cell surface. This is consistent with the independent origin of multicellular life in the two lineages, and with the independent evolution of mechanisms to cope with life on land. One example is the large number of receptor serine/threonine kinases found in Arabidopsis (so far well over one hundred, the total may exceed 300). None of these (yet) have exact counterparts in animals or fungi. The existence of several hundred uncharacterized receptor kinases implies that large numbers of plant signaling molecules are yet to be discovered. An opposite example is the family of nuclear hormone receptors that includes animal steroid receptors. While Caenorhabditis elegans has 270 genes for such receptors, Arabidopsis, with a genome similar in size, so far has none. The Arabidopsis genome also has large families of genes with no animal counterparts, and no known functions, showing that we have much yet to learn.

1-1

The Reveille (RVE) family of DNA binding proteins and the circadian clock

Carol R. Andersson, Stacey L. Harmer, Thomas F. Schultz and Steve A. Kay
Department of Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd BCC265, La Jolla CA 92037 USA

Circadian clocks allow organisms to anticipate environmental changes over the day/night cycle and to time physiological events to the appropriate phase of day. We are investigating the role of a newly defined family of DNA binding proteins in transducing information from the circadian oscillator to downstream target genes. Two Arabidopsis genes, CCA1 and LHY, have previously been shown to be associated with the circadian clock, as constitutive overexpression of either in transgenic plants causes arhythmicity of several clock controlled processes (Wang and Tobin, 1998; Schaffer et al, 1998). CCA1 and LHY contain a single Myb-like DNA binding domain and bind the CAB2 promoter in a region shown to be sufficient to confer circadian expression on a luciferase reporter gene. We have identified several genes that are related to CCA1 and LHY through the Myb-like domain. Like CCA1 and LHY, abundance of their transcripts varies with a circadian cycle, peaking at around dawn. As these DNA binding proteins are likely to signal "lights on" to downstream targets, we have designated this gene family Reveille (RVE). The RVE genes fall into three distinct subclasses, of which CCA1/LHY is one, based on both the predicted amino acid sequence and phenotypes of transgenic plants overexpressing each gene. The possible role of the RVE genes in circadian clock regulation will be discussed.

1-2

Molecular responses to drought stress in Arabidopsis

Kazuo Shinozaki (1), Kazuko Yamaguchi-Shinozaki (2).
(1) The Institute of Physical and Chemical Research (RIKEN), Tsukuba, (2) Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan

Under water deficit conditions, various genes are induced and are thought to function in stress tolerance and response. To understand molecular responses to drought stress, we have cloned more than 50 drought-inducible genes from Arabidopsis. We identified at least four independent signal transduction pathways in stress signaling. Two are ABA-dependent and two are ABA-independent. Transcription factors encoding MYC and MYB homologues as well as bZIP are involved in the ABA-dependent gene expression. A cis-acting element (DRE: TACCGACAT) is involved in dehydration- and cold-inducible gene expression in one of the ABA-independent pathways. We isolated five cDNAs (DREB1 and DREB2) for DRE binding proteins. They encode DNA binding proteins that contain a conserved DNA binding motif found in AP2 and EREBP proteins. DREB1 genes are induced by low temperature whereas DREB2 genes are induced by drought and high salinity. These results indicate that two DREB proteins, DREB1 and DREB2, function as transcription factors in two separate signal transduction pathways under low temperature and dehydration, respectively. Improvement of drought, salt, and freezing tolerance was performed by overexpression of DREB1A in transgenic Arabidopsis. We have shown a MAP kinase cascade (ATMEKK1-MEK1/ATMKK2-ATMPK4) by yeast two hybrid system. Rapid and transient activation of ATMPK4 MAP kinase by stress treatment suggests an important role of MAPK cascade in stress signaling. Recently, we isolated a cDNA (ATHK1) encoding a yeast osmosensor Sln1p homologue. ATHK1 complemented yeast sln1 mutant, suggesting an important role of a two component system in signal perception of osmotic stress.

1-3

Phytochelatin synthase genes from fungal, plant and animal species

Cobbett, C.S.(1), **Ha, S.-B.**(1), Smith, A.P.(2), Howden, R.(1), Dietrich, W.M.(1), Bugg, S.(3), O'Connell, M.J.(3), Goldsbrough, P.B.(2)
(1) Department of Genetics, University of Melbourne, Parkville, Australia, 3052; (2) Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907-1165, USA; (3) Trescowthick Research Laboratories, Peter MacCallum Cancer Institute, Melbourne, Australia, 3000.

Plants respond to heavy metals, such as cadmium, with the biosynthesis of a class of cysteine-rich, heavy metal-binding peptides called phytochelatins (PCs). PCs have been found in all plant species examined, in addition to some yeasts, and marine diatoms. To date they have not been reported in any animal species. Unlike metallothioneins, which are gene-encoded metal binding proteins, PCs are synthesised by an enzymatic process from the tripeptide, glutathione (GSH) and consist of repeated gamma-glutamylcysteinyl groups with a C-terminal glycine [(gammaEC)nG]. The enzyme, PC synthase, is constitutively expressed in plant tissues but is activated in the presence of a range of heavy metal ions. Cadmium-sensitive, PC-deficient mutants of cad1 mutants of Arabidopsis have wild type levels of GSH and are deficient in PC synthase activity. The CAD1 gene has been isolated using a positional cloning strategy. This has been confirmed through complementation of the mutant phenotype and the molecular characterisation of four independent cad1 mutant alleles. Database searches identified a similar gene in the yeast, S. pombe. To test the role of this gene a target deletion mutant was constructed and it too exhibited a cadmium-sensitive, PC-deficient phenotype. Expression of both the Arabidopsis and S. pombe genes in E. coli allowed the identification of GSH-dependent, metal ion-activated PC synthase activity. A similar gene was identified in the nematode, C. elegans, suggesting that perhaps some animal species may also synthesise PCs in response to heavy metal exposure. An alignment of the amino acid sequences of the Arabidopsis, S. pombe and C. elegans gene products identifies a highly conserved N-terminal region believed to be the catalytic domain and a variable C-terminal. A model for the role of these domains in the activation of the enzyme by metal ions will be proposed.

1-4

Analysis of Arabidopsis mutants that confer delayed senescence symptoms or prolonged longevity

Hye Ryun Woo, Joon-Hyun Park, Hae Young Kim, Deok Hoon Park, Hong Gil Nam
Department of Life Science, Pohang University of Science and Pohang, Kyungbuk, Korea

Senescence is a sequence of biochemical and physiological events that lead to death of a cell, organ, or whole organism. Senescence is now clearly regarded as a genetically determined and evolutionarily acquired developmental process comprising the final stages of development. However, in spite of the biological and practical importance, genetic mechanism of senescence has been very limited. We previously reported that three Arabidopsis mutants, ore1, ore3, and ore9 confer global delay of leaf senescence symptoms and prolonged longevity (Oh et al., 1997). When we examined senescence responses of these mutants to various senescence-promoting factors, such as age, dark, abscisic acid, ethylene, and methyl jasmonate, they showed delayed senescence response to all these factors at physiological and molecular levels. This suggests that ore1, ore3, and ore9 may function at a common step of senescence pathway: The ORE1, ORE3, and ORE9 genes may be required for proper progression of leaf senescence induced by the phytohormones, as well as age and darkness. In contrast to these mutations, the newly isolated ore4 and ore5 mutants cause a defect in the progression of in planta leaf senescence, but not in dark-induced senescence. Thus, the ORE4 and ORE5 genes may function specifically in age-dependent senescence pathway. ore4 was isolated from a T-DNA insertional pool, is a single recessive nuclear mutation, and shows cosegregation with a T-DNA insertion. We are in the process of cloning the gene.

1-5

Analysis of root phototropism mutant, rpt2, of Arabidopsis.
Tatsuya SAKAI, Takuji WADA, Sumie ISHIGURO and Kiyotaka OKADA
Graduate School of Science, Kyoto University, Kyoto, Japan

Roots of Arabidopsis seedlings are phototropic and grow away from a light source. To understand the mechanism of root phototropism, we isolated three mutants, rpt1, rpt2 and rpt3, with no response in primary roots. Based on the complementation tests, rpt1 and rpt3 were shown to be allelic with nph1 and nph3, nonphototropic hypocotyl mutants isolated by Liscum and Briggs (1995), respectively. In vitro analysis of a blue light-induced phosphorylation of a 120-kD protein indicated that RPT2 works on the downstream of NPH1, which is a blue light receptor involved in phototropic response. Interestingly, the green light-induced phototropism is normal in hypocotyls of rpt2, though that of rpt1 and rpt3 are not. Furthermore, the degree of phototropic curvature of rprt2 was decreased as the fluence rate of blue light was increased. These results suggest that at least two distinct signaling pathways, one RPT2 dependent and the other RPT2 independent, exist downstream of NPH1, and that the ratio of signaling to induce phototropic curvature through these pathways changes according to the quality and quantity of light. We cloned the RPT2 gene, which is light inducible, encodes a novel protein with putative phosphorylation sites and nuclear localization signal, and belongs to a large gene family in Arabidopsis.

1-6

Evolution of non-symbiotic hemoglobins and expression of two classes of non-symbiotic hemoglobins in Arabidopsis thaliana
Peter Hunt (1), J. Ben Trevaskis (1), Richard Watts (1), Mark Hargrove (2), John Olson (3), Liz Dennis (1) and J. Peacock (1).
(1) CSIRO Division of Plant Industry, Black Mountain Laboratories, Canberra, A.C.T. 2601(2) Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50010, U.S.A. (3) Department of Biochemistry and Cell Biology, Rice University, Houston TX 77005-1892, U.S.A.

We have identified 2 hemoglobin genes, AHB1 and AHB2, in Arabidopsis. The genes belong to different classes of hemoglobins (I & II) and have only 51% identity at the amino acid level. Northern blotting reveals that AHB1 is expressed primarily in roots and is induced by low oxygen and by sucrose. In contrast, AHB2 is expressed primarily in shoot tissues and is not induced by any treatments. Both hemoglobins have been expressed in E. coli and the recombinant proteins purified. In comparison with leghemoglobin A from Soybean (Lba), oxygen release from both proteins is 50 fold slower and overall oxygen binding affinities are 10 fold higher (AHB1) and three fold lower (AHB2) at pH 7. The pH dependence of oxygen affinity is reversed for the non-symbiotic hemoglobins in comparison with Lba. These data suggest that the non-symbiotic hemoglobins have a very different function from the leghemoglobins and that neither can act as an oxygen donor to any other known oxygen binding proteins from plants. We have analysed the expression of AHB1 and AHB2 using GUS reporter gene constructs. AHB1-GUS is expressed in germinating seeds in the cotyledons and hypocotyl. It is also expressed in roots and cotyledons in response to sucrose or hypoxia in older plants. AHB2-GUS is expressed in developing seeds and in roots of older (flowering) plants. A number of genes expressing homologues of either of these non-symbiotic hemoglobins have been identified from a wide variety of plants. Phylogenetic analyses of these two classes of hemoglobin genes indicates that both possibly existed in plants prior to the evolution of flowering. Symbiotic hemoglobins have evolved more than once in the dicotyledonae from either class I or class II hemoglobins.

1-7

Characterisation of a homologue of the phytochelatin synthase, CAD1, gene in Arabidopsis
Suk-Bong Ha (1), Aaron P. Smith (2), Ross Howden (1), Wendy M. Dietrich (1), Peter B. Goldsbrough (2), Christopher S. Cobbett (1)
(1) Department of Genetics, University of Melbourne, Parkville, Australia, 3052, (2) Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907-1165, USA.

Plants respond to heavy metals, such as cadmium, with the biosynthesis of a class of cysteine-rich, heavy metal-binding peptides called phytochelatins (PCs). PCs are synthesised by an enzymatic process from the tripeptide, glutathione (GSH) and consist of repeated gamma-glutamylcysteinyl groups with a C-terminal glycine [(gammaEC)nG]. The enzyme, PC synthase, is constitutively expressed in plant tissues but is activated in the presence of a range of heavy metal ions. Cadmium-sensitive, PC-deficient cad1 mutants of Arabidopsis have wild type levels of GSH and are deficient in PC synthase activity. In particular, no PCs were detected in the cad1-3 mutant in the presence of Cd, suggesting that CAD1 is the only active PC synthase gene in the Columbia ecotype. The CAD1 gene has recently been isolated using a positional cloning strategy. Database searches identified a predicted gene on chromosome 1 which is very similar in DNA sequence and deduced amino acid sequence (84% identical) to CAD1. The intron/exon structure of the CAD1-homologue is very similar to that of CAD1. Exons differ in size by, at most, a single codon, except for a 90 bp deletion in exon 8. The sizes and sequences of the corresponding introns of the two genes, however, are highly variable suggesting one is not a recent duplication of the other. The CAD1-homologue is transcribed and, like CAD1, was expressed in both root and leaf tissue regardless of Cd treatment, indicating that its expression is constitutive. No substantial PC synthase activity was detected when the CAD1-homologue was expressed in E. coli. Interestingly, when a region of about 30 amino acids of the CAD1-homologue was substituted by the corresponding region from CAD1, activity increased about10-fold. This suggests the CAD1-homologue gene product is not active. Variation of the CAD1-homologue across Arabidopsis ecotypes is being investigated.

1-8

Functional analysis of the tomato B1 and B2 phytochromes in Arabidopsis
Mamatha Hanumappa, Tamayo Kubota, Kate Harrison, Seiko Miura, Li Zhao, Marie-Michèle Cordonnier-Pratt*, Lee H Pratt* and Richard E Kendrick
Laboratory for Photoperception and Signal Transduction, Frontier Research Program, RIKEN, Wako-shi, 2-1 Hirosawa 351-0198 Japan

Similar to Arabidopsis, which contains two B-type phytochromes namely phyB and phyD, tomato also contains two B-type phytochromes. These are phyB1 and phyB2, which are highly homologous to each other and Arabidopsis phyB and phyD. A phylogenetic analysis indicates the progenitor PHYB gene duplicated independently in the Solanaceae and Brassicaceae after the families evolved. Therefore, the two phyB in tomato and Arabidopsis are not equivalent. In view of this, our main interest was to study if the tomato phyB1 and phyB2 are functional in Arabidopsis. We have expressed both PHYB1 and PHYB2 in the wild type (Ler) and the phyB-null mutant of Arabidopsis (hy3, ABRC # CS6213) to see if the wild-type overexpressors have a phenotype and if the mutant phenotype can be rescued. We have also produced specific antibodies to PHYB1 and PHYB2 to study their expression levels. Results show that both phyB1 and phyB2 are functional in Arabidopsis. While phyB2 appears to be more effective than phyB1 in rescuing the long-hypocotyl and pale-green phenotype of hy3, both appear equally effective when expressed in Ler, imparting the dwarf, dark-green phenotype that is typical of phytochrome overexpressors. To confirm that the rescue is due to the functionality of tomato phyB1 and phyB2 in Arabidopsis, we are investigating the degree of overexpression in several lines using single and double 35S promoters and comparing it to the phenotype of seedlings and adult plants.

1-9

Phytochrome mutants of Pisum sativum L.

Huub Kerckhoffs (1), Shona Batge (1), Nicola Beauchamp (1), Jim Weller (1,2) and Jim Reid(1)
(1) School of Plant Science, University of Tasmania, GPO Box 252C, Hobart, TAS 7001, Australia, (2) Department of Plant Physiology, Wageningen Agricultural University, The Netherlands

The generality of the conclusions drawn from the Arabidopsis phytochrome deficient mutants were examined by the use of similar mutants in pea. We have subsequently cloned the PHYB gene from pea and shown that the photomorphogenic fun1 and lv mutations in pea are in the PHYA and PHYB structural genes respectively. Both the fun1-1 and lv-5 alleles have been shown to contain stop codons in the predicted open reading frame. The fun1-1,lv-5 double mutant was therefore constructed to determine the residual responses to light in a plant devoid of phytochrome A (phyA) and phytochrome B (phyB). The seedling de-etiolation responses of the fun1-1,lv-5 double mutant are fully explained in far-red light by phyA deficiency and in red light by a redundancy of phyA and phyB in a similar fashion as described in Arabidopsis. However in blue light (B), data strongly suggest an interaction of an additional photoreceptor, possibly a B photoreceptor with evidence of a phyA-mediated promotion of elongation. This contrasts to Arabidopsis and tomato, where phyA inhibits elongation in B. The fun1-1,lv-5 double mutant under light-grown conditions in the greenhouse shows a dramatic phenotype with pale, split, twisted stems and peduncles, and poor leaflet development. This contrasts with a relatively minor phenotype in similar mutants in Arabidopis and tomato under similar conditions and may reflect the different (epigeous) growth habit of pea compared with Arabidopsis and tomato.

1-10

Dissecting signalling pathays in ozone-sensitive Arabidopsis mutant line

R. Kettunen, K. Overmyer, H. Tuominen & J. Kangasjärvi
Inst. of Biotechnology, Univ. of Helsinki, FIN- 00014 Helsinki, Finland

Ozone forms activated oxygen species (AOS) in the apoplast and causes the plant cell itself to produce AOS in an oxidative burst. In sensitive plants, this leads to the formation of hypersensitive response (HR) -like lesions, the formation of which has the characteristics of programmed cell death. This raises the questions of possible common mechanisms downstream of pathogen and ozone induced oxidative burst. We have isolated ozone-sensitive Arabidopsis mutants, in which line 2-20 exhibits spreading cell death after a single ozone exposure. We present the use of ozone induced damage formation as a model for the activation of cell death using ozone tolerant / sensitive (Col-0 / mutant line 2-20) pair, in combination with signal transduction mutants ein2-1, etr1-1, coi1 and the transgenic NahG plants. Ozone-induced changes in the mRNA levels of 92 different genes was determined using macro arrays. The role of SA, jasmonate and ethylene signalling pathways in the ozone-induced changes in gene expression will be discussed.

1-11

Characterization of Heme Oxygenase Genes for Phytochrome Chromophore Biosynthesis in Arabidopsis

Takuya Muramoto, Munehisa Masuda, and Takayuki Kohchi
Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan

Holophytochrome is composed of an apoprotein to which a chromophore is covalently attached, and is capable of existing in either of Pr and Pfr forms. The chromophore, phytochromobilin, is important for its photoreversibility. Although the biosynthetic pathway has been well-known, relatively little is known about the genes, their expression and the enzymatic properties for phytochrmobilin biosynthesis. The HY1 and HY2 genes in Arabidopsis are necessary for the chromophore biosynthesis and are defined by mutations confering the etiolated phenotype. We have cloned HY1 gene by its map position. Although the HY1 gene product showed weak similarity to heme oxygenase (HO), it is phylogenically distinct from algal HO. The HY1 protein was detected stroma fraction in plastids immunochemically. Recombinant protein for HY1 showed HO activity in vitro. Reduced ferredoxin is necessary for HO activity, and the presence of iron chelators and ascorbate is needed to show full HO activity. We concluded that the HY1 protein is a functional heme oxygenase for phytochrome chromophore biosynthesis in Arabidopsis and it could be named AtHO1 for Arabidopsis thaliana heme oxygenase-1. Blast analysis revealed that the Arabidopsis genome contains an additional sequence homologous to the HY1 gene, and we call it tentatively AtHO2 (the second gene for HO in Arabidopsis). The hy1 mutation is said to be "leaky" since trace levels of phytochrome action could be detected at late stage of plant development. We are currently analyzing AtHO2 expression and its enzymatic property since the presence of AtHO2 may explain leaky hy1 phenotype.

1-12

Molecular characterization and expression of a cDNA encoding copper/zinc superoxide dismutase from cultured cells of cassava (Manihot esculenta Crantz)

Haeng-Soon Lee, Kee-Yeun Kim, Soon-Hee You, Suk-Yoon Kwon, Sang-Soo Kwak
Plant Biochemistry Research Unit, Korea Research Institute of Bioscience and Biotechnology

The cDNA encoding cytosolic copper/zinc superoxide dismutase (CuZnSOD) mSOD1 was cloned and characterized from cell cultures of cassava (Manihot esculenta Crantz) which produced a high yield of SOD. mSOD1 encoded 152 amino acid polypeptides with a pI value of 5.84. Southern blot analysis using a mSOD1 specific probe indicated that a single copy of the mSOD1 gene was present in the cassava genome. The mSOD1 gene was highly expressed in cultured cells, as well as in intact stems and tuberous roots. It was expressed at a low level in leaves and petioles. Transcripts of mSOD1 were not detected in nontuberous roots. During cell growth, transcription activity was at a high level during the stationary growth stage and sharply decreased after further culturing. The mSOD1 gene in excised cassava leaves responded to various stresses in different ways. These stresses included temperature change and exposure to stress inducing chemicals. Transcript levels of mSOD1 increased dramatically a few hours after heat stress at 37¡É and showed a synergistic effect with wounding stress. Levels decreased in response to chilling stress at 4¡É and showed an antagonistic effect with wounding stress. The gene was induced by ABA, ethephon, NaCl, sucrose, and methyl violgen. These results indicate that the mSOD1 gene is involved in the antioxidative mechanism in response to oxidative stress induced by environmental change.

1-13

The Regulation of Superoxide Dismutase in Arabidopsis thaliana
Lee, Kuo-Wei, and Pan, Shu-Mei
Department of Botany, National Taiwan University, Taipei, Taiwan.

A number of environmental stresses can lead enhanced production of superoxide in plant tissues, and superoxide dismutase (SOD) is used to scavenge this reactive oxygen species. In this study, using native PAGE, SOD activity assay and western blot analysis, the SOD activity in the rosette leaves was observed during its growth and development. The fluctuation of SOD activity in Arabidopsis imposed to several abiotic stresses was also examined. Cycloheximide was used for the study of translational and posttranslational regulation of SOD in Arabidopsis. The regulation of SOD in Arabidopsis and its physiological role will be discussed.

1-14

New approaches towards the understanding of S-like RNase functions in Arabidopsis
Gustavo C. MacIntosh(1), Pauline Bariola (1,2), John Golz (3), Ed Newbigin (3) and Pamela Green (1).
(1) MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824, USA, phone #: 517-353-3264; FAX #: 517-353-9168; email: greenlab@pilot.msu.edu
(2)Present address: Institut de Biologie et the Physiologie Végétales, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland. (3) Plant Cell Biology Research Centre, University of Melbourne, Parkville, Victoria 3052, Australia.

The RNase T2 family of ribonucleases is broadly distributed in nature with members found in almost all organisms examined from bacteria to mammals. In Arabidopsis, three members of this family, RNS1, RNS2 and RNS3, have been identified. Although the regulation of the expression of these RNases in response to different stress conditions has been well characterized, their physiological function and specific substrates remain to be elucidated. As a new approach toward the identification of the function of the S-like RNases, and in a more general sense, of the T2 family of RNases, we initiated the characterization of RNY1, the only member of this family present in Saccharomyces cerevisiae. An analysis of the RNY1 promoter identified potential regulatory sequences including a minimal heat shock element, an element regulated by oxidative stress, and two stress response elements, suggesting that RNY1 is expressed in response to various stresses. Expression studies indicating that the RNY1 gene is regulated by heat-shock support this hypothesis. Mutant rny1- strains, constructed by insertional knockout, show different phenotypes depending on the wild type parental strain used. The most severe phenotype displays temperature sensitivity when grown at 37°C, and the mutant cells are five times larger than wild type. Complementation analysis of this mutant showed that expression of each of the three Arabidopsis RNS genes was sufficient to rescue the temperature sensitivity phenotype. This result suggests that the essential function of RNY1 in yeast is dependent on its RNase activity and indicates that the same function could be conserved in Arabidopsis.

1-15

Nuclear localization of phytochrome B

Akira Nagatani (1), R. Yamaguchi (1), M. Nakamura (1), K. Suita (1), T. Matsushita (1), N. Mochizuki (1) and S. A. Kay (2).
(1) Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan, (2) National Science Foundation Center for Biological Timing, Department of Cell Biology, Scripps Research Institute, La Jolla, California 92037, USA

Phytochrome is a major photoreceptor of plants. We have recently shown that a functional nuclear localization signal may reside within the C-terminal region of a member of the phytochrome family, phytochrome B (phyB) (Sakamoto and Nagatani. 1996). In the present study, a fusion protein consisting of full length phyB and the green fluorescent protein (GFP) was overexpressed in the phyB mutant of Arabidopsis. The resulting transgenic lines exhibited pleiotropic phenotypes reported previously for phyB overexpressing plants, suggesting that the fusion protein is biologically active. Immunoblot analysis with anti-phyB and anti-GFP monoclonal antibodies confirmed that the fusion protein accumulated to high levels in these lines. Fluorescence microscopy of the seedlings revealed that the phyB-GFP fusion protein was localized to the nucleus in light grown tissues. Interestingly, the fusion protein formed speckles in the nucleus. Analysis of confocal optical sections confirmed that the speckles were distributed within the nucleus. In contrast, phyB-GFP fluorescence was observed throughout the cell in dark-grown seedlings. Hence, phyB appears to be localized to specific sites within the nucleus upon photoreceptor activation. Kinetics of the light-induced nuclear accumulation was further examined in pea seedlings. The nuclei were isolated and probed with an anti-phyB antibody. In dark-grown seedlings, phyB was not detected in the nuclear fraction. However, treatment of the seedlings with either continuous or a pulse of red light induced stable accumulation of phyB in the nucleus. The level reached a plateau about 2 h after the onset of light treatment. Once phyB is accumulated in the nucleus, it remained there at least for 12 h in darkness. Conversely, far-red light accelerated disappearance of phyB from the neucleus. These observations suggest that the nuclear localization and retention of phyB is Pfr-dependent.

1-16

The roles of the two cis-acting elements, DRE and ABRE in the dehydration, high salt and low temperature responsive expression of the rd29A gene in Arabidopsis thaliana

Yoshihiro Narusaka(1), Zabta K. Shinwari(1), Kazuo Nakashima(1), Yoh Sakuma(1), Takashi Furihata(1), Setsuko Miura(1), Kazuko Yamaguchi-Shinozaki(1), Kazuo Shinozaki(2)
(1)JIRCAS, Tsukuba 305-8686, (2)RIKEN, Tsukuba 305-0074

To understand signal transduction pathways from the perception of osmotic and cold stress signals to gene expression, we have precisely analyzed regulation of a dehydration-responsive rd29A gene in Arabidopsis thaliana. The rd29A gene is also induced by low temperature stress and ABA treatment. We analyzed the rd29A promoter and identified a cis-acting element, DRE(Dehydration Responsive Element). DRE is involved in the induction by dehydration and low temperature, but does not function in ABA-responsive expression. ABRE(ABA Responsive Element) is thought to be one of cis acting elements in ABA-dependent expression of rd29A. We analyzed the promoter region (-190 -71) of rd29A, and examined the relationship between DRE and ABRE. We constructed a chimeric gene with the promoter region of rd29A fused to the GUS reporter gene, and then transformed tobacco or Arabidopsis plants with the constructs. Base-substitutions in DRE reduced the expression of GUS under dehydration, high salt and low temperature stress condition. In contrast, base-substitutions in the ABRE sequence of the promoter region significantly reduced the ABA responsive expression of GUS. Base-substitutions in both DRE and ABRE completely abolished expression of GUS under various stress condition. The results indicate that both DRE and ABRE are interdependent in the stress-responsive expression of the rd29A gene.

1-17

Characterization of rice cytosolic CuZnSOD protein in Arabidopsis--implication of a metal incorporating system for CuZnSOD protein in plants

Shu-Mei Pan, Ming-Kuan Chen, Ming-Hsun Chung, and Kuo-Wei Lee.
Department of Botany, National Taiwan University, Taipei, Taiwan.

Superoxide dismutases are a group of metalloenzymes that catalyze the dismutation of superoxide radical to molecular oxygen and hydrogen peroxide. We constructed a transgene using a CaMV 35S promoter to express a rice cytosolic CuZnSOD gene in Arabidopsis and generated 195 transformants. Only a polypeptide about the similar size of the rice CuZnSOD subunit, was detected in the transgenic Arabidopsis. Interestingly, two active rice CuZnSOD forms, rSODI and rSODII, having the same dimeric size were always detected in the independent transgenic plants. Western blot analysis also indicated several multiple proteins immunologically related to CuZnSOD as well as active CuZnSOD in the transgenic plants. In vitro, the addition of copper had effects on the rSODII form in the extract of transgenic plant. We propose that a putative metal incorporation system is required for proper binding of copper and zinc to the apoSOD or nascent SOD in higher plant. This is consistent with the report that a functional homolog of the yeast copper chaperone in Arabidopsis.

1-18

Disruptions to chloroplast development adversely affects plant fitness under light and temperature stress

Hyyounshin Park (1), Sarah S. Kreunen (1), Dalia Bishai (1) and **Barry J. Pogson** (1&2).
(1) Dept. Plant Biology, Arizona State University, Tempe AZ 85287-1601 USA (2) Div. Biochem. Molec. Biology, Australian National University, Canberra, ACT 0200, Australia.

The chloroplast is the site of photosynthesis and many metabolic pathways, including lipid and carotenoid biosynthesis. In the dark, the undifferentiated proplastid develops into an etioplast (skotomorphogenesis). During photomorphogenesis, etioplast to chloroplast differentiation is more rapid than direct proplastid to chloroplast development. We have identified a class of Arabidopsis mutants, ccr (ccr = chloroplast and carotenoid regulation) that are conditionally viable; apparently due to defects in specific aspects of chloroplast development. ccr1 and ccr2 were identified by aberrant carotenoid biosynthesis in chloroplasts (decreased lutein) and etioplasts, but they are not lesions in carotenoid biosynthetic genes. During photomorphogenesis in ccr1 there is a marked increase in cytosolic lipid bodies, arrested development at low temperatures or under prolonged red light, and partial male sterility: phenotypes that may be caused by aberrant lipid metabolism or trafficking. ccr2 etioplasts lack the prolamellar body and accumulate acyclic carotenoid precursors; and as an apparent consequence, the seedlings are slow to green. ccr2 chloroplasts have reduced thylakoid granal stacking and altered stromal lamellae. While ccr1 and ccr2 have pronounced effects on lipids, carotenoids and skoto- and photomorphogenesis they are not allelic to a range of fatty acid desaturase mutations, carotenoid biosynthetic enzymes, or photomorphogenic loci. Evidently, ccr1 and ccr2 are novel lesions differentially affecting key chloroplastic developmental and metabolic pathways, resulting in plants with compromised fitness.

1-19

Carotenoid biosynthesis and photoprotection in Arabidopsis
Rissler, Heather and **Pogson, Barry J.**
Dept. of Plant Biology, Arizona State University, Tempe, AZ, 85287-1601, USA.

The ability to protect against photo-oxidative damage is an almost daily occurrence for a plant exposed to full sunlight, and as a consequence there are multiple different mechanisms that serve to dissipate excess light. One of these mechanisms, nonphotochemical quenching (NPQ), dissipates excess light in the antenna prior to damage by utilizing xanthophylls, such as zeaxanthin and lutein (1). We have over-expressed a lutein biosynthetic enzyme, the e-cyclase, to study how carotenoid accumulation is regulated and how lutein contributes to photoprotection. Interestingly, the e-cyclase transgene construct using the CAMV promoter should result in a very large increase in mRNA, yet the maximum increase in lutein was about 180 % which suggests that mechanisms other than gene expression contribute to the regulation of carotenoid accumulation.

In addition, we are utilizing an antisense approach to examine how a reduction in the b-carotene hydroxylase enzyme will effect carotenoid biosynthesis, photoprotection, and assembly of the antenna. A series of transgenic lines with reduced accumulation of b-carotene derived xanthophylls have been established. However, the maximum reduction is 60 % and the plants are fully viable. Preliminary studies suggest that if the transgene is expressed in a e-hydroxylase defective background (lut1), then there is extensive photobleaching of the leaves. If confirmed, this implies that xanthophylls are required for plant viability and b-carotene alone is not sufficient and it implies that the two hydroxylase enzyems can act on the other's substrate. More definitive studies are in progress.
1. Pogson, et al. (1998) Proc. Natl. Acad. Sci. USA 95, 13324.

1-20

Analysis of uvi mutants induced by ion beams
Ayako Sakamoto (1), Atsushi Tanaka (1), Naoya Shikazono (1), Shigemitsu Tano (1), Yasuto Ishigaki (1), Osamu Nikaido (2), Hiroshi Watanabe (1)
(1) Plant Res. Lab, Japan Atomic Energy Research Institute, Watanuki-machi 1233, Takasaki, Japan.
(2) Fac. of Pham. Sci., Kanazawa Univ., Takaramachi 13-1, Kanazawa, Japan

UVB in sunlight penetrates into plant cells and causes a variety of biological reactions. Especially, UVB causes DNA damage and also induces several physiological responses such as flavonoid biosynthesis. Little is known, however, about the UVB photoreceptor and the UVB signal transduction process in plant cells. We previously reported the isolation of four Arabidopsis mutants, induced by carbon ion irradiation, namely, UVB insensitive 1 - 4 (uvi1 - uvi4, previously UV2-19 etc.). They can grow vigorously even under UVB and are insensitive to 7kJ of UVB irradiation to the roots. As a first step to elucidate the UVB resistant mechanism(s), we analyzed DNA damage and DNA repair activities in these mutants by means of ELISA using specific antibodies against CPD and 6-4 photoproducts. Although the amount of DNA damage just after UV-irradiation in the mutant plants were almost comparable to that of wild-type plant, one of the mutants, uvi2, repaired CPD photoproduct more effectively than the wild-type after 0.5 - 5 hour incubation under the light. In addition, this mutant also repaired 6-4 photoproduct when incubated without light. We then analyzed the expression patterns of two genes encoding DNA photolyases which repair DNA damages by photoreactivation. The expression of the CPD photolyase gene was more strongly induced by 6-hour UVB irradiation in the uvi2 mutant than in the wild-type. We speculate that the CPD photolyase gene may be regulated negatively as well as positively, and a deficiency in the negative regulation of the CPD photolyase gene may be involved in the uvi2 mutant phenotype.

1-21

The expression of RCI2A and RCI2B genes is developmentally regulated in Arabidopsis

Joaquin Medina, Rafael Catala and **Julio Salinas**
Depto. de Mejora Genetica y Biotecnolgia,Instituto de Investigacion y Tecnologia Agraria y Alimentaria (INIA)Carretera de la Coruña Km.7, 28040 Madrid, Spain

Most plants from temperate regions increase their ability to tolerate freezing in response to low-nonfreezing temperatures. This process, known as cold acclimation (CA), is associated with several physiological changes and alterations in gene expression. By screening a cDNA library from cold-acclimated etiolated seedlings of Arabidopsis with a substracted probe enriched in cold-inducible transcripts, we identified several clones corresponding to Rare Cold Inducible (RCI) genes which expression is induced during CA at medium-low levels. Two of these genes, named RCI2A and RCI2B, encode small, highly hydrophobic proteins containing two integral transmembrane domains. The expression of RCI2A and RCI2B was shown to be not only induced by low temperatures but also by water deficiency and ABA. Recently, genes showing high similarity to the RCI2s have been described in cyanobacteria, nematodes, yeast and E. coli. To investigate how RCI2 gene expression is regulated, we have isolated the promoter regions of RCI2A and RCI2B and generated Arabidopsis transgenic plants containing transcriptional fusions in which these regions are driving the GUS reporter gene. Results on the GUS expression patterns in different tissues of Arabidopsis and at different stages of development reveal that RCI2 genes are not only environmentally but also developmentally regulated. Furthermore, we will present results on the regulation of RCI2 gene expression by transcription factors from the CBF family. Based on these data, the potential roles of RCI2 proteins in cold acclimation will be discussed.

1-22

Binding Specificity of DRE Binding Proteins, DREBs, Involved in Dehydration-Responsive Transcription in Arabidopsis

Yoh Sakuma(1), Qiang Liu(1), Hiroshi Abe(1), Mie Kasuga(1), Setuko Miura(1), Kazuko Yamaguchi-Shinozaki(1), Kazuo Shinozaki(2).
(1)Japan Int. Res. Cent. Agri. Sci. (JIRCAS), Tsukuba 305 JAPAN, (2)Lab. Plant Mol. Bio., Inst. Phys. Chem. Res. (RIKEN), Tsukuba 305 JAPAN

One of the dehydration responsive genes, rd29A, is induced by low temperature stress and ABA treatment. We precisely analyzed the rd29A promoter and identified a cis-acting element named DRE (TACCGACAT) that is involved in dehydration-responsive expression. DRE is also involved in low-temperature responsive expression, but not in ABA-responsive expression. Two cDNA clones that encode DRE binding proteins, DREB1A and DREB2A, were isolated by yeast one-hybrid screening. Both DREB proteins specifically bind to DRE in the rd29A promoter and have conserved DNA-binding domains found in EREBP/AP2. The DREB1A mRNA is accumulated in response to dehydration and high-salt, whereas the DREB2A mRNA is accumulated in response to low temperature stress. In a transient transactivation experiment using Arabidopsis protoplasts, both DREB proteins activated the transcription of the rd29A promoter. Based on the gel shift assay with recombinant DREB proteins, both DREB proteins were shown to specifically bind to the six nucleotides, ACCGAC, of DRE. However, the two DREBs have different binding specificity to DRE sequence having a mutation in the second A or third C nucleotide position.

1-23

Promotor analysis of a gene encoding proline dehydrogenase in Arabidopsis thaliana
Rie SATOH (1), Kazuo NAKASHIMA (1), Kazuko YAMAGUCHI-SHINOZAKI (1) and Kazuo SHINOZAKI (2).
(1) Biological Resources Division, Japan International Research Center for Agricultural Sciences (JIRCAS), 1-2 Ohwashi, Tsukuba, IBARAKI, 305-8686 JAPAN, (2) Laboratory of Plant Molecular Biology, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, IBARAKI, 305-0074 JAPAN.

Proline is one of the most common compatible osmolytes in water-stressed plants. We obtained a cDNA clone for the proline dehydrogenase, ProDH (ERD5), by differential screening, and a promoter region of the ProDH gene from Arabidopsis thaliana. The expression of the ProDH gene was studied using Northern blot analysis and characterization of transgenic Arabidopsis having a fused gene consisting of the ProDH promoter and the reporter gene for ß-glucuronidase (GUS). We reported that the ProDH gene is upregulated by rehydration after 10-h dehydration, but downregulated by dehydration for 10-h in Arabidopsis. The ProDH gene is also induced by proline. For further understanding of the expression of the ProDH gene, we analyzed cis-acting elements involved in rehydration- and proline-induced expression using deletion or mutated fragments of the ProDH promoter fused to the LUC (luciferase) gene in transgenics. We found that a direct repeat sequence in the promoter may be involved in rehydration- and proline-induced expression of ProDH. The DNA fragment containing only one of the direct repeat sequence responded to rehydration and proline in transgenic tobacco.

1-24

Molecular markers in wheat: linkage to a starch granule distribution locus
Michael Tripet (1), Jennifer A. Saleeba (1) and Fred L. Stoddard (2).
(1) School of Biological Sciences, and (2) Plant Breeding Institute, The University of Sydney, Sydney, NSW 2006.

Bread wheat, Triticum aestivum, deposits starch in large A granules and small B granules in the developing endosperm. The distribution of starch is of interest because of the slower precipitation of the smaller B granules in the wet process of starch manufacture, resulting in the loss of a significant proportion of the total starch content. In an investigation of the genetics of the B-granule content, two cultivars differing for this trait have been crossed and doubled haploids have been derived from the F1 generation, so only one cycle of meiotic exchange has taken place. By screening this doubled haploid population, we will associate AFLP markers with the inheritance of the lower proportion of B granules. Molecular markers will also be used to estimate the relatedness of the two cultivars.

1-25

Molecular analyses of Arabidopsis Two-component systems

Takeshi Urao (1), Shinichi Miyata (1), Kazuko Yamaguchi-Shinozaki (1), Kazuo Shinozaki (2)
(1) JIRCAS, Min. Agr. Forest Fish., Tsukuba 305, Japan, (2) Lab. Plant Mol. Biol., Inst. Phys. Chem. Res. (RIKEN), Tsukuba 305, Japan

Two-component system is a simple and elaborate signaling module that transduces extracellular signals to cytoplasm through phosphotransfer between sensors and response regulators. To analyze the roles of two-component systems in the signal transduction pathways of plant, we have so far cloned cDNAs encoding a hybrid-type histidine kinase ATHK1, three phosphorelay intermediates ATHP1-3, and four response regulators ATRR1-4 from Arabidopsis. We have also demonstrated that ATHK1 has a potential ability to act as an osmosensor by analyzing both sensing (input) and catalytic (output) activities with yeast osmosensing-defective mutants. To determine which molecules constitute two-component signaling modules in Arabidopsis, we first examined protein-protein interactions among these proteins using pairwise yeast two-hybrid analysis. A specific interaction between ATHK1 and ATHP1 was detected. We also detected interactions between yeast SLN1 and ATHP1 or ATHP3, between CKI1 and ATHP1 or ATHP2, and between ETR1 and all ATHPs. However, ERS did not interact with any ATHPs. We further examined protein-protein interactions between ATHPs and ATRRs. The results showed that ATHP2 interacts with ATRR4, and that ATHP3 interacts with ATRR1 or ATRR4. Based on these results, possible signaling pathways of Arabidopsis two-component systems will be discussed.

1-26

Isolation and genetic analysis of low-K-tolerant (lkt) Arabidopsis mutants and chromosomal mapping of lkt1 gene*

Wei-Hua WU, Shu-Qing ZHAO, Chun LIN, & Xiao-He YANG
Dept. of Plant Sciences, College of Biological Sciences, China Agricultural University, Beijing 100094

Although higher plants are possessed of high affinity K+-uptake mechanism, most plants also require low affinity K+-uptake mechanism for their K+ nutrition. In the present study, low K+-tolerant (lkt) mutants of Arabidopsis were isolated and genetically analyzed with the aim of identifying "very-high affinity" K+-uptake mechanism(s) by which plants can take up sufficient K+ without requiring complement of low affinity K+-uptake mechanism. EMS-mutagenized M2 populations were screened in low-K+- medium using the root-bending assay. K+ concentration for lkt mutant screening was 100 micromolar because root growth of wild type seedlings was completely inhibited at or below this concentration. Forty-two low K+-tolerant mutants were selected from M2 seedlings, and 2 out of 42 M2 mutants maintained low K+-tolerance in their M3 generations. The seedlings of F1 generation of these two lkt mutants all showed wild-type low-K+-sensitive phenotype, and F2 progeny of these two mutants segregated 1,062:351 (wt:lkt; for lkt1-1) and 284:96 (wt:lkt; for lkt1-2), respectively, when cultured in low-K+ medium. The results of genetic analysis suggested that either mutant has a monogenic recessive mutation in a nuclear gene, and that two mutations of two independent mutants are allelic to each other. To determine the chromosomal map position of lkt1, F2 mapping populations (235 F2 plants) were generated from cross between lkt1 mutant and wild-type Columbia plants. The genetic linkage between lkt mutation and polymorphic DNA markers was determined using the codominant PCR-based CAPS markers or microsatellite markers, and the results showed that the lkt mutation is linked to the marker nga248 and UFO on chromosome I. (* Supported by the NSFC competitive research grants #39570074, #39525003, and #39873956 to WHW)

1-27

Characterization of *ecil*, an *Arabidopsis* mutant with lesion formation induced by environmental conditions on leaves

Fengling Li, Yuju Zhao and Hai Huang
Shanghai Institute of Plant Physiology, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China

An *Arabidopsis* mutant, *ecil* (environment-condition-induced lesion), has been recently isolated by Ac/Ds gene trap system. The genetic analysis demonstrated that the mutation was heritable and recessive. The mutant phenotype was due to a single gene defect, and was tightly linked to a Ds locus. The *ECIL* gene was mapped on the top part of chromosome V.
In comparison with previously identified lesion mimic mutants in *Arabidopsis*, the expression of *ecil* phenotypes was dependent on temperature, photo period of time and intensity of light. Under 19°C, *ecil* plant has the exactly same phenotypes as shown in wild-type plant. However, when *ecil* plants were grown at 22 °C, which is the common *Arabidopsis* growth temperature, lesions could form on cotyledons, rosette leaves and cauline leaves. The *ecil* plants grown at 24°C showed even severer phenotypes. Short photo period and low intensity of light also greatly reduced the symptoms on *ecil* leaves. When normally looking *ecil* plants grown at 19°C were removed to 24°C, lesions appeared within 2-3 days. If those plants were moved back to 19°C, the following appeared leaves were normal. In order to determine if the lesion was the defect caused by programmed cell death (PCD), we have analyzed cell structure by electron microscopy. In the course of high temperature induction, the plasma membrane was first separated from the cell wall, and then the protoplast was shrunken and condensed. These results indicated that environmental factors might cause PCD in *ecil* leaves. The possible functions of *ECIL* gene will be discussed.

2-1

Gene silencing in Arabidopsis

Hervé Vaucheret, Christophe Béclin, Richard Berthomé, Stéphanie Boutet, Mathilde Fagard, Jean-Benoit Morel, Philippe Mourrain, Florence Proux, Carine Remoué
Laboratoire de Biologie Cellulaire, INRA, 78026 Versailles Cedex, France

In eukaryotes, transgenes may undergo epigenetic silencing, i. e. heritable alterations in gene expression that can not be explained by changes in DNA sequence. When the introduced transgenes are homologous to host endogenous genes, gene silencing can affect both host and transgene expression. Gene silencing can occur either at the transcriptional level (TGS), i. e. transcription is blocked, or at the post-transcriptional level (PTGS), i. e. RNA are degraded. TGS is mainly associated with promoter-methylated multicopy transgenes whereas PTGS is usually associated with coding sequence-methylated (strongly expressed) transgenes. The identification of mutants affected in the control of gene silencing provides good tools to elucidate the underlying mechanisms. Mutants impaired in TGS have been isolated by three groups, (Furner et al, 1998; Mittelsten Scheid et al, 1998; Jeddeloh et al, 1998). One of these mutants was previously identified as being deficient for DNA methylation (Vongs et al, 1993). Mutants affected in PTGS efficiency were isolated by two groups. One group isolated mutants with an increase in PTGS efficiency (Dehio and Schell, 1994), whereas the other isolated mutants impaired in PTGS (Elmayan et al, 1998). The recent progress of these analyses will be presented.

2-2

Genetic Determinants of mRNA Degradation in Arabidopsis

Mark A. Johnson (1,2), Miguel A. Perez-Amador (1), James P. Kastenmayer (1,3), and Pamela J. Green (1,3)
(1) MSU-DOE Plant Research Laboratory, (2) Department of Microbiology, and (3) Department of Biochemistry, Plant Biology Building, Michigan State University, East Lansing, Michigan 48824-1312 USA

Genes which encode unstable mRNAs can be regulated very efficiently and allow the cell to make rapid changes in gene expression in response to internal and external stimuli. We have focused on the unstable small-auxin-up-RNAs (SAUR) as a model for rapidly degraded mRNAs. mRNAs encoded by this gene family share a sequence element called DST in their 3' untranslated region (UTR) which has been shown to target otherwise stable reporter transcripts for rapid turnover in plants. In order to understand the molecular basis of sequence-specific recognition and degradation of unstable mRNAs we devised an approach to isolate Arabidopsis mutants defective in DST-mediated mRNA degradation. Our strategy involved the generation of transgenic plants expressing HPH (hygromycin phosphotransferase) and GUS (b-glucuronidase) reporter genes. The transcripts from both genes were destabilized by insertion of a tetramer of the DST instability determinant into their 3' UTR. Mutants in the mRNA decay pathway mediated by the DST element are expected to have increased HPH and GUS mRNA abundance and therefore, it should be possible to isolate them on the basis of these increased expression levels. Using this approach, two mutants (dst1 and dst2) that elevate the levels of DST-containing mRNAs have been isolated. Characterization of dst1 and dst2 indicates that they harbor mutations in trans-acting factors involved in DST-mediated mRNA decay. These mutants should serve as powerful tools in our future explorations into the molecular mechanisms that govern rapid degradation of specific mRNAs. In addition, these mutants should help us address interactions between sequence-specific factors and potential components of the general mRNA decay machinery that have recently been isolated.

2-3

Protein-Protein interactions between Polycomb-group members in Arabidopsis.
Colin MacDougall (1), Christine Stock (1), Bill Crosby (2) and Justin Goodrich (1).
(1) Institute of Cell and Molecular Biology, University of Edinburgh, The King's Buildings, Edinburgh EH9 3JH, Scotland, UK. (2) Plant Biotechnology Institute, NRC Canada, 110 Gymnasium Place, Saskatoon, Canada.

Cell fate determination requires that the on or off states of homeotic genes are inherited through cell division during development. In animals, the Polycomb-group (Pc-G) and trithorax-group (trx-G) proteins play a key role in such maintenance, with Pc-G proteins acting as repressors and trx-G proteins as activators.

The Arabidopsis CURLY LEAF (CLF) gene encodes a protein with structural homology to the Drosophila Polycomb-group protein Enhancer of zeste (E[z]). Like E(z), CLF also acts to repress homeotic gene expression. Whereas in wild-type plants, expression of the homeotic C-function gene AGAMOUS (AG) is restricted to inner floral whorls, where it specifies stamen and carpel development, in clf mutants AG is ectopically expressed in leaves, stem and outer floral organs. Analysis of clf ag double mutants further suggests that the clf phenotype of leaf curling, early flowering and floral homeotic transformation is caused by AG mis-expression.

One approach we have been using to elucidate the mechanism of CLF function and the role of plant Polycomb proteins in general, is to identify proteins which directly interact with CLF protein using the yeast two-hybrid screening system.

2-4

Comparison of global gene expression profiles of herbicide-treated and antisense "knock-out" plants
Feng Zheng, Klaus Maleck, Leslie Friedrich, Sharon Potter, Jurg Schmid, Eric Ward, Marcus Law, Joshua Z. Levin
Novartis Agribusiness Biotechnology Research, Inc., Research Triangle Park, NC 27709 USA

We used Synteni's GEM (Gene Expression Microarray) technology to characterize the global differences in RNA levels due to inhibition of an enzyme by antisense or a chemical inhibitor. We chose to study adenylosuccinate synthetase (AdSS), an enzyme in the purine biosynthetic pathway, to evaluate the potential for this profiling method. Hydantocidin is a naturally occurring spironucleoside with potent herbicidal activity due to its inhibition of AdSS. Similarly, AdSS antisense plants exhibit severe growth retardation and lethality. Gene expression profiles resulting from AdSS inhibition by antisense and hydantocidin treatment were compared. PCR products generated from ~10,000 Arabidopsis ESTs (obtained from the ABRC) were sent to Synteni to create GEMs and ~600 ng of polyA RNA per sample was also provided to create probes. AdSS antisense plants showed induction of 115 ESTs and repression of 152 ESTs (threshold 2.5X). Hydantocidin-treated plants showed induction of 77 ESTs and repression of 104 ESTs. A comparison shows 10 genes were induced and 32 genes were repressed in both. Confirmation of these data is in progress. This profiling method could potentially be used to characterize plants with defects in genes of unknown function or inhibited by chemicals with unknown modes of action.

2-5

A 323 bp of rapeseed gene promoter region confers petal-specific expression in Arabidopsis transformants

Brocard I., Charlot F., Pelletier G., Guerche P. and Teoule E.
INRA Station de Genetique et d'Amelioration des plantes, Route de St-Cyr 78026 Versailles cedex France

Within the general theme of plant architecture study in our lab, an approach of isolating genes specifically expressed in the petal was conducted. Subtraction and differential hybridization has enabled us to isolate a cDNA (named 9.2) corresponding to an mRNA which is present in petals and, in lesser amounts, in stamens. The corresponding genes, which were isolated from a rapseed genomic library, belong to a family including at least 2 genes (named 4.1.1 and 8.1.1). Their respective homologous genes were also found in cabbage and rape. Some preliminary phylogenetic experiments in the Brassicaceae family (sequence comparison of ORF, expression patterns in flowers by northern blot analysis) were made. The expression specificity conferred by upstream regions of the 2 genomic clones was analyzed using GUS reporter gene fusions in the related model plant, Arabidopsis thaliana. A proximal 323 bp fragment of the 4.1.1 gene confers a petal-specific expression in 36 % of A.thaliana independant transformants. These genes encode for a putative cell-wall protein. A.thaliana transformed with constructions with the 4.1.1 coding sequence in sense and anti-sense orientations under the control of 323 bp of 4.1.1 promoter didnÌt show evident modified phenotype with regard to non transformed control. One homologous gene is present in A.thaliana. We are currently screening by PCR the Versailles T-DNA lines to find mutated transformants for this gene. We have isolated the A.thaliana upstream region and we would like to compare the expression specificity of the proximal fragment with 4.1.1 one.

2-6

Correlation between Superoxide dismutase activity and low-molecular-weight Antioxidants in Arabidopsis

Ing-Chien Chen, Ming-Hsun Chung and Shu-Mei Pan
Department of Botany, National Taiwan University, Taipei, Taiwan, R.O.C.

Superoxide dismutase (SOD) is one of the defense enzymes scavenging toxic active oxygen species. Several SOD-transgenic Arabidopsis were generated by expressing the sense and antisense construct of rice CuZnSOD and MnSOD gene. The transgenic plants express various levels of SOD activity in rosette leaves about several-fold to only half amount of that in the wild-type plants. In order to study the relationship between SOD and nonenzymatic antioxidant activity, acid-extraction was used to estimate the total antioxidant activity and total thiols in the various parts of different SOD expression lines. The correlation between SOD levels and low-molecular-weight antioxidants were discussed.

Shu-Mei Pan
886-2-23630231 ext 2366
Pan@ccms.ntu.edu.tw

2-7

Localization of Arabidopsis N-Myristoyltransferase and its Expression Pattern during Development
Raju Datla(1), Qungang Qi (1), Qun Xia (1), Rajala Raju (2), Rajendra Sharma (2) and Gopalan Selvaraj(1)
(1) National Research Council of Canada, Plant Biotechnology Institute, 110 Gymnasium Place,Saskatoon, Sask. S7N0W9, Canada; (2) Department of Pathology, University of Saskatchewan, Saskatoon, Sask S7N 4H4, Canada

Structural modifications of proteins play an important role in affording functionality to proteins. Covalent attachment of myristic acid to the N-terminal amino acid, catalyzed by myristoyl-CoA:protein N-myristoyltransferase (NMT, EC2.3.1.97), is one such modification. NMT has been studied well in yeast and mammalian cells but not in plants. Here we present our work on Arabidopsis thaliana NMT. NMT transcript was found in all organs that were examined by northern blot. These include roots, stem, flower and silique. In addition, pollen cells were also found to contain NMT transcripts. In general, youngest tissue contained more, and shoot apex contained the most steady-state level of NMT transcripts. In situ hybridization analysis also showed greater levels of NMT transcript in shoot apices and cambial cells of the vascular bundles, and immunocytochemistry further confirmed this. Developing siliques, again, showed a correlation of NMT transcript abundance and developmental (metabolic) activity: Immature green (young) siliques contained the most in contrast to yellow (maturing) or brown (near maturity) siliques. Further, mature seeds contained barely detectable amount of NMT transcript. Western blot analysis also showed that the NMT polypeptide level was highest in the younger siliques. Subcellular fractionation by differential ultracentrifugation and sucrose gradients showed ca. 50% greater level of NMT in the ribosomal fraction (58% of the total activity) in comparison with cytolsolic (37%) activity. NMT activity was not detectable in mitochondrial, chloroplastic or microsomal fractions.

2-8

Hypomethylation and hypermethylation of DNA at the SUPERMAN locus in METI antisense plants.
N. Kishimoto(1), E.S. Dennis , W.J. Peacock and **E.J. Finnegan**
CSIRO, Plant Industry, GPO Box 1600, Canberra, ACT 2601; (1)JST 4-1-8, Honcho, Kawaguchi, Saitama 331-0012, Japan

To investigate the role of DNA methylation in plant development we have transformed Arabidopsis with an antisense construct of METI, an Arabidopsis gene encoding a DNA methyltransferase. DNA methylation was reduced by up to 90% compared to untransformed C24 plants. Reduced methylation was associated with dramatic changes in plant morphology, including homeotic transformation of floral organs that resemble those of plants mutant in genes controlling flower development.

We have focussed on the superman (sup) mutant flowers to investigate the molecular basis for abnormal floral development in METI antisense plants. The sup mutant phenotype of the antisense plants was recessive, suggesting that it was due to loss of function of the SUP gene. Southern analyses demonstrated that there were changes in DNA methylation around the SUP gene. Sequencing of bisulphite treated genomic DNA showed that there was no methylation in the coding region of the SUP gene in untransformed plants but that this region was hypermethylated in the METI antisense plants (1). In a detailed analysis of the SUP locus we have shown that hypermethylation of SUP was associated with repression of transcription of SUP and was observed only in those antisense plants which had sup mutant flowers. The distribution and density of methylcytosine was heterogeneous between independent DNA molecules; some molecules had no methylcytosine while up to 30% of cytosines were methylated in other molecules isolated from the same plant. The frequency and severity of the sup phenotype increased between T3 and T4 generation antisense plants and this was correlated with increased methylation at SUP. 1. Jacobsen, S and Meyerowitz, E (1997) Science 277: 1100-1103

2-9

Multiple DNA methyltransferase genes in Arabidopsis thaliana

R.K. Genger(1,2), **K.A. Kovac**(1,2), W.J. Peacock(1), E.S. Dennis(1) and E.J. Finnegan(1)
(1) CSIRO, Plant Industry, Canberra, ACT 2601 (2) Division of Biochemistry and Molecular Biology, Australian National University, Canberra, ACT 0200

Methylation of plant DNA occurs at cytosines in any sequence context, and as the Arabidopsis methyltransferase, METI, preferentially methylates cytosines in CG dinucleotides [1], it is likely that Arabidopsis has other methyltransferases with different target specificities. We have identified three additional genes, which belong to two distinct families, encoding putative DNA methyltransferases. METII is approximately 80% identical to METI throughout the coding region; these two genes probably arose by a gene duplication event. Both METI and METII are expressed in most tissues, but the level of METII expression is about 10,000 fold lower than that of METI; the level of METII transcripts is not affected by the introduction of a METI antisense transgene, nor does METII substitute for the reduced activity of METI in methylating CG dinucleotides. Southern analyses suggest that there may be as many as three other genes in this family. Two other genes encode a second class of DNA methyltransferase with the conserved motifs characteristic of cytosine methyltransferases, but with little homology to the METI-like methyltransferases through the remainder of the protein. These two methyltransferases are characterized by the presence of a chromodomain inserted within the methyltransferase domain [2], suggesting that they may be associated with heterochromatin. Both genes have low levels of transcripts in vegetative and reproductive tissues.

1. Finnegan, EJ et al., (1996) Proc. Natl. Acad. Sci. USA 95: 5824-5829.
2. Henikoff, S. and Comai, L. (1998) Genetics 149: 307-318.

2-10

ARP1: a regulator of RNA metabolism in Arabidopsis

Nicole D. LeBrasseur, Michael L. Abler *, Debrah M. Thompson (2), and Pamela J. Green
Department of Energy Plant Research Labs, Michigan State University, E Lansing, MI 48824*Current address: Department of Biology, University of Wisconsin, LaCross, WI 54601 (2) Baylor College of Medicine, Houston, TX 77030.

Plant ribonucleases (RNases) are implicated in many physiological processes that occur during growth and development and during the plant's response to limiting nutrient availablity. Despite the abundance of correlative evidence linking the expression of RNases with such processes as senescence, phosphate starvation, and tracheary development, little is known about the genetic determinants that are involved in the regulation and function of these enzymes in higher plants. We searched for regulators of RNases in Arabidopsis by isolating mutants with altered RNase profiles (arp mutants). One of these mutants, arp1, overexpresses a 33 kD doublet of nuclease activity which degrades both RNA and ssDNA. Interestingly, arp1 also overexpresses a band which comigrates with RNS1, a previously characterized member of the RNase T2 family. The modification of RNase activities is seen only in arp1 stem tissue but not in leaves or seedlings of the mutant. Visible phenotypes of the mutant plant include shorter height and increased branching, also indicative of a stem-associated effect of the mutation. Because several RNase activities are increased, the mutation appears to affect a regulatory function possibly associated with stem growth or development. The mutant is not altered in the degradation of specific stable or unstable reporter mRNAs, nor does it appear to alter general turnover of mRNA. Further characterization of the mutant will be discussed.

2-11

Functional characterization of GT-element-binding transcription factors in Arabidopsis
José Le Gourrierec, Mira Ayadi, Virginie Delaporte, You-Fang Li & Dao-Xiu Zhou
Laboratoire de Génétique Moléculaire des Plantes, Association Biopôle Végétal de Picardie, France.Université de Picardie Jules Verne33, rue Saint Leu 80090 AMIENS, France

GT-elements are regulatory DNA sequences found in the promoter region of many different genes.They can have either a positive or a negative function, depending on the promoter and cellular contexts.They are bound by a class of transcription factors, namely,GT-factors.This class of transcription factors contains 1 or 2 trihelical DNA-binding domains.This type of DNA binding domain has been only identified in plants so far.In Arabidopsis, there exists a small family of GT-factors,with only a few identified members.Our laboratory is interested in the functional characterization of 4 GT-factors:GT-1,GT-1b,GT-3a and GT-3b.These GT-factors contain only 1 trihelix DNA-binding domain. GT-1 was previously cloned by its binding affinity to Box II,a promoter sequence found in pea RBCS-3A gene.We have shown that GT-1 contains a transcriptional activation domain in its C-terminal part, and that GT-1 can interact directly with the minimal pre-initiation complex containing TFIIA-TBP-TATA box.These results suggest that GT-1 may activate transcription through this interaction.The in planta function of GT-1 is currently under investigation using transgenic plants expressing sense, antisense and dominant negative constructs. GT-1b presents more than 80% of identity with GT-1 in the DNA-binding domain.Like GT-1, GT-1b appears to be ubiquitously expressed and contains a trans-activation activity in yeast.Results on its target binding sequences and its relationship with GT-1 in the regulation of plant gene expression will be presented. GT-3a and GT-3b are closely related each other.Their DNA-binding domain presents 40% identity to the trihelix region of GT-1.However, their expression appears to be organ-specific, with more mRNA found in stems and in roots.They can form homo-or heterodimers as detected in 2-hybrid system.However, no trans-activation domain could be found in these factors, suggesting that they may have a different regulatory function from what found in GT-1 and GT-1b

2-12

Network of interactions between Arg/Ser-rich splicing factors from Arabidopsis
Sergiy Lopato, Caroline Weber and Andrea Barta
Institute of Biochemistry, University of Vienna, Biocenter, Dr. Bohrgasse 9, A-1030 Vienna, Austria

Both plants and mammals have numerous evolutionary conserved splicing factors containing Arg/Ser-rich domains. This factors are involved in constitutive and alternative splicing and take part in recognition of splice sites. Earlier we have characterised three families of Arg/Ser-rich splicing factors from Arabidopsis. Members of the first family are close homologues of human SF2/ASF and consist of atSRp30 (1) and the previosly characterised SR1/atSRp34 (2). The second family includes three Zn finger containing proteins (atRSZp21, atRSZp22 and atRSZp22a) with a domain structure and RNA binding specificity similar to the human splicing factors 9G8 and SRp20 (3). Two of this proteins were found in two hybrid screen using the U1 70K protein as bait (4). The third family of Arg-rich proteins contains three members atRSP31, atRSp40 and atRSp41 (5); they do not have real homologues in mammalians and could therefore be responsible for difference in splice site recognition between plants and mammalians. We present here one more protein, atRSZp33 which contains two Zn fingers. No homologues in other species have as yet been found in the databases. One of our approaches to localize this splicing factors within the spliceosome is to search for interacting proteins. Therefore, we used atRSp31 and atRSZp33 as baits to screen a yeast two hybrid library. We report now on the identification of interacting proteins and present a network of interactions between Arg/Ser-rich proteins and their domains.
1. Lopato et al., 1999, Genes & Dev., 13: 987: 2. Lazar et al., 1995, Proc. Natl. Acad. Sci. 92: 7672
3. Lopato et al., 1999, Plant Mol. Biol. 39: 761; 4. Golovkin and Reddy, 1998, The Plant Cell, 10: 1634
5. Lopato et al., 1996, The Plant Cell, 8: 2255

2-13

The Arginine-rich Region of Arabidopsis U1-70K Protein Interacts with two Novel Serine/Arginine-rich Proteins: Modulation of Interaction by Protein Phosphorylation

Maxim Golovkin and **A.S.N. Reddy**
Department of Biology and Program in Cell and Molecular Biology, Colorado State University, Fort Collins, CO 80523

The U1 small nuclear ribonucleoprotein (U1snRNP) 70K protein (U1-70K), a U1 snRNP-specific protein, has been shown to have multiple roles in nuclear pre-mRNA processing in animals. We have recently isolated Arabidopsis U1-70K gene (Golovkin and Reddy, Plant Cell, 8, 1421-35, 1996). The plant U1-70K protein shares certain features with the animal U1-70K protein but differs in some. Using the C-terminal arginine-rich region part of Arabidopsis U1-70K protein in the yeast two-hybrid system, we have identified two novel plant serine/arginine-rich (SR) proteins that interact with the plant U1-70K. Both SR proteins are encoded by a single gene and expressed differentially in several tissues. One of the genes produces multiple transcripts by alternative splicing of its pre-mRNA whereas the second one produces a single transcript. The SR proteins share several features with the members of SR family proteins including modular domains typical of splicing factors in SR family of proteins. Using coprecipitation assays we further confirmed their interaction of plant U1-70K. Furthermore, in vivo and in vitro protein-protein interaction experiments have shown one of the proteins interacts with itself. A protein kinase from Arabidopsis phosphorylated all SR proteins that interact with plant U1-70K. Coprecipitation studies have confirmed the interaction of SR proteins with the kinase and their interaction is modulated by phosphorylation status of the proteins. These and our previous results (Golovkin and Reddy, Plant Cell, 10, 1637-47, 1998) suggest that the plant U1-70K interacts with several distinct members of SR family.

2-14

A geminivirus-based episomal vector replicates to high copy number in Arabidopsis

K. Richardson, A. Gleave, B-J. Janssen, R. Atkinson, J. Yao, E. Walton, B. Morris
Hort Research, Private Bag 92169, Auckland, New Zealand.

Tobacco yellow dwarf virus (TYDV), a monopartite, geminivirus that infects dicotyledonous plants has a 2580 nt circular, ssDNA genome containing two viral sense ORFs (V1,V2), two complementary ORFs (C1, C2) and large and small intergenic regions (LIR and SIR). We have developed a TYDV-based vector designed to integrate stably into the plant genome via Agrobacterium-mediated transformation and consisting of the elements (LIR, C1, C2 and SIR) essential for release and subsequent episomal replication. The vector is capable of high copy number episomal replication in tobacco, tomato, and petunia without the development of viral symptoms. Transgenic petunia plants containing a 35S-chalcone synthase A gene cloned into the episomal vector produce white spotted flower tissue as a result of post-transcriptional gene silencing of the endogenous and episomal chsA genes and the frequency and extent of gene silencing correlates with episome copy number. Sense and antisense copies of the ACC oxidase gene have been cloned into the episomal vector. Plants containing the antisense ACC oxidase gene display delayed flower senescence and fruit formation without abscission of petals and stamens - a phenotype previously observed for the ethylene insensitive Never Ripe mutant. We have introduced the TYDV vector into arabidopsis via in planta vacuum infiltration transformation. Here we show that the episomal vector is also capable of high copy number replication in arabidopsis. This demonstration that the TYDV-based vector replicates in arabidopsis offers an opportunity to utilise the vector for analysis of gene function in this host.

2-15

AtMRE11: a component of meiotic recombination and DNA repair in plants
Kevin L. Rozwadowski, Tricia D. Kreiser, Raghuram V. Hasnadaka and Derek J. Lydiate.
Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, S7N 0X2, Canada.

MRE11 is an essential component of the DNA recombination and repair process in eukaryotic cells. Originally identified in a yeast mutation screen for defects in recombination, it has been shown biochemically to participate in resection of double-stranded DNA. The single-stranded ends thus created are acted upon by RecA-like proteins to catalyse strand invasion and initiate recombination between DNA molecules. To investigate DNA recombination and repair in plants at the molecular level we have cloned an homologue of MRE11 from Arabidopsis. AtMRE11 shares extensive homology with proteins from yeast and animals at the N-terminal half but diverges in the C-terminus. The AtMRE11 transcript is most abundant in inflorescence tissues but is also detectable in siliques, leaves and roots. AtMRE11 is induced in response to UV irradiation but shows no response to gamma irradiation. We are conducting a two-hybrid screen to identify proteins interacting with AtMRE11. Identification and characterization of AtMRE11 and interacting components will provide a mechanistic understanding of DNA recombination and repair in plants.

2-16

A molecular genetic approach for studying gene silencing in Arabidopsis thaliana
R. Schmidt, M. Gils, D. Schubert, B. Lechtenberg and A. Forsbach
Max-Delbrueck-Laboratory in the MPG, Carl-von-Linné-Weg 10, D-50829 Cologne, Germany

Introduced genes often show variable expression levels in different transgenic lines. To study this we are taking a molecular genetic approach in Arabidopsis. We would like to analyse whether a tight correlation between the copy number of a transgene and its expression can be found. Constructs suitable for Agrobacterium-mediated plant transformation have been made. Either a single chimaeric glucuronidase (GUS) marker gene under the control of the 35S promoter has been cloned in a binary vector or three copies of this reporter gene in tandem. Only those transgenic plants were analysed which carry a single, intact copy of T-DNA. Quantitative GUS activity assays of homozygous lines revealed that plants carrying the single copy of the marker gene showed uniformly high expression. All transformants harbouring three copies of the reporter gene in tandem displayed much lower expression of the marker gene, in older plants almost complete silencing of the reporter gene was found. Homozygous and hemizygous plants showed clear differences in marker gene expression. Hence, a tight correlation of reporter gene copy number and its expression could be established. We are currently analysing to which extent the chromosomal environment of an integration event contributes to gene silencing. Transgenic plants carrying a single copy of the GUS reporter gene in a Ds element were crossed with plants carrying an Ac transposase source. Plants carrying a transposed Ds elements are currently analysed for their marker gene expression. For those lines which show a significantly different reporter gene expression level compared to that of the Ds-T-DNA line they were derived from, we will determine the site of Ds integration.

2-17

Analysis of an enhancer trap line with auxin upregulated expression

Pauline A Vincent (1), Anthony P Caryl (2), Justin FX Ainscough (3) and Ian J Furner (1)
(1) University of Cambridge, Dept of Genetics (2) University of Birmingham (3) University of Cambridge, Wellcome CRC Inst Canc and Dev Biol

Auxin has many roles in plant growth and development including cell elongation and division, lateral root initiation and apical dominance. Screening of enhancer trap lines has led to the identification of a line with an auxin responsive expression pattern. Expression of the GUS reporter gene is upregulated in the roots in response to growth on NAA, especially in the lateral root buds and root tips. This suggests the presence of an auxin responsive element in the vicinity of the insertion. This line has no visible phenotype. We have mapped the insertion to near the marker m251 on chromosome 2. It is within a predicted non-coding region flanked by sequences with homology to three genes: two casein kinase 2 active subunits and a putative N-acetyltransferase with a high degree of homology to the ethylene responsive gene italicize{HLS1}. We have made double homozygotes with known auxin mutants and the staining patterns in these lines will be presented.

2-18

Evolutionarily Conserved and Nucleus-encoded Multiple s Factors Promote Differential Transcription of Chloroplast Genes

Kohki Yoshimoto, Masanori Shimizu and Hirokazu Kobayashi
Lab. Plant Cell Tech., Grad. Sch. Nutr. Envir. Sci. , Univ. Shizuoka, 52-1 Yada, Shizuoka, 422-8526

The σ factors play essential roles in the transcriptional control of cell cycle and differentiation in prokaryotes. Although multiple homologues of bacterial σ factors, all belonging to the σ70 group, have recently been found in red algae and higher plants, their functions and necessity are entirely unknown. Three putative σ factors (SIGs) destined for the plastid of Arabidopsis thaliana were ectopically expressed in tobacco BY-2 culture cells and in leaves of A. thaliana adapted to the dark or grown in supplementation with the photosynthetic end product sucrose. Here we show that the products of the three SIG genes conferred different promoter specificities on the chloroplast RNA polymerase core enzyme. For the expression of psbA or psbD, encoding photosystem II components, SIG2 > SIG3 >> SIG1. For expression of rbcL, encoding the Rubisco large subunit, SIG1 • SIG2 > SIG3. In addition, removal of a phosphorylation site in SIG3 further relieved the dark-suppression and sugar repression. The expression of chloroplast photosynthetic genes has evolved to be under finer nuclear regulation through multiple σ70-type factors, in contrast to bacterial σ70-type factors with a single specificity to gene promoters on the same genome.

3-1

Whole Genome Shotgun Sequencing of Arabidopsis - analysis and insight.

Steve Rounsley, Sai Subramaniam, Yongwei Cao, David Bush, Craig DeLoughery, Casey Field, Cheryl Phillips, Oleg Iartchouk, Rob Last, Roger Wiegand, David Fischhoff and Bill Timberlake.
Cereon Genomics, 45 Sidney Street, Cambridge, MA 02139

Due to its physical and genome features, Arabidopsis thaliana has become a model organism for plant biology. As a result, it is the focus of many genomic studies in the public and commerical sectors. In 1996, the publicly funded Arabidopsis Genome Initiative (AGI) began an international and coordinated plan to sequence the genome of the Columbia ecotype and it is expected to be largely complete by the end of 2000. The goal of the public project is to produce a highly accurate and complete genome compiled by shotgun sequencing overlapping large insert clones (BACs, PACs etc) selected from a tiling path across the genome. As a complementary approach, Cereon Genomics has engaged in a whole genome shotgun approach to obtain sequence from most of the genome of the Landsberg erecta ecotype. While not producing the complete and accurate sequence that the AGI project plans, this approach has the advantage of being a cost-effective way of sampling an entire genome quickly. Genes along all five chromosomes are discovered at an equal rate. Many sequence differences are apparent between the two ecotypes, enabling the first genome-wide comparison of two varieties of a higher organism as well as providing a wealth of molecular markers. An overview of the data obtained from this project will be discussed, as well as insights obtained from its analysis.

3-2

Structural analysis of the Arabidopsis thaliana genome toward functional genomics

Satoshi Tabata, Yasukazu Nakamura, Erika Asamizu, Shusei Sato, Takakazu Kaneko, Hirokazu Kotani, Tomohiko Kato, Nobuyuki Miyajima
Kazusa DNA Research Institute, 1532-3 Yana, Kisarazu, Chiba 292-0812, Japan

In order to obtain information on the genome structure to perform comprehensive analysis of gene function and regulation, we initiated a large scale structural analysis of Arabidopsis thaliana genome including both genome sequencing and cDNA sequencing. We are focusing our target of genome sequencing on chromosome 5 and 3 along the line of the international agreement of the Arabidopsis Genome Initiative. As of May 1999, we have released 20.1 Mb of finished sequences through the public databases and our web database KAOS (Kazusa Arabidopsis data Opening Site) [http://www.kazusa.or.jp/arabi/]. In addition, we have developed a new database named Arabidopsis Genome Displayer (AGD) [http://www.kazusa.or.jp/arabi/displayer/] which provides the annotated sequences of the whole genome. As of May 1999, AGD contains 67.8 Mb sequences and 59.8 Mb are ready to browse. To complement the currently available cDNA information, we have been conducting large-scale EST collection project using normalized libraries and size-selected (>3 Kb) libraries from various sources. A total of 13000 5' and 20000 3' ESTs from normalized libraries of rosette leaves, roots, flower buds and siliques, and the size-selected library of rosette leaves have been obtained. The 3' ESTs were grouped into 8500 independent species, of which 24% did not have matched sequences in the registered Arabidopsis ESTs.

3-3

The Arabidopsis Genome Annotation Database: A Resource for Plant Biologists
Buell, C. R., Lin, X., Benito, M. I., Kaul, S., Carrera, A., Creasy, T., Ronning, C., Fu, L., Khalak, H., Town, C. D., Nierman, W. C., White, O., Fraser, C. M., and Venter, J. C.

The Institute for Genomic Research (TIGR) is one of five groups involved in the Arabidopsis thaliana Genome Initiative (AGI). Our current objectives include the sequencing of chromosome II as well as the development of a comprehensive database for Arabidopsis genomic sequence data. We have created the Arabidopsis Genome Annotation Database (AGAD; http://www.tigr.org/tdb/at/at.html) for this purpose. The database has been populated with all Arabidopsis genomic sequences available from Genbank and we will be re-annotating all AGI-derived sequences to provide a uniform, centralized repository for Arabidopsis genomic sequence data. Annotated sequences in AGAD can be viewed based on their map position and can be searched either by BAC clone name or by gene name. AGAD contains sequences from all five chromosomes and we will be incorporating additional sequence from the international AGI sequencing projects as it is released. We will also be updating the annotation in a semi-automated manner such that the annotation does not become stale, thereby maximizing the impact of having the complete genomic sequence of this model species.

3-4

The nature of the EST: Distribution across chromosome IV
L. Parnell (1), C. Schueller (2) P. Zaccaria (2) and K. Mayer (2)
(1)Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA
(2)Munich Information Center for Protein Sequences, Munich, Germany

The sequencing of the Arabidopsis thaliana genome provides for a thorough analysis of the quality and breadth of the publicly available EST dataset. We have undertaken an extensive analysis of EST matches over the entire length of chromosome IV. The publicly available EST dataset is highly redundant and biased towards certain genes: Approximately 50% of the ESTs match to about 10% of the genes. Only about one third of all annotated genes contain an exact EST match. The frequency with which genes containing EST matches are found varies greatly as one proceeds along the chromosome. EST matches are rare in areas of reduced recombination. Relationships between the locations of EST-containing genes and both chromosomal location and biochemical function of the encoded proteins will be presented. Full length cDNAs and/or ESTs from diverse tissue sources and developmental time points will be of immense use in verifying gene models and confirming that genes are indeed expressed.

3-5

A complete BAC-based physical map of the Arabidopsis thaliana genome

Teresa Mozo1, Ken Dewar2, Pat Dunn3, Joe Ecker3, Sabine Fischer1, Sebastian Kloska1, Hans Lehrach4, Marco Marra5, Robert Martienssen6, Sebastian Meier-Ewert7, and **Thomas Altmann**1
1Max-Planck-Institut für molekulare Pflanzenphysiologie, Karl-Liebknecht-Strasse 25, 14476 Golm, Germany. 2Whitehead Institute/MIT Center for Genome Research, Cambridge, MA 02141, USA. 3Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018, USA. 4Max-Planck-Institut für molekulare Genetik, Ihnestrasse 73, 14195 Berlin, Germany. 5Washington University School of Medicine, Genome Sequencing Center, St. Louis, Missouri 63108, USA. 6Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA. 7GPC Aktiengesellschaft, Lochhamer Strasse 29, 82152 Martinsried, Germany.

In order to supply the scientific community with a high quality resource to be applied to map-based gene cloning and genomic sequencing purposes, we present here a complete physical map of Arabidopsis using essentially the IGF BAC library. The map (http://www.mpimp-golm.mpg.de/101/mpi_mp_map/access.html) consists of 27 contigs which cover the entire genome except for the presumptive centromeric regions, nucleolar organisation regions (NOR) and telomeric areas. This is the first reported map of a complex organism entirely based on BAC clones and it represents the most homogeneous and complete physical map established to date for any plant genome. Furthermore, the analysis performed here serves as a model for an efficient physical mapping procedure using BAC clones that can be applied to other complex genomes.

3-6

Large scale Arabidopsis cDNA sequencing at Kazusa DNA Research Institute

Erika Asamizu, Shusei Sato, Yasukazu Nakamura and Satoshi Tabata.
Kazusa DNA Research Institute, Kisarazu, Chiba 292-0812, JAPAN.

Information on cDNA structures is essential for precise assignment of protein coding regions on the genomic sequence accumulated in the genome sequencing project. In Arabidopsis thaliana, more than 37000 ESTs have been deposited in the public databanks. However, these information are not sufficient for the purpose of identifying protein coding regions because of the high redundancy of the cDNA species in the libraries and the biased sequence direction. To complement the currently available cDNA information and to facilitate the gene modeling process, we have been conducting large scale cDNA sequencing using two types of cDNA libraries; normalized libraries and size-selected (>3 Kb) libraries. As of May 1999, we have obtained 13000 5’ and 20000 3’ end sequence data from normalized libraries of rosette leaves, roots, flower buds and siliques, and the size-selected library of rosette leaves. These 3’ end sequences were grouped into 8500 independent species, of which 50% showed similarity to sequences in the protein database, 26% matched only to Arabidopsis ESTs, and the remaining 24% were novel genes. Search against Arabidopsis genomic sequence showed that among the clones matched to annotated regions, 20% matched to unassigned regions. The project goal is to accumulate end sequence data of 40000 cDNA clones. To overcome the saturation of the libraries and to continuously obtain novel genes, a subtraction library is under construction.

3-7

The Arabidopsis Biological Resource Center: New stocks and stock usage
Randy Scholl, Keith Davis, Doreen Ware, **Deborah Crist**, Emma Knee, Luz Rivero, Nasser Assem and Sakti Pramanik.
Depts. of Molecular Genetics and Plant Biology, Ohio State Univ. and Computer Science Dept., Michigan State Univ.

The Arabidopsis Biological Resource Center (ABRC) cooperates with the Nottingham Arabidopsis Stock Centre (NASC) to collect, preserve and distribute seed and DNA stocks of Arabidopsis. ABRC stock information is maintained in the AIMS database and the ABRC Web Catalog (both found at http://aims.cse.msu.edu/aims/). The Seed and DNA Catalog is published electronically and can be accessed in three ways, including: 1) a series of Web pages; 2) formatted PDF files which are downloaded/viewed using Adobe Acrobat; and 3) PostScript files which can be downloaded and printed with various PostScript-handling software.

The status of recent acquisitions to the collection will be highlighted. Included are: A) NEW MUTANT SEED STOCKS: More than 100 new mutant stocks. B) NEW ECOTYPES: Strains from Russia, Tadjikistan and other locations. C) T-DNA LINES: More than 30,000 lines are now represented in the available T-DNA collection and many. D) NEW RECOMBINANT INBRED POPULATION AND A TETRAD POPULATION: A very polymorphic recombinant inbred population from M. Koornneef is now available as is the tetrad mapping population from D. Preuss and G. Copenhaver. E) POOLED DNA FROM T-DNA POPULATIONS: DNA from 6,000 line from K. Feldmann and 6,000 lines from T. Jack for PCR screens. F) TAC LIBRARY: The transformable BACs from Mitsui. G) LARGE INSERT LIBRARIES: Includes those used for genome sequencing. H) FILTER SETS OF NON-REDUNDANT ESTs: Filter sets for hybridization by users to probes of interest are available for ESTs and BAC libraries.

We appreciate the support of the Arabidopsis community. ABRC is supported by the National Science Foundation.

3-8

Progress in Sequencing Chromosome 1 of Arabidopsis thaliana by the SPP Consortium.
Nancy A. Federspiel (1), Joseph R. Ecker (2), Athanasios Theologis (3), and Ronald W. Davis (1).
(1) Stanford DNA Sequencing & Technology Center(2) University of Pennsylvania(3) Plant Gene Expression Center

The SPP Consortium was formed in 1996 as a collaborative effort between the Stanford DNA Sequencing & Technology Center, the University of Pennsylvania, and the Plant Gene Expression Center, to sequence chromosome 1 (~30 Mb) of Arabidopsis thaliana. The SPPC has made great progress toward this goal: at the time of submission of this abstract, 8.5 Mb has been completed, with an additional 3 Mb in either shotgun or finishing phase for a total of 11.3 Mb. Seventy-five seed points have been established along the length of chromosome 1. As the sequence of each seed BAC is completed, it is compared to the database of BAC end sequences to find overlapping BACs for contig extension. After checking the BAC fingerprints to verify their integrity, minimally overlapping BAC clones are chosen for the next round of sequencing. Currently the largest contig is 1.1 Mb in size, and several others are ~900 kb. The immediate data release policy of the SPPC directs that all contigs > 2 kb, prescreened for vector and E. coli contamination, be released in the HTGS division of GenBank as Phase 1 for unordered contigs and Phase 2 for ordered contigs. This allows researchers in all fields, not just Arabidopsis investigators, to access the sequence data in one location at the earliest possible time. The final edited sequence is then released as Phase 3 data. Annotation is added to the GenBank entries as soon as possible after release. The most recent sequencing progress by the SPPC on chromosome 1 will be discussed.

3-9

MIPS Arabidopsis thaliana Database (MATDB) - a comprehensive database for chromosome IV sequence and analysis data

Klaus F.X. Mayer*, Christine M.E. Schueller*, Lawrence D. Parnell,Paolo Zaccaria*, Kai Lemcke*, Gertrud Mannhaupt* and H.W. Mewes**
MIPS, at Max-Planck-Institute for Biochemistry, Martinsried, Germany L.D.P. is at Cold Spring Harbor Laboratory, USA

Arabidopsis thaliana chromosome 4 sequence has been completed by a joint effort of the ESSA consortium, CSHL and Washington university (WashU). MIPS did a detailed analysis of chromosome4 distal arm sequence. For analysis and annotation intrinsic data (various geneprediction alghorithms) and extrinsic data (protein and EST similarities) are combined. Analysis data are graphically depicted using a JAVA applet, enabling the user to retrieve information in a dynamic way.

ESSA sequences and analysis data as well as data stemming from our collaborators at CSH and WashU were integrated into the chromosome IV database. Sequence analysis and annotation data are displayed in a comprehensive and multidimensional way (http://www.mips.bichem.mpg.de/proj/thal). Several opportunities to explore the information are provided. From a chromosome overview, including BAC and YAC tilling paths as well as markers it is possible to navigate via positional clues to regions of interest. Data and related information, such as EST hits, literature links, DNA and AA sequence, functional classification etc. on each gene/protein entry are being given. Additional options to query the database include searches for keywords, gene and clone names and summaries of genes involved in specific pathways and functions. In addition the deduced protein sequences are analysed using the PEDANT system. PEDANT is a fully automated system using a whole spectrum of sequence analysis and structure prediction techniques such as attribution of proteins to protein superfamilies, secondary structure, transmembrane regions, coiled-coil regions etc.

3-10

Genomic sequence analysis and display systems at Kazusa, its status in 1999

Yasukazu Nakamura and Satoshi Tabata.
Kazusa DNA Research Institute, Yana 1532-3, Kisarazu, Chiba 299-0812 Japan.

The multi-national Arabidopsis genome sequencing project coordinated by the AGI has already deposited more than 65 Mb of genomic sequence to the international DNA database. To provide a standard view of Arabidopsis genome sequence information, we have constructed a web-site: Arabidopsis Genome Displayer. This site enables users to browse the annotated data with following re-computational results. Nucleotide sequence produced by the AGI are collected from GenBank DNA database and subjected to similarity searches by BLASTX and BLASTN against non-redundant protein database and in-house EST or RNA gene datasets extracted from GenBank, respectively. Local alignments which showed E-value of 0.001 or less to known protein sequences are extracted and stored. Potential exons are predicted by the computer programs Grail and GENSCAN. For localization of exon-intron boundaries, donor/acceptor sites for splicing are predicted by NetGene2 and SplicePredictor. All the outputs are parsed and stored in the same format specified as GFF (Gene-Finding Format). Gene structures proposed on the annotated sequences as well as those predicted by computer programs are presented by graphics made on-demand, and each graphic item has a hyperlink to detailed information of the corresponding area. Therefore, annotation bases of deduced genes can be examined by any researcher in a user-friendly manner. As of May 1999, we have finished re-computation and opened ca. 60 Mb for 736 clones (90 % of the AGI total). URL of Arabidopsis Genome Displayer: http://www.kazusa.or.jp/arabi/displayer/ Also, the display system has made fast composition and/or refinement of predicted gene structures possible. Our new annotation process and the latest status of the system will be presented.

3-11

Genome-wide Analysis of Arabidopsis Protein Sequences
C.J. Palm, N.A. Federspiel and R.W. Davis.
Stanford DNA Sequencing and Technology Center855 Califorina Ave, Palo Alto, CA

A database of genomic Arabidopsis thaliana protein sequences was created to enable genome-wide analysis of A. thaliana proteins and coding sequences. The database was constructed using all completed A. thaliana genomic sequence in GenBank. Protein data was extracted from annotated GenBank records and an automated annotation process for unannotated GenBank records. In addition, functional motifs are documented in the database, by searching all protein sequences with eMofif/Identify(Nevill-Manning, C.G. Wu, T.D. & Brutlag, D.L.(1998). Proc. Natl. Acad. Sci USA, In Press). The database is periodically updated to include new GenBank submissions. A web interface (http://baggage.stanford.edu/group/arabprotein/)to the database provides easy access to summary annotation information for the entire sequenced A. thaliana genome. Through the web interface, the data can be searched by name, comment or motif field. In addition, a browse option lists either all the protein names or motifs present in the sequenced A. thaliana genome. Analysis of the current sequenced A. thaliana genome shows a gene density of one gene per 4.5 kb and an average gene size of 2.1 kb. Approximately 25% of the A. thaliana genes have matching ESTs. 20% of the Arabidopsis proteins contain identifiable motifs. The function of 48 % of the proteins is not known. The hypothetical proteins differ significantly from the other A. thaliana proteins. The hypothetical genes are about 200 bp shorter than the other genes, while the hypothetical introns are 30 bp larger. On average, the hypothetical proteins contain 4.1 introns/gene, compared to 5.3 introns/gene for the rest of the proteins. This database provides an excellent resource for web-based searches for protein function and for genome-wide analysis of A. thaliana proteins.

3-12

Arabidopsis chromosome IV
European Scientists Sequencing Arabidopsis, Cold Spring Harbor Laboratory Genome Sequencing Center, Washington University Genome Sequencing Center, Applied Biosystems

The Arabidopsis Genome Initiative is an international collaboration to determine the complete sequence of the Arabidopsis genome. Two consortia, one European, the other American, have been collaborating to sequence and analyze Arabidopsis chromosome IV. This task is now essentially complete. Using a map-based approach, the chromsome arms are completely sequenced and over 2 Mb of the predicted 4 Mb pericentromeric heterochromatin has been sequenced. The short arm contig is 3.5 Mb and the long arm contig is 16 Mb. The chromosome contains approximately 3,800 genes. We will describe ongoing efforts to analyze the centromeric region at the sequence level. The sequence has shown a fascinating relationship between chromosome structures and biological activity.

Using a number of analysis methods, we have estimated the gene content of chromosome IV. The gene density overall is about as was expected. Surprisingly, the percentage of matches to Arabidopsis ESTs is low. Using the PEDANT system developed by the staff at the Munich Institute of Protein Science, we have cataloged the genes into functional categories. In addition to basic housekeeping genes, several surprises have been found. As an example, Arabidopsis genes that are similar to those that encode mammalian NMDA neurotransmitter receptors have been found, as well as genes related to insect virus inhibitors of apoptosis.

The systematic sequencing of the entire chromosome has now revealed the structure of chromosome IV as a biological entity. We can now begin to determine how that structure relates to the functioning of this chromosome in the plant cell life cycle.

3-13

Progress in sequencing the Arabidopsis bottom arm of chromosome 3

M. Salanoubat, F. Quétier (coordinators, Genoscope, Evry, France) N. Choisne (Genoscope), W. Ansorge (EMBL), H. Blocker (GBF), M. Boutry (Université de Louvain), M. Delseny (LPBMP), V. De Simone (CEINGE), B. Fartmann (MWG-Biotech GmbH), L. Grivell (Universiteit van Amsterdam), R. Mache (Université Joseph Fourier), H-W. Mewes (MIPS), B. Obermaier (MediGenomix GmbH), M. Perez-Alonso (Universitat de Valencia), P. Puigdomenech (Consejo Superior de Investigaciones Cientificas), M. Rieger (Genotype), G. Valle (Universita degli Studi di Padova), H. Voss (Lion Bioscience AG).

Genoscope is one of the groups participating in the Arabidopsis Genome Initiative (A.G.I.). Genoscope is coordinating an EU project aiming to sequence the 9 Mb of the chromosome 3 bottom arm by the year 2000. The strategy used for sequencing this region combines two different approaches: laboratories with limited sequencing capacity are sequencing the BACs chosen according to a minimal tiling path. This minimal tiling path has been constructed over a region of approximately 3.5 Mb, using three resources : 1) the BAC contigs constructed with fingerprinting by Washington University, 2) the BAC end sequences database released by TIGR, Genoscope and University of Pensylvania and 3) reiterative hybridizations. The groups with a larger sequencing capacity will determine the next BACs to be sequenced using the STC approach. Since October 1998 we have finished about 2.5 Mb and an additional 2.5 Mb are currently being sequenced. Sequencing progress is available through our Web site (http://www.genoscope.cns.fr).The results of the annotation of the BAC sequences are presented on the MIPS web site at http://www.mips.biochem.mpg.de/proj/thal. We will report the mapping strategies that we have used, the sequencing progress and our plans for the completion of the sequence of the chromosome 3 bottom arm by the year 2000 at the latest.

3-14

Analysis of microsynteny in Arabidopsis thaliana, Brassica oleracea and Capsella rubella

R. Schmidt (1), A. Acarkan (1), K. Boivin (1), M. Koch (2) and M. Rossberg (1)
(1) Max-Delbrueck-Laboratory in the MPG, Carl-von-Linne-Weg 10, D-50829 Cologne, Germany (2) present address: Institute of Botany, University for Agricultural Science Vienna, Austria.

We are aiming to elucidate the degree of microsynteny in three related cruciferous plant species, Arabidopsis thaliana, Capsella rubella and Brassica oleracea. To analyse conservation of gene repertoire, order and spacing in these species we have chosen a region located on the long arm of chromosome 4 of Arabidopsis thaliana. At least 17 different genes were found in the 200 kbp region. Cosmid clones carrying Brassica oleracea genes corresponding to five of the Arabidopsis thaliana genes were isolated. Genetic mapping experiments showed that all cosmids are mapping to Brassica oleracea chromosome 1. These cosmid clones together with clones identified in chromosome walking experiments were used to establish a cosmid contig for the region in Brassica oleracea. The five genes are arranged in the same order in Brassica oleracea and in Arabidopsis thaliana. A characterisation of the intergenic regions revealed that the genes are generally further apart in Brassica oleracea than in Arabidopsis thaliana. All 17 different Arabidopsis thaliana genes were used to identify corresponding cosmid clones in Capsella rubella. So far two cosmid contigs could be assembled for Capsella rubella. The gene order in both species is similar if not identical. Furthermore, the intergenic regions in Capsella rubella and Arabidopsis thaliana are similar in size. The comparative analysis has recently been extended to another genomic region located on Arabidopsis thaliana chromosome 4, also in this case a high degree of microsynteny could be established for the three species.

3-15

Arabidopsis thaliana Chromosome II: Sequencing and Analysis

C.D. Town, J.C. Venter, S. Rounsley, S. Kaul, X. Lin, T.P. Shea, C.Y. Fujii, T.Mason, C.L. Bowman, M. Barnstead, M. Adams, T. Feldblyum, H. Koo, K. Moffat., L.Cronin, M. Shen, G. Pai, S. Van Aken, L. Umayam, L. Tallon, J. Gill, K.A. Ketchum, C.M.Ronning, M-I. Benito, C.R. Buell, A.J. Carrera, T.H. Creasy, H.M. Goodman, W.C. Nierman, O. White, J.A. Eisen, S. Salzberg and C.M. Fraser.
The Institute for Genomic Research, Rockville, MD 20850, USA

At TIGR, the sequencing of Arabidopsis Chromosome II is almost complete. 228 BACs have been sequenced for a total of 21.8 Mb of finished sequence (19.6 Mb unique). The sequence can be assembled as two continuous regions that represent the two arms of the chromosome. The short (upper) arm extends from rDNA (presumably part of the Nucleolar Organizer Region, NOR) to a block of 180 bp repeats believed to be centromeric, while the lower arm extends from another block of 180 bp repeats almost to the telomere. At 19-20 Mb (excluding the NOR) the chromosome is about 40% longer than the YAC-based estimate of 13-14 Mb. Our analysis reveals 4,131 genes of which 51% can be identified by database similarity. A further 19%, represented by ESTs that have no database match, encode unknown proteins. The remaining 30%, identified by gene prediction programs, represent "hypothetical" genes for which there is neither experimental evidence nor database similarity. Many of the genes belong to gene families, some of the most abundant being protein kinases, DNA binding proteins/transcription factors and cytochrome P450s. The sequence of the entire chromosome allows an evaluation of genetic to physical map distances along the chromosome and the correction of the locations of certain incorrectly assigned genetic markers. We have also identified many sequences related to transposable elements, especially retroelements, that occur more frequently close to the centromere. An unexpected discovery was the insertion of a large fragment of the mitochondrial genome into the chromosome near the centromere. Many regions of both inter- and intra-chromosomal duplication are observed, the largest being a region of ca. 500 kb that is duplicated between Chromosomes I and II. Supported by NSF, DOE and USDA.

3-16

Production and Preliminary Analysis of a Minimized Library of MSU ESTs Arrayed in 96-well Microtiter Plates

Emma M. Knee, **Doreen H. Ware**, Randy Scholl, Thomas Newman, Keith R.Davis.
Department of Plant Biotechnology, The Ohio State University

The Arabidopsis Biological Resource Center (ABRC) at The Ohio State University produced and distributed a collection of 11,600 cDNAs from the Michigan State University Expressed Sequence Tag (MSU EST) project. The ESTs were selected from a group of 13,500 potentially unique clones identified from the MSU and French CNRS EST projects by Dr. Steve Rounsely at The Institute for Genome Research (TIGR). ESTs were grouped using a TIGR assembly program. The program built a table of all possible 10-mers in the EST data set, then used a modified Smith-Waterman algorithm to group sequences based on their 10-mer content. Ungrouped ESTs, or the longest EST in a group, were identified as non-redundant, potentially unique clones. The ABRC arrayed 11,600 of the MSU ESTs identified by TIGR in 96-well microtiter plate format. Dr. Rod Wing at the Clemson University Genome Center arrayed the clones in 384-well microtiter plates and generated high-density filters. The arrayed collection of ESTs is a valuable new resource. Bacterial stocks in microtiter plates are easily replicated, distributed and stored compactly. They may be utilized as a template for DNA micro-arrays or colony blot filters. The filters can be used in conventional library screens with cDNA or genomic clones to identify Arabidopsis genes homologous to genes from other organisms. BACs, YACs or cosmids may be used to identify ESTs for synteny mapping. The filters may also be used to conduct 'full screen' analyses of physiological processes. We will present a preliminary analysis of the collection and a sample filter. In the future we plan to array clones from the French CNRS and new potentially unique clones generated by the MSU EST projects.

4-1

T-DNA insertional mutagenesis for reverse genetics in Arabidopsis

David Bouchez (1), Fabienne Granier (1), Nicolas Bouché (1), Michel Caboche (1), Nicole Bechtold (2), Georges Pelletier (2).
(1) Laboratoire de Biologie cellulaire(2) Station de Génétique et Amélioration des PlantesINRA Centre de Versailles78026 Versailles

We have established a population of T-DNA insertion lines in ecotype Wassilevskija (WS), using infiltration of adult plants with Agrobacterium. Sofar, 46,000 independent lines have been produced, all of them propagated individually as families. On average, each T-DNA family contains 1.5 insertion locus, which gives a total of 70,000 independent T-DNA inserts (one insertion every 1.7 kb on average). The in planta T-DNA transformation process itself has been investigated, and there is good genetic evidence that T-DNA is transferred to the oocyte in the female gametophyte, after meiosis and presumably during fertilization. In order to allow reverse genetics screening of this population for insertions in genes of interest, we have prepared DNA pools from 35,000 lines (55,000 inserts), in pools of 48 lines. PCR screens for specific insertions have been performed for a variety of genes. We are investigating methods to increase the efficiency of screening by the use of high-density hybridization filters, or systematic sequencing of insertions sites. Most mutants isolated do not show clear phenotypes in standard growing conditions. This can be taken as an indication for widespread functional redundancy in Arabidopsis, and/or for prevalence of genes required only in specific physiological situations. Combination of knock-out mutations and phenotype analysis in a battery of environmental conditions may solve some of these problems. However the characterization of knock-out mutants remains a labour intensive, low throughput process, and we have to design new and efficient tools for molecular and physiological analysis of such mutants.

4-2

Characterization of the powdery mildew disease resistance locus, RPW10, using DNA microarrays

Wilson I.W. and S. C. Somerville
Department of Plant Biology, Carnegie Institution of Washington, Stanford, California USA 94305.

The genome era has provided large amounts of gene sequence information, however, the ability to adequately characterize these genes beyond mere sequence similarity is challenging. One technology that is able to aid in gene assignment is DNA microarrays. DNA microarrays consist of hundreds to thousands of amplified DNAs robotically arrayed on specially treated glass slides. In a reverse northern procedure, an mRNA-derived fluorescent probe is hybridized to the microarray and then scanned with a fluorescence laser scanning device.

This technology can quantitate the expression level of large numbers of genes simultaneously, producing expression profiles that can aid in the assignment of gene function. We are interested in utilizing DNA microarrays to understand the Arabidopsis responses to the biotrophic pathogen, powdery mildew (Erysiphe cichoracearum UCSC1). Our DNA microarray (~ 2,300 genes) is specifically enriched for genes thought to be involved in plant pathogen interactions, but also contains genes involved in other processes, as well as many genes of unknown function. We collected mRNA from powdery mildew-infected near-isogenic lines, which carry either resistance or susceptibility alleles at the RPW10 locus. Samples were harvested at eight time points, reflecting different stages in the infection cycle. Results from the microarray experiments and global insights into the infection and disease resistance processes derived from the microarray analysis will be presented.

4-3

Analysis of flanking sequences from 1000 independent Ds-transposon insertion lines in Arabidopsis thaliana

Serguei Parinov, Mayalagu Sevugan, De Ye, Wei-Cai Yang and Venkatesan Sundaresan.
Institute of Molecular Agrobiology, The National University of Singapore, 1 Research Link, Singapore 117604.

Current progress in Arabidopsis thaliana sequencing project opens new perspectives for plant molecular biologists. With more than half of the genome sequence completed and almost 40000 ESTs available from Arabidopsis GenBank, the number of genes with clearly described biological function is less than a thousand.

We have been utilizing maize transposable Ds elements to generate a library of transposon insertions spread throughout the Arabidopsis genome. Transposons carry a reporter to identify gene expression patterns. Most of the transposans carry a single Ds element, which is stable, but can also be remobilized, by crossing back to Ac. We have amplified DNA flanking Ds elements using TAIL-PCR to characterize the disrupted sequence. For 78% of the 1000 independent lines useful Ds-flanking sequence information has been obtained. They include 354 disrupted protein genes. 46% of the lines has insertions in annotated BAC clones with known map positions that allow as evaluating the distribution of Ds-insertions throughout the genome. We discovered the interesting fact of substantial preference of Ds-transposition to the regions adjacent to NOR2 and NOR4 comprising about 10% of all insertions. The rest of mapped insertions are dispersed randomly in genome without considerable preference. Data also points to possible slight preference to 5'-ends of genes.

Sequence information from at least 500 lines will soon be made available on the Internet. We hope that our database is potentially interesting for researchers looking for ready knock-outs of particular genes, as well as it already presents a good database for targeted mutagenesis.

4-4

Global analysis of environmental stress and developmentally regulated genes in Arabidopsis.

N. V. Fedoroff (1&2), F. Chen (2), N. A. Eckardt (2), A.-M. Gomez-Buitrago (1), and R. Raina (1).
(1)Life Sciences Consortium and (2) Biology Department, Penn State University, University Park, PA. USA 16802.

Identifying genes that work together to produce a developmental change or a response to an environmental stimulus is essential to understanding, characterizing, & manipulating the change or response. Our aim is to add new sequences corresponding to environmentally & developmentally regulated genes to the EST database, & to perform large-scale microarray gene expression analyses to identify stress- and development-specific gene expression patterns in Arabidopsis. We are using PCR-based suppression subtractive hybridization (SSH), designed to enrich a cDNA library for rare differentially expressed transcripts, to isolate genes upregulated in response to developmental & environmental stimuli. Subtractive hybridization experiments were carried out with mRNA isolated from the following tissues: 1)ovaries collected within 24 hrs of pollination from wildtype plants versus a male-sterile mutant; 2)leaf tissue from plants exposed to ozone stress versus clean air controls, and 3)leaf tissue from plants treated with salicylic acid versus water-treated controls. Sequences were obtained for over 1000 putatively upregulated genes, about 40% of which are not currently represented in the Arabidopsis EST database. Sequences corresponding to known functions fall across most of the major classes of gene functions. Among the new ESTs are rare transcripts likely to be involved in signal transduction during early embryogenesis & plant defense responses. Our results demonstrate that SSH is an efficient method for identifying genes missing from the Arabidopsis EST database. Novel clones will form part of a larger collection of ESTs to be used in cDNA microarray gene expression analysis expts.

4-5

Analysis of grape EST's: investigation of gene function in Arabidopsis.
Effie M. Ablett, George G. Seaton, L. Slade Lee, Timothy A. Holton and Robert J. Henry
Centre for Plant Conservation Genetics, Southern Cross University, P.O.Box157, Lismore, NSW 2480, Australia.

The Australian Agriculture Research Institute is embarking on a large-scale grape EST analysis project. cDNA libraries are being prepared from a range of tissues, developmental stages and cultivars with the aim of producing a data base of up to 50,000 sequences, covering a high proportion of grape genes. 2,466 sequences have been obtained from a leaf cDNA library, and 2,438 from a berry library. Analysis of the leaf and berry EST's indicate 59% of clones have significant homology with known plant genes, 16% with more distant organisms and 25% may represent novel genes (no BLAST 2 match or match to EST's of unknown function). Redundancy levels in the leaf library are about 42%, and 39% in the berry library. Overall both libraries may yield more than 3,000 unique sequences. We are using expression in Arabidopsis to investigate the function of 58 EST's that match to 26 different transcription factors with unknown function in plants, and 330 EST's with no homology to known genes or other plant EST's. The inserts from the cDNA clones of interest are being cloned into pKMB and pSMB (1). Arabidopsis plants will be transformed with each construct to either over-express or knock out the cloned grape gene of interest. Transformed seeds will be germinated and the plants studied to determine phenotypic changes produced by the altered expression of the grape gene. The functional analysis in Arabidopsis of novel grape genes identified as EST's will provide a powerful new resource for discovering genes with the potential to improve table and wine grapes. (1) Mylne, J., and Botella, J.R. (1998) Binary Vectors for sense and antisense expression of Arabidopsis EST's. Plant Mol. Biol. Reporter 16, 257-262.

4-6

Arabidopsis arabinogalactan-proteins: Their function and their membrane anchors.
Yolanda Gaspar, Paul Gilson and Anthony Bacic.
Plant Cell Biology Research Centre,School of Botany,University of Melbourne, Australia.

Arabinogalactan-proteins (AGPs) are a family of high molecular weight proteoglycans secreted by plant cells into the plasma membrane and into the cell wall and extracellular matrix. Although, the exact function(s) that AGPs perform are unknown, they are implicated in diverse developmental roles such as tissue differentiation, cell growth and somatic embryogenesis. We are attempting to address AGP function by using Arabidopsis thaliana as a model organism. Screening of T-DNA tagged lines has identified an AGP insertional mutant. A T-DNA tag upstream of the AtAGP6 gene perturbs Agrobacterium tumefaciens binding to Arabidopsis roots. We are currently examining the role AtAGP6 may have in the development and construction of root cell walls. Virtually all Arabidopsis putative AGP genes, identified to date (+20 genes), have a C-terminal signal sequence that is thought to direct the attachment of a glycosylphosphatidylinositol (GPI) membrane anchor. In other eukaryotes these anchors provide an alternative to transmembrane domains for attaching proteins to the cell surface, implying that these AGPs may reside on the surface of plant cells. Database searches have identified many putative GPI-anchored proteins of which AGPs form a major class. We are currently identifying components of the GPI-anchor synthesising pathway in plants. Several candidate genes have been identified and we are intending to perturb their expression to assess the effects this has on GPI-anchoring and hence AGP function.

4-7

Transposon-mediated activation tagging in Arabidopsis thaliana

Thomas Kirch, Ruediger Simon and Wolfgang Werr.
Institut fuer Entwicklungsbiologie, Universitaet zu Koeln, Gyrhofstr. 17, 50923 Koeln, Germany

We have constructed a transposon-mediated activation tagging system for Arabidopsis, where dominant gain-of-function alleles shall be created by insertion of a 35S enhancer tetramer. A defective I/D Spm element carries a Basta resistance gene and a tetramer of the strong enhancer of the cauliflower mosaic virus to promote constitutive expression of genes in its vicinity after transposition. In addition, the T-DNA is supplied with a transposase source and a counterselectable marker gene. Transpositions and reintegrations of the modified Spm-element are selected by Basta resistance and counterselection against the T-DNA donor. This isolates transposition events, that are genetically unlinked and stable, due to the loss of the transposase source, but still carry the enhancer tetramer. Remobilisation of the element can be achieved by crossing to plants carrying a transposase gene. As 350.000 plants have been cultivated so far, 1.800 plants with stable transposition events could be created. Among these, two plants with mutant phenotypes have been detected and will be analysed further.

4-8

Taking Off With TAIL PCR: Rapid Identification of Essential Genes from Lethal Insertion Mutants of Arabidopsis

John McElver, Carl Ashby, Carla Thomas, David Patton, and Marcus Law
Novartis Agricultural Biotechnology Research, Inc., Research Triangle Park, North Carolina, 27709 USA

To derive utility from our mutant screening program at Novartis, we must identify genes corresponding to mutant phenotypes. Efficient gene identification from a large collection of insertional mutants requires rapid and facile methods of obtaining plant DNA flanking the T-DNA or transposon insert. To that end, we have utilized plasmid rescue, adapter-mediated PCR, and TAIL PCR. With only minor modifications to the original protocol (Liu et al, The Plant Journal (1995) 8(3), 457-463), we have found TAIL PCR to be robust and amenable to high throughput border isolation from insertional mutants. We will present an examination of our results, focusing on the number of product bands obtained per reaction, and the ability to directly sequence products on a per mutant and per degenerate primer basis.

4-9

Telomerase Structural and Regulatory Genes in Arabidopsis

Matt Fitzgerald(1), Karel Riha(1), Shuxin Ren(2), Feng Gao(1), Dorothy Shippen(1), and **Thomas D. McKnight**(2).
(1)Department of Biochemistry and Biophysics and (2)Department of Biology, Texas A&M University, College Station, TX 77843 USA.

Telomeres, complex nucleoprotein structures at the ends of eukaryotic chromosomes, are essential for stability of the genome. Telomeres are synthesized by telomerase, an unusual reverse transcriptase that carries its own RNA template. We have identified and characterized the Arabidopsis gene encoding the telomerase reverse transcriptase (At-TERT). The At-TERT protein contains all of the conserved motifs found in other telomerases. Expression of the At-TERT mRNA is at least 20-fold higher in callus than in leaves, which correlates with telomerase activity in these tissues. The function of the gene was confirmed by isolating plants carrying a T-DNA insertion within the gene. Plants homozygous for this insertion lack all detectable telomerase activity. The T-DNA disruption segregates in a Mendelian pattern, indicating that both male and female gametogenesis proceed normally in the absence of telomerase activity. The visible phenotype of these plants is normal, for at least the first two generations. Telomeres progressively shorten, however, suggesting that the telomerase- deficient plants cannot propagate indefinitely. Telomerase activity is abundant in reproductive organs but absent from most vegetative organs. We are using activation tagging to identify genes that regulate this differential expression. We have screened 1,200 tagged lines and found two that ectopically express telomerase in their leaves. The activation tag in the first of these lines has been recovered and mapped to a relatively uncharted region of Chromosome III. It is not physically linked to the AtTERT gene. We are curently attempting to identify the activated genes in these lines.

4-10

Transgenics Galore: High throughput transformation to assign functions to novel arabidopsis genes

Josh S. Mylne (1), Jimmy R. Botella (1)
(1) Plant Genetic Engineering Laboratory, Department of Botany, University of Queensland, QLD 4072.

Using six custom made binary vectors, in planta transformation and Basta selection on soil, a high throughput transformation technique is being used to produce 20 lines for 98 different antisense or sense gene constructs. All Basta vectors have the CaMV 35S promoter and nos terminator flanking one of six different multiple cloning sites. Cloned into this polylinker are uncharacterized genes chosen from the MSU PRL-2 EST library. The EST's include 66 protein kinases, 3 protein phosphatases and 27 EST's of unknown or interesting putative function. This catalogue of antisense and sense transgenic plants will provide unique opportunities in mutant screening and in some ways, is a favorable alternative to T-DNA mutants.

4-11

Random antisense mutagenesis as a new functional genomic approach in Arabidopsis
Hong Gil Nam, Ji Hyung Chun, Chul Soo Kim, Haujung Shi, Chan Man Ha.
Dept. of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, South Korea.

Large scale functional assignment of expressed sequences is crucial for ultimate understanding of cellular processes. Toward this goal in plants, we designed "random antisense mutagenesis" approach. In a pilot experiment, 1,000 transgenic Arabidopsis plants that express a random antisense cDNA(s) were generated with Agrobacterium culture harboring an Arabidopsis antisense cDNA library. Visual screening showed 12% of the transgenic plants have mutant phenotypes. Genetic and molecular evidences showed that ~30% of the mutations are due to antisense effect. Through this approach, it should now be possible to assign a large number of expressed sequences with known in vivo functions in plants.

4-12

Use of Insertional Mutagenesis for the Identification of Essential Genes in Arabidopsis
David Patton(1), Joshua Levin(1), Carl Ashby(1), George Aux(1), Joanna Barton(1), Greg Budziszewski(1), Eddie Cates(1), Mary Ann Cushman(2), Linda Franzmann(2), Amy Gregoski(1), Rebecca King(2), John McElver(1), Gary Jones(1), David Meinke(2), Todd Nickle (2), Beat Nyfeler(1), Sharon Potter(1), Jennifer Reineke(1), Kelsey Smith(2), Amy Schetter(2), Lisa Schlater(1), Lyn Wegrich(1), and Marc Law(1)
(1)Novartis Agribusiness Biotechnology Research Inc., 3054 Cornwallis Rd., Research Triangle Park, NC 27703-2257 USA (2)Department of Botany, Oklahoma State University, Stillwater, OK 74078 USA

The goal of this project is to examine the cellular basis of plant growth and development by saturating for insertional mutants defective in essential genes. Over 50,000 T-DNA lines generated at Novartis and 10,000 Ds transposon lines from Cold Spring Harbor Laboratory have been screened for embryonic and seedling lethal phenotypes. Approximately one-third of the flanking sequences from tagged mutants recovered through plasmid rescue or TAIL PCR have homology to genes with known functions. Another third have homology to genes encoding hypothetical proteins, and the remainder have no significant homology to sequences in GenBank. Essential genes identified to date fall into all but two of the 15 major functional categories outlined by Bevan et al. [Nature (1998) 391:485]. As expected many of the identified genes have basic cellular functions. Based on sequence similarities, several identified genes fall into the disease/defense category, consistent with an important role for such genes in growth and development. Examples of particularly interesting mutants will be presented in some detail and the relationship between the gene function and mutant phenotypes discussed.

4-13

Use of an En-1 tagged Arabidopsis thaliana population for the characterisation of seed colour mutants
Martin Sagasser, Klaus Hahlbrock and Bernd Weisshaar
Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln, Germany

The Arabidopsis thaliana transparent testa (tt) mutants are defined by yellow to pale brown seeds in contrast to the dark brown seeds produced by wild type plants. These mutants can be grouped into two classes depending on whether pigmentation of the whole plant or only the colour of the seeds is affected. Most members of the first group have been cloned. They encode structural genes of flavonoid biosynthesis leading on the one hand to flavonols and anthocyanins that accumulate in seeds and in the plant body, and on the other hand to condensed tannins which are responsible for seed coat pigmentation. Not much is known about mutants which are affected in seed colour only (tt1, 2, 8, 9, 10). In an attempt to assign functions to this group, 27 lines producing yellow seeds were isolated from an A. thaliana population mutagenised with the autonomous maize transposable element En-1. Test crosses confirmed one of these lines to be allelic to tt1. Southern analysis of a population of sister plants segregating for yellow seeds identified one band hybridising to an En-1 probe that co-segregated with the phenotype. Sequences flanking the insertion were cloned using rapid amplification of genomic ends (RAGE) and used to isolate genomic BAC clones hybridising to these sequences. These BAC clones map to the region assigned to tt1, i.e. chromosome I close to the centromere. A subclone of one of the BACs was used for Agrobacterium mediated transformation of the original tt1 line to complement the mutation. Sequencing of this subclone should allow cloning of the tt1 gene.

4-14

Construction of high-content, full-length Arabidopsis cDNA libraries by biotinylated CAP trapper and monitoring gene expression pattern under dehydration and cold stress using cDNA microarray
Motoaki Seki (1), M.Narusaka (1), K.Yamaguchi-Shinozaki (2), P.Carninci (3), Y.Hayashizaki (3), and K.Shinozaki (1)
(1) Lab. of Plant Molecular Biology, Tsukuba Life Science Center, RIKEN, 3-1-1 Koyadai, Tsukuba 305-0074, Japan, (2) Biological Resources Division, JIRCAS, 1-2 Ohwashi, Tsukuba, IBARAKI, 305-8686 Japan

Full-length cDNAs are essential for functional analysis of plant genes. We constructed a high-content, full-length cDNA library from leaves and stems of Arabidopsis using a thermoactivated reverse transcriptase and chemical introduction of a biotin group into the diol residue of the CAP structure of eukaryotic mRNA, followed by RNase I treatment, to select full-length cDNA (M.Seki, et al., Plant J. 15: 707). More than 90% of the total clones obtained were of full length; recombinant clones were obtained with high efficiency (2.2 X 10^6 /9 ug starting mRNA). Sequence analysis of 111 random clones indicated that 32 isolated cDNA groups were derived from novel genes in the A. thaliana genome. We also constructed full-length cDNA libraries from the following 3 types of material: 1) dehydration-treated rosette plants; 2) cold-treated rosette plants; 3) plants at various developmental stages from germination to mature seeds and those that are treated with dehydration and cold. Until now, we have done single-pass sequencing of ca. 2800 clones from the 5' end. They were grouped as ca. 1700 cDNA groups. We will present characterization of the cDNAs in the libraries. We also prepared a cDNA microarray using ca. 1400 full-length cDNAs including RD and ERD genes. We are analyzing expression pattern of A. thaliana genes under dehydration and cold stress.

4-15

Progress of ABRC in obtaining new T-DNA stocks and preparing DNA from T-DNA populations for PCR screening

Randy Scholl, Luz Rivero, Deborah Crist, Doreen Ware and Keith Davis;
Arabidopsis Biological Resource Center and Department of Plant Biology, The Ohio State University, 1735 Neil Ave., Columbus, OH 43210 USA

The Arabidopsis Biological Resource Center (ABRC) maintains many stocks relevant to genome exploration. Among these, T-DNA lines and associated DNA are highest demand with users. The Center is rapidly expanding its holding in this area. The progress in this endeavor will be updated, and the success of users in identifying knockout mutations utilizing the presently available DNA samples will be evaluated.

The collection of 6000+ T-DNA lines donated by K. Feldmann has been distributed for the past seven years. In the past two years, the Center has expanded its holdings of available lines to 30,000+, including enhancer trap lines from T. Jack, activation tagging lines from D. Weigel, enhancer trap lines from INRA (France) and lines from C. Koncz. In addition, new lines have been received from D. Weigel (7000+), K. Feldmann (4000), J. Ecker and J. Alonso (20,000) and C. Somerville and W. Scheible (50,000+). Additional, expected donations should raise the number of these stocks to 200,000+.

DNA of 6,000 Feldmann lines has been distributed for two years, DNA from 6,000 T. Jack lines has been available for one year. DNA for PCR screening will be extracted from additional lines so that the population sampled will number 80,000. Laboratories were surveyed regarding their results, both in late 1998 and late 1999. Results with these two populations indicate that the rate at which users are finding insertions and their phenotypic effects is similar for the two populations and is as expected based on probability projections. Data of both surveys will be presented. ABRC is supported by a grant from the National Science Foundation.

4-16

A multidisciplinary approach to determining the function of arabinogalactan-proteins in plants using Arabidopsis

Carolyn J. Schultz (1), Kim L. Johnson (2) James McKenna (2) and A. Bacic (1&2).
(1) CRC for Industrial Plant Biopolymers and (2) Plant Cell Biology Research Centre, School of Botany University of Melbourne, Parkville, VIC 3052.

Arabinogalactan-proteins (AGPs), a class of proteoglycans, are implicated in many fundamental cellular processes such as cell growth, differentiation and somatic embryogenesis, but proof of function is difficult using classical techniques. A combined proteomics and genomics approach was chosen to characterise the AGPs in Arabidopsis and we are now using reverse-genetics to address the function of AGPs.

Initially we identified five Arabidopsis expressed sequenced tags (ESTs) that likely encode the protein backbones of distinct classical AGPs (Schultz et al, Trends in Plant Sc, 3: 426-431). AGPs were then purified from Arabidopsis tissues and deglycosylated to enable N-terminal sequencing of the protein backbones. We identified six distinct AGP protein backbones by N-terminal protein sequencing and all of these were matched to proteins encoded by EST or genomic sequences in the DNA sequence databases. In total, 15 distinct genes encoding ‘classical’ AGPs were identified.

To address the function of ‘classical’ AGPs we are using two reverse-genetics approaches; the first is a mapping approach and the second is screening of T-DNA tagged lines to search for mutants in AGP genes. The map position of 9 of the 15 AGPs genes was determined and these genes map to within 2cM of a variety of developmental and structural mutants in which the affected gene is unknown. In the second approach, we have identified plant lines with T-DNA tags in or near 3 of the AGP genes. We are currently in the process of identifying homozygous tagged plants to determine the phenotypic effect (if any) of these insertions.

4-17

Implementing the micro-array technique in the Lab of Plant Genetics

Cristian Chaparro, Raimundo Villaroel, Marc Van Montagu and **Nancy Terryn**
Lab of Genetics, Departement of Plant Genetics, Flemish Interuniversity Institute for Biotechnology, University Gent, K.L. Ledeganckstraat 35, 9000 Gent, Belgium
contact : nater@gengenp.rug.ac.be fax: 32 9 264 5349

The Departement of Genetics (VIB-RUG) has been involved from the start in the Arabidopsis Genome project. This resulted in a thorough automatisation within the sequencing team. DNA labstations, able to prepare DNA from bacterial cultures and perform different pipeting and spotting steps were purchased (Qiagen Biorobot, Biomek 2000). This allowed us as well to prepare DNA micro-arrays when there was a need in the department for that. Initially micro-array nitrocellulose filters have been prepared spotted with for example BAC clones, genes of interest to certain projects, subclones of larger clones, etc that had to be screened. Now we want to enter the field of micro-arrays as a tool for gene expression, thus using an RNA based probe. The DNA is spotted on a solid support, either filter or glass, and hybridised to labelled cDNA. Till now we worked on nitrocellulose filters and used both radioactive and one non-radioactive method for the probe labelling. We have now purchased a Nanoplotter (GeSim, Dresden, Germany), which allow us to spot nanoliter volumes in a dense array on microscope glass slides or membranes. This will allow us to use fluorescent probes, furthermore this technique permits the use of two dyes (cy3 and Cy5) that can be screened simultaneously. Thus the hybridisation pattern of genes to RNA from two different tissues, or samples from different conditions can be compared. As a pilotproject we want to study the expression of 103 genes present in a contig around the Ap-2 gene on chromosome 4.

4-18

N.A.S.C. (Nottingham Arabidopsis Stock Centre)

Fiona Wilson, Penny Dennis, Clair Hall, Jamie Kincaid, Stephanie Kitchingman, Karen Searle, Sean Walsh and Sean May.
Nottingham Arabidopsis Stock Centre, Plant Science Division (University Park), School of Biological Sciences, University of Nottingham, NG7

The Nottingham Arabidopsis Stock Centre (NASC), in collaboration with the Arabidopsis Biological resource Center (ABRC), currently curate and distribute stocks of Arabidopsis representing a minimum of 50,000 insertion mutagenesis events. It is expected that within a year the number of lines supplied by the stock centres will be close to representing saturation of the Arabidopsis genome with insertions. Access to these materials, along with the sequence data generated by the Arabidopsis Genome Initiative (AGI), will facilitate the reverse genetics revolution in Arabidopsis research.

NASC and ABRC also receive, bulk and distribute seeds of characterised ecotype populations and mutants of Arabidopsis which will continue to be essential for forward screens and gene expression analysis in the post genome era. The availability of mapping resources has increased with the release of 3 novel recombinant inbred populations this year.

To support the archiving and distribution of genetic resources NASC has upgraded its on-line catalogue http://nasc.nott.ac.uk/ which will provide more frequent catalogue updates, more efficient catalogue browsing and searching and a more user friendly ("shopping cart") ordering system.

Based at NASC, Arabidopsis Genome Resource (AGR), the Arabidopsis node of UK CropNet, is available from http://synteny.ac.uk/. The AceBrowser web interface provides access to integrated Arabidopsis sequence and map data. AGR is freely available to download.

NASC is funded by the BBSRC, EU, Gatsby Foundation and the University of Nottingham.

4-19

Gene expression profiling in Arabidopsis using DNA microarrays.
Ellen Wisman, Jeffry Landgraf, Robert Schaffer, Matt Larson, Monica Accerbi, John Ohlrogge, Ken Keegstra, Pam Green.
MSU-DOE Plant Research Laboratory, Michigan State University, 310 Plant Biology, East Lansing, Mi 48824-1312, USA

The microarray facility at MSU is part of the Arabidopsis Functional Genomics Consortium (AFGC), a collaboration recently funded by National Science Foundation. Together with Shauna Sommerville at Carnegie Institute and Mike Cherry at Stanford University our goal is to make DNA microarray technology available to the academic community. This will be accomplished through the establishment of a service facility and by providing the data generated to the community through the WWW. The first year of our project will be devoted to optimization of the technology and preparation of microarrays containing thousands of Arabidopsis cDNAs. The DNA fragments spotted on the microarray will be amplified from the collection of EST clones sequenced by Tom Newman. By hybridizing these arrays with probes corresponding to mRNA extracted from different tissues and organs or from plants subjected to different stimuli, global gene expression patterns can be investigated. We will also examine the expression of plant specific genes of unknown function under a set of standardized conditions. The microarray facility is scheduled to begin providing service to academic researchers in year two of our project and the service will be continued in year three. Other investigators of the AFGC will establish a gene knockout facility. Together these technologies should provide the scientific community with a set of powerful tools that can be used in their efforts to understand the function and interrelationships of the genes of Arabidopsis and other plants.

4-20

Functional Analysis of Small Subunit of RubisCO in Arabidopsis thaliana
Minsoo Yoon 1,2, Jo Putterill1, Gavin Ross 2, and William Laing 2
1 Plant Sciences, The School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand and 2 The Horticulture and Crop Research Institute of New Zealand Limited Private Bag 92169, Auckland, New Zealand

Rubisco (ribulose-1,5-bisphosphate carboxylase oxygenase) is a critical enzyme in carbon metabolism. In higher plants, Rubisco consists of eight large (LSU) and eight small subunits (SSU), and the small subunit genes (rbcS) are encoded in the nuclear genome in the form of a multigene family. Each rbcS gene encodes slightly different versions of the mature protein, which are differentially expressed. The aim of this research is to elucidate the role of rbcS genes and their proteins in photosynthesis by dissecting the non-overlapping functions of each member of the rbcS gene family. This research will also provide insights into the roles of multigene families, which is prevalent in higher plants. Currently, the available Arabidopsis T-DNA and transposon mutant lines are being screened for insertional mutations in rbcS genes. The initial mutant screening and Rubisco characterisation will be presented.

5-1

Various mechanisms of vesicular trafficking to the plant vacuole

Natasha.V. Raikhel, Sharif U. Ahmed, Diane Bassham, Valentina Kovaleva, Enrique Rojo, Anton Sanderfoot, Sridhar Venkataraman, and Haiyan Zheng
DOE Plant Research Lab, Michigan State University, E. Lansing, MI 48824, USA

Cargo proteins destined for the plant vacuole contain positive sorting signals that lead to their segregation from the default secretory pathway to the cell surface. Two of these sorting signals, an N-terminal propeptide (NTPP) and a C-terminal propeptide (CTPP) have been found to be trafficked to the vacuole by distinct pathways. We have characterized several components of the machinery involved in the sorting of NTPP-type cargo, including a trans-Golgi network (TGN) localized cargo receptor and several SNARE components involved in vesicular traffic between the TGN and the prevacuolar compartment of the model plant Arabidopsis. We believe that the CTPP pathway may be unique to plants. We have initiated a genetic screen for mutants defective in the vacuolar targeting of CTPP-bearing protein reporters. We are now working to characterize mutants derived from this screen.

5-2

Understanding the complexities of metal uptake: a true test of one's mettle

Mary Lou Guerinot (1), Erin Connolly (1), Elizabeth Rogers (1), Catherine Procter (2), Nigel Robinson (2)
(1)Department of Biological Sciences, Dartmouth College, Hanover, NH 03755 USA,
(2) Department of Biochemistry and Genetics, University of Newcastle, Newcastle, NE2 4HH UK

Iron deficiency afflicts 2.7 billion people worldwide and plants are the principal source of iron in most diets. Low iron availability often limits plant growth because iron forms insoluble ferric oxides leaving only a small, organically-complexed, fraction in soil solution. Fe(III) chelate reductase activity is essential for the acquisition of this soluble iron by most plants and is induced in response to iron deficiency. Previously, we identified two Arabidopsis mutants, frd1 and frd3, that show no induction or constitutive expression, respectively, of Fe(III) chelate reductase activity. frd1 is allelic to FRO2, a gene that encodes a protein belonging to a superfamily of flavocytochromes that transport electrons across membranes. The frd3 mutants are allelic to the manganese overaccumulator man1 and, like man1, have elevated levels of iron, manganese and other metals. frd3 constitutively expresses the FRO2 Fe(III) chelate reductase gene and the iron-responsive transporter IRT1. IRT1 belongs to a novel family of metal transporters, the ZIP family, with representatives from a diverse array of eukaryotes. We have constructed site-directed mutations in IRT1 to investigate transporter function. While many of the mutants lack transporter activity, the substitution of alanine for a charged residue in certain positions in IRT1 yields a protein with altered metal specificity. In wild type Arabidopsis, IRT1 mRNA accumulates only in iron-deficient roots, whereas transgenic plants carrying a CaMV 35S::IRT1 construct accumulate IRT1 mRNA in roots and shoots under both iron-deficient and iron-sufficient conditions. However, IRT1 protein is detected only in the roots of iron-deficient transgenic plants, indicative of post-transcriptional control. Ongoing studies with other members of the ZIP transporter family are focused on uptake of another essential micronutrient, zinc

5-3

Analysis of T-DNA-Disrupted K+ Channel Genes in Arabidopsis thaliana
Whitney R. Robertson and Michael R. Sussman,
Program in Cellular and Molecular Biology and UW Biotechnology Center, 425 Henry Mall, University of Wisconsin, Madison, WI 53706 USA

Potassium channels are involved in a number of fundamental physiological processes in plants, including regulation of turgor and uptake of K+ from the surroundings. To directly test the function of cloned K+ channels, it is important to identify mutants in these genes and examine the phenotypes of the mutant plants. A reverse-genetic screen of a T-DNA-transformed Arabidopsis population (Krysan et al., PNAS 93:8145-50) was used to identify a plant with a disruption in the K+ channel gene AKT1. Phenotypic analysis of the akt1 "knockout" plant revealed that AKT1 is responsible for the absorption of K+ from micromolar concentrations (Hirsch et al., Science 280:918-21). In addition to AKT1, there are currently fifteen genes in Arabidopsis known to encode K+ channels and K+ transporters. Focusing mainly on transporters likely to be in the plasma membrane, we have used a reverse-genetic strategy to identify disruptions in six of eight of these genes. The T-DNA-tagged insertionally mutagenized population of Arabidopsis we are using contains over 90,000 independent insertional events. This population theoretically contains at least one mutant in each of the plant's average sized genes. Once individual mutant lines are identified, appropriate crosses will be performed to create plants containing multiple mutant K+ transporter genes. The relative contribution of each gene to K+ transport will be assessed using biochemical and electrophysiological techniques.

5-4

Molecular genetic manipulation of very-long-chain fatty acid biosynthetic pathways in Arabidopsis
Anthony Millar, Sabine Clemens, Sabine Zachgo, Tanya Hooker and Ljerka Kunst
Deptartment of Botany, University of British Columbia, Vancouver, B.C., V6T 1Z4, Canada

In Arabidopsis, very-long-chain fatty acids (VLCFAs > C20) are found in the seed oil (C20-C22), in sphingolipids of the plasma membrane (C24-C26) and as precursors of cuticular waxes (C26-C32). VLCFAs are synthesised by microsomal fatty acid elongation (FAE) systems by sequential addition of two carbon units to pre-existing C18 fatty acids. This process requires four enzymatic steps. We have recently demonstrated that microsomal FAE is regulated through the expression of the first enzyme of the pathway, the condensing enzyme, which determines the levels of VLCFA accumulation and the acyl chain lengths of the VLCFAs produced (Millar and Kunst, Plant J., 1997, 12:121). Thus, condensing enzymes are key targets for metabolic engineering. We have ectopically expressed the seed-specific condensing enzyme FATTY ACID ELONGATION1, required for the synthesis of C20-22 fatty acids in seed oil, using the 35S promoter in Arabidopsis. This resulted in the synthesis of VLCFAs throughout the plant. VLCFAs accumulated to up to 30% of total plant fatty acids, at the expense of C16 and C18 acyl groups. The incorporation of VLCFAs into membrane lipids resulted in unique alterations in chloroplast membrane structure and had a profound effect on the overall plant morphology. In addition, we recently identified a gene, CUT1, which encodes an epidermis-specific condensing enzyme required for wax biosynthesis. Co-suppression of CUT1 resulted in a waxless phenotype and male sterility. The wax load on CUT1-suppressed plants is only 6-7% of wild-type plants, which is lower than in any of the eceriferum mutants isolated to date. CUT1 represents the first gene in wax biosynthesis with a known biochemical function. Its isolation will allow the study of wax deposition, as well as the genetic manipulation of wax composition in plants.

5-5

Expression of phage-derived antibody genes to modulate flavonoid biosynthesis in transgenic Arabidopsis

Michael C. O. Santos and Brenda Winkel-Shirley
2119 Derring Hall, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA

As part of an on-going effort to characterize the flavonoid biosynthetic pathway, we are attempting to modulate flavonoid biosynthesis in Arabidopsis by expressing phage-derived antibodies in transgenic plants. To this end, we have isolated one anti-CHS and four anti-CHI antibody genes in the scFv format (i.e. single chain fragment variable) through the phage display technique. Each of these genes has been cloned behind the double-enhanced CaMV 35S promoter and TEV translation enhancer in pRTL2, and subsequently into the T-DNA region of the plant transformation vector, pBI121. These constructs were used to transform Arabidopsis using the vacuum infiltration method. We have recovered at least 10 independent transgenic plants for each construct. T2 progeny that are homozygous for the transgene are being identified based on inheritance of the linked kanamycin resistance gene in the T3 generation. In addition, the expression of the scFv gene is being analyzed by protein immunoblot analysis using a mouse monoclonal antibody directed against the c-myc tag on the carboxy-terminus of each scFv antibody. Several transgenic lines exhibiting high-level expression of the antibody genes have been identified and the effects of these antibodies on flavonoid biosynthesis are being analyzed. This work will determine the feasibility of using phage-derived scFv antibodies to disrupt or redirect metabolic processes in the plant cytosol. This system may also provide a powerful approach to probing the subcellular organization of biochemical pathways in living plant cells.

5-6

Aminoacyl-tRNA synthatases, a set of essential proteins in translation distributed between cytosol, mitochondria and plastids.

N. Peeters, K. Akama, A. Chapron, N. Choisne, A. Dietrich, L. Drouard, A-M. Duchene, A. Giritch, D. Lancelin, G. Souciet, I. Small, H. Wintz.
Station de Genetique et Amelioration des Plants, INRA, Versailles, France Shimane University, Japan Genoscope - Centre National de Sequencage, Evry, France Institut de Biologie Moleculaire des Plantes, Strasbourg, France

Thanks to the progress of the large scale genome sequencing of Arabidopsis, our lab, in collaboration with the three other labs cited, has been able to set up a database devoted to listing and describing the transfer RNAs (tRNAs) and aminoacyl-tRNA synthetases (aaRSs) from Arabidopsis. These two sets of macromolecules are key components of the translation machinery in all cells or compartments where protein synthesis occurs. Prokaryotes generally contain 30-60 tRNAs to read the 61 sense codons and 18-20 aaRSs to aminoacylate these tRNAs specifically with one of the 20 primary amino acids. In plants, the situation is more complex, as three translation systems coexist – in the cytosol, in the mitochondria and in plastids. Our work shows that in Arabidopsis the tRNAs and the aaRSs in each compartment can have different phylogenetic origins, indicating multiple transfers of genes or gene products between the three compartments. The best example is the mitochondrial translation system, which contains a mixture of tRNAs and aaRSs of mitochondrial, plastid and nuclear origin. To investigate the cellular location of the aaRSs, we used fusions with the Green Fluorescent Protein (GFP) as a reporter in a transient expression system. In addition to enzymes specific to mitochondria or chloroplasts, we have identified four enzymes (histidyl-, methionyl-, cysteinyl-and asparaginyl-tRNA synthetases) that are dual-targeted to both mitochondria and chloroplasts. Other examples have been found of aaRSs that are present in the mitochondria and the cytosol, resulting in a sort of “overlap” of the three translation systems of the plant cell.

5-7

Comparison of frequencies of individuals resistant to imazethapyr, chlorsulfuron, and glyphosate in EMS-mutagenized populations of Arabidopsis thaliana (cv. Col-0)

Scott R. Baerson, Minhtien Tran, Damian J. Rodriguez, Kathleen A. Gonzalez, David E. Schafer, Daniel M. Krupa, Kenneth J. Gruys, David M. Stalker, Nancy B. Taylor, Gerald M. Dill
Monsanto Company, 700 Chesterfield Parkway, St. Louis, Missouri. 63198, USA

A soil based method of screening large populations of EMS-mutagenized Arabidopsis seedlings was devised to quantitate and contrast the probabilities of spontaneous resistance occurring in three different herbicides - glyphosate, imazethapyr, and chlorsulfuron. Both imazethapyr and chlorsulfuron are herbicides inhibiting the enzyme acetolactate synthase (ALS), which catalyzes the first step in the branched chain amino acid biosynthetic pathway. There have been reports of spontaneous resistance occurring in at least 15 weed species for these two herbicides (Saari et al., 1994). In contrast, the number of reports of spontaneous resistance occurring among weeds to glyphosate have been significantly lower.

Previous mutant screens conducted using the same or similar herbicide chemistries were based upon germination of surface-sterilized Arabidopsis seeds in media supplemented with these herbicides (Haughn and Somerville, 1986; 1990). The novel soil-based method utilized in the present study was designed to more accurately mimic the selective pressure faced by weed populations subjected to repeated over-the-top applications. Using this approach, one million EMS-mutagenized Arabidopsis seedlings were screened for each herbicide at an established 2X-I100 rate. Surviving plants were then allowed to self and produce seeds for use in secondary screens to confirm or deny "true" resistance. One chlorsulfuron and five imazethapyr-resistant lines were confirmed via these secondary screens, however no glyphosate-resistant lines were isolated.

5-8

Characterisation of an Arabidopsis mutant, mto2, with altered methionine and threonine accumulation

Derek Bartlem, Ingrid Lambein, Yuko Tamaki and Satoshi Naito
Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, JAPAN

We have isolated an Arabidopsis mutant, mto2, in which over-accumulation of methionine is accompanied by a reduction in threonine levels. In higher plants, O-phosphohomoserine (OPH) represents a branch point between the methionine and threonine biosynthetic pathways. It is believed the enzymes threonine synthase (TS) and cystathionine gamma-synthase (CGS) actively compete for the OPH substrate for threonine and methionine biosynthesis, respectively. A single base pair mutation was identified within the coding region of the mto2 threonine synthase gene. Expression of the threonine synthase gene was normal in mto2 plants, whereas functional complementation with an E. coli (thrC) mutant indicated that the activity of the encoded enzyme is impaired. Our data suggests that the single base pair mutation within the threonine synthase gene is responsible for the mto2 phenotype, resulting in decreased threonine synthesis and a channelling of OPH to methionine biosynthesis. Analysis of temporal changes in threonine and methionine levels, along with respective changes in TS and CGS gene expression, have also suggested that the competitive ability of the methionine branch is reduced in older rosettes of both wild-type and mto2 plants, allowing for a marked increase in threonine accumulation. Data on spatial changes in threonine and methionine accumulation will also be presented and discussed with reference to developmental changes in gene expression of the TS and CGS genes.

5-9

Genetic analysis of dormancy, soluble oligosaccharide content and storability in seeds of Arabidopsis thaliana

Leónie Bentsink (1), Carlos Alonso-Blanco (1), Dick Vreugdenhil (2), Karine Tesnier (3), Steven Groot (3) and Maarten Koornneef (1).
(1) Lab. of Genetics, Wageningen Univ., Dreijenlaan 2, 6703 HA Wageningen, The Netherlands; (2) Lab. of Plant Physiology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen The Netherlands

A set of 162 Recombinant Inbred Lines (RILs) between the ecotypes Landsberg erecta (Ler) and Cape Verde Islands (Cvi) were developed and characterised with molecular markers. These RILs have been used to detect and locate Quantitative Trait Loci (QTLs) for seed dormancy, seed soluble oligosaccharide content and seed storability. The control of seed dormancy differences seems to involve multiple mechanisms as shown by the presence of different QTLs at different time points after harvesting. Among the 7 loci identified two exhibit major effects. In order to analyse the most interesting regions in detail, Near Isogenic Lines (NILs) with Cvi alleles at one single QTL are being prepared. It is generally presumed that seed soluble oligosaccharides play an important role in the acquisition of desiccation tolerance and consequently seed storability. To determine the role of soluble oligosaccharides in this seed storability we have genetically analysed the relation between the two traits. Seed soluble oligosaccharide content seems to be controlled by one major QTL located chromosome 1, and three loci with minor modifing effects. Candidate genes based on this locus are galactinol synthase and raffinose synthase. For seed storability six putative QTLs have been identified and comparison with the QTLs for seed oligosaccharide content showed no co-location for the main QTLs of the two traits.

5-10

Molecular dissection of protein-protein interactions within the cysteine synthase complex

Oliver Berkowitz, Markus Wirtz, Ruediger Hell
Institute of Plant Genetics and Crop Plant Research Gatersleben, Dept. Of Molecular Mineral Assimilation, 06466 Gatersleben, Germany

Sulfur metabolism in plants includes uptake of the macronutrient sulfate from the environment and assimilation into a variety of functionally essential sulfur compounds. The regulation of cysteine biosynthesis plays a key role since cysteine is the first stable organic compound in the pathway. Cysteine synthesis takes place in the cysteine synthase (CS) complex that consists of serine acetyltransferase (SAT) and O-acetylserine(thiol)lyase (OAS-TL). Since SAT is labile, has rate-limiting activity and can be feedback regulated by cysteine, SAT is the primary target to understand the function of the CS complex in cysteine biosynthesis. To this end we analyzed the structural and the catalytic domains of SAT from Arabidopsis thaliana by the two-hybrid system, mutagenesis and computer aided modelling. These data show that SAT consists of structurally distinct domains for the different protein-protein interactions that overlap with the catalytic domain. A partial three-dimensional structure of the conserved SAT/OAS-TL interaction region which includes the proposed catalytic center was obtained through homology modelling. This prism-like structure is formed by a repeated hexapeptide motif. In order to study expression and interaction of defined recombinant plant SAT and OAS-TL proteins in a background-free system, an E. coli chromosomal null mutant of both cysteine synthase enzymes was constructed. Coexpression of Arabidopsis SAT and OAS-TL leads to complementation of cysteine auxotrophy in this strain and to the reconstitution of the plant CS complex in vivo. The combination of these tools will allow to dissect the structure-function relationsship in the plant CS complex.

5-11

Characterization of an Arabidopsis phosphoglucose isomerase mutant

Jychian Chen (1), Tien-Shin Yu (1), Wei-Ling Lue 91) and Shue-Mei Wang (2).
(1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, (2) Department of Botany, National Taiwan University, Taipei, Taiwan.

A nuclear encoded Arabidopsis mutant, pgi1-1, with a reduced amount of starch in leaves was isolated and found to have a decreased chloroplastic phosphoglucose isomerase activity. We cloned and sequenced the Arabidopsis PGI1 gene. Sequence analysis indicated that the Arabidopsis PGI1 gene encoding the chloroplastic PGI is closely related to that of Synechocystis, a photosynthetic cyanobacterium. The pgi1-1 allele was found to have a single nucleotide substitution causing a serine to phenylalaine transition. The decrease of starch content in leaves of the pgi1-1 mutant indicates that cytosolic glucose 6-phosphate may not be efficiently transported into chloroplasts to complement the mutant's deficiency of chloroplastic phosphoglucose isomerase activity for starch synthesis. Whereas the flowering time of the pgi1 mutant was similar to that of the wild type under long day-length conditions, it was significantly delayed under short day-length conditions. This late flowering phenotype was also observed in Arabidopsis starch deficient mutants, pgm1 and adg1. The pleiotropic phenotype of late flowering confered by these starch metabolic mutations suggests that carbohydrate metabolism plays an important role in floral initiation.

5-12

Characterization of Arabidopsis membrane-associated acyl-CoA binding protein ACBP1

Mee-Len Chye, Bing-Quan Huang and S.Y. Zee.
Department of Botany, The University of Hong Kong, Pokfulam Road, HONG KONG.

Until recently, only cytosolic acyl-CoA binding proteins (ACBPs) have been characterized. They are small, highly-conserved 10kD-proteins that bind acyl-CoAs in lipid metabolism. In plants, fatty acids are transported from the chloroplast as acyl-CoA esters to the endoplasmic reticulum for lipid biosynthesis. Plant ACBPs could be involved in the transport and storage of long-chain acyl-CoAs. The isolation of an Arabidopsis cDNA encoding a predicted 24.1kD membrane-associated ACBP, designated ACBP1, has suggested that membrane-associated ACBPs occur in plants (Chye, 1998; Plant Mol. Biol. 38, 827-838). To elucidate the role of ACBP1 in Arabidopsis, we have used anti-ACBP1 antibodies in western blot analysis, in immunolocalization studies using light microscopy and in immunoelectron microscopy. Results from western blot analysis revealed that ACBP1 accumulates in developing seeds and its expression coincides with lipid deposition. Using light microscopy, we showed that ACBP1 is strongly expressed in the embryo at the cotyledons, the hypocotyl, the procambium of the axis and in most peripheral cells of the cotyledons and the hypocotyl. Immunogold labelling localized ACBP1 to vesicles, to the plasma membrane especially at the epidermal cells of developing embryos and to the cell wall of the outer integument cells at the seed coat. These results suggest that ACBP1 is involved in intermembrane lipid transport from the endoplasmic reticulum via vesicles to the plasma memmbrane where it could maintain a membrane-associated acyl pool. Its immunolocalization to the cell wall of the outer integument cells at the seed coat suggests a possible role in cuticle and cutin formation.

5-13

Regulation of iron uptake in Arabidopsis

Erin L.Connolly (1), Nigel J. Robinson (2), Catherine M. Proctor (2) &Mary Lou Guerinot (1)
(1) Department of Biological Sciences, Dartmouth College, Hanover, NH, 03755, USA,
(2) Department of Biochemistry and Genetics, The Medical School, University of Newcastle, Newcastle, NE2 4HH, UK

In response to iron deprivation, plants induce both ferric chelate reductase activity and ferrous iron transport activity. We have now identified genes that encode ferric chelate reductase (FRO2) and a ferrous iron transporter (IRT1) in Arabidopsis. Previously, we identified an Arabidopsis mutant, frd1, which shows no induction of Fe(III) chelate reductase activity. frd1 is allelic to FRO2, a gene that encodes a protein belonging to a superfamily of flavocytochromes that transport electrons across membranes. Transformation of frd1 mutant plants with FRO2 complements the frd1 phenotype. The IRT1 gene is predicted to encode an integral membrane protein with eight transmembrane domains and it belongs to the ZIP family of metal transporters. IRT1 mRNA is only expressed in iron-deficient roots in wild-type plants. IRT1 mRNA is expressed constitutively (i.e. under both iron-deficient and iron-sufficient conditions) in roots and shoots of transgenic plants that carry a CaMV 35S-IRT1 construct. However, IRT1 protein is only detected in the roots of iron-deficient transgenic plants, indicating that expression of IRT1 is subject to post-transcriptional control. Metal analysis of the transgenic plants showed that these lines accumulate various metals as compared to wildtype and 35S-IRT1 plants show an enhanced sensitivity to cadmium under iron-deficiency conditions. Time course experiments show that expression of both IRT1 and FRO2 is induced in the roots within 24 hours of exposure to iron deprivation conditions. Conversely, both transcripts disappear within 24 hours when plants are transferred from iron-deficient to iron-sufficient growth conditions. In addition, the two transcripts disappear when plants are transferred from iron-deficient to iron-deficient plus cadmium growth conditions. These results indicate that the two genes are regulated coordinately.

5-14

Reversibly Glycosyalted Polypeptides of Arabidopsis thaliana

Ivan J Delgado (1), Kenneth Keegstra (1) and Natasha V. Raikhel (1).
(1) Michigan State University - Department of Energy, Plant Research Laboratory, Michigan State University. East Lansing, MI 48824-1312

Proteins that reversibly bind nucleotide sugars may be intermediates in the synthesis of various polysaccharides. Arabidopsis thaliana cDNAs encoding reversibly glycosylated polypeptides (AtRGPs) have been identified in the Expressed Sequence Tags (ESTs) database based on sequence similarity to a pea 41-kDa protein thought to be involved in hemicellulose biosynthesis. RGPs contain domain A, but not domain B, of b-glycosyltransferases, suggesting that they may be non-processive b-glycosyltransferases. To study the function of these proteins, polyclonal antibodies have been raised against a glutathione-S-transferase (GST) fusion of AtRGP1 (GST-AtRGP1) expressed in E. coli (Delgado et al. [1998] Plant Physiol 116: 1339-1349). These antibodies detect RGP homologs in both dicot and monocot species. GST-AtRGP1 retained the reversible glycosylation activity of AtRGP1 and was used to show that the reversible nature of this reaction is nucleotide specific (uridine diphosphate, UDP) but independent of sugar-type, at least in vitro. Purified GST-AtRGP1-[3H]Glc, incubated with enriched Golgi vesicles, preferentially transferred [3H]Glc to ethanol precipitatable material, possibly a polysaccharide. Finally, an Arabidopsis cell wall mutant has been identified that may also be an atRgp mutant. A model for the possible role of RGP in cell wall polysaccharide biosynthesis will be presented.

5-15

Conservation of the bicistronic message structure and rapid processing characteristics of the S-adenosylmethionine decarboxylase family between monocots and dicots.
Marina Franceschetti, Colin Hanfrey, Sonia Scaramagli and Anthony J Michael
Division of Food Safety, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA, United Kingdom

We have characterised the mRNAs and genes of the two S-adenosylmethionine decarboxylases (SAMDC) from Arabidopsis and rice. Each mRNA is bicistronic and the amino acid sequence of the small uORF of the SAMDC leader sequence is more conserved than that of the main open reading frame. There are no introns in the main ORFs of either of the two SAMDC genes from Arabidopsis or from rice although the small uORF of the leader sequence is flanked by introns in a manner reminiscent of exon capture. The eukaryotice SAMDC protein is post-translationally processed to produce a pyruvoyl prosthetic group necessary for the active site. We used in vitro transcription/translation to show that the processing is very rapid in both the Arabidopsis and rice SAMDCs and this strongly suggests that post-translational processing of the proenzyme is not regulatory and implies that the small uORF is the main site of post-transcriptional regulation.

5-16

Characterisation of Arabidopsis transformants containing genes from Agrobacterium rhizogenes
Eugene B. Fredericks and John D. Hamill
Department of Biological Sciences, Monash University, Clayton, 3168, Australia

Polyamines (PAs) are essential, ubiquitous molecules whose precise roles are unclear. In plants, they are associated with growing tissues, and are suggested to influence development. In tobacco, PA metabolism can be inhibited by the specific enzyme inhibitor difluoromethyl ornithine (DFMO). The stunted phenotype of treated plants is similar to that caused when the Ri T-DNA of A.rhizogenes is expressed. DFMO treatment and Ri T-DNA transformation reduces PA levels in tissues, but watering with free amines ameliorates their effects. The transformed phenotype may thus be caused by the depressive effect of Ri T-DNA upon PA metabolism (Martin-Tanguy et al., '96; Pl. Physiol. 111:131 & refs). To explore this further in a species outside the Solanaceae, Arabidopsis was transformed with Ri T-DNA and the rolABC genes from A.rhizogenes. Results suggest a link between altered PA metabolism and the transformed phenotype, but it is not a cause-and-effect relationship. PA levels in transformed shoots and roots are reduced, but exogenous PAs does not restore the normal phenotype, although high levels of PAs are measured in these plants. In addition, Northerns detect similar transcript levels for several important PA biosynthetic genes in wt and transformed roots. Moreover, DFMO is inhibitory to the growth of transformed root lines but not to that of wt controls. Leaf tissue of transformants containing rol genes show enhanced auxin sensitivity with respect to adventitious root formation. This may be due to altered auxin perception or metabolism. The stem structure of transformants show altered vascular organisation, is similar to that of wt plants treated with an auxin transport inhibitor, or possessing a mutation in the AtPIN1 locus (Gälweiler et al.,'98; Sci. 282:2226).

5-17

Molecular characterisation and expression analysis of a putative arabinosidase gene from Arabidopsis thaliana.

Lynette Fulton, Enmore Lin, and Christopher Cobbett
Department of Genetics, University of Melbourne, Parkville, Australia.

Arabinosidases belong to a large family of hydrolytic enzymes that are known to mediate modifications to the cell wall during plant development. Their exact roles in cell elongation and differentiation, tissue ripening and senescence, however, remain to be elucidated. An Arabidopsis EST has been isolated which exhibits homology to known bacterial arabinosidase genes. Screening of a cDNA library with this EST sequence yielded a full-length cDNA clone encoding a putative arabinosidase. A 23kb genomic clone was subsequently isolated, from which a 5.5kb EcoRI subclone containing the entire arabinosidase gene sequence was obtained. Comparative analysis of sequences enabled the resolution of the gene structure. The predicted protein comprises 678 amino acids, which exhibit 31.7% amino acid identity to the ASD1 arabinosidase in Bacteroids ovatus. Southern analysis has revealed this gene exists as a single copy in the Arabidopsis genome. We have demonstrated that crude protein extracts derived from Arabidopsis seedlings are capable of hydrolysing r-nitrophenyl arabinose glycoside substrates. In vitro studies of enzyme activity in heterologous expression systems such as Sacchromyces cerevisiae or E.coli will be used to confirm the identity of this gene and to define substrate specificities of the enzyme encoded. An analysis of the spatial and temporal expression of the gene is currently proceeding using three experimental strategies; the introduction of promoter:GUS reporter gene constructs into Arabidopsis plants via Agrobacterium-mediated transformation, qRT-PCR and northern analysis. In the absence of a mutant, antisense studies may allow us to characterise the effect an arabinosidase deficiency has on plant morphology and development.

5-18

Characterisation of Arabidopsis genes involved in the Golgi secretory pathway

Leigh Gebbie, Joanne Burn and Richard Williamson
Plant Cell Biology Group, Australian National University, Canberra, ACT 0200.

Several different classes of proteins are involved in the secretion of proteins and cell wall polysaccharides through the Golgi to either the vacuole or the cell surface. While it has been fairly well established that the same classes of proteins are involved in Golgi transport in all eukaryotic organisms, the molecular mechanisms controlling the process in plants are only starting to be understood. A number of Arabidopsis cDNA clones (Expressed Sequence Tags (ESTs)) show high homology to genes encoding proteins involved in secretion in other eukaryotic species as well as plants. Six of these have been chosen for study. Three are similar to synaptobrevins or v-SNAREs (vesicle compartment-soluble N-ethylmaleimide-sensitive factor attachment protein receptors)which are thought to associate with syntaxins or t-SNAREs (target membrane SNAREs) to ensure vesicle-to-target specificity. Two other ESTs are highly homologous to clones encoding Rab proteins, small GTP-binding proteins thought to be required in vesicle formation and targeting. Also under study is an ADP-ribosylation factor (ARF) homologue. In other systems this protein has been shown to be essential for recruitment of coat proteins onto forming COPI type vesicles. These six genes were characterised at the molecular level by examining their tissue specific expression patterns and gene copy number in Arabidopsis. Furthermore, in an attempt to elucidate functions for these gene products, an antisense approach has been used. Complementation of yeast secretory mutants and antibody studies are also underway and should provide interesting information about the role these proteins may be playing in the Golgi secretory pathway.

5-19

Sugar-Insensitive Mutants of Arabidopsis with Defects in Phytohormone Metabolism and/or Response
Susan I. Gibson, Ron J. Laby and Donggiun Kim.
Department of Biochemistry & Cell Biology, Rice University, Houston, TX USA 77005-1892

Soluble sugar levels have been shown to affect the expression of many plant genes and have been suggested to play an important role in regulating several developmental processes. To help characterize plant responses to sugar, and to aid in identifying components of the plant sugar sensing/response pathways, we have isolated two groups of Arabidopsis mutants that show an impaired ability to sense and/or respond to sugar. The first group of mutants was isolated by screening EMS-mutagenized and T-DNA tagged plants for those that were able to germinate and develop relatively normal shoot systems on media containing 0.3 M glucose or sucrose. At these sugar concentrations, wild type Arabidopsis plants germinate, but show little or no shoot development. 19 sugar-insensitive (sis) mutants have been isolated using this screen. Of these 19 mutants, 8 exhibit an abscisic acid insensitive (abi) phenotype in addition to their sis phenotype, with at least two of these mutants carrying defects in the ABI4 gene. Three more sis mutants have been shown to be allelic to previously identified mutants with defects in abscisic acid biosynthesis (aba2) or ethylene response (ctr1). The remaining 8 sis mutants, which appear to fall into at least 2 complementation groups, have not been found to be defective in abscisic acid or ethylene metabolism or response. Currently we are cloning the affected genes from these last two sis complementation groups, and are investigating possible connections between abscisic acid, ethylene, gibberellin and sugar signaling pathways. The second group of sugar-response mutants was isolated by screening for plants that showed an impaired ability to induce beta-amylase expression in response to increases in endogenous sugar levels. The eight reduced beta-amylase (ram) mutants identified using this mutant screen fall into at least 4 complementation groups. This research was supported by Department of Energy Biosciences Program Grant #DE-FG03-98ER20300.

5-20

An insertion in a sucrose transporter gene of Arabidopsis thaliana inhibits carbohydrate movement out of leaves
Jennifer R. Gottwald (1)(2), Ray F. Evert (2) and Michael R. Sussman (1)
(1) Biotechnology Center and (2) Department of Botany, Unversity of Wisconsin-Madison, Madison WI 53706 USA

The evolution of specialized organs in higher plants necessitated the development of unique means of moving carbohydrates from source organs, such as photosynthetic leaves, to sink organs, such as roots or flowers. The pathway of carbohydrate movement consists of the sieve tubes of phloem tissue, and its driving force is the concentration differential along a sieve tube between a source and a sink. Sugars in source leaves must be actively loaded into sieve elements to a higher concentration than that in the surrounding cells. The proteins that mediate this process include sucrose transporters. These integral plasma membrane proteins couple the transport of sucrose across the membrane against its concentration gradient to the favorable transport of protons. In Arabidopsis, at least one sucrose transporter, SUC2, is known to be expressed in the phloem (Stadler and Sauer 1996). Using a reverse genetic screen of a large population of T-DNA insertionally mutagenized plants, we have identified plants containing insertions in the SUC2 gene. Plants heterozygous for the insertion phenotypically resemble wild type plants during vegetative growth. In plants homozygous for the insertion, embryo lethality is often observed, and the seedlings that escape and germinate grow poorly and produce very few seeds. The leaves of these plants also contain chloroplasts with a much higher starch content than comparable wild type leaf chloroplasts. Taken together, our observations indicate that the SUC2 transporter is required for efficient loading of carbohydrates into sieve elements and therefore plays a central role in carbon partitioning in higher plants.

5-21

The Arabidopsis S-adenosylmethionine decarboxylase is translationally regulated by the encoded product of an upstream

Colin Hanfrey and Anthony J Michael
Division of Food Safety, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA, United Kingdom

S-adenosylmethionine decarboxylase (SAMDC) is a key enzyme in polyamine biosynthesis. In plants the message for SAMDC is abundant but the enzyme activity is very low. The activity of SAMDC is repressed by an excess of spermidine or spermine. A long 500 bp leader sequence is present in the two SAMDC messages of Arabidopsis and other plants and it contains a highly conserved small uORF potentially encoding a peptide of 50 amino acids. We have mutated the Arabidopsis SAMDC1 small uORF so that the initiator ATG is removed thereby abolishing the small uORF; we have also introduced a premature TAG stop codon into the small uORF so that the carboxy terminus is abolished. The SAMDC leader sequence variants were introduced between the CaMV 35S promoter and the GUS reporter gene. The constructs were introduced into tobacco and the translational efficiency of the leader sequence constructs was determined. Abolition of the small uORF relieves translational repression; the encoded product and not the nucleotide sequence of the small uORF is critical; the carboxy terminus of the small uORF is essential for translational repression.

5-22

Glucosinolate Biosynthesis

Carsten H. Hansen, Michael D. Mikkelsen, Ute Wittstock, Bent L. Petersen & Barbara A. Halkier
Dept. of Plant Biology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark

Glucosinolates are natural plant products found in the Capparales order. They have a wide range of biological effects e.g. as defence and flavour compounds. Glucosinolates are related to cyanogenic glucosides. Both groups of plant products are derived from amino acids and have oximes as intermediates. In the biosynthesis of cyanogenic glucosides in Sorghum bicolor a multifunctional cytochrome P450, CYP79A1, catalyzes the conversion of tyrosine to the corresponding oxime (1). In the glucosinolate containing plants Sinapis alba (2) and Tropaeolum majus (3) the conversion of amino acids to oximes has been shown biochemically to be catalyzed by cytochrome P450s. At least 6 different CYP79 homologs have been identified in Arabidopsis. They are presumably involved in the conversion of precursor amino acids to their corresponding oximes in the biosynthesis of glucosinolates. The 23 different glucosinolates in Arabidopsis are synthesized from tryptophan and chain elongated homologs of methionine and phenylalanine. The function of the Arabidopsis CYP79s is being studied by heterologous expression and construction of sense and anti-sense plants. Overexpression of CYP79B2 gives a dwarfed phenotype, but no change in the glucosinolate profile, which suggests that CYP79B2 is not rate limiting. The expression pattern of CYP79B2 has been investigated by construction of a promoter-GUS fusion. High GUS activity is measured in the leaves of young plants, in the roots and after mechanical wounding. Some activity is detected in the ends of the siliques. Additional analysis data will be presented.

1) Sibbelsen et al. (1995) J. Biol. Chem., 270, 3506
2) Du et al. (1995) Proc. Natl. Acad. Sci. USA, 92, 12505
3) Du & Halkier (1996) Plant Physiol. 111, 831

5-23

Characterization of Plant Peroxisomal Long-chain and Short-chain Acyl-CoA Oxidase
Hiroshi Hayashi1, Luigi De Bellis1,2, Akira Kato1, Makoto Hayashi1, Maki Kondo1 and Mikio Nishimura11
Department of Cell Biology, National Institute for Basic Biology, Okazaki, 444-8585, Japan, 2 Dipartimento di Biologia delle Piante Agrarie

We have characterized two acyl-CoA oxidases in fatty acid beta-oxidation of higher plants, namely long-chain and short-chain acyl-CoA oxidase with different substrate specificity. Long-chain acyl-CoA oxidase is synthesized as a large precursor containing a cleavable N-terminal presequence. This presequence has similarity to a peroxisomal targeting signal. However, short-chain acyl-CoA oxidase has a non-cleavable peroxisomal targeting signal localized at the carboxyl-terminus. Cell fractionation and immunocytochemical analyses revealed that the long-chain and short-chain acyl-CoA oxidases are localized in peroxisomes. Short-chain acyl-CoA oxidase was expressed by employing a baculovirus expression system. Although the molecular structure and the amino acid sequence of short-chain acyl-CoA oxidase are similar to those of mammalian mitochondrial acyl-CoA dehydrogenases, the purified short-chain acyl-CoA oxidase has no activity of acyl-CoA dehydrogenase. Thus, short-chain acyl-CoA oxidase actually oxidizes short-chain acyl-CoAs in plant peroxisomes and is not present in mammalian peroxisomes. During post-germinative growth, the expression patterns of long-chain and short-chain acyl-CoA oxidase were similar to that of 3-ketoacyl-CoA thiolase, one of peroxisomal fatty acid beta-oxidation enzymes. The enzymes of fatty acid beta-oxidation are cooperatively expressed during post-germinative growth and plant peroxisomes are able to completely degrade from fatty acids to acetyl-CoAs by the cooperative action of long-chain and short-chain acyl-CoA oxidases, whereas mammalian peroxisomes are not.

5-24

Overexpression of CAX1 in Tobacco: Altered Calcium Homeostasis and Increased Stress Sensitivity
Kendal D. Hirschi
Baylor College of Medicine, Texas Medical Center, Houston, TX, 77030, USA

Plants must maintain a precise balance of cytosolic and extracellular ion concentrations. Vacuolar ion transporters, such as CAX1 (CAlcium eXchanger 1), are thought to be key mediators of cytosolic ion concentrations. In order to clarify the role of CAX1 in general ion homeostasis, I examined CAX1 expression in response to various stimuli. CAX1 is highly expressed in response to exogenous Ca. I then produced transgenic tobacco plants that overexpress CAX1. These plants displayed necrotic lesions on leaves and blackening of the apical meristem. Transgenic plants demonstrated increased tonoplast-enriched Ca/H+ antiport activity as well as increased calcium accumulation. CAX1-overexpressing plants were hypersensitive to cold shock and an array of ion imbalances. Increased Ca in the media abrogated the ion sensitivity of CAX1-expressing plants and delayed the onset of necrosis. These results suggest that dysregulated CAX1 expression perturbs normal Ca homeostasis and markedly increases stress sensitivity.

5-25

Arabidopsis dynamin-like protein 2 is involved in the biogenesis of chloroplast in Arabidopsis
Sung Hee Kim(2), Jeong Hwa Lim(1), Soo Jin Kim(1), Shin Gene Kang(1), **Inhwan Hwang**(1,2)
(1) Center for Plant Intracellular Trafficking and (2) Department of Molecular Biology, Gyeongsang National University, Chinju, 660-701, Korea

Dynamin is a high molecular weight GTPase that is known to play an important role in the endocytosis in animal cells. We have isolated homologs of dynamin from Arabidopsis and characterizing the biological role of these proteins. Previously we have shown that ADL1 is localized at the thylakoid membranes and plays a important role in biogenesis of thylakoid membranes. Here we report that ADL2 was localized at the chloroplast envelope membrane and exists as a high molecular weight complex in vivo. Various chemicals such as nucleotides and Ca2+ affect the membrane-binding property of ADL2. We isolated a T-DNA insertion mutant from Felderman's T-DNA insertion library. The mutant had yellow leaves occasionally and germination rate of the mutant was reduced to approximately 50%. The presence of a highly homologous gene, ADL2-2 is likely a possible explanation for the incomplete penetration of the mutant phenotype. However transgenic plants with various deletion mutant ADL2 cDNA showed much clear phenotypes such as yellow caulin leaves, yellow siliques, and reduced fertility. These results suggest that ADL2 may play an important role in the biogenesis of chloroplast and in the embryogenesis. A model for the role of ADL2 in the chloroplast will be presented.

5-26

Post-translational regulation of polyamine biosynthesis in plants; isolation of ornithine decarboxylase-binding protein from Arabidopsis.
Crista Illingworth and Anthony J Michael
Division of Food Safety, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA, United Kingdom

Polyamines (PAs) are small polycations found in all cells. They play a vital role in cell proliferation in mammalian cells where the biosynthetic pathway is a target for chemotherapeutic intervention in carcinogenesis and tumour growth. PAs are essential for general protein synthesis and the key biosynthetic enzymes ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase are regulated at both the translational and post-translational level. In animal cells ODC is post-translationally regulated by a small protein termed antizyme whose abundance is determine by PA-mediated programmed frame-shifting of the antizyme mRNA. Plant cells also contain ODC-binding proteins: a cytoplasmic protein of 16 kDa and a chromatin-associated protein of 9 kDa (Koromilas and Kyrikiadis) which exist in an inactive complex with ODC.

We have used the yeast two hybrid system to search for ODC-binding proteins from Arabidopsis using the Datura stramonium ODC as bait. Employing the three reporter gene (HIS,ADE, LACZ) stringent two hybrid yeast strain J69-4A, all positive clones contained a partial cDNA potentially encoding the 9 kDa carboxy half of a small subunit ribosomal protein of 16 kDa. The biological significance of the binding is suggested by the importance of PAs in general translation and the prevalence of translational mechanisms in regulating PA biosynthetic genes.

5-27

Geranylgeranyl diphosphate synthases are distributed into four subsellular compartments in Arabidopsis.

Yuji Kamiya (1), Kazunori Okada (1), Takeshi Saito (2), Tsuyoshi Nakagawa (2), and Makoto Kawamukai (3)
(1) Plant Hormone Function, Frontier Research Program, RIKEN, (2) Res. Inst. of Mol. Gen., (3) Dept. of Appl. Bios. Biot, Univ. of Shimane.

Geranylgeranyl diphosphate (GGPP), which is 20-carbons isoprenoid, is well known for the precursors of gibberellins, carotenoids, chlorophylls in plant, and GGPP synthase (GGPPS) is thought to be one of the key enzyme for producing such kinds of diterpenes. In Arabidopsis, six GGPPS genes have been reported on data base, and all GGPPSs have possible transit peptides to translocate these proteins into proper subcellular locations. So far, we have examined subcellular localization of three of these proteins in Arabidopsis by using Green Fluorescent Protein (GFP). As a result, GGPS1-GFP and GGPS3-GFP protein were translocated into chloroplast, GGPS4-GFP and GGPS5-GFP proteins were localized in ER, and GGPS6-GFP protein was translocated into mitochondria. In northern analysis, GGPS andGGPS1 mRNA were constitutively expressed at all parts of plant. GGPS5 and GGPS4 mRNA were specially expressed at flower cluster and were slightly expressed at root, whereas GGPS3 was expressed at only root. GGPS6 mRNA expressions could not be detected even though we used RNA probe. Promoter-GUS assay revealed that GGPS1 was expressed at whole part of the plant except root, and GGPS5 was expressed at tapetum and root cap. GGPS6 was slightly expressed at veins in rosette leaf. From these results, we concluded that every GGPS gene must be expressed at the different organisms and at the different developmental stages, and GGPP could be synthesized by the organelles themselves rather than being distributed to different subcellular from one particular location.

5-28

Characterization of a starchfree mutant in A.thaliana

H. Kofler (1), R.E. Häusler (1), U.I. Flügge (1) and A. Weber (1).
(1) Botanisches Institut, LSII, University of Cologne, Gyrhofstr. 15, D-50931, Germany

Mutants in starch metabolism provide a good tool to investigate the reaction pathways as well as the regulatory aspects of starch biosynthesis and degradation. T-DNA insertion mutants and transposon tagged lines of A.thaliana were screened by iodine staining for mutants with altered starch phenotypes. One Ac/Ds transposon line was identified being starchfree in any plant tissue at all developmental stages. Crosses to already characterized starchfree lines revealed that the mutant of interest is allelic to mutants defective in the plastidial phosphoglucomutase (PGM-p). Genetic data were confirmed by native PAGE. Gels stained for phosphoglucomutase activity showed that leaf extracts of the mutant lack the activity of the plastidial isoform. The transposon insertion did not cosegregate with the starchfree phenotype. The mutation was mapped to chromosome V between the markers DFR and EG7F2 (91-118 cM). The Arabidopsis Genome Initiative published a P1 clone homologous to a phosphoglucomutase (chromosome V, 108 cM). A corresponding EST clone was identified and sequenced. The deduced protein sequence showed the presence of a plastid targeting sequence. In vitro protein import experiments with isolated spinach chloroplasts confirmed the plastid localization. Sequencing of the mutated allele revealed a 55 bp deletion including the translational start codon. A complementation assay of the mutant phenotype with the wildtype allele is in progress.

5-29

Functional analysis of cellulose-synthase-like genes in Arabidopsis
T. Konishi, F. Sakai and T. Hayashi
Wood Research Institute, Kyoto University

A putative gene for cellulose 4-beta-glucosyltransferase was isolated from cotton and rice as a homolog of Acetobacter BcsA and also identified in the recessive mutant gene rsw1 of Arabidopsis. Arabidosis genome projects have led to the discovery of more than 19 cellulose-synthase-like genes for so far. Why are there so many genes for cellulose synthesis? We believe that some of these genes encode other related glycosytransferases, rather than that all the genes are required for tissue-specific expression. In order to define and identify the activity of the gene products, we have developed a heterogeneous expression system in Pichia pastoris and COS cells. This system is useful to determine directly the activity of callose, xyloglucan, xylan or cellulose synthase from the recombinant proteins, and also to examine the interaction between sucrose synthase and glucosyltransferase during glycan synthesis. The expression vectors constructed contains a complementary T base for TA cloning at the cloning site and signals for secretion and antibody recognition attached to the structural gene inserted. In an attempted expression study for sucrose glucosyltransferase cDNA, the gene product was detected by Western blot analysis and showed sucrose synthase activity.

5-30

Plant responses to phosphate deficiency: a molecular approach
Julie C. Lloyd, Oksana Zakhleniuk, Matthew Jackson and Christine Raines
Department of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK

Phosphate is an important macronutrient for plant growth, however, little is known about how plants manage their phosphate economy at a cellular level, including the pathways for sensing and responding to phosphate levels and regulating phosphate distribution. In order to address the regulatory processes involved in phosphate metabolism we have taken a mutagenesis approach, developing a screening protocol to isolate mutants defective in their response to low phosphate. The screen is based on the ability of plants grown in low phosphate to induce APase secretion from roots and involves the use of an agar overlay to stain roots for the presence of this enzyme. We have used this method to screen pools of T-DNA-tagged Arabidopsis and isolated 3 mutants, which we have designated apd1, apd2 and apd3 (acid phosphatase deficient). The three mutants are phenotypically distinct from one another and show a range of features which are characteristic of phosphate deficient plants, even when grown in Pi sufficient conditions. Measurements of total phosphate indicate that the apd mutants all have reduced P content in comparison with wild type plants. Other effects observed in the apd mutants include retarded growth rates, delayed flowering and reduced fertility. We are continuing to characterise these mutants at a molecular level.

5-31

Characterisation of mutants of the gamma-glutamylcysteine synthetase gene of Arabidopsis
Maughan, S.C.(1), Wilson, R.C.(2), May, M.(3), Sung, Z.R.(2), Cobbett, C.S.(1)
(1) Department of Genetics, The University of Melbourne, Parkville, Victoria, Australia 3052.. (2) Department of Plant and Microbial Biology, 11 Koshland Hall, University of California, Berkeley, California, USA 94720. (3) Laboratorium voor Genetica, Universiteit Gent, KL Ledeganckstraat 35, B-9000, Gent, Belgium

Glutathione (GSH, gamma-glutamylcysteinyl glycine) is an ubiquitous low MW thiol in plant cells which is believed to play a significant role in various responses to stress. GSH is synthesised in a two step pathway from its constituent amino acids by two enzymes, gamma-glutamylcysteine synthetase (GCS) and glutathione synthetase. Previously we have described the cad2-1 mutant of Arabidopsis which exhibits 20-30% of wild type levels of GSH. This deficiency is due to a 6 bp deletion in the GCS gene. In addition, the rml1 mutant of Arabidopsis also contains a substitution mutation in the GCS gene which results in less than 5% of wild type levels of GSH being produced. Interestingly, both of these mutations reside in close proximity to a conserved cysteine residue within the proposed glutamate binding site of the GCS enzyme. In order to investigate this important region of the GCS enzyme we are undertaking a structure-function analysis focusing especially on the conserved cysteine residue.

5-32

Identification of T-DNA insertions in genes involved in nitrate transport or nitrate reductase regulation
Christian Meyer (1), Gil Savino (1), Sophie Filleur (1), Fabienne Granier (1), David Bouchez (1), Alain Gojon (2) and Françoise Vedele (1)
(1) Laboratoire de Biologie Cellulaire, INRA Versailles, 78026 Versailles, France, (2) Biochimie et Physiologie Moléculaire des Plantes, INRA/ENSAM, Place Viala, 34060 Montpellier, France

In most higher plants the nitrate assimilatory pathway provides the reduced nitrogen needed for plant growth and development. Nitrate is taken up from the soil by an active process and is first reduced into nitrite by nitrate reductase (NR). Genes coding for putative high affinity nitrate transporters have been isolated from Arabidopsis based on their homology with fungal and algal nitrate transporters. Evidence that these genes actually participate in nitrate transport is, however, still lacking. The nitrate reduction by NR is known to be a highly regulated process. Indeed it has been shown that NR is phosphorylated in the dark and that the phosphorylated enzyme is inactivated by subsequent binding of proteins belonging to the 14-3-3 family. There are at least 10 members of the 14-3-3 protein gene family in Arabidopsis and the question of whether there is an isoform specificity for particular partners among the 14-3-3 proteins remains open. Thus in order to determine which gene(s) of these families are actually involved in nitrate uptake or NR inactivation, we have identified T-DNA tagged Arabidopsis lines in the Versailles collection carrying an insertion in one the putative high affinity nitrate transporter gene (AtNrt2:1) or in some 14-3-3 genes. In both cases null mutants were obtained. The consequences on nitrate transport of the disruption of the AtNrt2:1 gene and the impact on NR inactivation in the dark of the disruption of 14-3-3 isoforms has been investigated. This work was partly supported by the EU contract n° BIO4CT97-2231.

5-33

Site-directed mutagenesis of the Arabidopsis arginine decarboxylase indicates that the active site is formed across the interface of the subunits.

Anthony J Michael and Susanne Sommer
Division of Food Safety, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA, United Kingdom

Arginine decarboxylase (ADC) is a key enzyme in plant and bacterial polyamine biosynthesis but it is not found in animal cells. ADC, bacterial meso-diaminopimelate decarboxylase (involved in lysine biosynthesis) and the eukaryotic ornithine decarboxylase (ODC) may have arisen from the same ancestor. The ADC preprotein of oat is processed to give a 42 kDa N-terminal peptide and a 24 kDa carboxy terminus. It has been suggested that the processing of the enzyme is necessary for enzyme activity and is achieved by a regulatory and distinct protease. We have expressed the Arabidopsis ADC in yeast, which does not contain the ADC pathway. By aligning conserved regions of the mouse ODC and the Arabidopsis ADC, we were able to putatively identify the N-terminal pyridoxal 5' phosphate cofactor binding site and the C-terminal substrate binding site. Co-expression of N and C-terminal site-directed mutants indicated that the active site is in trans between subunits, and expression in a general protease deficient yeast strain suggests that activity of ADC in yeast may be due to general proteolytic cleavage of the heterogeneously expressed Arabiopsis protein rather than a specific regulatory ADC cleavase.

5-34

Use of a "smart" genetic screen in yeast to isolate Arabidopsis suppressors of the growth defect caused by low levels of polyamines.

Elodie Morlon and Anthony J Michael
Division of Food Safety, Institute of Food Research, Norwich Research Park, Norwich, NR4 7UA, United Kingdom

In order to understand the biological function of polyamines (PAs) we employed a "smart" genetic screen to investigate polyamine function. Most cells contain both a PA biosynthetic pathway and a PA uptake transport system.
We took a PA biosynthetic mutant of yeast mutated in the S-adenosylmethionine decarboxylase gene and employed one-step gene deletion using the kanMX4 approach knockout out the PTK2 serine/threonine protein kinase that regulates high and low affinity PA uptake in yeast. This double biosynthesis/uptake mutant can take up only low levels of PAs. Without added PAs the yeast cannot grow as PAs are essential for cell proliferation but with 10 micromolar spermidine added to the medium there is sluggish growth. We transformed the double mutant with the pFL61 Arabidopsis cDNA yeast expression library of Minet and Lacroute and after a selective growth enrichment process obtained colonies encoding only a few different cDNAs. In the presence of low PA levels,selective growth advantage was obtained by the over-expression of an Arabidopsis ATP-dependent DEAD-box RNA helicase as well as a nucleoside diphosphate kinase, suggesting that RNA processing and translation may be the sensitive steps when PA levels are depleted.

5-35

Point Mutations at the 3' End of omr1, the Gene Encoding Threonine Dehydratase/Deaminase of Arabidopsis thaliana, Alter Isoleucine Feedback Inhibition

George Mourad, Richard Emerick, Alan Smith
Department of Biology, Indiana-Purdue University, Fort Wayne, IN 46805, USA

In Arabidopsis thaliana, we isolated an L-O-methylthreonine (MTR)-resistant mutant line GM11b overproducing free Ile by 20-fold due to an Ile feedback insensitive threonine dehydratase/deaminase (TD) encoded by a single gene omr1 (Mourad and King, 1995, Plant physiol 107:43-52). In order to reveal the molecular basis of Ile feedback insensitivity in GM11b, we isolated and molecularly characterized cDNA clones of omr1 and its wild type allele OMR1. A homologous probe generated by PCR amplification using degenerate primers was used for probing the omr1 cDNA library. DNA sequencing revealed that the full length omr1 was 1779 nucleotides encoding a deduced polypeptide of 592 amino acids. omr1 differed from OMR1 by two base substitutions. One was a C to T transition at nucleotide 1495 predicting a change from Arg (wild type) to Cys (mutant) at amino acid residue 499. The other was a G to A transition at nucleotide 1631 predicting a change from Arg (wild type) to His (mutant) at amino acid residue 544. Both amino acid changes resided in a highly conserved region in the regulatory domain of TD. Upon transformation, omr1 was able to revert an auxotrophic strain of E. coli lacking TD to prototrophy. When omr1 was driven by the CaMV 35S promoter and expressed in A. thaliana Columbia wild type plants, it conferred upon transgenic plants resistance to MTR. omr1 has two important applications. It could be used in genetic engineering of plants for Ile overproduction and also as a stringent dominant biochemical selectable marker in the genetic engineering of plants and bacteria.

5-36

Autoregulation of Cystathionine gamma-Synthase mRNA Stability in Response to Methionine

Y. Chiba, F. Kijima, M. Ishikawa, H. Tyson(1), J. Kim(2), A. Yamamoto, **E. Nambara**, T. Leustek(2), R. Wallsgrove(1), S. Naito
Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan. (1) IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK. (2) Center for Agricultural Molecular Biology, Rutgers University, New Brunswick, NJ 08903-0231, USA.

Regulation of Met biosynthesis was studied using mto1 mutants that overaccumulate soluble Met. In wild type, accumulation of mRNA for cystathionine gamma-synthase (CGS), the key enzyme of Met biosynthesis, was downregulated by Met application. On the other hand, the mutant had an elevated level of CGS mRNA regardless of Met treatment, indicating wild-type has a mechanism to downregulate the level of CGS mRNA in response to Met (or its metabolite), whereas this regulation is impaired in the mto1 mutant. Nuclear run-on experiments suggested this regulation is post-transcriptional. Sequence analysis revealed that 5 independently isolated mto1 mutants are clustered in a small region of 8 amono acids within the first exon of CGS gene. Each mutation altered the amino acid sequence. Wild-type and mutant exon 1 was joined in-frame to reporter gene(s) and placed under the control of CaMV 35S promoter. Electroporation experiments showed that the reporter activity of wild-type exon 1 construct was repressed by Met, while that of the mto1 constructs were not appreciably affected, indicating that the exon 1 has sufficient information to downregulate the reporter activity in response to Met. In vitro mutagenesis showed that amino acid sequence is responsible for this regulation. Co-electroporation experiments showed that neither wild-type nor mutant exon 1 affects the reporter activity of the other, indicating the mutaion acts in cis. We propose a model in which the nascent polypeptide of CGS exon 1 is responsible to destabilize its own mRNA.

5-37

Characterisation of Arabidopsis T-DNA mutants affected in flavonoid metabolism in the seed coat.
NESI, N. (1), DEBEAUJON, I. (1), JOND, C. (1), KOORNNEEF M. (2), CABOCHE, M. (1) and LEPINIEC, L.(1)
(1) Laboratory of Seed Biology, INRA Versailles France; (2) Laboratory of Genetics, Univ. of Wageningen, NL.

The project aims to analyse seed coat flavonoid nature and content in Arabidopsis and the influence of these pigments on seed quality (dormancy, longevity and seedling vigour). For this purpose, we are studying mutants affected in seed coat pigmentation. Such mutants are isolated from the Versailles T- DNA insertion collection (G.Pelletier). Previous works have demonstrated that seed viability and dormancy depend to a great extend on the structure and the chemical composition of the seed coat. Particularly, a correlation has been established between testa flavonoids and seed longevity, probably due to their impermeable and antioxidant properties. However, phenolic compounds have been demonstrated to delay germination. Although 20 loci implicated in flavonoid biosynthesis have been already identified (including transparent testa mutations), only a few genes involved have been cloned. Finally, there is a few biochemical and molecular data concerning the precise nature of the flavonoid compound(s) (and therefore enzymes or regulatory factors) involved in seed quality. We identified 22 seed colour mutants of which physiological, genetic and molecular studies are underway and will be presented. Four of these mutants represent new tt loci. Besides, for three other lines the corresponding mutations are genetically linked to the T-DNA insertion and we isolated genomic and cDNA clones using usual methods for T-DNA tagging. The corresponding predicted aa sequences show similarities with transcription factors and a putative glucosyltransferase. Thorough analysis of these mutants will provide effective means to identify new genes involved in the flavonoid metabolism.

5-38

Regulated nuclear localization of homeodomain proteins
Stephanie Ohnesorge and Artur Pfitzner
Institute for Genetics, General Virology University for Hohenheim; steffio@uni-hohenheim.de

For their function as transcription factors homeodomain proteins have to be directed to the nucleus. Recently it was shown several times that the transport of transcription factors into the nucleus could be regulated. For the homeodomain protein KN1 it has been postulated that it is apart from the nuclear localization transported from cell to cell via plasmodesmata. To investigate further the subcellular localization of KN1 like and other homeodomain proteins the homeodomain proteins KNAT4 and BEL1 from A. thaliana were characterized in their subcellular distribution. The proteins were fused to GFP and transiently expressed in protoplasts and onion cells. In both systems the proteins showed a bipartie localization, to the nucleus and / or to cytoplasmic structures. By means of deletionmutants the target signal to the nucleus could be restricted to the C-terminal part and the signal for cytoplasmic localization to the N-terminal part of these proteins.

5-39

A subunit of a chlorophyll biosynthetic enzyme, the magnesisum chelatase, is encoded by a single gene in the large Barley genome and a multi-gene family in the small Arabidopsis genome.

Heather Rissler1, Eva Collakova2, Dean DellaPenna2 and **Barry J. Pogson**1
1Dept. of Plant Biology, Arizona State University, Tempe AZ 85287-1601, U.S.A., 2Dept of Biochemistry, University of Nevada, Reno, NV 89557, U.S.A.

The magnesium chelatase catalyzes a key reaction in the chlorophyll biosynthetic pathway, the insertion of magnesium into protoporphyrin IX to form magnesium-protoporphyrin IX. Studies of the enzyme, reconstituted in vitro, have shown that all three subunits of the enzyme complex, CHLI, CHLD, and CHLH, are required for enzymatic activity and the CHLI subunit is reported to be encoded by a single gene in Barley and tobacco. Yet a null mutant of the chlorina, ch42 (chlI), locus in Arabidopsis (ch42-3) is still able to accumulate some chlorophyll despite the apparent absence of chlI message and protein and a T-DNA insertion 6 bp downstream of the start methionine that results in the absence of any promoter or transcription start site. We identified a BAC open reading frame with 82% similarity to the known chlI sequence that apparently encodes a second chlI gene, chlI2. Significantly, this gene is expressed, yet the amount of chlorophyll biosynthesis not sufficient for plant viability, except on sucrose supplemented media. In general, Arabidopsis has fewer genes per protein in a gene family that most other plant species, so this results is curiously atypical. It is also curious as to what is the functional significant of this second gene.

5-40

Interactions of Arabidopsis acetolactate synthase (AtALS) catalytic subunit with two novel proteins, C1 and C3, as revealed by in vivo interaction experiments

W. Donald, **R. Pokatong** (1&2), P. Schorr (1), R. T. Tyler (2), W. L. Crosby (1)
(1) Gene Expression Group Plant Biotechnology Institute, 110 Gymnasium Place Saskatoon, SK Canada S7N 0W9, (2) Dept. of Applied Microbiology and Food Science University of Saskatchewan 51 Campus Drive, Saskatoon, SK Canada S7N 5A8

Acetolactate synthase (ALS, EC4.1.3.8) is the first enzyme in the ilv biosynthetic pathway leading to the formation of isoleucine, valine and leucine. The enzyme is post-translationally regulated by direct feedback inhibition of the end products of the pathway. Previous work in our lab using the yeast two-hybrid system revealed that Arabidopsis ALS catalytic subunit interacted with 10 classes of proteins. Two of these (C1 and C3) exhibit significant deduced sequence similarity (365%) to several ALS regulatory and catalytic subunits from prokaryotes and eukaryotes, respectively. The objective of this work was to verify by independent biochemical assessment the existence of ALS-mediated protein interactions in plants as revealed by the yeast 2-hybrid system. We have generated transgenic lines of Arabidopsis expressing N- and C-terminal His6-tagged derivatives of the chlorsulfuron (CS) resistant mutant of ALS (AtALS-CSR1-1 mut.). Transgenic lines exhibited resistance to CS indicating that the modified ALS was functional were obtained. To assess the interaction of C1 and/or C3 with the tagged ALS catalytic subunit IMAC cobalt affinity batch chromatography was employed to specifically immobilize the gmALS catalytic subunit. The results indicated that C1 and C3 bound to the Co+2 bead matrix via a catalytic subunit protein interaction. The results suggest that the Arabidopsis ALS catalytic subunit interacts with two novel potential regulatory proteins, C1 and C3, and that this interaction persists in vivo.

5-41

Identification of putative galactosyltransferases from Arabidopsis thaliana
Yongmei Qu, Paul Gilson, and Antony Bacic
Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, VIC 3052, Australia

Galactosyltransferases (GalTs) are a group of enzymes that catalyse the addition of galactosyl residues to glycan chains, peptide or lipid acceptor molecules. Although GalT activity has previously been reported from plants, to date no GalT genes have been cloned and characterised from plants. To identify putative plant GalTs, we have searched the Arabidopsis sequence database with the genes encoding animal GalTs and have identified several candidates. Analysis of the secondary structures of these plant proteins reveals they are similar to animal GalTs. These enzymes are type II transmembrane proteins, containing a short N-terminal cytoplasmic tail, a transmembrane domain, and a large C-terminal globular catalytic domain that projects into the lumen of endoplasmic reticulum or Golgi apparatus. Putative plant GalT genes are currently being expressed in Escherichia coli where their enzyme activity will then be assayed. It is anticipated that once the acceptor specificity and the type of linkage synthesized by GalTs are known, then it may be possible to predict their precise enzymatic function in the various glycosylation pathway of plants.

5-42

Using Mutants to Elucidate Iron Uptake Responses in Arabidopsis
Elizabeth E. Rogers(1), David J. Eide(2) and Mary Lou Guerinot(1)
(1)Dept. of Biological Sciences, Dartmouth College,Hanover, NH, USA and (2)Dept. of Food Science and Human Nutrition, University of Missouri, Columbia, MO, USA

In response to iron deficiency, all plants except the grasses induce Fe(III) chelate reductase activity, Fe(II) transport activity and proton release into the rhizosphere. Previously, we identified an Arabidopsis mutant, frd3, that shows high levels of Fe(III) chelate reductase activity under both iron sufficient and deficient conditions. The frd3 mutant is allelic to the manganese overaccumulator man1 and, like man1, has elevated levels of iron, manganese and other metals in its tissues. Consistent with this phenotype, frd3 constitutively expresses the FRO2 Fe(III)chelate reductase gene and the iron-responsive transporter IRT1. Cu(II) reductase activity is also increased in frd3. The tomato mutant chloronerva also exhibits constitutive iron deficiency responses and, like frd3, is chlorotic. chloronerva does not synthesize the non-protein amino acid nicotianamine and its phenotypes are reversed by application of nicotianamine. frd3 is unaffected by nicotianamine. frd3 maps to chromosome 3 and a positional cloning effort is underway. The iron-regulated transporter IRT1 belongs to a novel family of metal transporters from a diverse array of eukaryotes. Arabidopsis family members variously transport iron, zinc and manganese when expressed in yeast. In plants, three of the family members, ZIP1, ZIP3 and ZIP4, are up-regulated by zinc deficiency and one, IRT1, is up-regulated by iron deficiency. We have constructed site directed mutations to elucidate how these transporters function. Three IRT1 mutant transporter alleles transport only a subset of the metals that the wild type IRT1 can transport.

5-43

Isolation and characterisation of a Magnesium Transporter from Arabidopsis thaliana
Ana F. Tutone and Richard C. Gardner
School of Biological Sciences, Auckland University, Private Bag 92019, Auckland, New Zealand

Magnesium (Mg) is one of the most abundant and important divalent cations in biological systems, yet its biology is perhaps the most poorly understood. Mg is an essential ingredient in a wide range of crucial cellular reactions including photosynthesis in plants. To date no Mg transport genes have been cloned from higher eukaryotes and therefore little is known about Mg accumulation and the regulation of intracellular Mg levels by eukaryotic cells. The goal of this research is to isolate and characterise genes for transport of Mg using a yeast complementation approach. A yeast expression library made from Arabidopsis thaliana cDNA was screened in a mutant yeast strain in which the Mg transport system (ALR) has been deleted. This strain requires high levels of Mg for growth. An Arabidopis thaliana clone (L8) was identified as an effective suppressor of the alr mutant phenotype. L8 encodes a protein of unknown function which contains structural features similar to bacterial (CorA) and yeast (ALR1 and ALR2) Mg transporter genes. Comparison of the L8 sequence with the DNA sequence database has identified several homologous EST's derived from human, mouse, yeast and Arabidopsis thaliana. I am presently using the Xenopus oocyte heterologous expression system for functional analysis of this potential Mg transporter (L8). Voltage clamping and Mg ion sensitive electrodes are used to measure the movement of Mg across the cell following injection of the complementary RNA of L8 into oocytes.

5-44

Characterization of a xyloglucan-specific cell wall mutant mur2
Gary F. Vanzin (1), Ken Keegstra (2) Natasha Raikhel (2), and Wolf-Dieter Reiter (1)
(1) University of Connecticut, Department of Molecular and Cello Biology, Storrs, CT, (2) MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI

The plant cell wall plays an integral role in the maintenance of cell structure and function. To better understand the multiple roles of the plant cell wall a screen was devised to identify Arabidopsis mutants with altered cell wall monosaccharide composition. One mutant isolated, mur2, was found to be 50% deficient in cell wall L-fucose. Fucose feeding studies indicate that mur2 is not affected in the biosynthesis of GDP-L-fucose, but most likely in a downstream event. Recently an Arabidopsis gene termed AtFT1 was identified and characterized as a a-(1,2) fucosyltransferase. This enzyme utilizes GDP-L-fucose for the addition of L-fucose to the 2-position of galactose in xyloglucan, the major hemicellulosic polysaccharide in dicot cell walls (Perrin, DeRocher, Bar-Peled, Zeng, Narambuena, Orellana, Raikhel and Keegstra, Science, in press). AtFT1 has been shown to genetically map to the mur2 locus, and sequencing data indicate that there is a point mutation changing an aspartic acid to an asparagine, creating a potential N-glycosylation site (NXS/T). Complementation of mur2 plants with the AtFT1 gene restores wild type levels of fucose within the cell wall, further verifying that AtFT1 and MUR2 represent the same gene. In plants L-fucose is found within three different cell wall polysaccharides: xyloglucan, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). Chemical fractionation and mass spectrometry analysis of wild type and mur2 cell wall polysaccharides indicate that mur2 xyloglucan is completely lacking fucose, while RG-I and RG-II contain wild type levels of fucose. Although the mur2 mutation is predicted to abolish a xyloglucan-derived growth regulator, mur2 plants do not show any obvious visible phenotype.

5-45

Subcellular localization of plant hexokinases and their possible role in a sugar sensing pathway
A. Wiese (1), F. Gröner (1), J. Lerchl (2), U. Sonnewald (2), H. Deppner (2), U. Hebbeker (1), U.I. Flügge (1) and A. Weber (1).
(1) University of Cologne, LS Botany II, Gyrhofstr. 15, 50931 Cologne, Germany (2) IPK, Correnstr. 3, 06466 Gatersleben

Sugars have an influence on growth and development as well as on metabolism and gene-expression in plants. In analogy to the sugar sensing system in yeast, plant hexokinases seem to be involved in the generation or transmission of the sugar signal. In a DDRT-PCR of glucose and water fed spinach leaves, we identified a differentially expressed hexokinase. After isolation of a full length cDNA (SoHxK1) and sequence analysis, a hydrophobicity plot revealed a putative n-terminal membrane anchor. A specific antiserum against recombinant SoHxK1 was raised and used to determine the subcellular localization of SoHxK1. A possible function of this hexokinase with regard to its subcellular localization will be discussed. A function of SoHxK1 as a sugar sensor will be investigated in SoHxK1-overexpressing Arabidopsis thaliana and potato plants. Further screenings of cDNA libraries allowed us to identify a hexokinase from tobacco (NtHxK) which shows ca. 70% sequence identity with SoHxK1 and a predicted membrane anchor in the same position. Under less stringent screening conditions we identified soluble hexokinase isoforms in a potato tuber and a spinach leaf cDNA library. Detailed analysis of subcellular localization, substrate specifities and possible sugar sensing function of these hexokinase isoforms is in progress.

5-46

Vacuolar Processing System in the Lytic Vacuoles of Vegetative Tissues During Senescence andunder Various Stressed Conditions
Kenji Yamada, Tetsu Kinoshita, Nagako Hiraiwa, Maki Kondo, Mikio Nishimura, Ikuko Hara-Nishimura (Department of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan)

Vacuolar processing enzyme (VPE) has been shown to be responsible for maturation of various seed proteins in protein-storage vacuoles. Arabidopsis has three VPE homologues; beta-VPE is specific to seeds and alpha-VPE and gamma-VPE are specific to vegetative organs. To investigate the activity of the vegetative VPE, we expressed the gamma-VPE in a pep4 strain of the yeast Saccharomyces cerevisiae and found that gamma-VPE has the ability to cleave the peptide bond at the C-terminal side of asparagine residues. An immunocytochemical analysis revealed the specific localization of the VPE in the lytic vacuoles of Arabidopsis leaves that had been treated with wounding. This result supports the possibility that a VPE-mediated system similar to that in seeds might function in the lytic vacuoles. The vegetative VPE promoters were found to direct the expression of the b-glucuronidase reporter gene in senescent tissues, but not in young intact tissues. This is supported by the results of the immunoblot analysis of the tissues with gamma-VPE-specific antibodies. The mRNA levels of the vegetative VPEs were increased in the primary leaves during senescence. The results raise the question of what are the target proteins of the vegetative VPEs. The target proteins of vegetative VPEs might also be senescence-inducible hydrolytic enzymes localized in the vacuoles. We found that the vegetative VPE was accumulated in leaves during senescence in parallel with the increase of the level of RD21A, a proteinase of papain family. Treatment with wounding, ethylene and salicylic acid up-regulated the expression of alpha-VPE and gamma-VPE, while jasmonate slightly up-regulated the expression of gamma-VPE. These gene expression patterns of the VPEs were associated with the accumulation of vacuolar proteins that are known to respond to these treatments. Taken together, the results suggest that vegetative VPE might regulate the activation of some functional proteins in the lytic vacuoles.

5-47

AtVTI1a, a v-SNARE possibly involved in vesicle trafficking between the Golgi and the prevacuolar compartment

Haiyan Zheng(1), Gabriele Fischer von Mollard(2#), Valentina Kovaleva1, Tom H. Stevens (2), and Natasha V. Raikhel(1*)
(1) DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 USA(2) Institute of Molecular Biology, University of Oregon, Eugene OR 97403-1229 USA# Present address: Georg-August-University, Biochemie II, Göttingen, Germany

Protein targeting to the vacuole in the plant cell is complex due to multiple vacuoles and multiple pathways for targeting. Characterization of components involved in these complex processes helps us to start to understand the cellular machinery of vacuolar protein targeting. Previously, we have identified AtELP, a putative cargo receptor on the TGN and AtPEP12p, a t-SNARE on the prevacuolar compartment (PVC). Data from studies of yeast and mammalian cells shows that Vti1p is a v-SNARE involved in multiple routes for delivering different vacuolar proteins. We have identified AtVTI1a and AtVTI1b, two Arabidopsis homologues of Vti1p. These two proteins share 60% sequence identity. Both AtVTI1a and b complemented the yeast vti1 mutant. Among three pathways to the vacuole in which yVti1p is involved, AtVTI1a restored one route while AtVTI1b functioned in the other two. To study these two proteins separately in plant, we epitope tagged AtVTI1a with a T7-tag and AtVTI1b with a HA-tag. Using T7-AtVTI1a transgenic plants, we showed that AtVTI1a formed a SNARE complex with AtPEP12p and AtVAM3p (another homologue of AtPEP12p) in vivo. Electron micrographes showed that AtVTI1a co-localized with AtELP on the TGN and the PVC, and with AtPEP12p on the PVC. We propose that AtVTI1a is a v-SNARE involved in a subset of cargo proteins transport from the TGN to the PVC. The characterization of AtVTI1b is in progress.

6-1

Perception, Signaling and Responses to a Simple Hydrocarbon

Joseph R. Ecker et al.,
Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018 USA

Ethylene regulates plant growth, development and responsiveness to a variety of stresses. The isolation of the genes corresponding to ethylene-related mutants in Arabidopsis has substantially improved our understanding of the processes of ethylene biosynthesis, perception, signaling and response to this gas. Recent progress on understanding the function of these genes/proteins in the ethylene response pathway will be presented.

6-2

Gibberellin Signal Transduction in Arabidopsis

A.L. Silverstone, A. Dill, H-S. Jung & **T-P. Sun**
Dept. of Botany, Duke University, Durham, North Carolina 27708, USA

The recessive rga mutation is able to partially suppress phenotypic defects of the Arabidopsis gibberellin (GA) biosynthetic mutant ga1-3. This indicates that RGA is a negative regulator of the GA signal transduction pathway. Cloning and sequencing of the RGA gene revealed that it is a member of the GRAS protein family, which includes the radial patterning gene SCARECROW and another GA signal transduction repressor, GAI. The presence of several structural features, including homopolymeric serine and threonine residues, a nuclear localization signal and leucine heptad repeats, indicates that the RGA protein may be a transcriptional regulator that represses the GA response. In support of this, we showed that a green fluorescent protein-RGA fusion protein is nuclear localized in transgenic Arabidopsis plants. Comparing the GRAS family proteins, we have identified 3 conserved domains (named after motifs of highly conserved amino acid residues): the N-terminal DELLA domain, the central VHIID domain, and the C-terminal RVER domain. The DELLA domain is unique to RGA, GAI and RGL (for RGA-like). Peng et al. (1997) found that the semi-dominant gai mutant contains a 17-amino acid in-frame deletion within the DELLA domain, and proposed that this deletion turns the gai protein into a constitutively active repressor of GA signaling. Because the sequences that are deleted in gai are identical between GAI and RGA, we tested whether an identical deletion mutation (rga-delta 17) in the RGA gene would have the same effect as the gai allele. Our results showed that the rga-delta 17 allele is semi-dominant and causes a GA-unresponsive dwarf phenotype in transgenic Arabidopsis plants. Therefore, the DELLA domain is likely to be important for the inactivation of both RGA and GAI by the GA signal.

6-3

Regulating cellular interactions by modifying extracellular properties
Robert E. Pruitt, Katherine Krolikowski, Sara E. Ploense and Susan J. Lolle.
Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA

Our research is concerned with how the epidermal cells found on the surface of all plants regulate their communication with the outside world. These cells must interact with and respond to a wide variety of signals which come from the external environment. These signals include those coming from other plant cells, from other beneficial or pathogenic organisms, as well as signals coming from the environment itself. We have used large scale genetic analysis to look at two independent types of interactions (pollen/stigma interactions and inter-organ fusion) and have found that both appear to be regulated by a common mechanism: the cuticle found on the outside surface of the epidermal cells controls what signals can be sent or received by those cells. This is regulated by subtle changes in the biochemical composition of the cuticle itself which are at present poorly understood. Our research has identified a series of genes which are responsible for controlling the permeability of the cuticle and we are in the process of characterizing those genes at the molecular level. In addition to understanding the role these genes play in regulating epidermal cell interactions, an understanding of the biochemical function of these genes could lead to our ability to manipulate the cuticle through biotechnology, allowing us to engineer plants which are more or less sensitive to chemical compounds provided from the outside (herbicides, pesticides or plant growth regulators, for example) as well as providing a potential new mechanism for engineering resistance to plant pathogens.

6-4

A plant homolog of mammalian ionotropic glutamate receptors controls light- and auxin-induced opening of stomatal aperture
J.M. Kwak (1&2), S.A Kim (1), S. Thomine(2), E.S Shin (1), S-K. Jae (1), C.H Goh (1), J.I. Schroeder (2) & H.G Nam (1)
(1)Dept. of Life Science, Pohang Univ. of Science & Technology, Hyoja Dong, Pohang, Kyungbuk 790-784, Republic of Korea, (2) Dept of Biology and Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

Guard cells form stomata, microscopic pores in the epidermis of plant leaves, through which carbon dioxide intake and water evaporation occur. To identify molecular components involved in stomatal regulation, we previously generated expressed sequence tags (ESTs) of guard cells of Brassica campestris [Kwak, J.M., et al, (1997) Planta 202, 9]. One of the ESTs, BnGluR1, encodes a protein related to mammalian glutamate receptor (GluR) ion channels and this protein is required for stomatal opening. The deduced peptide (BnGluR1) is 912 amino acids long and contains all of the signature domains of mammalian ionotropic GluRs. The predicted secondary structure and membrane topology are also very similar to those of mammalian GluR ion channels. The gene is expressed in guard cells as revealed by in situ hybridization. Transgenic tobacco plants expressing antisense BnGluR1 transcripts exhibit impairment in light- and auxin-induced but not fusicoccin-induced stomatal opening. Inward-rectifying K+ channel and R-type anion channel currents were of similar magnitude and characteristic in control and antisense plants. The antisense plants showed reduced stomatal conductance in well-watered conditions and drought tolerance. These results show that BnGluR1 plays an important role in regulation of light- and auxin-induced stomatal opening as an upstream signaling component. The results also indicate that water loss during drought stress can be modulated through regulation of the BnGluR1 gene.

6-5

Do heterotrimeric G-proteins regulate translation in plants?

Jose R. Botella and Andrew M. Plume
Plant Genetic Engineering Laboratory Department of Botany University of Queensland Brisbane, Qld 4072 Australia

Heterotrimeric G proteins (G proteins) are members of the GTPase superfamily, a group of related proteins with diverse cellular roles. G proteins consist of Ga, Gb, and Gg subunits. In animals, G proteins mediate transmembrane signal transduction from receptors to intracellular effectors, but their role in plants is currently unknown. We have used the yeast two-hybrid system (Y2H) to identify proteins that interact with the Arabidopsis thaliana Ga subunit, GPa1. We have isolated a partial cDNA (AtSUP35) encoding a protein with significant homology to the SUP35 (eRF3) protein family. SUP35 proteins are also G proteins which enhance the efficiency of translation termination by the eukaryotic ribosome. Southern blot analysis suggests that AtSUP35 is a low copy gene. AtSUP35 mRNA transcripts were detected at similar levels in all tissues tested. Deletion analysis shows that the central 'switch' region of GPa1 is sufficient for interaction with AtSUP35. Conversely, the N-terminal region of AtSUP35 is responsible for the interaction with GPa1. We have also used the Y2H to prove the interaction between AtSUP35 and its interaction partner AtSUP45. Functional complementation of a yeast SUP35 mutant strongly suggests that AtSUP35 acts as a translation factor in vivo. We propose a model for the involvement of Ga in transmembrane signalling in plants.

6-6

Analysis of the promoter of a plant defensin gene for dissection of a jasmonate-mediated systemic signalling pathway

Rebecca Brown(1), Kemal Kazan(1), Donald Maclean(1), Willem Broekaert(2) and John Manners(1)
(1)Cooperative Research Centre for Tropical Plant Pathology, John Hines Building, University of Queensland, St Lucia, QLD Australia 4072 (2)F.A. Janssens Laboratory of Genetics, Katholieke Universiteit Leuven, Belgium

The PDF1.2 gene of Arabidopsis encodes a plant defensin that may be involved in plant defence against fungal pathogens. This gene is induced by fungal attack, both locally at the site of infection and systemically in unchallenged regions of the plant. PDF1.2 is also induced systemically by the application of jasmonates and ethylene, but not by salicylic acid (SA), suggesting that a SA-independent systemic signalling pathway is responsible for the induction of this gene. The PDF1.2 promoter fused to the GUS reporter gene behaves very similarly to the native gene in transgenic plants, and is pathogen, jasmonate and ethylene inducible. Thus, this construct provides a valuable tool for the dissection of the jasmonate-mediated systemic signalling pathway leading to the induction of PDF1.2. In order to identify cis regulatory elements involved in local and systemic induction by pathogens and chemical signal compounds, a 5' deletion analysis of the PDF1.2 promoter has been undertaken. Translational fusions of the promoter deletion sequences were made to the GUS reporter gene, and these constructs stably introduced into Arabidopsis. Analysis of second generation transgenic plants has indicated that the GCC box, which has been show to be involved in ethylene sensitivity in other genes, is required for induction of PDF1.2 by methyl jasmonate. Local and systemic induction are also being assessed, to determine if these responses can be uncoupled. It is envisaged that these approaches may lead to the isolation of transcription factors involved in the regulation of the jasmonate-mediated systemic defence responses in Arabidopsis.

6-7

Gene disruptions of calcium-dependent protein kinases in Arabidopsis thaliana: Towards understanding their in vivo functions

Catherine W. M. Chan and Michael R. Sussman
Departments of Horticulture and Genetics, University of Wisconsin-Madison, Biotechnology Center, 425 Henry Mall, Madison, Wisconsin 53706 USA.

A typical calcium-dependent protein kinase (CPK) consists of a serine/ threonine protein kinase domain fused to a series of calcium-binding motifs. Therefore, CPKs are capable of being directly activated by intracellular calcium and can potentially play important roles in mediating calcium signaling in a variety of cellular processes. To date, CPKs are found only in plants, algae and protists. The result from the Arabidopsis thaliana genome sequencing project suggests that the CPK gene family may contain as many as 40 or more different members, and possibly represent the largest group of serine/ threonine protein kinases. To gain insights into the in vivo functions of CPKs, reverse genetic analyses are carried out on A. thaliana, a plant model system. Taking advantage of the ability of the transferred DNA (T-DNA) of Agrobacterium tumefaciens to integrate into the plant genome and act as an insertional mutagen, plants with disrupted CPK genes are isolated using a PCR-based technique, essentially as reported by Krysan et al. (Proc. Natl. Acad. Sci. USA (1996) 93: 8145-8150). We have identified T-DNA insertions in seven different CPK genes, and are in the process of finding additional CPK mutants. We are phenotypically analyzing plants with one or more disrupted CPK genes under different growth conditions. These mutant plants represent valuable resources for physiological and biochemical studies aimed at revealing the in planta functions of CPKs.

6-8

Differential Expression of Different Annexins in Arabidopsis

Greg B. Clark (1), Allen Sessions (2), Dennis Eastburn (1), and Stanley J. Roux (1)
(1) Department of Botany, University of Texas, Austin, TX 78713 USA (2) Department of Biology, University of California at San Diego, La Jolla, CA 92093 USA

Annexins are a diverse, multigene family of Ca2+-dependent, membrane-binding proteins. They appear to be multifunctional playing a role in essential cellular processes such as membrane trafficking, ion transport, mitotic signaling, cytoskeleton rearrangement, and DNA replication. In plant cells annexins have been immunolocalized at the cell periphery of highly secretory cell types and in the tip region of polarly growing cells of plants. Based on these localization results it has been hypothesized that annexins play a role in the Golgi-mediated secretion of new wall materials and plasma membrane in plant cells. Most plant species have at least two annexin genes, and Arabidopsis appears to have four. We have isolated and sequenced two annexin cDNAs, AnnAt1 and AnnAt2, by screening plant ESTs for annexin-like sequences. Recently, chromosome II from Arabidopsis was found to contain two new annexin sequences aligned next to each other. We have isolated the cDNAs corresponding to these two annexins, AnnAt3 and AnnAt4, using RT-PCR. We have characterized the expression patterns of AnnAt1 and AnnAt2 at the tissue and cellular level using Northern blot analysis and in situ hybridization techniques. Gene-specific probes were used in Northern blot analyses of various tissues and revealed distinct expression patterns. AnnAt1 is expressed in all tissues and is most abundant in stems, whereas AnnAt2 is expressed mainly in roots. For in situ hybridization experiments we have constructed full length sense and antisense digoxigenin-labeled probes for AnnAt1 and AnnAt2 which were used to assay germinating seedlings, vegetative plants, and flowering plants. The Northern results are confirmed by the in situ hybridization analyses which show that AnnAt1 and AnnAt2 are developmentally regulated, tissue specific, and mainly non-overlapping in their expression patterns. These results suggest that these two annexin genes have specialized functions in plants. (Supported by NASA: NAGW 1519).

6-9

atdrg1: The Arabidopsis member of a new class of GTP-binding proteins
Naomi Etheridge (1), Yuri Trusov (1) and Jose Ramon Botella (1)
(1) Plant Genetic Engineering Laboratory, Department of Botany, University of Queensland, Brisbane, Qld, 4072

We report the characterisation of an Arabidopsis thaliana cDNA (atdrg1), a member of a new class of GTP-binding proteins in plants, as well as several proteins found to putatively interact with ATDRG1. The predicted ATDRG1 protein contains all five structural motifs characteristic of the G-protein superfamily with specific homology to members of the recently discovered sub-family named Developmentally Regulated G-proteins (DRGs). The proteins of the DRG sub-family show remarkable evolutionary conservation even between quite different species (eg. 80% amino acid similarity between human DRG and atdrg1), which suggests an important, but as yet unclear role. Phylogenetic analysis of the available homologous sequences strongly suggests a diphyletic origin of the eukaryotic DRG proteins. Northern analysis shows high levels of atdrg1 mRNA in all Arabidopsis tissues and developmental stages studied and in situ hybridisation reveals that atdrg1 is expressed in actively growing cells and reproductive organs. Western analysis of the ATDRG1 protein shows varying protein expression levels in different Arabidopsis tissues. Immunolocalisation studies show that the protein is located to cytoplasmic vesicles found mainly in actively growing tissues. The yeast-two-hybrid procedure was used to isolate proteins that putatively interact with the ATDRG1 protein. Several of the putative interacting proteins were independently isolated more than once, with one protein isolated 12 times. We present here northern analyses showing the mRNA expression patterns of several of these interacting proteins in various tissues and developmental stages of Arabidopsis.

6-10

HYL1, a factor necessary for normal plant growth, is a novel double strand RNA binding protein.
Cheng Lu (1), Ryuji Tsugeki & **Nina V. Fedoroff.**
(1)Biotechnology Institute, Pennsylvania State Uni, University Park, PA 16802, USA. (2)Dept of Botany, Kyoto Uni, Sakyo-ku, Kyoto 606-8502, Japan

We've identified a Ds insertion mutant of Arabidopsis designated hyl1 (hyponastic leaves1) which has pleiotropic defects including slow root growth, reduced root gravitropic response, narrower & hyponastic leaves, reduced fertility & delayed flowering. The mutation in the HYL1 gene also impairs responses to plant hormones. Mutant plants are less sensitive to auxin & cytokinin than wild type, but more sensitive to ABA. We've cloned the HYL1 gene using a TAIL-PCR (Thermal Asymmetric Interlaced PCR) based cloning strategy. Based on Southern analysis, HYL1 is a single copy gene. Consistent with the pleiotropic phenotype of the mutant, HYL1 is ubiquitously & constitutively expressed in roots, leaves, stems, & flowers. The results of Northern blot analysis show that the HYL1 gene is down-regulated by the plant hormone ABA. The expression levels of several ABA responsive genes (cor47, kin2 & ABI1) are likewise enhanced in hyl1 mutant seedlings. The HYL1 gene encodes a polypeptide of 419 amino acids with 2 putative dsRNA binding motifs, 6 consecutive perfect repeats and a nuclear localization motif. Both in stable & transient experiments (transgenetic Arabidopsis & bombardment of oinon epidermal cells), we've demonatrated that the HYL1 protein targets GFP or GUS fusion proteins to the nucleus. These results suggest that the native HYL1 protein functions in the nucleus. The nucleic-acid-binding activity of a truncated HYL1 fusion protein containing the 2 putative dsRNA-binding motifs was analyzed using a gel mobility shift assay. The truncated HYL1 protein has a much higher affinity for dsRNA than for ssRNA, dsDNA & ssDNA. We believe that HYL1 is a regulator in plant development.

6-11

The expression pattern of the SHI gene is consistent with a role of SHI in the GA signal transduction pathway

Ingela Fridborg, Sandra Kuusk and Eva Sundberg
Dept. of Physiological Botany, Uppsala University, Villav. 6, S-752 36 Uppsala, Sweden

shi (short internodes) is a semidominant dwarfing mutation in Arabidopsis, caused by a transposon insertion leading to overexpression of the SHI gene (Fridborg et al., 1999, Plant Cell 11:6). The shi mutation confers a phenotype typical of mutants defective in the biosynthesis of gibberellin, GA. However, application of exogenous GA on shi does not correct the dwarf phenotype, which suggests that shi is defective in the perception of, or the response to, GA. The expression pattern of SHI was examined using RT-PCR analysis and promoter::GUS fusion constructs. SHI was shown to be expressed in meristematic and expanding tissue, e.g. root tips, emerging lateral roots, developing shoots, young expanding leaves and developing flowers. This expression pattern is very similar to that of the GA biosynthesis gene GA1 (Silverstone et al., 1997, Plant Journal 12:1), which is consistent with a role of SHI in the GA signal transduction pathway. As most of the dwarfed putative GA signal transduction mutants are isolated in the semidwarfed Ler background, we introduced shi into Ler+ plants (lacking the erecta mutation). In this wild-type ERECTA background the shi mutation did not show a strong dwarf phenotype, suggesting that the erecta mutation is necessary for the penetrance of the shi mutant phenotype.

6-12

Isolation and characterization of novel mutations within the phytochrome A light signal transduction pathway

Rebecca Fry (1) and Xing Wang Deng (1)
(1) Department of MCDB, Yale University New Haven, CT 06520-8104, U.S.A.

The phytochrome family of photoreceptors plays a vital role in mediating light regulation of plant development. The signaling mechanisms of the phytochromes are just beginning to be revealed. In the case of phyA, genetic screens have identified several putative downstream components including FHY1, FHY3, FIN2, and SPA1. To define possible new components of this pathway, an extensive genetic screen for Arabidopsis mutants with phenotypes similar to that of phyA mutants was carried out. In addition to the identification of new alleles of previously reported mutations, two novel and non-allelic mutations specifically defective in phytochrome A signal transduction pathway have been identified. These mutations exhibit a long hypocotyl phenotype in response to continuous far-red light and were designated as fry1 (far-red elongated hypocotyl) and fry2. Both fry1 and fry2 seedlings showed defects in inhibition of hypocotyl elongation in far-red but not red light, blue, and white light. fry1 was found to be a dominant mutation, whereas fry2 is recessive. Immunoblot analysis indicated that the expression level of phytochrome A apoprotein in the fry1 mutant was comparable to that in wildtype. Similar to phyA mutants, fry1 mutant seedlings also showed defects in the far-red block of greening response, anthocyanin accumulation and phyA-regulated germination. Further characterization of theFRY1 and FRY2 loci may reveal novel insights regarding the phytochrome A signal transduction pathway.

6-13

EIN2, a Bifunctional Transducer of Ethylene and Stress Responses in Arabidopsis
Jose M. Alonso, **Takashi Hirayama**, Gregg Roman, Saeid Nourizadeh and Joseph R. Ecker
Plant Science Institute, Department of Biology, University of PennsylvaniaPhiladelphia, Pennsylvania 19104-6018, USA

Ethylene is an important regulator of plant growth, development and responsiveness to a variety of stresses. We report cloning and characterization of the Arabidopsis ETHYLENE-INSENSITIVE2 (EIN2) gene, a central component of the ethylene signal transduction pathway. EIN2 encodes a dimorphic protein in which the amino-terminal integral membrane domain shows similarity to the disease-related Nramp family of divalent cation transporters, whereas the large hydrophilic carboxyl-termius (CEND) contains a coiled-coil domain but is otherwise novel. Expression of the EIN2 CEND in plants is sufficient to evoke constitutive activation of downstream ethylene responses, and restores responsiveness to jasmonic acid and paraquat-induced oxygen radicals. Demonstration of the requirement of EIN2 for transmission of multiple volatile/gaseous signals may provide a molecular link between previously distinct hormone response pathways, and suggest that plants use a combinatorial mechanism for assessing a variety of stresses by employing a common set of signaling molecules.

6-14

Identification of ABA-responsive genes during heterophyllous switch in Marsilea quadrifolia
Ting-Chang Hsu, Jang-Shiun Wang, Ying-Chuan Wang and Bai-Ling Lin
Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, Taiwan, Republic of China

The plant hormone abscisic acid (ABA) plays a significant role in inducing the responses to adverse environmental stimuli, such as drought stress. In many aquatic plants, application of ABA simulates changes in the environment and causes heterophyllous switch, from the development of submerged characteristic to the formation of aerial morphology. Using Marsilea quadrifolia as a model system, we examined genes regulated by ABA in shoot apical meristem containing the tissues responsive to heterophyllous induction. Differential display techniques were used to isolate cDNAs corresponding to mRNAs present at different levels in shoot apical meristems untreated or treated with ABA. Sequence analysis shows significant homology between some of the cDNAs we isolated and known sequences in databanks. To confirm ours results, specific primers were made for each cDNA clone and were used in RT-PCR. The expression level of individual mRNA species was compared in meristematic tissues treated with ABA for various periods of time. Based on these results, we identified cDNAs of genes that are induced at early stages during heterophyllous induction. We are investigating the function of these genes in heterophyllous determination and their involvement in the responses to ABA.

6-15

The sugar sensing mutant sun6 is insensitive to abscisic acid; involvement of abscisic acid and the ABI genes in sugar sensing

J.C. Huijser, J. Pego, A. Kortstee and S. Smeekens
University of Utrecht, Department of Molecular Cell Biology, Padualaan 8, 3584 CH Utrecht, The Netherlands

Sun6 is a sugar sensing mutant. Expression of several sugar responsive genes is altered in this mutant. Photosynthesis of three week old sun6 mutants shows a reduced snsitivity to the glucose analog 2-deoxy glucose. Furthermore, in contrast to wildtype plants, this mutant is able to germinate on medium containing 7.5 mM mannose. It can also grow on medium with 6% glucose. This suggests that SUN6 is part of a hexokinase dependent signalling pathway. We exploited the phenotype that sun6 exhibits on media containing mannose or glucose to search for tagged alleles in the collection of Ellen Wisman. We isolated two allelic mutants and cloned the DNA flanking to the transposable element. SUN6 turned out to be a gene involved in abscisic acid signal transduction. The sun6 mutant is abscisic acid insensitive (abi). This prompted us to investigate the role of ABA and the ABI genes in sugar sensing. All abscisic acid deficient (aba) and abi mutants appeared to respond different to sugars as wildtype plants do. Some of these mutants have a more pronounced phenotype than others. We conclude that ABA and the ABI genes are necessary for a normal response to sugars.

6-16

Characterisation of a Putative G Protein Coupled Receptor from Arabidopsis

Tania V Humphrey and Jose R Botella
Plant Genetic Engineering Laboratory Department of Botany University of Queensland St Lucia, Queensland, Australia 4072

As a means for sensing the environment and communicating between cells, receptors occupy an important role in the signaling systems of any organism. Receptors coupled to heterotrimeric G proteins are found in many organisms ranging from yeast through to humans and recognise a variety of signal molecules. So far, there have been no G protein coupled receptors (GPCRs) found in plants despite considerable evidence for their existence. We present here the characterisation of a putative G protein coupled receptor from Arabidopsis called ATMP1. Sequence analysis reveals the characteristic seven transmembrane structure common to all GPCRs as well as some homology to GPCRs from other organisms. The gene is expressed at extremely low levels throughout the plant and expression of an ATMP1/green fluorescent protein fusion has revealed a plasma membrane location. Overexpression of the gene in Arabidopsis is currently being investigated as to possible phenotypic effects relating to receptor function. A yeast two hybrid screen has identified a small protein of unknown function that interacts with ATMP1.

6-17

Phytochrome Regulation of Gibberellin 3ß-Hydroxylase Genes in Germinating Arabidopsis Seeds
Yuji Kamiya (2), Shinjiro Yamaguchi (1&2), Maria Smith (2), Robert Brown (2), Tai-ping Sun (1)
(1) DCMB Group, Dept. Botany, Duke University, Box 91000, Durham, NC27708, USA, (2) Frontier Research Program, RIKEN, Hirosawa 2-1, Wako-shi 351-0198, Japan

Despite extensive studies on the roles of phytochrome in photostimulated seed germination, the mechanisms downstream of the photoreceptor to promote germination are basically unknown. Previous studies have indicated that light-induced germination of Arabidopsis seeds is mediated by the hormone gibberellin (GA). Using RNA gel blot analyses, we studied the regulation of two Arabidopsis genes, GA4 and GA4H (for GA4 homolog), both of which encode GA 3ß-hydroxylases that catalyze the final biosynthetic step to produce bioactive GAs. The newly isolated GA4H gene was predominantly expressed during seed germination. We showed that expression of both GA4 and GA4H genes in imbibed seeds was induced within 1 hr after a brief red (R) light treatment. This result suggests that the elevated expression of GA 3b-hydroxylase gene may result in an increase in biosynthesis of active GAs to promote seed germination. In situ hybridization showed that both GA4 and GA4H transcripts are predominantly localized in cortical cells in embryo axis during seed germination. Interestingly, in the phytochrome B-deficient phyB-1 mutant, GA4H expression was not induced by R light, whereas GA4 expression still was, indicating that R light-induced GA4 and GA4H expression is mediated by different phytochromes. In contrast to the GA4 gene, the GA4H gene was not regulated by the feedback inhibition mechanism in germinating seeds. Therefore, the GA4H gene may play an important role in the maintenance of a high level of active GAs, which may be required for light-induced seed germination.

6-18

Characterization of Arabidopsis Dof Transcription Factors, a Novel Zinc Finger Protein Family in Plants
Hong-Gu Kang and Karam Singh (1)
(1) Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90066, U.S.A.

Dof proteins are a novel family of plant transcription factors with a single zinc finger DNA binding domain. Three AtDof clones were isolated and characterized in addition to AtDof1 that was previously isolated through its interaction with OBF4, an ocs element binding protein. No significant differences were found between the AtDofs in terms of their DNA and protein binding properties and all the AtDof proteins contain transcriptional activation domains in their C-terminal region. Distinct expression patterns for each AtDof were found. For example, AtDof3 and AtDof4 were expressed highest in leaves and roots respectively. Some of the AtDofs showed auxin, salicylic acid (SA) and cyclohexamide induced expression patterns. Interestingly, there is a strong correlation between the expression of some of the AtDofs and the ocs element, a stress response element which is also induced by auxin, SA and cyclohexamide. To analyze the function of Dof proteins in plants, transgenic lines overexpressing AtDof4 were generated. Five lines out of eight showed a severe growth phenotype that resulted in early death or retarded growth with distinct root morphologies and yellowish leaves, suggesting that some AtDof proteins may play important roles in plant growth and development.

6-19

Ethylene signaling is involved in the spread of oxygen radical-induced cell death in ozone sensitive Arabidopsis mutants

Jaakko Kangasjärvi (1), Kirk Overmyer (1), Reetta Kettunen (1), Tero Kuittinen (1),Mart Saarma (1), Christian Betz (2), Christian Langebartels (2)
1) Institute of Biotechnology, University of Helsinki, POB 56, FIN-00014 Helsinki, Finland, 2) Institute of Biochemical Plant Pathology, GSF Research Center, D-85764 Oberschleissheim, Germany

EMS mutagenized seed of the ozone tolerant Col-0 was screened for O3-induced lesions. The appearance of cellular markers of programmed cell death in the lesions suggests that the damage is similar to HR and programmatic in nature. When the O3-induced lesions were expanding in the mutants, superoxide was produced in the apoplast of adjacent healthy plant tissue. The mutants showed also either significantly elevated, or prolonged ethylene emissions relative to Col-0. Superoxide production, lesion spread, and increased ethylene emissions all occurred at the same time. The leaf damage correlated with the increased ACC levels and ethylene emission. When Col-0, ethylene insensitive mutant (ein2-1), and the ozone-sensitive mutant line 2-20 were exposed first to O3 and subsequently to either ethylene gas or clean air, the ethylene exposed Col-0 and line 2-20 mutant plants, but not the ein2-1, showed more damage than controls that had been exposed to O3 alone. Exposure to O3 concentrations that resulted in visible damage in Col-0 caused no damage in the ein2-1. Addition of the ethylene precursor, ACC, with an artificial superoxide generating system increased the radical-induced damage in both O3 sensitive mutant and Col-0, but not in the ein2-1 mutant suggesting that the enhancement of superoxide-induced cell death is dependent on ethylene perception. These results suggest a role for ethylene in regulating the spread of extracellular radical-induced cell death during oxidative stress generated with ozone.

6-20

Transgenic Analysis for the Functions of an Arabidopsis Phosphatidic Acid Phosphatase (PAP)

Takeshi Katagiri and Kazuo Shinozaki.
Laboratory of Plant Molecular Biology, The Institute of Physical and Chemical Research (RIKEN), Tsukuba, 305-0074, Japan.

The phospholipid metabolism system has been suggested to play important roles in signal transduction pathways in higher plants as well as animals. Recently, several genes that are involved in the phospholipid metabolism system have been isolated including phosphoinositide-specific phospholipase C (PI-PLC) and phospholipase D (PLD). Phosphatidic acid (PA) is produced from phosphatidylcholine (PC) by PC-PLD while diacylglycerol (DAG) is produced from PIP2 by PI-PLC. Phosphatidic acid phosphatase (PAP) produces DAG by dephosphorylating PA. While diacylglycerol kinase (DGK), which converts DAG into PA, a reaction opposite to that catalyzed by PAP. PA and DAG play variety roles in cellular signal transduction in animals. To understand biological roles of PA and DAG in plant signaling, we cloned cDNAs for DGK(1) and PAP. We reported that cloning and characterization of a cDNA clone, AtPAP1, encoding PAP in Arabidopsis thaliana in this meeting last year. We report here the functional analysis of AtPAP1 using transgenic plants which overexpress the AtPAP1 sense/antisense RNA. Root-length of the AtPAP1-antisense plants were significantly shorter than those of control plants on agar medium containing neomycin, which is known as a PLC inhibitor. The internode-length of AtPAP1-antisense plants is frequently shorter than those of control plants. These results suggest that AtPAP1 may be involved in root and shoot elongation through phospholipid metabolism system. Possible functions of PA/DAG as second messengers and phospholipid metabolites in higher plants will be discussed. (1) Katagiri et al. Plant Mol. Biol. 30: 647-653, 1996

6-21

Sugar/osmoticum levels modulate differential ABA-independent expression of two stress-responsive sucrose synthase genes in Arabidopsis

Annabelle Déjardin, Lubomir N. Sokolov and **Leszek A. Kleczkowski**
Department of Plant Physiology, Umeå University, 901 87 Umeå, Sweden

Sucrose synthase (Sus) is a key enzyme of sucrose metabolism. Two Arabidopsis thaliana Sus-encoding genes, Sus1 and Sus2, were found to be profoundly and differentially regulated in leaves exposed to environmental stresses (cold stress, drought, O2 deficiency). Transcript levels of Sus1 increased upon exposure to cold and drought, while Sus2 mRNA was induced specifically by O2 deficiency. Both cold and drought exposures induced accumulation of soluble sugars and caused a decrease in leaf osmotic potential, whereas O2 deficiency was characterised by a near complete depletion in sugars. Feeding abscisic acid (ABA) to detached leaves or submitting Arabidopsis ABA-deficient mutants to cold stress conditions had no effect on Sus1 or Sus2 expression profiles, whereas feeding metabolisable sugars (sucrose, glucose) or non-metabolisable osmotica (polyethylene glycol, sorbitol, mannitol) mimicked the effects of osmotic stress on Sus1 expression in detached leaves. Using various sucrose/mannitol solutions, we demonstrated that Sus1 was upregulated by a decrease in leaf osmotic potential rather than an increase in sucrose concentration itself. We suggest that Sus1 gene expression is regulated via an ABA-independent signal transduction pathway that is related to perception of a decrease in leaf osmotic potential during stresses. On the other hand, the expression of Sus2 was independent of sugar/ osmoticum effects, suggesting the involvement of a signal transduction mechanism that is distinct from that regulating Sus1 expression. The differential stress-responsive regulation of Sus genes in leaves may represent a part of a general cellular response to the allocation of carbohydrates during acclimation processes.

6-22

Two new genes encoding zinc finger proteins with potential roles in stem elongation control

Sandra Kuusk, Ingela Fridborg and Eva Sundberg
Dept. of Physiological Botany, Uppsala University, Villav. 6, S-752 36 Uppsala, Sweden

We have cloned two new Arabidopsis genes designated SHI RELATED SEQUENCE 1 and 2, (SRS1 and SRS2), which encode proteins with a single zinc finger motif. The genes are members of a small gene family of putative transcription factors in which the SHORT INTERNODES (SHI; Fridborg et al., 1999, Plant Cell 11:6) gene is found. High level expression of the SRS2 gene in transgenic Arabidopsis plants confers dwarfing, reduced apical dominance, partial sterility and narrow, dark green rosette leaves. All these traits are found among mutants with reduced GA response. Since the 35S::SRS2 plants show a phenotype similar to the SHI overexpressor (the shi mutant) plants we propose that some of the genes in the family may have overlapping or redundant functions in negative regulation of GA-induced stem cell elongation, possibly through transcriptional control. We will report on additional characterisation of transgenic plants with altered expression levels of SRS1 and SRS2 and on the expression patterns of SRS1 and SRS2 in wild-type Ler. Furthermore, analysis of a T-DNA tagged srs1 loss-of-function mutant is under progress.

6-23

Dominant negative guard cell K+ channel mutants inhibit light-induced stomatal opening in Arabidopsis

J. M. Kwak, Y. Murata, V.M. Baizabal-Aguirre, J. Merrill and J.I. Schroeder,
Dept of Biology and Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

Inward-rectifying K+ (K+in) channels in guard cells have been suggested to provide a major pathway for K+ influx into guard cells during stomatal opening. To test this role of the guard cell K+in channel KAT1 in vivo, transgenic Arabidopsis plants were generated that expressed dominant negative point mutations in KAT1. When the dominant negative KAT1 mutant was expressed under control of the single CaMV 35S promoter, the reduction in K+in channel activity had no effect on light-induced stomatal opening. To more strongly inhibit K+in currents, a tandem-repeat CaMV 35 promoter was used. Analyses with guard cells from two independent lines showed that steady state K+in channel currents were reduced by 75% compared to controls at -180 mV, which resulted in significant inhibition of light-induced stomatal opening. Another transgenic plant line in which K+in currents were reduced by only 58% did not exhibit significant inhibition in light-induced stomatal opening. Analysis of intracellular K+ contents showed that potassium uptake was significantly reduced in guard cells of the two strongest suppressor lines during light-induced stomatal opening. Transpiration rate measurements showed that these transgenic K+in suppressor lines lost less water than control plants. These data provide direct molecular evidence for the model that K+in channels function as a central pathway for K+ uptake in vivo during light-induced stomatal opening. Furthermore, comparison of K+in channel activities among different transgenic and control plants allow us to quantitatively determine the range of activities of K+in channels that are required for K+ uptake during light-induced stomatal opening.

6-24

Isolation and characterization of a novel Arabidopsis cell death mutant

Jiayang Li, Zhonglin Mou, Yikun He, Ya Dai and Xinfang Liu.
Institute of Genetics, Chinese Academy of Sciences, Beijing 100101, China.

A novel Arabidopsis thaliana mutant was isolated from M2 seeds derived from mutagenesis with ethylmethane sulfonate (EMS). The mutant plants displayed multiple developmental abnormalities ranging from leaf curling, chlorotic leaf patches, early senescence of inflorescence, distorted siliquae to semidwarfism and reduced fertility. Histochemical staining revealed that in the mutant plants many clusters of cells underwent cell death in multiple organs. The nuclear DNA in the mutant was also found to be fragmented. High temperature and continuous light exacerbated the mutant phenotype. However, low temperature plus photoperiod treatment led to a drastic suppression of the mutant phenotype. Furthermore, the mutant phenotype could be induced or reversed by imposing or withdrawing temperature and light stress. These findings indicate that temperature and light stress may be an important trigger of cell death in the mutant plants. Genetic analysis showed that the mutant phenotype was the consequence of a single recessive mutation and that the locus was tightly linked to the molecular marker mi421 on the top part of chromosome 2. Our phenotypic and genetic studies demonstrated that it is a novel cell death mutant in higher plants. Molecular cloning of the gene is under way.

6-25

Regulation of germination and seedling growth by ethylene and polyamines.
Jacquiline M. Locke and Peter C. Morris
Department of Biological Sciences,Heriot-Watt University,Edinburgh, Scotland, UK.

Germination and seedling growth depends upon endogenous and environmental factors. The levels of endogenous plant growth regulators and the sensitivity of plant tissues to these regulators are major factors in these processes. The plant growth regulator ethylene appears to play an important role in the germination process, since inhibitors of ethylene perception (silver ions, NBD) blocked germination and seedling growth in barley plants. Paradoxically, inhibitors of ethylene production (AVG, cobalt ions) stimulate seedling growth. It was found that this stimulated growth could be attributed to increased levels of polyamines. We have demonstrated that not only are the ethylene and polyamine biosynthetic pathways are biochemically linked, these compounds also show strikingly similar physiological effects on germination and seedling growth. In particular, nanomolar levels of polyamines can overcome the inhibitory effects of ethylene perception blockers. The effects of polyamines were investigated in germinating Arabidopsis seedlings. Arabidopsis roots were found to be very sensitive to exogenously applied polyamines, showing stimulated growth at concentrations even as low as 10 pMolar. Polyamines will also improve the poor germination shown by the ethylene insensitive etr1 mutant. These findings have provided us with an interesting basis to screen for polyamine insensitive root mutants. We are screening the Feldman T-DNA insertion mutant collection for plants which show insensitivity to 1µM putrescine. Several candidate insensitive mutants have so far been identified, which are currently undergoing further testing. Although polyamines can overcome the inhibitory effects of silver ions or NBD, polyamines are not equivalent to ethylene in all respects. For example, dark grown Arabidopsis seedlings in the presence of ethylene produce the characteristic triple response; however the triple response is not induced by polyamines, either in wildtype nor in etr1 mutant plants

6-26

An Arabidopsis mutant with altered Hsp90 accumulation patterns during heat stress
Jutta Ludwig-Müller (1) and Christoph Forreiter (2).
(1) Botanisches Institut, Goethe-University, Siesmayerstr. 70, 60054 Frankfurt, Germany, (2) Department of Molecular Cell Biology, Goethe-University, Marie Curie-Str. 9, 60439 Frankfurt, Germany.

The TU8 mutant of Arabidopsis thaliana previously described to be deficient in glucosinolate metabolism and pathogen induced auxin accumulation showed markedly reduced thermotolerance compared to wild type plants when exposed to elevated temperatures. While moderately increased temperatures only affected shoot growth of the mutant, severe heat stress led to a dramatic decay of mutant plants while wild type seedlings were only slightly damaged. Analysis of heat stress proteins (Hsps) produced by the mutant revealed that cytoplasmic Hsp90 protein expression decreased under heat stress as shown by Western analysis and immunofluorescence, whereas expression of other Hsps was essentially comparable to wild type. Northern analysis showed that the expression of Hsp90 protein in mutant cells coincided with a reduced transcript level under heat stress. Transient transformation of TU8 protoplasts with a Hsp90 construct restored thermotolerance to the wild type level, whereas untransfomred protoplasts of the mutant were still more thermosensitive. This mutant will allow us to further study the role of Hsp90 under heat stress and for plant development.

6-27

DET1 Responsive Elements on the CAB2 Promoter

Bridey B Maxwell, Daniel Poole, Joanne Chory
The Salk Institute for Biological Studies10010 N. Torrey Pines Rd., La Jolla, California 92037

The Arabidopsis mutant det1 grows as a light-grown plant in the dark with expanded cotyledons, a short hypocotyl, developed chloroplasts, and derepressed expression of several light-regulated genes. In the light, det1 mutants misexpress light-regulated genes in inappropriate cell types. DET1 is thus a core signal transduction component linking the perception of light to a switch in developmental program . In previous studies of det1 mutants, the light-regulated CAB2 gene has been shown to be overexpressed in the dark and underexpressed in the light compared to wild type. In this study, we aimed to define elements of the CAB2 promoter that respond to the DET1 transcriptional control signal. We constructed a set of CAB2 promoter truncations and mutations fused to the reporter gene luciferase and introduced them into wild type and det1 mutant plants. We have discovered that the DET1 responsive element (DtRE) in the dark lies between -195 and -155 base pairs 5' of the CAB2 mRNA start site and does not require an intact CUF-1 or CGF-1 binding element for repressive action. In the light, the underexpression of the CAB2 promoter in the det1 mutant requires the CUF-1 element but not sequences between -199 and -142. In roots, DET1 mediates repression of CAB2 expression via both the CUF-1 element and sequences between -199 and -142 . These studies indicate that there are two DET1 responsive elements on the CAB2 promoter; DtRE which is shared between dark-specific and root-specific repression, and CUF-1 which exerts opposite effects on expression depending on tissue type. In addition, we have discovered that there are factors present in Arabidopsis extracts which specifically bind the DtRE and that the binding of these factors is altered in det1 mutant extracts.

6-28

Characterization of mutants altered in circadian phase in Arabidopsis thaliana

C. Robertson McClung, Patrice A. Salome, Janet E. Painter, Ellen V. Kearns, Arthur G. Fett-Neto and Dorota B. Balaban
Dartmouth College, Department of Biological Sciences, 6044 Gilman Laboratory, Hanover, NH, USA 03755-3576

We have identified 65 putative mutants impaired in circadian timing based on a circadian rhythm in sensitivity to exogenously applied toxic gases (sulfur dioxide). This screening method can potentially yield mutants altered in period length or phasing of circadian rhythms. The previously described short period mutant ctd1 (circadian timing defective) was isolated from this screen. Here we report the characterization of another class of mutants: oop for out-of-phase. oop1 and oop2 show a long hypocotyl under red and blue light, respectively, implicating oop1 in phytochrome-, and oop2 in cryptochrome-mediated signaling. The defects could be either in photoperception, or in the circadian-regulated inhibition of hypocotyl elongation. Both mutants also have altered flowering time relative to the wild-type Columbia, suggesting that OOP1 and OOP2 may be involved in the clock-dependent pathway leading to flowering induction. The oop mutants display a novel circadian phenotype: although the period length seen in the leaf movement, carbon dioxide assimilation or gene expression oscillations is relatively unchanged when compared to Columbia, the phase of several of these rhythms is significantly shifted by 2 to 4 hours. Initial mapping located oop1 centromere proximal on the bottom arm of chromosome 2, while oop2 is centromere proximal on the bottom arm of chromosome 4. High resolution mapping of the two mutations is underway. The refined mapping of the OOP loci should lead to the identification and cloning of genes participating in the phasing of different rhythms to distinct times of day. This work was supported by a grant from the National Science Foundation (MCB 9723482).

6-29

Characterization of auxin signal transduction mutants in Arabidopsis.
Jane Murfett, Xiao-Jun Wang, Gretchen Hagen and Tom Guilfoyle.
Dept. Biochemistry, University of Missouri-Columbia117 Schweitzer Hall, Columbia MO 65211

The long term goal of this project is to use a genetic approach to elucidate as many steps as possible in the auxin signal transduction pathway(s) leading to expression of genes that are rapidly upregulated in response to auxin. We have developed a mutant screen involving natural and synthetic Auxin Response promoter Elements (AuxREs), linked to a selectable marker gene encoding hygromycin resistance (HPH), and the GUS reporter gene. The AuxREs were derived from the soybean GH3 promoter, which is rapidly and specificially induced by biologically active auxins. The chimeric genes are expressed at very low levels in untreated transformed plants, but after application of endogenous auxin, they are induced in all organs and tissues, with highest expression in the roots. Arabidopsis plants transformed with these constructs were mutagenised, and mutants that express the transgenes at elevated levels in the absence of exogenous auxins were selected on hygromycin - containing media. Plants with elevated levels of GUS expression (i.e., plants with trans-acting mutations rather than mutations within the HPH gene) were identified. Eight independent mutant lines were selected for further analysis. Preliminary mapping data indicated that five of the mutant lines map to the bottom of chromosome 5, and allelism tests indicated that these mutant loci are allelic. Another line, which maps to chromosome 2, has a small bushy phenotype. The remaining two lines map to chromosomes 1 and 4 respectively, and have no obvious visible phenotypes. The chromosome 5 locus was chosen for detailed mapping and cloning. Progress with this work will be presented.

6-30

NDK genes are contributed to the gamma-induced signal transduction cascade
Toshifumi Nagata1, Setsuko Todoriki2, Toru Hayashi2, Setsuko Komatsu3, Yuriko Shibata4, Du Zeji3, Masaki Mori3, Shoshi Kikuchi3
1 JST.Nagasaki, 2 Food Research Institute, 3 National. Inst. Agrobiol. Resouces, 4 Univ. Tsukuba

Massive dosage of gamma-radiation induces accumulation of anthocyanin, formation of trichomes, radial expansion of root epidermal and cortical cells and extension of root hairs (Plant Physiology 120: 113-120(1999)). To know the signal transduction system of these phenomena, protein analyses were carried out. In-gel assay of the MAP kinase activity revealed that the 17.5kDa-protein kinase activity elevated after irradiation in the membrane fraction (105k x g ppt). The similarity of the molecular weight(17.5kDa), pI(5.2) and the protein kinase activity suggest that the 17.5kDa protein is a nucleoside diphosphte kinase (NDK). Anti-NDK antibody from rice interacted with the 17.5kDa protein. It has been reported that NDK is required not only for the synthesis of NTPs but also for the signal transduction in plant photosystems. NDK activity increased after lighting and supposed to regulating the photo signal transduction pathway by activation of the G protein and/or COP1 family genes(Hamada et al. 1994, 1996). Phytochrome A protein is reported to interact with NDK2 gene product (Song et al. in ESOP meeting in 1999 Berlin). In-gel assay of the cryptochrome mutants were carried out and found that the hy4 (cry1) mutant did not elevate the kinase activity in the membrane fraction after gamma irradiation and the fha-1 (cry2) mutant indicates high NDK activity in the membrane fraction in nonirradiated plants. Therefore, the NDK signal transduction pathway involving NDK possibily regulate the photo and/or electron energy mediated reactions. Now we have started the cloning of the genes interacting with NDK by yeast two-hybrid system and obtained some candidates.

6-31

Regulation of a plant MAPK cascade signaling by a kinesin-like protein, NACK1

Ryuichi Nishihama (1), Takashi Soyano (2), Masaki Ishikawa (1), Tetsuhiro Asada (3), Kenji Irie (4), Hiroharu Banno(5), Kunihiro Matsumoto (4), Yasunori Machida (1)
(1) Group Plant Dev., (4) Group Signal Transduction, Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ., Japan, (2) Grad. Sch. Sci., Kyoto Univ., (3) Sch. Sci., Osaka Univ., (5) Lab. Plant Mol. Biol., The Rockefeller Univ.

The mitogen-activated protein kinase (MAPK) cascades play important roles in various signal transductions in eukaryotes. They consist of three families of the protein kinase: the MAPK, the MAPK kinase (MAPKK), and the MAPKK kinase (MAPKKK) families. Diverse protein factors are involved in activations of MAPKKKs. Many plant species including tobacco and Arabidopsis have a plant-specific member of the MAPKKK family that is called NPK1. The NPK1 protein is present in S to M phase of cell cycle and disappears after cytokinesis, suggesting its role in regulation of plant cell cycle. In a search for an activator of NPK1, we have identified a novel kinesin-like microtubule-based motor protein, NACK1, as such a factor. The NACK1 protein binded to NPK1 and was accumulated only at M phase, suggesting that the activation of NPK1 by binding to NACK1 occurs during M phase. Both NPK1 and NACK1 were localized to the phragmoplast where the cell plate is formed. We have also isolated tobacco cDNAs named NQK1 and NRK1 encoding a putative MAPKK and MAPK downstream of NPK1, respectively. The yeast two-hybrid analysis predicted protein-protein interactions in combinations of NPK1 and NQK1, and NQK1 and NRK1. NPK1 and NQK1 were shown to phosphorylate NQK1 and NRK1 in vitro, respectively. These data suggest that NACK1 activates the MAPK cascade through NPK1 and targets the signaling complex to the phragmoplast to regulate some process of cytokinesis, such as formation of the cell plate and the organization of the phragmoplast.

6-32

Two Arabidopsis cinnamoyl coA reductase genes are differentially regulated by abscisic acid and throughout development

Lars Østergaard, Morten Petersen, Henrik Næsted, Ole Mattsson, and John Mundy
Department of Plant Physiology, Institute of Molecular Biology, University of Copenhagen, Ø.Farimagsgade 2A, DK-1353 Copenhagen K, Denmark

Cinnamoyl CoA Reductase (CCR) is the first committed enzyme in lignin biosynthesis. Previous studies in Eucalyptus and tobacco have implicated a key role for this enzyme in regulating lignin production. Two tandemly repeated genes were identified on Arabidopsis chromosome II encoding CCRs (Atccr1 and Atccr2) with 87% of amino acid identity. The promoter regions, however, are highly divergent indicating alternative regulation of the ccr genes. We initially analysed their expression by Northern blots and found strong Atccr induction by abscisic acid (ABA), drought, and heat shock, and a high expression level in seeds, thus resembling the expression pattern of late embryogenic abundant (lea), ABA-responsive genes. Differential regulation of the two CCR promoters was observed in transgenic plants expressing GUS behind the promoters and the regulation of both was found to be under tight developmental control: No activity of the AtCCR1 promoter was detected in lignified tissue of GUS-stained stem sections. However, the promoter was highly responsive to ABA and heat shock and showed a dramatically high activity in embryos of dormant seeds. Atccr2 on the other hand did not respond to the above mentioned stimuli and was not expressed in seeds; however, GUS-staining of 10 days old plants and stem sections of four weeks old plants revealed constitutive expression in lignified tissue. We therefore propose that whereas AtCCR2 is involved in lignification of the vascular tissue in older tissue, AtCCR1 is involved in synthesis of the vascular tissue of germinating embryos.

6-33

Analysis of GA signalling by a negative regulator, HvSPY

Masumi Robertson (1), David Bagnall (1), and Steve Swain (2)
(1) CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601; (2) CSIRO Plant Industry, Horticulture Unit, Private Mail Bag, Merbein VIC 3505

Gibberellins (GAs) are involved in controlling many aspects of plant growth and development, such as germination, elongation growth, flowering and fruit development. SPINDLY is a negative regulator of GA response whose function in GA signalling was demonstrated by a barley homologue, HvSPY, in a functional assay using a well-defined GA response (a-amylase expression) in barley aleurone (Robertson, Swain, Chandler, Olszewski, 1998 Plant Cell 10:995-1007). Its structure suggests that the molecular mechanism of negative regulation may involve protein-protein interaction through the tetratricopeptide repeat (TPR) domain and a dynamic protein modification by O-linked N-acetylglucosamine (O-GlcNAc) glycosylation by the enzyme O-GlcNAc transferase (OGT). The role of HvSPY in controlling other GA responses was examined in Arabidopsis plants transformed with HvSPY under the control of the 35S promoter. The loss-of-function spy-3 mutant showed increased response to many GA-regulated characteristics. In the spy-3 mutant background, transgenic plants exhibited reduced GA response, consistent with increased negative regulation of GA signalling by 35S::HvSPY. The whole plant phenotype included altered germination, rosette growth and flowering responses. The specific effects of HvSPY and spy-3 mutation on the expression of a number of GA- and ABA-regulated genes showed a complex outcome, suggesting direct and indirect effects on GA signalling.

6-34

WRKY6 - a regulatory gene functioning in senescence and plant defense

Silke Robatzek, Klaus Hahlbrock, Imre E. Somssich
Max-Planck-Institut fuer Zuechtungsforschung, Department of Biochemistry,Carl-von-Linne-Weg 10, D-50829 Koeln, Germany

WRKY proteins are zinc-finger type plant transcription factors which form a large multigene family with over 50 presently idendified members in Arabidopsis. A common feature of all WRKY proteins is the occurrence of either one or two copies of a highly conserved WRKY domain consisting of approximately 60 amino acids. This domain has been shown to bind specifically to W-box elements with the consensus motif (T)TGAC(C). WRKY6 shows clear expression in roots and flowers, but is strikingly strong in senescing leaves. Transgenic Arabidopsis plants carrying WRKY6 promoter::GUS fusions show high GUS expression in roots, in senescing leaves and in mature sepals. In these lines, rapid and local GUS expression is observed after pathogen infection or wounding, and is induced upon treatment with SA, MeJA and ethylene. Since many pathogen-induced genes are up-regulated during both the defense response and senescence, WRKY6 may be involved in the cross-talk between these two signal transduction pathways. To address this point, a WRKY6 knock-out mutant in Arabidopsis has been identified. A novel differential display technique has been applied to the isolation of several putative target genes of WRKY6. Furthermore, reintroduction of WRKY6 in the knock-out background under the control of an inducible expression system enables the classification of primary and secondary target genes. These studies should allow us to gain important information concerning the in vivo role of an interesting member of the WRKY transcription factor family.

6-35

TSL-like protein kinases: a conserved family

Judith L. Roe (1), Xiaofen Liu (1), Jolanta Jacobs (1), Tim Durfee (2), Jennifer Nemhauser (2), Pat Zambryski (2).
(1)Kansas State Univ., Manhattan, KS 66506, (2) Univ. of CA, Berkeley, CA. 94720.

Mutations at the TOUSLED (TSL) locus cause abnormal leaf and flower development in Arabidopsis. The TSL gene encodes a nuclear serine/threonine protein kinase expressed in all the major organs of the plant. The Arabidopsis TSL gene is a member of a highly conserved family of protein kinase genes, the Tousled-like kinase (TLK) family. Mammals to date have two closely related TLK genes, and maize has at least 2 genes that encode TLK members. A TLK member has been found in both Caenorhabditis elegans and Drosophila melanogaster. The yeast Saccharomyces cerevisiae does not have a recognizable TLK gene in its genome, suggesting that TLKs may function in some aspect of multicellularity. The TLK family is most closely related in sequence to the STE11/MEKK family of protein kinases, a family of kinases involved in transmission of extracellular signals through a kinase cascade. TLKs contain a highly conserved C-terminal catalytic domain and a less well conserved N-terminal regulatory domain which contains a nuclear localization signal(s) and a coiled/coil region. The function of TSL (or any TLK member) during development is unknown, but the tsl loss-of-function phenotype and the expression pattern of a mitotic marker gene in tsl mutants suggest that TSL may be involved in the control of cell divisions during morphogenesis in the wild type floral meristem. Regulators of cell division that are known to be controlled by phosphorylation are being analyzed as potential TSL substrates including Arabidopsis Rb (RBR). Also, genetic studies have suggested that TSL interacts with the ETTIN gene which encodes a member of the Auxin Response Factor family of transcription factors, and we are testing whether ETT protein is a substrate for TSL phosphorylation. Fusion proteins are being produced and purified from yeast and E. coli, phosphorylated in vitro by TSL fusion proteins, and analyzed by two-dimensional peptide mapping after tryptic digestion.

6-36

Mutants impaired in sucrose induced expression of a starch biosynthetic gene

Fred Rook, Caroline Smith and Mike Bevan
Dept. of Molecular Genetics, John Innes Centre, Colney Lane, Norwich, United Kingdom

Plant growth and productivity are determined by the photosynthetic activity of the plant, and by the way the products of photosynthesis are allocated. Plants assess their carbohydrate status to co-ordinate sugar supply and demand through the control of gene expression in source and sink tissues. The understanding of these sugar sensing mechanisms is limited. We use a genetic approach to identify components of these sugar sensing and signalling pathways in Arabidopsis. Specifically the genes that are required for the regulation and sugar induction of starch biosynthesis. Starch is the major reserve carbohydrate in most higher plants. ADP-glucose pyrophosphorylase (AGPase) controls the key enzymatic step in starch biosynthesis. A sugar inducible AGPase promoter was fused to a negative selection marker, a bacterial cytochrome P450. This cytochrome P450 catalyses the conversion of a sulfonylurea proherbicide to a highly phytotoxic form. A transgenic Arabidopsis line containing the AGPase::P450 construct was EMS mutagenised. Mutants were isolated which survive the presence of the proherbicide under sugar inducing conditions. These mutants show reduced sucrose induction of the endogenous AGPase gene, and we have named these mutants isi for impaired sucrose induction. Mutants unique to this genetic screen as well as mutants having additional phenotypes on other sugar sensing screens, have been isolated.

6-37

Cytokinin-responsive kinases and phosphatases

Thomas Schmuelling, Silke Schaefer, Jan Wohlfahrt and Michael Riefler
Centre for Plant Molecular Biology, General Genetics, University of Tuebingen, Auf der Morgenstelle 28, D-72076 Tuebingen, Germany

Changes in transcript abundance of protein kinases and phosphatases in response to a signal can be an indication that the respective proteins play a role in transducing this signal. We have identified by different means (EST microarrays, Representational difference analysis) kinases and phosphatases in Arabidopsis thaliana and Nicotiana tabacum whose steady state mRNA levels change in response to cytokinin treatment. One of the responsive kinase genes shows high sequence homology to receptor-like kinases of plants. The steady state transcript level of this cytokinin-regulated receptor kinase homologue (CRK1) was reduced 30 min after cytokinin treatment of a tobacco cell culture. The kinetics of downregulation was dose-dependent and could be a desensitization step. Physiological cytokinin concentrations were sufficient to induce the down-regulation. Studies with kinase and phosphatase inhibitors revealed that a phosphatase is likely involved in signaling processes upstream of CRK1. The predicted CRK1 protein has a single transmembrane domain, an extracellular domain with features of cAMP and ATP binding sites and a cytoplasmic Ser/Thr kinase domain. The extracellular domains of the Arabidopsis and tobacco proteins share 40% homology. We hypothesize that CRK1 is involved in an early step of cytokinin signaling. Gain of function and loss of function experiments are under way in Arabidopsis.

6-38

Light in darkness: genes that regulate the light/dark signalling cascade in plants.

Kim Snowden (1), Pedro Gil (2), Joanne Chory (2) and Jo Putterill (1).
(1) Plant Sciences, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand (2) Plant Biology Laboratory, Salk Institute, La Jolla, CA 92037 USA

Light energy drives photosynthesis and light signals have a profound effect on gene expression, with a resulting impact on plant developmental responses such as seed germination, seedling morphogenesis and the timing of flowering. To isolate genes in downstream branches of the light-regulated signal transduction pathway, a genetic screen was devised to identify mutants with increased expression of the photosynthetic gene CAB (chlorophyll a/b-binding protein) in darkness. The DOC1 gene (for dark overexpression of CAB) is one candidate for a further step in the light signal transduction pathway. Progress towards the cloning and characterisation of the DOC1 gene will be presented.

6-39

Negative Regulation of Gibberellin Signal Transduction by SPINDLY

S. Swain*, T-S. Tseng & N. Olszewski
Dept. Plant Biology, Univ. of Minnesota, St. Paul, MN 55 108, USA.* Present address: CSIRO Plant Industry, PMB, Merbein, VIC 3505, Australia.

The SPINDLY (SPY) protein is involved in gibberellin (GA) signal transduction and resembles animal serine (threonine)-O-linked N-acetylglucosamine transferases (OGTs). This type of dynamic protein glycosylation has been found on a wide range of eukaryotic nuclear and cytosolic proteins and may regulate their biological activity by preventing serine (threonine) phosphorylation by kinases. Similar to animal OGTs, the N-terminal half of SPY contains 10 tetratricopeptide repeats thought to mediate protein-protein interactions, suggesting that SPY may interact with other proteins to regulate GA response.

Recessive spy mutations exhibit a range of phenotypes, some of which are consistent with increased GA signaling. Several lines of evidence suggest that SPY is a negative regulator of GA response. For example, loss-of-function spy mutations can partially suppress the effects of chemical inhibitors of GA biosynthesis (paclobutrazol and uniconazole), the GA-deficient ga1 mutation, and the reduced GA-response mutation, gai.

To study the role of SPY in plant development, we have analyzed spy mutants and constructed transgenic Arabidopsis plants that overexpress SPY under the control of the 35S promoter of CaMV. 35S:SPY lines exhibit a subtle but complex phenotype that has led to new models for GA signal transduction. Some 35S:SPY phenotypes may result from the action of SPY in other signaling pathways. In addition, overexpression of SPY in adult plants may titrate RGA and/or GAI, two additional negative regulators of GA response. This model predicts that one function of SPY is to form a protein complex with RGA and/or GAI to negatively regulate GA signal transduction by O-GlcNAc-modifying unknown target protein(s).

7-1

Systemic acquired resistance is controlled by both positive and negative regulators

Xin Li, Yuelin Zhang, Joe Clarke, Mark Kinkema, Weihua Fan, and **Xinnian Dong**
DCMB Group, Department of Botany, Duke University, Durham, NC 27708, USA

The NPR1 protein of Arabidopsis thaliana has been shown to be an important regulatory compoment of systemic acquired resistance (SAR). Mutations in the NPR1 gene block the induction of SAR by the signal molecule salicylic acid (SA). The sequence of NPR1 shows that NPR1 contains ankyrin repeats and is involved interaction with other protein(s). To further study the function of NPR1 and the regulatory mechanism of SAR, both a molecular and a genetic approaches were taken to identify additional SAR regulatory components through interaction with NPR1 or their ability to suppresss the npr1 mutation. The results of these projects will be represented

7-2

Molecular genetic Dissection of R gene-mediated Disease Resistance Pathways

Jane E. Parker, Bart J. Feys, Nicole Aarts, Mark Austin, Lisa Moisan, Louise N. Frost, and Michael J. Daniels
Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

We are using molecular genetic, and increasingly biochemical, approaches to unravel components of a disease resistance pathway in Arabidopsis. Mutational screens revealed four non-allelic genes, EDS1, PAD4, RTR1 and RTR2, that are required for the function of RPP5, conferring downy mildew resistance. RPP5 belongs to a major subclass of nucleotide binding-leucine rich repeat (NB-LRR) type R proteins that has amino-terminal Toll/Interleukin-1 receptor (so called "TIR") homology. Genetic studies show that both EDS1 and PAD4 are central components of a signalling pathway conditioned by the TIR-NB-LRR R protein structural type, against multiple pathogen classes. Both components precede the function of salicylic acid-mediated plant responses, although their activities are not synonymous. EDS1, encoding a putative lipase, was used as bait in a yeast two-hybrid assay and found to interact with PAD4 that encodes a quite distinct protein that also has lipase homology. PAD4 was also isolated by positional cloning in Dr Jane Glazebrook's lab at The University of Maryland, USA. Our studies are now focusing on understanding the mode of expression of these and other disease resistance signalling components in response to pathogen attack. We are investigating the anticipated enzymatic activities of EDS1 and PAD4 and their possible association in the plant cell. Progress here and in genetic analyses of eds1 combinations with other putative disease resistance signalling mutations will be described.

7-3

Identification of Arabidopsis proteins that interact with the movement protein of cauliflower mosaic virus (CaMV)

Stephen H. Howell, Huang Zhong and Vyacheslav Andrianov.
Boyce Thompson Institute, Cornell University, Ithaca NY, USA.

Cauliflower mosaic virus (CaMV) spreads systemically from local sites of infection in Arabidopsis plants. The cell-to-cell and long-distance movement of CaMV are mediated by a movement protein (MP) encoded by the virus (gene I). There is great interest in identifying host proteins in Arabidopsis that interact with and link the viral MP to the cellular trafficking machinery. The CaMV MP was used in a yeast 2-hybrid system to screen an Arabidopsis cDNA library (provided by William L. Crosby, Plant Biotechnology Institute, Saskatoon, Saskatchewan) for cDNAs encoding MP-interacting proteins (MPIPs). Three different clones encoding proteins MPIP1, -2 and -7 were recovered repeatedly using the N-terminal third of the CaMV MP as bait in the 2-hybrid system. The interaction with one of the Arabidopsis proteins, MPIP7, correlated with the infectivity properties of known MP mutants. For example, a non-infectious MP mutant, ER2A, with two amino acid changes in the N-terminal domain failed to interact with MPIP7, while a MP from an infectious second-site mutant, which differed from ER2A by only a single amino acid change, interacted positively. The CaMV MP binds to residues neighboring one of two central hydrophobic domains in MPIP7. MPIP7 is a member of small gene family in Arabidopsis, is expressed widely in uninfected plants and is related in sequence, size and hydropathy profile to a rat protein (PRA1) described as a dual prenylated rab and VAMP2 receptor associated with the vesicle transport machinery in mammalian cells. MPIP7:GFP fusions were constructed to locate the MPIPs in uninfected Arabidopsis cells and in gene I-transfected protoplasts which form long tubules that project from their surface. These findings are consistent with a role of MPIPs in viral movement and suggests that the viral MP may interact with a component of the macromolecular trafficking machinery in plant cells.

7-4

Trans-splicing ribozymes for potential virus resistance in plants and for use as "RNA sensors"

Uwe Köhler (1), Brian G. Ayre (1,2), and Jim Haseloff (1)
(1) Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, U.K. and (2) Section of Plant Biology, Division of Biological Sciences, Cornell University, Ithaca, NY

We have recently described modified trans-splicing ribozymes with improved biological activity which work well in E.coli and yeast (Köhler et al., JMB 285:1935-1950 (1999); Ayre et al., PNAS 96:3507-3512 (1999)). These ribozymes allow accurate splicing of a new 3' exon sequence into a chosen site within any target RNA and in frame fusion of the exon results in expression of a new gene product. We have targeted the ribozymes against mRNAs of chloramphenicol acetyltransferase, human immunodeficiency virus, and cucumber mosaic virus (CMV), and demonstrated both trans-splicing and delivery of a marker gene in E. coli cells. The ribozymes targeted against the coat protein mRNA of CMV, a widespread plant pathogen, were also engineered to trans-splice the coding sequence of the diphtheria toxin A chain (DTA) in frame with the viral initiation codon of the target sequence. In Saccharomyces cerevisiae ribozyme expression was shown to specifically inhibit the growth of cells expressing the virus mRNA. These trans-splicing ribozymes are now being tested in plants: the anti-CMV ribozymes were introduced into Arabidopsis thaliana and tobacco. The transgenic plants were challenged with CMV and analyzed for trans-splicing and virus resistance. Also, this new class of ribozymes might be useful as "RNA sensors". Trans-splicing ribozymes could be used for the delivery of reporter gene activity in living cells, conditional upon the presence of a chosen mRNA species. For example, we have constructed ribozymes capable of splicing the GAL4-VP16 transcription factor coding sequence into the KNAT3 or CDC2A mRNA of Arabidopsis. Trans-splicing would result in GAL4-VP16 protein driving a (reporter) gene of choice under control of the UAS promoter. These trans-splicing ribozymes may provide a new class of agents for engineering virus resistance and might be a useful tool in functional genomics.

7-5

Analysis of resistance sources towards Leptosphaeria maculans in Arabidopsis thaliana, and its use for rapeseed

Bohman Svante, Dixelius Christina
Dept of Plant Biology Swedish University of Agricultural Sciences (SLU), Sweden

Leptosphaeria maculans is a fungal facultative parasite, living mainly on wild and cultivated genera of Brassicaceae, particularly on Brassica, Sinapis and Raphanus. L. maculans have a worldwide distribution, but causes damage as a pathogen only in temperate regions or at high altitudes in the tropics. Resistance to L. maculans has been found in several rapeseed related Brassica but also in Arabidopsis thaliana ecotypes. Due to the relatively close relationship between Arabidopsis and Brassica, production of somatic hybrids between the species have been accomplished. Furthermore, the DNA transferred from A. thaliana mediating resistance for cotyledons and adult leaf in Brassica napus are different for the different resistance. For adult leaf resistance a co-segregation with chromosome 3 has been shown and for cotyledon resistance a segregation with chromosome 1 are under investigation. To establish a more exact position of the transferable resistance loci / locus on chromosome 1 and 3, asymmetric somatic hybrids between A. thaliana and B. napus containing various parts of chromosome 1 and 3 have been utilised. The hybrids have been backcrossed to B. napus two to three times before being selfed. To identify the transferred DNA, Arabidopsis total DNA on BAC filters from Mozo et al (1996) will be utilised.

7-6

Characterization of mutants defective in pathogen-mediated signal transduction.

Katherine J. Denby (1), Laure Jason (1) and Robert L. Last (2)
(1) Dept. of Biochemistry, University of Cape Town, Private Bag Rondebosch 7701 South Africa(2) Cereon Genomics, 14 Sidney Street, Cambridge MA 02139

We are interested in how plant defence responses are regulated during pathogenesis and particularly in identifying components of pathogen-mediated signal transduction pathways. The enzymes of tryptophan biosynthesis are induced, at both the mRNA and protein level, in response to infection with a virulent bacterial pathogen and have been used as markers for analysis of signalling events. The major phytoalexin in Arabidopsis, camalexin, is produced from an intermediate of the tryptophan pathway and accumulates co-ordinately with induction of the tryptophan biosynthetic enzymes. These responses to pathogen infection have been shown to require salicylic acid, as well as the COI1 protein. COI1 is a protein involved in regulating gene expression in response to jasmonic acid (JA). However, experiments with the fad3,7,8 triple mutant unable to synthesise JA, suggested that JA itself is not required for induction of the trp enzymes and accumulation of camalexin. It appears that the COI1 protein is being activated independently of JA during pathogen infection. Mutants have been isolated which are defective in induction of the tryptophan enzymes and accumulation of camalexin after Pseudomonas infection. These ups (Underinducer for Pathogen and Stress) mutants are currently being characterised with respect to induction of other defence responses and sensitivity to pathogens.

7-7

Mlo-like genes represent the only family of 7TM proteins in plants

Alessandra Devoto, Andreas Hartmann, Pietro Piffanelli, Erik Wallin, Gunnar von Heijne and Paul Schulze-Lefert
The Sainsbury Laboratory, Norwich Research Park, Colney lane, Norwich NR4 7UH, England, UK

Lack of wild type Mlo protein leads to broad-spectrum disease resistance to the pathogenic fungus, Erysiphe graminis f. sp. hordei, and deregulated cell death in leaf tissues. We have shown that Mlo is anchored in the plasma membrane via seven transmembrane helices such that the N-terminus is extracellular and the C-terminus intracellular. In the A. thaliana databases we identified 16 family members of which 11 are full length and are distributed on the various chromosomes. We estimate the total number of Mlo homologues to be approximately 35 in the Arabidopsis genome. Analysis of the full-length sequence-related proteins revealed almost identical hydrophobic segments predictions. As these predictions match the 7 TM topology determined experimentally for the barley Mlo, it is likely that all Mlo family members share the same membrane topology. This common topology of the Mlo sequence-related proteins enabled us to interprete stretches of sequence conservation and variability on a common 7 TM scaffold. A statistical genome-wide analysis of multi-spanning integral membrane proteins of the Arabidopsis database identified the Mlo gene family as the single most abundant class of 7 TM proteins. The 7TM topology is reminiscent of mammalian G-protein coupled receptors (GPCRs). To examine the possible involvement of the Mlo homologues in plant defense responses we are currently screening A. thaliana seedlings of the Sainsbury Laboratory transposon-tagged population for mlo-like phenotypes upon E. cichoracearum infection. We are also screening transposon-tagged populations and a collection of "Ds launching pads" for insertion in the A. thaliana Mlo homologues to define their function.

References

1. Büschges, R. et.al (1997) Cell 88, 695-709

7-8

Activation of Disease Resistance and Enhanced BTH Efficacy in Transgenic Plants Expressing NIM1.

Bob Dietrich, Leslie Friedrich, Laura Weislo, Mike Willits, Kay A. Lawton, John Salmeron
Novartis Agribusiness Biotechnology Research, Inc. Research Triangle Park, NC 27709 USA

NIM1 (for non-inducible immunity) is a gene that is essential for biological and chemical activation of systemic acquired resistance (SAR) in Arabidopsis. High level NIM1 expression in wild type plants results in constitutive resistance to a fungal pathogen. In addition, NIM1 overexpressing plants respond to treatment with subclinical concentrations of BTH and various fungicides, enhancing the capacity of these plants to resist attack by a variety of pathogens. In order to understand the mechanism by which this resistance is conferred, we examined changes in NIM1 gene expression as well as known SAR markers (PR1) and other genes shown to be involved in disease resistance. Since salicylic acid (SA) is critical for the establishment of SAR in response to a biological inducer, we assayed SA levels in the transgenic plants and we also crossed them to NahG plants (which prevent SA accumulation). SA levels are not elevated in the NIM1 overexpressers relative to wild type. However, the resistance is suppressed in double homozygous plants produced from the NahG crosses indicating that SA accumulation is required for the resistance phenotype.

7-9

Early response of Arabidopsis to Agrobacterium transformation
Renata F. Ditt (1), Luca Comai (1) and Eugene Nester (2).
(1) Dept. of Botany and (2) Dept. of Microbiology, University of Washington, Seattle, USA.

Agrobacterium tumefaciens is an invaluable tool to create transgenic plants for basic studies and for plant breeding. Many bacterial genes necessary for plant transformation have been characterized. Although studies on plant-Agrobacterium interaction suggest the involvement of plant cell-cycle components and DNA-repair enzymes, little is known about plant factors that participate in the process. Some host factors could be differentially regulated in response to the transformation process. To identify such factors we have been using a differential screen approach. The AFLP-cDNA pattern of roots co-cultivated with Agrobacterium for 12 hours was compared to that of the control. Differential products were isolated and sequenced. Three of these products have similarity to known A. thaliana sequences in the database and their differential expression is being confirmed by RT-PCR. We also established a transformation time-course for Arabidopsis thaliana root and leaf tissue using different Agrobacterium strains and GUS-intron gene expression as a reporter. In roots the GUS mRNA was first detected 24 hours after inoculation, although the plant response might have initiated earlier. Leaf tissue infiltrated with Agrobacterium and collected at different time-points is also being analyzed by AFLP-cDNA. Work sponsered by CNPq, Brazil (R. F. Ditt fellowship) and by NSF, USA.

7-10

Two-hybrid analysis reveals interactions between EDS1 and PAD4, two essential components of R-gene mediated signaling in Arabidopsis
Bart J. Feys, Lisa Moisan and Jane E. Parker
Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

The Arabidopsis EDS1 gene has previously been identified as a central signaling component in a resistance pathway that depends on recognition through R genes of the TIR-NBS-LRR class. In addition, EDS1 also functions in the containment of compatible pathogens. EDS1 has recently been cloned and is the first member of a novel class of putative lipase-like proteins in plants. In order to identify interacting protein partners, a Two-Hybrid screen was performed. A second, quite distinct lipase-like protein that interacted specifically with EDS1 was isolated, and found to correspond to PAD4. PAD4 has previously been described as a regulator of the defence response in Arabidopsis (Zhou et al, Plant Cell 10, 1021). EDS1 was also shown to interact with itself, suggesting the existence of homo- and/or hetero-oligomerization in planta. PAD4 mRNA is induced strongly in both compatible and incompatible interactions, and also by application of salicylic acid. Only the former induction of PAD4 is dependent on EDS1 function. As a prelude to a biochemical in planta approach, EDS1 polyclonal antisera have been generated and used to analyse EDS1 protein levels. No EDS1 protein was detected in any of the eds1 mutants analysed, but EDS1 protein was detected at wild-type levels in a pad4 null mutant background. We are currently generating anti-PAD4 antibodies to analyse protein-interactions in planta and survey the composition of EDS1/PAD4 protein complexes by gel-filtration after various plant-pathogen challenges.

7-11

The Function of NPR1 in Regulating Plant Defense
Mark Kinkema, Yuelin Zhang, Weihua Fan, and Xinnian Dong
Developmental, Cellular, and Molecular Biology, Duke University, Durham, NC 27708

NPR1 is a key regulator in the signal transduction pathway(s) leading to plant disease resistance. Mutations in NPR1 result in plants that are highly susceptible to pathogen infection while transgenic plants that overexpress the NPR1 protein have an enhanced plant defense response. Here we describe experiments aimed at determining the role of NPR1 in plant defense. Analysis of the subcellular localization of a

functional NPR1-GFP fusion protein reveals that NPR1 accumulates in the nucleus in response to biological and chemical inducers of plant defense responses. Increased levels of the signal molecule salicylic acid enhance the nuclear accumulation of NPR1-GFP and result in elevated levels of PR1 gene expression. Nuclear targeting of NPR1 requires a bipartite nuclear localization signal consisting of five basic amino acids. The NPR1 protein was found to interact with specific bZIP transcription factors both in vitro and in the yeast two hybrid assay. One of these factors was shown to bind to an element in the PR1 promoter that is known to be required for PR1 gene activation. Our results suggest that NPR1 may regulate the expression plant defense genes through its interaction with specific transcription factors in the nucleus.

7-12

Expression of bacterial avirulence genes form Xanthomonas campestris pv. malvacearum in cotton
Helen McFadden, Rob de Feyter and Liz Dennis
CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

Xanthomonas campestris pv. malvacearum (Xcm) infection in cotton gives rise to the disease, bacterial blight. Resistance to most blight strains is present in cotton and is controlled by the interaction of resistance (R) and avirulence (avr) genes. Transient expression of Xcm genes in cotton leaves was achieved by inoculation with cultures of Agrobacterium containing avr gene constructs. An artificial hypersensitive response was observed when corresponding R genes in the host and avr genes in the bacterium were present. Stable expression of 35S::avr gene constructs in transgenic cotton gave a range of phenotypes depending on the nature of the R and avr genes present. In some instances, phenotypes usually associated with disease resistance, such as elevated expression of PR proteins were observed in the absence of known resistance genes.

7-13

Transgenic Arabidopsis reporter lines showing local induction of defense genes as a sreenable system for the identification of mutants with constitutive defense gene expression
Norbert Nass, Claudia Köhler, Astrid Patzlaff, Susanne Berger and Dierk Scheel
Institute for Plant Biochemistry (IPB),Weinberg 3, 06120 Halle/Saale Germany

Promoter fragments of three putative defense genes (pal2, eli3, prxCA) have been isolated and fused to firefly luciferase in a binary transformation vector. Transgenic Arabidopsis lines have been generated by Agrobacterium mediated transformation. In these transgenic lines, the luciferase reporter gene is activated upon pathogen attack and wounding as visualized by using a photon counting video system, however, timing and pattern of these responses depend on the promoter used. The reporter constructs are not activated by the application of known inducers of systemic aquired resistance such as salicylic acid or jasmonate. Therefore, the gene activation observed here depends on a different mechanism. To isolate mutants that are compromised in these signal transduction pathways we generated and screened EMS-mutagenized M2-populations for constitutive expression of the transgenes and a variety of putative mutants could be isolated. Based on Northern blot-analysis most of these “mutants” show overexpression of the authentic genes and in some cases the expression of other defense genes is also altered. Also, a variety of morphological alterations seems to be associated with overexpression of the reporter genes in some of the mutant lines, however, a subset of the putative mutants shows no significant reduction in vigour. Therefore, the genes involved might be good candidates for engineering plant resistance.

7-14

Characterization of an Arabidopsis mutant which exhibit runaway cell death.
Morten Petersen, Anders Jensen, Susanna Cirera and John Mundy
Institute of Molecular Biology, Copenhagen University, Oesterfarimagsgade2A, 1353, Copenhagen, Denmark

We are using a transposon-tagging system (Sundaresan et al. 1995 Genes Develop. 9, 1797-810) to study genes mediating physiological and developmental processes in Arabidopsis. During the past year we have identified several recessive mutants and cloned the tagged genes and corresponding cDNAs. . One of the genes encodes a receptor kinase with leucine rich repeats (LRRK; Kobe & Deisenhofer 1994 TIBS 19, 415-21). The LRRK-knockout produces a hypersensitive-like reaction leading to runaway cell death. Since many plant LRR-containing proteins are resistance genes mediating the hypersensitive reaction in response to pathogen attack, the recessive LRRK mutation suggest that this LRRK is a negative regulator of cell death (Dietrich et al. 1997 Cell, 88, 685-94). We have to date performed several types of experiments to determine the nature of the LRRK mutation. For example, northern hybridizations showed that the LRRK mutant ectopically expresses several pathogenesis related proteins. This is accompanied by accumulation of reactive oxygen species such that the LRRK mutant undergo a massive oxidative burst compared to wild type. As an oxidative burst is believed to be sufficient to trigger apoptosis (Penell & Lamb 1997 Plant Cell, 9, 1157-68), we assayed LRRK mutant protoplast for apoptotic effects using a fluorescence-assisted cell sorter. This showed that LRRK mutant protoplasts exhibit 25% higher levels of apoptosis compared to controls. Furthermore, the apoptotic characteristics could be decreased 33% by treatment with DPI, an inhibitor of the neutrophil oxidase, whereas cytokinin treatment did not reduce apoptosis. These results indicate that the spread of necrosis in LRRK mutants is an active process and is not due to senescence.

7-15

Isolation of differentially expressed genes involved in clubroot disease
Astrid Schuller, Oliver In, Jutta Ludwig-Müller
J.W. Goethe-Universität, Institute of Botany, Siesmayerstr. 70, 60323 Frankfurt/M, Fed. Rep. of Germany

The interaction between the fungus Plasmodiophora brassicae, the causal agent of the clubroot disease in crucifers, and its hosts is investigated by two strategies. Using healthy and infected roots of Brassica rapa and a series of subtractive hybridisations and amplification reactions, we have constructed a cDNA library highly enriched in cDNAs expressed in infected roots. The analysis of a sample of 25 clones from this library identified three cDNA clones corresponding to genes expressed at increased levels in infected roots. Sequence analysis and comparison with database entries of these cDNAs revealed that one clone possesses high homology to small heat shock proteins. Hybridisation with a labelled 17,6 class one small heat shock clone from Arabidopsis thaliana showed a strong signal with our fragment. Southern blot analysis should reveal whether the gene is from fungal or plant origin. The remaining two clones showed no similarity to any sequence in the databases. In addition, root hypertrophy in club root disease is dependent on increased auxin levels and these could result from auxin-conjugate hydrolysis. Therefore by PCR amplifications of genomic DNA and RT-PCR strategy based on the design of degenerate oligonucleotides resembling conserved domains of amidohydrolases, we isolated two genomic fragments from Brassica rapa, one cDNA clone from seedlings of Brassica napus and one cDNA clone from seedlings of Brassica rapa. Comparison of these clones with database entries revealed high identity to IAR3, ILL1/2 and ILL3 genes from Arabidopsis thaliana. In Southern blot analysis each individual clone recognised a different specific band. To determine the expression in the course of development of healthy and infected roots of Brassica rapa RNA gel blots were analysed. First results indicate differential expression of the genes.

7-16

Suppressors of the npr1-5 mutant identify new loci involved in plant defense
Jyoti Shah (1), Pradeep Kachroo (2) and Daniel F. Klessig (2)
(1) Kansas State University, Manhattan, KS(2) Rutgers University, Piscataway, NJ

Salicylic acid (SA), jasmonic acid (JA) and ethylene are important signal molecules in plant defense against disease. These molecules induce expression of several defense-associated genes. For example, SA activates expression of the pathogenesis-related (PR) genes, some of which possess antimicrobial activity. Likewise, JA and ethylene activate expression of the antifungal Defensin gene (PDF1.2). The Arabidopsis npr1-5 mutant was identified in a screen for SA non-responsive mutants. npr1-5 plants are unable to express the PR (PR-1, BGL2 and PR-5) genes at elevated levels in response to SA. Furthermore, npr1-5 plants show increased susceptibility to pathogens. We have isolated three suppressor mutants (ssi1, ssi2-1 and ssi2-2) which constitutively express elevated levels of PR genes in npr1-5 plants. These mutants also accumulate very high levels of SA and spontaneously develop HR-like lesions. Furthermore, the dominant ssi1 mutation restores resistance to pathogen in npr1-5 plants. Interestingly, PDF1.2 is also expressed at elevated levels in ssi1 plants. The mutant phenotypes of ssi1, including PDF1.2 expression, are dependent on its ability to accumulate and respond to SA. Furthermore, SSI1 regulates cross talk between the SA-dependent and ethylene/JA-dependent defense pathways.
In contrast to ssi1, the recessive ssi2-1 and ssi2-2 mutants do not constitutively express PDF1.2, nor do they require elevated levels of SA for the expression of BGL2 and PR-5, and development of spontaneous lesions. However, elevated levels of SA are necessary for the expression of PR-1 in ssi2-1 npr1-5 plants. Hence, PR-1 expression in the ssi2-1 occurs via a SA-dependent but NPR1-independent pathway.

7-17

Analysis of the Arabidopsis susceptible response to Pseudomonas syringae pv. tomato.
Roger Thilmony, Julie Zwiesler-Vollick, Paula Lee, and Sheng Yang He.
Department of Energy Plant Research Laboratory, Michigan State University, East Lansing MI, USA.

We are studying the compatible interaction between Arabidopsis thaliana and the bacterial plant pathogen, Pseudomonas syringae pv. tomato (Pst) DC3000. Virulence in Pst DC3000 is controlled by the hypersensitive response and pathogenicity (hrp) island, which contains regulatory genes, genes that encode a type III protein secretion system and virulence genes. In order to dissect the hrp gene-controlled pathogenesis in host plants, we are studying both global gene regulation in the host during Pst DC3000 infection, as well as the plant response to expression of individual bacterial secreted proteins. We have isolated total RNA from plants infiltrated with water, Pst DC3000 and a hrpA- polar mutant which is not virulent on Arabidopsis. The RNA samples were used to make cDNA probes, which were then hybridized with approximately 10,000 unique A. thaliana cDNAs using DNA microarray technology (in collaboration with Monsanto, St. Louis MO, USA). We hope that such a genome-wide host expression analysis will reveal specific signaling and/or metabolic pathways that are affected by hrp-dependent virulence factors. Data from the analysis will be presented. In an effort to understand the roles of individual hrp-secreted proteins, we are creating transgenic plants that express avrB, avrE, avrPto, hrpZ or hrpW under control of a glucocorticoid-inducible promoter. Each of these genes encode putative virulence proteins secreted via the type III secretion apparatus. Characterization of these plants and their response following transgene induction will be presented.

7-18

Isolation Of Plant Host Factors Required For Geminivirus Infection
Doreen H. Ware, Kenneth J. Buckley, Keith R. Davis,
Plant Biotechnology Center, Ohio State University, Columbus OH.

Viruses represent a unique class of pathogenic agents because of the extremely intimate associations formed between the virus and its host. Although specific interactions of viruses with their host can vary widely, the requirement for host factors is ubiquitous. Host factors play critical roles throughout the virus life cycle, including the expression of viral genes, genome replication and the movement of virus throughout the plant. Our studies focus on isolating and characterizing specific plant proteins required for infection of Arabidopsis by a geminivirus, beet curly top virus (BCTV). A yeast two-hybrid library was screened, using BCTV-CFH ORF L4, a protein previously identified to be involved with symptom development. A partial cDNA clone, YC58 was identified and tested against the ORF L4 of second BCTV virus, Logan. Given that these two strains of BCTV have only 47% sequence identity of the L4 proteins, this result strongly suggests that this interaction is functionally relevant. YC58 is a homologue to the Drosophila shaggy (sgg) and mammalian GSK3 genes. The sgg/GSK3 are key components of the wingless (wng) /wnt pathway, one of the major families of developmentally important signaling molecules in animals (Trevor, 1998). The loss or inappropriate activation of Wnt expression has been shown to alter cell fate, morphogensis and mitogenesis. Shaggy is a downstream component of this pathway. In vertebrates, C. elegans and Drosophila shaggy is a 1 or 2 gene family. The Arabidopsis shaggy kinases (ASK) comprise a family of at least 10 members (Dornelas, 1998). Our results have identified a novel interaction between a viral protein required for symptom development with a specific kinase in a conserved evolutionary signaling pathway.

7-19

A single locus leads to resistance of Arabidopsis thaliana to bacterial wilt caused by Ralstonia solanacearum through an interaction similar to the hypersensitive response
Chang-Hsien Yang and Gan-Der Ho
Graduate Institute of Agricultural Biotechnology National Chung Hsing UniversityTaichung, Taiwan 40227 ROC

Strains of Ralstonia solanacearum have been shown to cause bacterial wilt in some but not all ecotypes of Arabidopsis thaliana. Using leaf inoculation procedure in this study, wilt symptom development was identical to that observed after inoculation of bacteria into the inflorescence stem in the susceptible plants as reported previously. This method thus provides an easy and efficient way to study the interactions between A. thaliana and R. solanacearum. We demonstrate here that after inoculation with R. solanacearum Ps95 of leaves of resistant ecotype S96, necrosis around the inoculation site rapidly appeared and no further symptoms developed in the plants. Leaves of susceptible ecotype N913 completely wilted 7 days after inoculation with Ps95, and symptoms spread systemically throughout the whole plant within two weeks after inoculation. These results suggest that the resistance of Arabidopsis to R. solanacearum is due to a response similar to the hypersensitive response (HR) observed in other plant disease. Northern blot analysis of the expression of defense-related genes, known to be differentially induced during the HR in Arabidopsis, indicated that pathogenesis-related protein PR-1, glutathione S-transferase (GST1) and Cu,Zn superoxide dismutase (SOD) mRNAs increased significantly in S96 leaves between 3 to 12 hours after infiltration with Ps95. The induction of these genes in susceptible ecotype N913 by Ps95 was clearly delayed. Genetic analysis of crosses between resistant ecotype S96 and susceptible ecotype N913 indicated that resistance to Ps95 is due to a single, dominant locus.

8-1

Patterning and cell morphogenesis: Trichomes in Arabidopsis as a model system
Martin Huelskamp
LS für Entwicklungsgenetik, Auf der Morgenstelle 1. 72076 Tübingen, Germany,

Leaf trichomes in Arabidopsis are large polarized single epidermal cells with a predictable three-dimensional structure. Based on a large collection of trichome mutants trichome development can be dissected into different regulatory steps: trichome initiation, endoreduplication, cell polarization, branching and cell elongation. We study the role of four genes, GL1, TTG, TRY and CPC, in regulating the regular distribution of trichome on the leaf surface (called spacing pattern). Using genetic mosaics, we demonstrate that the formation of trichome clusters in try mutants is not correlated with cell lineage favoring a model in which the spacing pattern is established via cell-cell interactions. Our genetic analysis leads to a scenario in which protodermal cells compete with each other with TRY and CPC acting as negative regulators of GL1 and TTG. For the analysis of cell morphogenesis we focus on the analysis of branching mutants. Genetic, cell biological and molecular data on the regulation of trichome branching will be presented.

8-2

Role of intercellular signaling in the cell fate specification
Kiyotaka Okada, Yasuhiro Koshino, Takuji Wada1, Ryuji Tsugeki
Department of Botany, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan 1 Present address: Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA92307, USA

The fate of plant cells is largely determined by extercellular signals. Young cells generated at meristems develop to mature cells following temporal and spatial regulations directed by neighboring mature cells, by signal molecules supplied from distant cells, or by environmental conditions. Root hairs are structurally distinct feature of root epidermal cells, and therefore, root hair development has been studies as a model system to study cell specification. In Arabidopsis, root epidermal cells to develop either to root hair-forming cells or to hairless cells. Former studies revealed that a homeobox gene, GL2, works as negative regulator, and a myb gene, CPC, as positive regulator of root hair formation. In situ hybridization and transgenic study of the promoter:GUS staining showed that both CPC and GL2 were strongly expressed in the hairless cells of wild type roots. However, in the cpc mutant, expression of GL2 was observed in all epidermal cells. In addition, expression of GL2 and CPC was promoted by the ectopically expressed maize R gene, but the transcription of CPC was repressed by itself. These results indicate that CPC and GL2 are controlled by the same regulatory system. Our data indicate that the intimate communication between the hair-forming cell and the hairless cell is involved in the fate determination of root epidermal cells. We are now investigating one fascinating possibility that CPC protein moves to the neighboring hair-forming cells from hairless cells where CPC is transcribed.

8-3

Arabidopsis SPIRAL1 gene is required for directional control of cell elongation.
Ikuyo FURUTANI, Keiji MATSUBARA and **Takashi HASHIMOTO**.;
Grad. school Biol. Sci., NAIST, Ikoma 630-0101, Japan

Mutations in Arabidopsis SPIRAL1 (SPR1) locus, cause isotropic expansion of endodermal and cortical cells in root, etiolated hypocotyl, and dark-grown influorescent stem, and induce right-handed spiral in epidermal cell files of these organs. Interestingly, addition of a low dosage of either microtubule-depolymerizing drug propyzamide (PPM) or microtubule-stabilizing drug taxol in the culture medium was found to cause isotropic expansion of endodermal and cortical cells at the root elongation zone of wild-type Arabidopsis seedlings, resulting in left-handed spiral. Exogenous application of PPM or taxol to spr1 seedlings reverted the direction of spiral, on the dose-dependent manner, from right-handed to left-handed and PPM at 1μM completely suppressed the cell expansion defects. We propose that a microtubule-

dependent process and SPR1 act antagonistically to control directional cell elongation by preventing elongating cells from twisting to skewed direction. SPR1 was cloned by a map-based approach, and found to encode a small novel protein. Deletions in spr1-1 and spr1-4 covered a promoter region and the first exon of SPR1, whereas spr1- 2 contained a T-DNA insertion just before a transcription start site. spr1-3 produced a SPR1 protein truncated at the C-terminus. Expression analysis involving RNA gel blot, in situ hybridization, and SPR1 promoter::GUS transgenic plants indicated that SPR1 is expressed in various cell types of wild-type Arabidopsis plants. SPR1-specific antibodies detected SPR1 in the peripheral region of microsomal fraction, in keeping with its presumed interaction with cortical cytoskelton.

8-4

Members of the YABBY gene family specify abaxial cell fate in Arabidopsis

John L. Bowman, Kellee R. Siegfried, Yuval Eshed, and Stuart F. Baum
Section of Plant Biology, University of California Davis, Davis, California 95616, USA

Lateral organs produced by the shoot apical and flower meristems exhibit a fundamental abaxial-adaxial asymmetry. We describe three members of the YABBY gene family, FILAMENTOUS FLOWER, YABBY2, and YABBY3, isolated on the basis of homology to CRABS CLAW. Each of three genes is expressed in a polar manner in all lateral organ primordia produced from the apical and flower meristems. The expression of these genes is precisely correlated with abaxial cell fate in mutants in which abaxial cell fates are found ectopically, reduced or eliminated. Ectopic expression of either of two members of the YABBY gene family, FILAMENTOUS FLOWER or YABBY3, is sufficient to specify the development of ectopic abaxial tissues in lateral organs. Conversely loss of polar expression of these two genes results in a loss of polar differentiation of tissues in lateral organs. That these organs often fail to develop blades is consistent with the proposal that juxtaposition of abaxial and adaxial cell fates is required for blade outgrowth. Taken together, these observations indicate that members of this gene family are responsible for the specification of abaxial cell fate in lateral organs of Arabidopsis. Furthermore, ectopic expression studies suggest that ubiquitous abaxial cell fate and maintenance of a functional apical meristem are incompatible.

8-5

Isolation and characterization of a gene encoding alpha subunit of F-actin capping protein in Arabidopsis

Yiqun Bao (1, 2), Ueli Klahre (2), and Nam-Hai Chua (2)
(1) Laboratory of Plant Cell Biology, Institute of Molecular Agrobiology, 1 Research Link, National University of Singapore, Singapore 117604, and (2) Laboratory of Plant Molecular Biology, The Rockefeller University

Actin-filament capping protein (CP) occurs as a heterodimer of α and β subunits, both of which are necessary for the function. In vitro studies have shown that CP binds tightly to the barbed ends of actin filaments, thereby preventing the addition and loss of actin monomers at this end. CP nucleates actin filament formation, but, unlike other capping protein, CP does not sever actin-filaments and does not require Ca^{2+} for activity. *In vivo* studies in many organisms such as nematode, yeast and *Drosophila* suggest that this protein is involved in organizing actin filaments. Knockout mutants of either α or β subunit of CP in *S. cerevisiae* have a deficit of actin cables and an increased amount of actin spots in the mother. Mutant cells are round and enlarged.

We have isolated two genes encoding the α subunit of the CP in *Arabidopsis thaliana* (AtCPα1 and AtCPα2) which shows high homology with the α subunit from other organisms. The two genes show over 99% homology in their coding region, differ only 1 amino acid, but intron number and size are different. Southern blot analysis also indicated that there are two closely related genes. Transgenic *Arabidopsis* plants overexpressing partial cDNA of AtCPα1 are generally bigger than Wt and have defects in fertility which is caused by co-suppression of endogenous genes. 7 day dark grown transgenic seedlings are 1.5 time of Wt in their root length, hypocotyle width, And the flowers and seeds of transgenic plants are bigger than Wt. These differences might be due to a increasement of cell size rather than cell number. We will also describe transgenic plants expressing antisense or overexpression AtCPα1 full length RNA, and experiments of yeast mutant complementation with the *Arabidopsis* AtCPα1 protein.

8-6

Spatial and temporal expression of the TTG1 (Transparent Testa Glabra 1) gene

Agnese C. Bolognesi-Winfield, John C. Gray, Amanda R. Walker.
Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, United Kingdom.

The TTG1 (Transparent Testa Glabra 1) gene plays a regulatory role in various developmental and biochemical pathways in Arabidopsis thaliana. Mutations in the gene affect the production of anthocyanin throughout the plant and the formation of hairs on roots, stems and leaves. We report on the analysis of the TTG1 transcript. The size of TTG1 mRNA, observed by northern blot analysis, was 1.5 kb; this was confirmed by mapping the 5' end of the transcript. The TTG1 mRNA was detected in all the major organs of Arabidopsis. Transcript analysis was complemented by a study of promoter-reporter gene constructs in transgenic plants. Strong GUS expression was revealed in the anthers, both in flower buds and in older flowers, in the stigma, in the ovules and on the edges and tips of the leaves. Different promoter-GUS constructs, carrying sequences of various length found at the 3' end and at the 5' end of the TTG1 gene, have also been used to generate transgenic plants. The patterns of their GUS expression are currently being characterised. The function of a putative pollen-specific sequence (AAATGA) in the TTG1 promoter is being investigated in transgenic plants following site-directed mutagenesis of the sequence.

8-7

Molecular genetics of a leaf development mutant, asymmetric leaves 1, in Arabidopsis thaliana.

Mary Byrne, Mark D. Curtis, Maitreya Dunham and Robert A. Martienssen
Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA

Leaves arise as lateral organs from primordia initiated on the flank of the shoot apical meristem. Fundamental to leaf development is the early establishment of two axis of symmetry. The dorso-ventral axis is first evident in the flattening of the emerging leaf primordia and is subsequently defined by differences in cell and tissue types. The proximo-distal axis is reflected in the progressive cessation of cell division and expansion from the tip to the base of the leaf. Differentiation of many tissue and cell types also occurs basipetally, starting at the leaf tip and proceeding toward the leaf base. Although many leaf developmental mutants have been described the genetic basis of leaf initiation, determination and patterning is largely unknown. One such mutant in Arabidopsis is asymmetric leaves 1 (as1). Wild type Arabidopsis adult rosette leaves are spatulate in shape with marginal serrations. In comparison as1 leaves are variably lobed with a drastically altered proximo-distal pattern, characterized by altered spacing of hydathodes, trichomes and veins relative to the leaf margin. We are using a gene trap (GT) and enhancer trap (ET) system to further define this mutant phenotype and have thus far identified several genes whose expression pattern is altered in as1 mutants. Also we have used positional cloning to isolate the As1 gene. Results of these studies will be presented.

8-8

An Arabidopsis dwarf mutant containing a Ds transposon insertion within a sterol methyltransferase gene

Amy N. Chin-Atkins, Wei Cai Yang, De Ye, Venkatesan Sundaresan
Plant Molecular Genetics, Institute of Molecular Agrobiology, National University of Singapore, 1 Research Link, SINGAPORE 117604.

Brassinosteroids and plant sterols are closely related in both structure and biosynthesis. An increasing number of brassinosteroid mutants have recently been described, providing some understanding of the role of this hormone. However, much less is known about the action of the plant sterols, although their presence is related to the efficiency of developing specific interactions with phospholipids in reinforcing and stabilising the bilayer architecture. Sterols and brassinosteroids share the same precursors until the pathway diverges at 24-methylene lophenol, after which parallel pathways produce sterols (sitosterol and stigmasterol) and brassinosteroids (brassinolide). Critical to the common pathway is the conversion of cycloartenol to 24-methylene cycloartenol by a sterol methyltransferase. We have identified a Ds insertion line (SGT 1866) containing an insertion within the untranslated leader of an S-adenosyl-methionine sterol-C-methyltransferase gene, that shows high homology with genes encoding putative orthologues from other plant species.
The sterol methyltransferase (SMT) mutant exhibits phenotypes similar to the brassinosteroid mutants; it is dwarfed, dark green, has reduced apical dominance, reduced male fertility and has cells of reduced size. In addition, the SMT mutant shows stunted root growth and polycotyly. Homozygotes are mostly lethal, and the penetrance is variable. In the dark, the mutants have short thick hypocotyls, open cotyledons and accumulate anthocyanin. The mutant line also expresses the GUS reporter gene in most tissues of the plant except the sepal, petal and anther. Further characterisation of this mutant is in progress.

8-9

Molecular Cloning and Characterization of Arabidopsis ADF genes

Chunhai Dong(1) Guixian Xia*(1) and Nam-Hai Chua(2)
(1)Plant Cell Biology, Institute of Molecular Agrobiology, National University of Singapore, Singapore, 117604; (2) Plant Molecular Biology, The Rockefeller University, New York NY USA 10021

It is known that the actin cytoskeleton plays an essential role in various cellular processes including cell division, cell morphorgenesis and intracellular trafficking. In response to internal stimuli and external signals, the actin cytoskeleton is dynamically reorganized. A number of actin binding proteins including profilin, ADF, fimbrin, villin etc are known to participate in the regulation of this process. ADF, Actin-Depolymerizing-Factor, is thought to be a key modulator of actin organization. The ADF/cofilin family is conserved in yeast, Dictyostelium, Drosophila, mammals and higher plants. Although some progress has been made in the last few years, the organization of the ADF gene family in Arabidopsis, the expression patterns of the different Arabidopsis genes, and the in vivo functions of the ADF proteins during Arabidopsis growth and development are largely unknown. The major aim of the present study is to address these questions.

We found that Arabidopsis contains at least nine ADF isoforms. Six clones, designated as AtADF1, AtADF2, AtADF3, AtADF4, AtADF5 and AtADF6, were obtained from EST databases. AtADF7 and AtADF9 were found in Arabidopsis genomic sequence databases. AtADF8 was identified by Ds tagging (Sundaresan et al. unpublished). We used the cDNAs of AtADF1, AtADF5 and AtADF6 as probes to screen an Arabidopsis genomic library. The full-length clones were obtained and sequenced. Comparison between cDNA sequences and genomic sequences revealed that all three ADF genes contain two introns. In order to study the expression patterns of AtADF1, AtADF5 and AtADF6, we cloned the promoter regions upstream of the GUS reporter gene and tranformed the resulting constructs into Arabidopsis. Staining analysis of the transgenic plants revealed that AtADF1 is mainly expressed in the vascular tissue of seedlings and flowers;

AtADF5 is exclusively expressed in root tip meristem; AtADF6 is constitutively expressed in all the parts of seedlings and flowers.

8-10

Characterisation of a plant DNA helicase gene analogous to a human helicase responsible for the advanced ageing disorder, Werner's Syndrome.

Kroon, J.T.M., *Markham, J.E., Wright, E.L., **Elborough, K.M**.
Plant Molecular Biology, Biological Sciences, University of Durham, DH1 3LE, U.K. *Present address: Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, U.K.

Highly conserved cDNA and genomic clones have been isolated from Arabidopsis, Brassica napus and Zea mays. The derived amino acid sequence shows greatest homology to the human DNA helicase gene who's pertubation is responsible for the human autosomal recessive genetic disorder Werners Syndrome (WS). WS is characterized by early ageing in adulthood and an ageing phenotype in tissue culture. Affected cells have a reduced replicative life-span, alterations in DNA synthesis and increased mutation rates predominantly due to large deletions. The plant homologue (2.8kb transcript) appears to be expressed only in rapidly dividing tissue i.e. root and embryo tissue, but at low levels. A small multigene family exists in both Brassica napus and Zea mays but evidence suggests there is only one gene in Arabidopsis. Arabidopsis plants are being generated with antisense downregulation in order to determine the role of this gene in plant development.

8-11

Gibberellin 2-oxidation

Diane R. Lester (2), John J. Ross (1), Jennifer J. Smith (1), **Robert C. Elliott** (1), and James B. Reid (1)
(1) School of Plant Science, University of Tasmania, GPO Box 252-55, Hobart, Tasmania, 7001, Australia
(2) Institute for Molecular Biology, Swedish University of Agricultural Sciences, Box 590, 75124 Uppsala, Sweden

Deactivation of GAs occurs via the activity of gibberellin 2-oxidases. Genes coding for these enzymes have recently been cloned from Phaseolus (1), Arabidopsis (1), and Pisum (2). Using degenerate PCR primers and an in vitro functional assay, we have isolated two gibberellin 2-oxidases from pea, one of which, PsGA2ox1, is encoded by the SLENDER (SLN) gene. The importance of gibberellin 2-oxidases is demonstrated by the elongated growth habit (due to elevated levels of GA1) found in sln plants. Similar mutants may not have been isolated in Arabidopsis because of it's small seed size, it's different growth habit, or maternal effects on the expression of the gene. The expression pattern of the two genes, their differing substrate specificities, and the effects of the sln mutation indicate that PsGA2ox1 plays a major role in GA20 deactivation in both shoots and maturing seeds, while PsGA2ox2 might be important for deactivation of the biologically active GA1 in the shoot. Southern blots and sequence comparisons suggest a diverse gene family. While both pea gibberellin 2-oxidases share the conserved motifs common to the Arabidopsis and Phaseolus gibberellin 2-oxidases, one of the pea genes, PsGA2ox2, is distinct, suggesting that it does not correspond to any of the Arabidopsis genes cloned so far.

(1) Thomas et al 1999 Proc. Natl. Acad. Sci. U.S.A. 96 (8), 4698-4703.
(2) Lester et al 1999 Plant J. in press

8-12

Mutations in PROCUSTE (PRC) cause a conditional light-dependent defect in primary wall assembly and cell elongation in the Arabidopsis hypocotyl.

M. Fagard (1), T. Desnos (1), M. McCann (2), T.Desprez (1), S.Vernhettes (1) and H.Höfte (1)
(1) Laboratoire de Biologie cellulaire, INRA, 78026 Versailles cedex, France, (2) Department of Cell Biology, John Innes Institue, Colney Lane, Norwich NR4 7UH, UK

As a part of a molecular-genetic study of cell wall assembly in growing cells, we have identified thirteen allelic mutations (prc1-13) causing reduced cell elongation and increased radial expansion in roots and dark-grown hypocotyls. In the mutant, the primary cell walls of dark-grown hypocotyl cells have pronounced structural defects as shown by TEM. Fourier Transform Infrared (FTIR) spectroscopy indicates a reduced cellulose content in these walls. In addition, many cortical cells accumulate which is calcofluor-stainable material, and therefore presumably of cellulosic nature. Surprisingly, the hypocotyl phenotype is conditional since it was not observed in the presence of light. The reversion of the phenotype is under phytochrome control. In the hope to identify a molecular link between phytochrome and cellular processes controlling the expansion of cell walls, we are attempting to isolate the gene by map-based cloning. A 60 kb window has been delimited genetically and candidate cDNAs for PRC have been identified.

8-13

Root hair diameter, growth rate and the effect of hyperosmotic media on normal and mutant root hairs of Arabidopsis thaliana

M. E. Galway, D. C. Lane and D. L. MacLellan.
Department of Biology, St. Francis Xavier University, Antigonish, Nova Scotia.

Plant root hairs are tip-growing cylindrical outgrowths of root epidermal cells that interact with soil microorganisms and assist in water and nutrient uptake. Their simple form, accessibility and relative isolation from the root make them useful models for investigating plant cell growth and morphogenesis. We used light microscopy and motion analysis of growing wild type and mutant hairs of Arabidopsis thaliana (Columbia ecotype) to investigate the relationship between hair growth rate and hair diameter, both of which vary during normal hair growth. Seedlings homozygous for the recessive mutation designated rhd4 (Schiefelbein and Somerville, Plant Cell 2:235-243, 1990) produce shorter hairs of larger average diameter than wild type hairs; rhd4 hair diameter also varies more than wild type hair diameter. Motion analysis indicated that as a group, rhd4 hairs grow more slowly and hair growth rates also vary more than wild type hairs. During periods of slower growth, the diameter of rhd4 hair tips increases, and the volume of cytoplasm in the hair tips diminishes. It has recently been shown that calcium ion concentrations in the tips of Arabidopsis hairs vary with hair growth rate, and are lower in more slowly growing hairs (Wymer et al., Plant J. 12:427-439, 1997). Growing wild type hairs in a hyperosmotic medium increases hair diameter and causes hair bulging such that they resemble rhd4 hairs, while growing rhd4 hairs in hyperosmotic medium increases hair diameter further. The results indicate that the rhd4 mutation and osmotic stress may act through a similar pathway, perhaps involving changes to the internal calcium ion concentration gradient, to alter tip growth and morphology in Arabidopsis root hairs.

8-14

Effect of Arabidopsis wus (jam) mutation on leaf development

Susumu Hamada (1), Mitsuko Kudo (1), Daisuke Shibata (2), Chiyoko Machida (1&3) and Yasunori Machida (1)
(1) Division of Biological Science, Graduate School of Science, Nagoya University, (2) Mitsui Plant Biotech Research Institute, (3) Central Laboratory for Key Technology, Kirin Brewery Co., Ltd

Arabidopsis vegetative development is divided into two phases, juvenile phase and adult phase. In Landsberg erecta ecotype, first and second leaves (juvenile leaves) have characteristics such as round leaf blade, long petiole, and lack of abaxial trichome, while the leaf blade of later leaves (adult leaves) is longer and has several trichomes on the abaxial side. Petioles of adult leaves are shorter than the juvenile ones. We have isolated the juvenile-leaf-less and misshapen-apical-meristem (jam) mutant which produces no juvenile leaves. We performed quantitative analysis of the number of leaf trichomes and the number of branching points in leaf veins. The results showed that the first and second leaves of the jam mutant had adult leaf characteristics in terms of morphological features as inspected above. The leaf initation of the first and second leaves of the jam mutant was also delayed, as in the wuschel (wus) mutant which was alellic to the jam mutant. From these results, we propose two possibilities for the lack of juvenile leaves: the wus (jam) mutation may affect the developmental programme for the juvenile phase so that the mutant skips this phase; or the primordium formation may be arrested in the wus (jam) mutant when the juvenile programme normally functions, and the programme for the adult phase may be expressed when the first and second leaves can be initiated.

8-15

Expression studies of Atmyb32 in Arabidopsis.

Joshua L. Heazlewood, Jeremy D. Preston, Song F. Li and Roger W. Parish.
Botany Department,LaTrobe University,Bundoora Campus,Melbourne.

Myb genes encode DNA-binding proteins that regulate gene expression at the transcriptional level. Myb genes were first characterised in animals and have recently been shown to form a large gene family in plants. Myb proteins have been implicated in the regulation of a number of important developmental processes in plants. More than one humdred myb genes have now been identified in Arabidopsis alone. Expression studies on the Atmyb32 gene from Arabidopsis using the GUS reporter gene have shown tissue specific expression in developing anthers, primary and lateral roots. Northerns and in situ hybridisation have confirmed these expression patterns. Primary and lateral root expression has also been demonstrated using green fluorescent protein. We are currently undertaking a study on the effects of hormones on the expression levels of Atmyb32, both through differential promoter::GUS deletions and real-time hormonal induced GFP production. The functional properties of Atmyb32 are being determined through the use of over-expression, anti-sense, transcriptional repressers and co-suppression constructs. The success of these methods will be discussed.

8-16

Characterisation of the Arabidopsis ent-kaurene oxidase gene, GA3
Chris A. Helliwell, W.J. Peacock and E.S. Dennis
CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

We isolated the Arabidopsis GA3 gene using a map based cloning strategy (PNAS 95, 9019-9024) and showed that it encodes a cytochrome P450 enzyme which defines a novel class of this group of enzymes, designated CYP701A. Introduction of either a copy of the wildtype gene or the GA3 cDNA under the control of the CaMV 35S promoter is sufficient to complement the ga3-2 mutant phenotype. Expression of the GA3 cDNA in yeast has shown that it encodes an enzyme with ent-kaurene oxidase activity (Plant Physiol. 119, 507-510), catalysing the three step oxidation of ent-kaurene to ent-kaurenoic acid. We are currently studying the expression of the GA3 gene to find when and where it is expressed in Arabidopsis using promoter-GUS fusions and RNase protection assays. These expression studies will show whether the expression of GA3 correlates with the expression of other GA biosynthesis genes.

8-17

Expression and Structural Analysis of a Myb-like gene: Atmyb103
Higginson, T. A., Li, S. and Parish R. W. P.
Botany Department. Latrobe University. Bundooora. 3083. Victoria. Australia.

As previously reported (Li et al., 1999) Atmyb 103 was isolated from a genomic library of Arabidopsis thaliana. Arabidopsis plants transgenic for chimeric Atmyb 103 / GUS genes expressed the enzyme during early anther development. In-situ hybridisation of flower sections revealed Atmyb103 mRNA is localised to the tapetum and middle layers of developing anthers. Further in-situ-hybridisation experiments revealed that Atmyb 103 mRNA is also located in trichomes. Arabidopsis plants transgenic for over expression / co-supression and antisense double 35S / Atmyb 103 genes revealed that Atmyb 103 effects trichome branching. Li, S., Higginson, T. A. and Parish, R. W. (1999). A novel Myb-like gene from Arabidopsis thaliana expressed in developing anthers. Plant Cell Physiology. 40 (3) 343-347.

8-18

Functional analysis of a ribosomal protein S13 gene using a Ds-insertion mutant of Arabidopsis thaliana.

Takuya Ito, Gyung-Tae Kim and Kazuo Shinozaki
Laboratory of Plant Molecular Biology, RIKEN, Tsukuba 305-0074, Japan

Maize transposon Ac/Ds is active in Arabidopsis genome. We have produced Ds-transposed Arabidopsis lines by two element system between Ds-containing and Ac transposase gene-containing lines (1). We recovered genomic DNAs flanking the Ds element by using TAIL-PCR from the 100 lines and determined their partial sequences. By comparison with sequences in the database, we identified Ds insertion mutants of various genes. We describe here functional analysis of a ribosomal protein S13 gene homologue (AtRPS13A) using a Ds insertion mutant of this gene. Ribosome is a large complex consisting of many ribosomal proteins and ribosomal RNAs, and indispensable for cell maintenance. The Ds insertion mutant of AtRPS13A showed smaller leaf size and aberrant leaf shape at early vegetative phase, and also showed bolting retardation. Southern and Northern analyses showed that AtRPS13A is a member of a small expressed gene family. These results suggest that maximal RPS13 gene expression from all the copies is necessary for maximal growth in shoot apical meristem during vegetative phase and transition phase from vegetative to reproductive. Using enhancer trap system, GUS staining was observed in shoot apical region, vascular tissue in young hypocotyl, axillary buds and young flower buds. These tissues are meristematic, where ribosomal protein genes are considered to be expressed. On the other hand, we could not detect any staining in root tissues, but inducible staining was observed in lateral root primordia by IAA addition. These results suggest that AtRPS13A gene is regulated differently between shoot and root tissues. We discuss the function of the ribosomal protein genes on growth and development. 1) Ito et al. (1999) Plant J., 17, 433-444.

8-19

Expression of the maize TERMINAL EAR1 gene alters Arabidopsis morphology.

Dan Jeffares, Carmel Gillman, Nena Alvarez and Bruce Veit.
Institute of Molecular Biosciences, Massey University, New Zealand.

The maize terminal ear1 gene appears to be involved in the positioning of leaf primordia in the peripheral zone of the shoot apical meristem (SAM). TE1 null mutants show an increased frequency of leaf initiation, irregularly shortened internodes, unusual phyllotaxy, and altered leaf form (1). In maize TE1 is expressed in the SAM in semicircular domains opposite sites of leaf initiation. Our model is that the expression of TE1 restricts the initiation of leaf primordia to certain precise positions in the SAM. TE1 encodes an RNA binding protein similar to S.pombe Mei2 and an Arabidopsis Mei2-like gene (AML1) which, like TE1, contain three RNA recognition motifs (2). The MEI2 protein is transported to the nucleus by virtue of its binding to a non-translated RNA, and is required for meiosis (3). Our degenerate PCR and library screening experiments indicate that Arabidopsis contains a TE1-like gene, that differs from those previously cloned. We have transformed Arabidopsis with the TE1 cDNA, and we observe that these plants irregularly develop very extended internodes. This phenotype is consistent with the model that TE1 constrains leaf primordia position. Since maize TE1 appears to confer an altered phenotype to Arabidopsis which is consistent with its proposed function in maize it appears that the function of TE1-like proteins has been conserved through evolution. 1.Veit et al (1998) Regulation of leaf initiation by the terminal ear1 gene of maize. Nature 393:166-168 2.Hirayama et al (1997) Functional cloning of a cDNA encoding Mei2-like protein from Arabidopsis thaliana using a fission yeast pheromone receptor deficient mutant. FEBS Letters 413:16-20 3.Yamashita et al (1998) RNA-Assisted Nuclear Transport of the Meiotic Regulator Mei2p in Fission Yeast. Cell 95:115-123

8-20

Expression of five sigma factors for a plastidic RNA polymerase

Kengo Kanamaru, Makoto Fujiwara, Kan Tanaka and Hideo TakahashiI
MCB, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-Ku, Tokyo 113-0032, JAPAN

Plastid DNA is transcribed by at least two types of RNA polymerase. One is plastid-encoded RNA polymerase homologous to eubacterial transcription machinery. However, the regulatory subunit, sigma factor, is not encoded on the plastid DNA. We have cloned the nuclear-encoded three sigma factor genes, sigA, sigB, and sigC, from Arabidopsis for the first time (FEBS Lett. 1997, Vol.413, 309-313). sigA and sigB were mapped on the chromosome I, whereas sigC was mapped on the chromosome III. Transient expression assay of GFP fusions in Arabidopsis protoplasts showed that N-termini of the three sig gene products could function as plastid-targeting signals, respectively. We constructed transgenic Arabidopsis lines harboring the sigA promoter- or the sigB promoter-uidA fusion. The GUS activities of the fusion genes were similarly detected at cotyledons, hypocotyls, rosette leaves, cauline leaves, sepals and siliques. In young seedlings, initial transcriptional activation of sigB was prior to that of sigA, then both sigA and sigB genes appeared to be synchronously activated in an oscillated fashion (Plant Cell Physiol. 1999 in press). In addition to the three sig genes, we recently isolated another two sig genes, sigD and sigE, on the chromosome V. SigD and SigE share conserved regions 1.2 to 4.2, and also have predicted plastid-targeting signals at the N-termini. sigD promoter-uidA fusion in transgenic Arabidopsis plants was activated in green tissues as well as sigA and sigB. Gene structures of the five genomic sequences implicate the evolutional relevance among the sig genes. We will also discuss about an alternative splicing involved in undefined functional variety or switching of a sig gene products.

8-21

The AXR3 gene of Arabidopsis

Stefan Kepinski, Dean Rouse, Mimi Tanimoto, Audrey Sarps, and Ottoline Leyser.
Plant Laboratory, Department of Biology, University of York, York, YO10 5YW, UK.

The Aux/IAA family of genes are central to auxin response and their significance is exemplified by the AXR3 gene of Arabidopsis. Mutations in AXR3 affect so many auxin mediated phenotypes that the defect presents as a quite general over-response to auxin. The cloning of AXR3 and other Aux/IAA family members has enabled us to begin to unravel the complexities of auxin signalling through the molecular dissection of the pathways in which these genes are involved.

The Aux/IAA genes are rapidly and specifically induced by auxin to encode ephemeral and low abundance, nuclear proteins which share a typical molecular architecture consisting of four highly conserved domains. EMS-mutagenesis resulted in two semi-dominant point mutations in domain II of AXR3 (axr3-1 and axr3-3), both gain-of-function alleles. As domain II is the putative site of the destabilization sequence that gives Aux/IAA proteins their characteristically short half-lives we have been assessing the comparative stability of the mutant protein using reporter-fusions. Further to these experiments, we are examining the longevity of both mutant and wild-type AXR3 protein in the auxin-related mutant backgrounds axr1 and tir1 as both mutants are affected in pathways involved in protein degradation.

We will also report our latest findings on the expression of AXR3 promoter-GUS fusions in wild-type and axr3-1 backgrounds and in a second Aux/IAA family mutant, shy2-2.

8-22

Changes in the Shape of Leaves and Flowers by Overexpression of the ROT3
Kim, G.-T. (1), Tsukaya, H. (2), Saito, Y. (3), and Uchimiya, H.
Inst. Mol. Cell. Biosci., Univ. Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan (1) Present address: Tsukuba Life Science Center, Inst. Phys. Chem. Res. (RIKEN), Tsukuba, Ibaraki 305-0074, Japan, (2) Additional post: Form and Function, PRESTO, JST, Japan, (3) Inst. Biomol. Sci., Gakushuin Univ., Mejiro 1-5-1, Tokyo 171-8588, Japan.

Leaves are morphologically diverse and are the basis for floral organs, such as petals. The two-dimensional expansion of leaves is regulated via the polarized elongation of cells in Arabidopsis (Tsukaya et al., 1994; Tsuge et al., 1996; this meeting). The ROTUNDIFOLIA3 (ROT3) is involved in polar-elongation process and regulates leaf length. We succeeded in the molecular cloning of the ROT3 gene and showed that the ROT3 gene encodes a novel cytochrome P450, which might be involved in steroid biosynthesis (Kim et al., 1998). Previous studies suggest that the ROT3 gene might be a key factor in leaf growth to the length direction. We constructed transgenic plants carrying a chimeric gene, ROT3p::GUS, and analyzed histochemically. Transgenic plants showed non-organ-specific GUS expression. Histochemical analysis showed that the ROT3 promoter expressed stronger in distal, matured part than in proximal, immature part of a leaf. We also constructed transgenic Arabidopsis by introducing a wild-type version or a rot3-2 type of variant of the ROT3 gene into the null mutant, rot3-1, in order to test the hypothesis for controlling the leaf shape. Transgenic plants that overexpressed wild-type ROT3 had longer leaves than parent plants, without any changes in leaf width. The shapes of floral organs were also altered. Elongation of stem, roots and hypocotyls was unaffected. Transgenic plants overexpressing a rot3-2 gene had enlarged leaf blades but leaf petioles of normal length. Morphological alterations in the above transgenic plants were associated with change of leaf-cell shape. Together, these results suggest that ROT3 is key regulator of the polar elongation of leafy organs and controls genes required for the morphogenesis.

8-23

Isolation and characterization of arabidopsis mutants defective in vascular pattern formation.
Koji Koizumi (1), Munetaka Sugiyama (2), Hiroo Fukuda (1).
(1) Department of Biological Sciences, Graduate School of Science, The University of Tokyo.(2) Botanical Gardens, Graduate School of Science, The University of Tokyo.

The formation of the vascular system is regulated temporally and spatially during development. In a project to understand regulatory mechanisms underlying vascular formation, we discovered 16 mutants that were defective in vascular pattern formation in the cotyledon among EMS and T-DNA mutagenized populations. Genetic analysis indicated that all of these mutations were monogenic and recessive. Complementation test of eight mutants in which lateral vein was disconnected showed that their abnormality in the vascular system was caused by mutations in seven genetic loci. We designated these mutants van1*`7 (vascular network defective). The van3 mutant out of these eight mutants was further characterized. The VAN3 locus was mapped to a region between SSLP markers nga249 and nga151 on chromosome 5. Phenotypic analysis revealed that the van3 mutation affected vein patterns in the cotyledon and the rosette leaves but did not hypocotyl and root vascular organization. In the rosette leaves of this mutant, most of veinlets did not connect with other veins whereas midveins and lateral veins formed a rather continuous network. The expression pattern of Athb-8-GUS, a molecular marker of the provascular tissue, suggested that provascular cells as well as mature vascular cells were absent at the disconnection points of the vascular network in the van3 mutant. Thus, effect of van3 mutation may be traced back prior to the stage of provascular tissue differentiation.

8-24

The DIRECTIONLESS mutation affects the pattern formation in Arabidopsis.
Mande Kumaran, De Ye, Wei-Cai Yang and Venkatesan Sundaresan.
Institute of Molecular Agrobiology, National University of Singapore.1, Research Link, Singapore-117604.

We are using the Ac/Ds maize transposable elements for insertional mutagenesis of Arabidopsis (Sundaresan, et al. (1995) Genes Dev. 9, 1797-1810). Here we describe a transposant line named SGT2467 that segregates a mutation called directionless. This mutation confers defects in the pattern formation in Arabidopsis. Plants which are homozygous for this mutant gene germinate normally but do not develop into adults. Defects in pattern formation can be observed from the very early stage of seedling development. Although the mutant phenotypes vary in severity, most of the seedlings lack roots and hypocotyls. In the severe phenotype the cotyledons are fused together without producing any postembryonic tissues such as leaves. Some seedlings produce maximum of two leaves with defective trichomes. None of the mutant seedlings survive. Scanning electron microscopy analysis of the mutant seedlings reveals severe defects in cell shape in the epidermis of the cotyledons and unbranched trichomes on the leaf surfaces. To identify the cell types which are present in the directionless mutant seedlings, we performed crosses to gene trap lines with specific GUS marker gene expression in tissues such as cotyledons, root apical meristem and shoot apical meristem. This analysis reveals that the mutants have cotyledons and shoot apical meristem cell types but not root apical meristem. Segregation analysis shows that directionless mutant phenotype is linked to a Ds transposable element. By Southern blotting we have confirmed that there is a single Ds insertion and the Ds flanking sequences were obtained by PCR. The flanking DNA was used as a probe to isolate genomic clones. Further characterization of this mutant line is being carried out and will be presented.

8-25

Siamese, a regulator of the endoreplication cell cycle during trichome development
John C. Larkin (1), David G. Oppenheimer (2), and Jason D. Walker (1)
(1) Dept. of Biological Sciences, Louisiana State University, Baton Rouge, LA (2) Dept. of Biology, University of Alabama, Tuscaloosa, AL

Endoreplication is a variant of the cell cycle that occurs in a wide variety of organisms. During endoreplication cycles, DNA is replicated without cellular or nuclear division. One Arabidopsis cell type exhibiting endoreplication is the trichome (leaf hair). Trichome nuclei have an average DNA content of approximately 32C. The SIAMESE (SIM) locus on chromosome 4 was detected due to a recessive mutation resulting in clusters of adjacent trichomes that appeared to be identical “twins” in morphology. Upon closer inspection, the sim mutant was found to produce multicellular trichomes in place of the unicellular trichomes produced by wild-type plants. Trichomes consisting of up to 15 cells have been observed. Individual nuclei of a multicellular trichome have a reduced level of endoreplication. SEM analysis of trichome development indicates that cell divisions occur very early in the development of mutant trichomes. Double mutants of sim with two other mutants affecting endoreplication, try and gl3, exhibit primarily additive phenotypes, suggesting that these genes function in a different pathway than sim. SIM appears to function as a repressor of mitosis in the endoreplication cell cycle. Additionally, the relatively normal morphology of multicellular sim trichomes indicates that trichome morphology is not dependant on the type of cell cycle occurring in the developing trichome. Further study of sim should give insight into endoreplication cell cycle and its role in trichome development. We are currently investigating candidate genes mapping near the position of SIM.
Supported by NSF grant IBN-9728047.

8-26

NtDSK1 encoding a dual-specificity protein kinase is involved in gibberellin signaling in Nicotiana tabacum

Hyun-sook Pai Lee, Hye-Sun Cho and Gyeong-Mee Yoon
Plant Cell and Molecular Biology Research Unit, Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yusong, Taejon 305-600, Korea.

NtDSK1 cDNA was isolated by screening a tobacco flower cDNA library. NtDSK1 contains the conserved kinase domain at the C-terminus and a putative regulatory domain at the N-terminus. NtDSK1 mRNA expression was developmentally regulated in different plant tissues. NtDSK1 gene expression was strongly stimulated by gibberellin. The recombinant kinase domain of NtDSK1, but not the mutant form, underwent autophosphorylation of serine, threonine, and tyrosine residues, indicating that NtDSK1 encodes a functional dual-specificity protein kinase. To determine the function of NtDSK1 kinase, "loss of function" approach, using both antisense RNA technique and co-suppression, was taken. Transgenic tobacco plants which express either the antisense NtDSK1 gene or a part of the NtDSK1 cDNA corresponding to the kinase domain showed the phenotypes similar to those of the "increased gibberellin signaling" mutants characterized in other plant system. Seeds from the transgenic plants were able to germinate with high frequency in the presence of 37mg/L paclobutrazol (PAC), a specific inhibitor of gibberellin biosynthesis, while germination of the control seeds was completely inhibited under the same condition. The plant growth was more strongly affected by PAC than was seed germination in the transgenic plants, but the degree of growth inhibition was less than that of the control plants. The transgenic plants were able to respond to exogenous gibberellin. Also the response to abscisic acid appears not to be affected in the transgenic seeds. The height of mature transgenic plants and their hypocotyl length were longer than those of the control plants. The antisense and sense transgene were highly expressed in the corresponding plants, while the endogenous NtDSK1 mRNA expression was greatly reduced. These results suggest that NtDSK1 kinase is involved in gibberellin signaling in tobacco, and that it may normally function as a negative regulator in the pathway.

8-27

Characterization of the FAT ROOT mutant in Arabidopsis

Keisuke Matsui (1), Takuji Wada (2), Sumie Ishiguro (1) and Kiyotaka Okada (1).
(1) Department of Botany, Graduate School of Science, Kyoto University, Japan (2) The Salk Institute for Biological Studies, 11010 N. Torrey Pines Rd, La Jolla, CA92037, USA

The determination of the axis of cell expansion is a key process in the morphogenesis of plant organs, but little is known about its regulatory factors. Here we report a mutation, fat root (ftr) which affects an axis determination of the cell expansion in all types of the cells. Mutant seedlings displayed abnormal increase of root diameter caused by irregular lateral cell expansion. Moreover, shapes of the other organs were shortened or round owing to the irregular lateral cell expansion. Since in FTR mutant, tip growth of the trichome and the root hair was almost normal, FTR mutant only affect diffusion growth and the FTR gene product might participate in fundamental process of the cell elongation. To confirm whether these defect of cell expansion are accompanied by aberrant hormonal control, we treated FTR seedlings with gibberellin and AVG as ethylene synthesis inhibitor. Since the seedlings showed no significant effect in root diameter and cortical microtubule orientation of the FTR mutant cells did not differ from that of wild type, FTR function is not involved in hormone-dependent cell expansion pathway that regulates cortical microtubule orientation. We have mapped FTR at the bottom of chromosome 1 near ADH marker and found that FTR is allelic to the botero mutant. Progress in the molecular and genetic analysis of ftr will be presented.

8-28

The two-component transposon system Ac/Ds as a mutagen in Arabidopsis: Identification of albino mutation induced by Ds insertion.

REIKO MOTOHASHI(1,2), Takuya ITO(1), Kazuko YAMAGUCHI-SHINOZAKI(2) and Kazuo SHINOZAKI(1).
1; Lab. Plant Mol. Biol., RIKEN, Tsukuba 305-0074. 2; Japan. Int. Res. Cent. Agri. Sci. (JIRCAS) Tsukuba 305-0851

Mutant analysis is a useful approach to understand biological functions of plant nuclear genes. To study functions of nuclear genes involved in chloroplast development, we used a two-component transposon system based on the Ac/Ds element of maize as a mutagen in Arabidopsis thaliana. To induce transposition, plants homozygous for the Ac24 plant were crossed to those homozygous for the Ds plant, and their progenies were obtained by self-fertilization. We isolated about 1500 Ds-transposed lines that contained both an excision maker and a transposon maker but that did not contain the Ac transposase gene. Approximately 90% of the selected F2 seedlings were expected to contain a transposed Ds element, in either the heterozygous or homozygous state. The progeny of F3 plants carrying independent transposition events were screened for mutants with albino or pale green phenotypes. Some albino and pale green mutations were shown to be closely linked to insertions of the Ds element. We then carried out Tail-PCR to obtain Ds-flanking regions in F2 plants, and determined their nucleotide sequences. Homology search of the Ds flanking sequences were performed with DNA data base. The Ds flanking sequences contained partial sequence similarity to chloroplast inner envelope membrane polypeptide, LEA-like protein and GABA permease genes. To recover plants carrying their revertant alleles, albino and pale green seedling variegated cotyledons were grown to maturity. We have obtained revertant plants derived from somatic reversion of the pale green mutations. We are now analyzing the pale green mutant genes and their functions in chloroplast development.

8-29

Stomatal Clusters in four lips Result from the Reiteration of the Guard Mother Cell Program

Jeanette Nadeau, Liming Zhao, Matt Geisler, Tsuyoshi Nakagawa and Fred Sack
Department of Plant Biology, Ohio State University, 1735 Neil Ave., Columbus, OH 43210

Stomata are a valuable system for investigating the mechanisms of cell patterning and differentiation in plants. Wild-type stomata are separated from each other by at least one intervening epidermal cell. The four lips mutation of Arabidopsis violates this spacing pattern. The previously-described flp-1 allele causes stomata to form in laterally-aligned pairs, or as unpaired guard cells. Six additional alleles ranging in severity have now been isolated. The most severe, flp-7, has clusters of up to 18 guard cells. Sequential dental resin impressions indicate that all guard cells in a cluster are derived from a single precursor cell, the guard mother cell. Normally the GMC divides symmetrically to produce two guard cells that exit the cell cycle. In contrast, electron microscopy reveals that in flp, the two daughter cells produced by the GMC acquire cytological characteristics of guard mother cells, rather than guard cells. Many of these GMC-like cells divide symmetrically to produce stomata or additional generations of GMCs. These data suggest that the four lips patterning defect results from a reiteration of the guard mother cell program in the stomatal cell lineage. This might be caused by a failure to positively regulate guard cell identity or to negatively regulate guard mother cell identity in progeny cells, or by a defect in exiting from the cell cycle. Analysis of the timing of the onset of guard cell-specific gene expression relative to the cell cycle is being used to address these hypotheses. It is likely that this mutation will provide insight into the coordination between cell cycle regulation and differentiation.

8-30

Atmyb5: its possible role in trichome and seed development
Olga Nicolaou, Song, F. Li and Roger W. Parish
Department of Botany, La Trobe University, Bundoora, Vic 3083, Australia

Atmyb5 is a myb-like gene isolated from a genomic library of Arabidopsis thaliana. It is expressed in a developmentally and tissue specific manner, with expression being observed in developing trichomes of rosette and cauline leaves, in stipules, on the margins of young leaves and in seeds.

To determine the function of Atmyb5 in Arabidopsis thaliana development, a number of overexpression and antisense chimeric constructs were made and transformed into wild type plants. These plants were screened for phenotypic changes, concentrating on the tissues in which Atmyb5 expression was observed. Phenotypic changes in both overexpression and antisense plants were similar. Transformed plants were affected in both trichome and seed development.

Trichome development in Arabidopsis has been chosen as a model system for studying plant cell morphogenesis. A number genes have been implicated in the initiation of trichomes from leaf epidermal cells, and others have been found to be involved in branch development, endoreplication and the morphology of trichomes. Atmyb5 may act as a negative regulator of trichome branching, since transformed plants possess trichomes with four to seven branches, instead of the normal three branches. Atmyb5 also appears to play a role in the correct formation of the seed coat and possibly the formation the underlying endosperm layers.

8-31

Quantitative detection and applications of nontoxic green fluorescent protein in living Arabidopsis
Y. Niwa
Lab. of Plant Cell Tech, Univ. of Shizuoka, 52-1 Yada, Shizuoka, 422-8526, Japan

Although GFP has emerged as a powerful new tool, it has been suggested that high levels of GFP expression could be toxic to plant growth and development, especially in Arabidopsis. An engineered sGFP(S65T) sequence containing optimized codons of highly expressed eukaryotic proteins has provided up to 100 fold brighter fluorescence signals than the original jellyfish GFP sequence in plant transient expression systems. To evaluate the toxicity of sGFP(S65T), I introduced the constitutive 35S promoter-sGFP(S65T) fusion gene into the Arabidopsis genome. I could achieve high levels of GFP expression and obtain morphologically normal and fertile plants at the usual frequency. This result indicates that sGFP(S65T) is not toxic in Arabidopsis. Then I demonstrate that the fluorescence intensity of whole plants can be measured under non-disruptive, sterile conditions using a quantitative fluorescent imaging system. Homozygous plants can be distinguished from heterozygous plants, and fully fertile progenies can be obtained from the analyzed plants. In the case of cultured tobacco cells, GFP-positive cells can be quantitatively distinguished from non-transformed cells under non-selective conditions. This system will be useful in applications such as mutant screening, analysis of whole-body phenomena, including gene silencing. To facilitate the elucidation of protein targeting and organelle biogenesis in planta, I also generated transgenic Arabidopsis that stably express the plastid- or mitochondria-targeted sGFP(S65T). Etioplasts in dark-grown leaves and mitochondria in dry seed embryos could be visualized for the first time in transgenic Arabidopsis plants under normal growing conditions.

8-32

Isolation and characterization of the mutant, uns1, which shows abnormal responses to sugars.
Kiyoshi Onai (1), Masa-aki Ohto (1) and Kenzo Nakamura (1&2).
(1) Division of Developmental Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan,
(2) Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-0814, Japan

When WT Arabidopsis plants are grown on medium with sugars in vitro, the chlorophyll content of leaves is increased by increasing sugar concentration in medium, suggesting that sugar-signalling such as transport and metabolism of sugars and regulation of sugar-regulated genes might be positively involved in leaf development with the photosynthetic activity. To understand the role of the sugar-signalling in the leaf development, mutants with the reduced level of chlorophyll content in leaves were screened in our enhancer-tagged mutant lines. Among such low-chlorophyll mutants, we isolated recessive mutant, uns1, which shows unusual sugar response. The sugar-induced amylase activity and anthocyanin accumulation in the uns1 leaf-cuttings were significantly lower than those of WT plants. The expression of sugar-modulated genes tested other than b-amy and CHS genes were generally normal, and sugar-induced accumulations of starch and sugars were also normally occurred. These results suggest that the uns1 mutation affects on the specific sugar-signalling pathway, not on the sugar-transport itself. Furthermore, the uns1 mutant showed late-flowering phenotype in LD on soil, but did not show in SD. On the other hand, this mutant showed early-flowering phenotype on medium with high concentration of sugar in LD. Therefore, the uns1 mutation also affects positively and negatively on the floral transition in LD. Southern analysis revealed that the mutated gene is not tagged by T-DNA. We have already determined the map position of the uns1 on chromosome 4, and positional cloning of this gene is in progress.

8-33

Interactions among the trichome branch number genes may identify a multiprotein complex involved in trichome branch initiation
David G. Oppenheimer, Sujatha Krishnakumar, Mary A. Pollock, and Danlin Luo
Department of Biological Sciences and Coalition for BioMolecular ProductsUniversity of AlabamaTuscaloosa, AL 35487-0344USA

The processes that control cell expansion in plants are largely unknown. We are using the initiation of branches on Arabidopsis trichomes to understand the regulation of specific cell expansion events. Mutational analysis of the trichome developmental pathway has led to the identification of 18 genes that control trichome branch number. We have focused on 12 of these genes and have constructed all pairwise combinations of the double mutants. Using the results of the double mutant analyses, we have constructed a model for the control of trichome branch initiation. This model successfully predicts all the phenotypes of the single and double mutants, and indicates points of control of the branching process. To test the hypothesis that some of the gene products encoded by the branch number genes interact, we looked for synthetic phenotypes and intergenic noncomplementation between different allele combinations of the branch number genes. We identified a synthetic gene interaction between the frc1-1 allele and the zwi-3 allele. Plants homozygous for the recessive frc1-1 allele produce predominantly 2-branched trichomes. Plants homozygous for the zwi-3 mutation produce exclusively 2-branched trichomes, whereas zwi-9311-11 mutants produce unbranched or 2-branched trichomes. The frc1-1 zwi-9311-11 double mutants produce exclusively unbranched trichomes. However, the frc1-1 zwi-3 double mutants produce trichomes that are short and "nubby", and appear to initiate but not expand their branches. Interestingly, plants of the genotype frc1-1/frc1-1 zwi-9311-11/zwi-3, have an unexpected phenotype: the trichomes are wildtype in appearance. This result suggests that zwi may act as a dimer, and supports the hypothesis that the products of ZWI and FRC1 interact. We will also report the sequence of the alleles of zwi used in this study as well as the phenotypes of other allele combinations of the trichome branch number genes.

8-34

Interactions between COP1 and HY5 may regulate seedling development through the targeted degradation of the HY5 protein

Mark T. Osterlund, Christian S. Hardtke, and Xing Wang Deng
Department of MCDB, Yale University New Haven, CT 06520-8104, U.S.A.

HY5, a bZIP type transcription factor, is a positive regulator of photomorphogenic development and a physiological target of the key repressor COP1 (1). It has been shown to bind to light-responsive promoter elements and activate light-inducible genes (2). With a newly available antibody specific to HY5, we identified that the abundance of HY5 protein is regulated by light. Depending on the light conditions in which seedlings are grown, multiple photoreceptors (PhyA, PhyB, CRY1, and CRY2) influence the light dependent regulation of HY5. Molecular and genetic evidence suggests that distinct signalling pathways are utilized to regulate mRNA levels and protein stability. All pleiotropic cop/det/fus mutants lack the light dependent regulation of HY5. The sequence similarity between many of these COP/DET/FUS gene products and subunits of the proteasome make it attractive to consider protein degradation as a mechanism to regulate the abundance of HY5 and thus seedling development. Correlations between the subcellular localization of COP1 and the cellular abundance of HY5 suggest that COP1 may regulate HY5 activity via targeted degradation.

References:

(1). Ang et. al, "Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development" Molecular Cell, Jan 1998 (2), 213-222.
(2). Chattopadhyay et. al, "Arabidopsis bZip Protein Hy5 Directly Interacts with Light-Responsive Promoters in Mediating Light Control of Gene Expression" The Plant Cell, May 1998 (10), 673-683

8-35

GLABROUS3 is a bHLH transcription factor that interacts with other regulators of trichome development.

Thomas Payne, Fan Zhang and Alan Lloyd
Molecular Cell and Developmental Biology Section University of Texas at Austin Austin, TX 78712, USA

The gl3-1 mutant produces reduced numbers of trichomes, and these are abnormal in DNA content and branching. Our lab has complemented the gl3-1 mutation with a genomic fragment encoding a bHLH transcription factor (Payne et al., submitted) highly homologous to the myc-like anthocyanin regulators delila, from Antirrhinum, and R, from maize. Like R, GL3 suppresses the ttg1-1 mutation when overexpressed, albeit weakly. Antisense transcription of GL3 reduces both trichome number and degree of branching. The gl3-1 mutation creates a premature stop codon. Two-hybrid constructs which fuse GL3 or GL3-derived fragments to the Gal4 activation or DNA binding domains have been used to demonstrate physical interactions in yeast between the GL3 protein and other known regulators of trichome development. Complementary overexpression and co-overexpression experiments in various mutant and wild-type backgrounds substantiate that these same interactions occur in Arabidopsis and that they are significant to trichome development.

8-36

Trichome patterning altered by manipulating expression of the TRANSPARENT TESTA, GLABRA 1 gene

Nicola A. Ramsay, John C. Gray and Amanda R. Walker
Dept of Plant Sciences, University of Cambridge, Downing St, Cambridge, CB2 3EA, UK.

The TRANSPARENT TESTA, GLABRA 1 gene encodes a WD40 repeat protein which regulates trichome initiation and anthocyanin biosynthesis in Arabidopsis. A similar gene, An11, in petunia controls anthocyanin biosynthesis only. TTG1 controls the activity of myb and/or myc transcription factors which together regulate many processes in plants. TTG1 might interact with the transcription factors directly or act through a signal transduction pathway. Genetic studies using mutants have shown that the relative levels of TTG1 and the myb factor GL1 are important in producing plants with a normal trichome distribution (Larkin et al, 1999, Genetics 151, 1591-1604; Schnittger et al, 1999, Plant Cell 11, no.6). To establish if the normal level of TTG1 is saturating (as might be expected if TTG1 is a component of a signal transduction pathway), we altered the level of TTG1 transcript in Arabidopsis. The expression of the TTG1 gene in tobacco was manipulated to establish if this gene regulates anthocyanin biosynthesis only in this species. Transgenic plants are currently being analysed. The effect of putative downstream myc transcription factors on trichome initiation is being investigated.

8-37

A Floral meristem- and floral organ-identity gene, FIL, regulate the determination of abaxial cell fate in leaves.

Shinichiro Sawa (1), Keiro Watanabe (2), Koji Goto (3), and Kiyotaka Okada (2)
(1) Department of Biology, Graduate School of Science, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan, (2) Department of Botany, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan, (3) Institute for Chemical Research, Kyoto University, Uji, Kyoto, 611-0011, Japan

Genetic analyses of filamentous flower (fil) mutants have indicated that FIL is required for the maintenance and growth of inflorescence and floral meristems, and floral organs of Arabidopsis thaliana. FIL encodes a protein carrying a zinc finger and a HMG-box like domains, which are known to work as a transcription regulator, and the FIL protein was shown to be localized at nucleus. In situ hybridization clearly showed that FIL is expressed only at abaxial side of primordia of leaves and floral organs. Transgenic plants, ectopically expressing FIL, formed filament-like leaves, suggesting the abaxialization of the filament-like leaves. Transgenic plants of mild phenotypes formed 5-10 wrinkled leaves. The adaxial surface of the wrinkled leaves showed several islands of small cells with many stomata, supporting our model that the ectopic expression of FIL promotes abaxialization. fil mutant did not show the abnormalities in the abaxial-adaxial development. This reason must be due to a presence of the FIL gene family. Database search also shows presence of the FIL gene family in rice genome. One might speculate that recessive mutants with defects in abaxial cell fate have not been isolated because of redundancy of the FIL gene family. Our results suggest that the FIL gene could regulate the determination of abaxial cell fate. References; (1) Sawa et al., 1999, The Plant Cell, 11, 69-86. (2) Sawa et al., 1999, Genes and Development, 13, 1079-1088.

8-38

ALE1: Endosperm protease that is required for proper development of the cotyledons and juvenile leaves

Hirokazu Tanaka, Hitoshi Onouchi, Chiyoko Machida, Yasunori Machida
Division of Biological Sciences, Nagoya University, 464-8602, Japan

Double fertilization in angiosperms leads to the formation of the embryo and endosperm. While endosperm in monocot seeds plays a prominent role in seed development, endosperm in dicot seeds are transient structure and their role has been unclear. Recently, several study implied that the endosperm in dicot seeds are important for proper development of the embryo. Arabidopsis plants homozygous for the abnormal leaf shape1 (ale1) mutation often produce wrinkled and fused cotyledons and leaves with less convoluted epidermal pavement cells. These abnormalities are the most apparent at cotyledons and juvenile leaves. The ale1 mutants are also defective in proper organization of cotyledons in a developing embryo, suggesting that the ALE1 gene functions in embryogenesis. We isolated the ALE1 gene and showed that it encodes a member of the subtilisin-like serine protease family. The predicted protein sequence contains a potential signal peptide that may direct secretion. To know distribution of ALE1 messenger RNA during development of cotyledons and leaves, we performed RNA in situ hybridization. ALE1 messenger RNA accumulates at cellularized endosperm surrounding the developing embryo, but not in embryos nor shoot apeces of young plants. These results suggest that ALE1 protease, synthesized in the endosperm cells, is involved in production of a signal(s) for the proper development of the cotyledons and juvenile leaves.

8-39

Evidences for a dominant mutation of IAA19 that disrupts hypocotyl growth curvature responses and alters auxin sensitivity

Kiyoshi Tatematsu, Masaaki K. Watahiki, Kotaro T. Yamamoto
Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, 060-0810 Japan

Unilateral application of auxin-containing lanolin paste to Arabidopsis hypocotyls induces growth curvature response. Utilizing this response, we isolated nph4/tir5/msg1 mutants of Arabidopsis that were defective in gravitropism, phototropism and hook formation of hypocotyl (Watahiki & Yamamoto, 1997; Watahiki et al., 1999). To further isolate new mutants of differential growth, we screened 74,000 M2 seeds, and obtained a new dominant mutant that were defective in auxin-induced hypocotyl growth curvature, msg2. msg2 hypocotyls did not show growth curvature upon unilateral application of IAA at any concentrations from 1 to 300 uM. msg2 hypocotyls lost gravitropism completely. msg2 showed weaker phototropism and weaker hook formation in hypocotyls than wild type. msg2 hypocotyls were resistant to 2,4-D. MSG2 was mapped on chromosome 3, about 19 cM north of GL1. Fine mapping showed that it was located on a P1 clone, MJK13. Arabidopsis Genome Initiative reveals that MJK13 includes IAA19, a member of the Aux/IAA gene family (Kim et al., 1997). Subcloning and sequencing of IAA19 showed that msg2-1 had a nucleotide change predicted to cause a substitution from Pro-69 to Ser, which had been reported to occur in a dominant mutation in SHY2/IAA3 (Tian & Reed, 1999); msg2-3 contained a nucleotide change corresponding to a change from Pro-69 to Leu, like another dominant mutation in AXR3/IAA17 (Rouse et al., 1998); msg2-2 had a nucleotide change predicted to cause a change from Gly-67 to Arg, which was a novel dominant mutation in Aux/IAA genes. Since phenotype of msg2 was very similar to that of nph4/msg1, products of the mutated MSG2/IAA19 gene may interact with NPH4 in a negative-dominant manner. Considering the recent finding by E. Liscum (personal communication) that NPH4 is ARF7 (Ulmasov et al., 1999), the present results suggest that Aux/IAA genes play a central role in differential growth responses of hypocotyl.

8-40

AN gene regulates leaf-cell morphogy via regulation of the arrangement of cortical microtubules.
Tsukaya, H. (1,2), Kim, G.-T. (3), Tsuge, T. (4) and Uchimiya, H. (1)
(1) Inst. Mol. Cell. Biosci., Univ. Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan, (3) Present address: Tsukuba Life Science Center, RIKEN, Tsukuba, Ibaraki 305-0074, Japan (4) Present address: Dept Biol., Yale Univ., New Haven, CT 6520-8104, USA (2) Additional post: Form and Function, PRESTO, JST, Japan

In the course of attempts to understanding of the details of leaf morphogenesis (Tsukaya, 1995), we showed previously that two genes of Arabidopsis are responsible for the polarity-specific expansion of leaves (Tsukaya et al., 1994; Tsuge et al., 1996): the ANGUSTIFOLIA (AN) gene regulates polar elongation in the leaf-width direction, and the ROTUNDIFOLIA3 (ROT3) gene regulates polar elongation in the leaf-length direction. We succeeded in the molecular cloning of the ROT3 and showed that the gene encodes a new cytochrome P450 (Kim et al., 1998; this meeting). On the other hand, the AN gene regulates width of leaf cells independently from the function of the ROT3. Since the AN gene and the ROT3 gene regulate the polar elongation of leaf cells, we postulated that cytoskeletal structures might be affected by these genes. Immunohistochemical analysis of cytoskeletons of the mutants revealed that orientation of cortical microtubules was specifically altered in leaf cells of the an mutant. This alteration can explain all the leaf-specific phenotype of the an mutant, by postulating that the AN gene regulates the shape of leaf cells by controlling of the orientation of cortical microtubules. We also attempted to isolate the gene for AN by map-based cloning. As a result we identified a genomic clone from chromosome I that complements the phenotypes of the an mutation. Based on the above results, we shall discuss on molecular genetic regulation of leaf expansion process in Arabidopsis.

8-41

Phenotypic characterization of the Arabidopsis srb (shoot and root branching) mutant
Karin van de Sande and Ottoline Leyser.
The Plant Laboratory, Department of Biology, University of York, York, United Kingdom.

The plant hormones auxin and cytokinin are involved in determining plant architecture. In a genetic approach to study their roles in plant architecture, several shoot branching mutants were isolated. One of them is srb, the Arabidopsis shoot and root branching mutant. A phenotypic characterization of the srb mutant will be presented. srb forms shoots from all rosette leaf axils, which show strong lateral branching. It also forms more crown roots, and more laterals in the first 2 cm of the primary root. Seedlings are often monocotyledonous, with a deformed calluslike cotyledon. Part of the seedlings are deformed and cannot continue their development. Reminiscent of monopterus, vascular tissue formation is disturbed in srb. Flower development is slightly disturbed: there is a reduced number of stamens, one or two of the filaments can be fused with a petal, and production of pollen is low. Hypocotyls of srb are shorter than wildtype hypocotyls due to shorter cell length. Hypocotyl elongation and primary root growth show some resistance to auxin. Polar auxin transport studies are underway.

8-42

An Arabidopsis Root-specific myb Gene: Expression Pattern and Ectopic Expression in Arabidopsis
Qing Wang, Aviva Pri-Hadash and Carl Douglas
Department of Botany, University of British Columbia, Vancouver, BC, Canada

The proto-oncogene c-myb encodes a transcription factor that regulates cellular proliferation and differentiation in animals. Structurally related myb genes are also found in plants. It is estimated that there are over a hundred myb genes in Arabidopsis. However, the functions of most of these genes are unknown. We have identified an Arabidopsis myb gene, provisionally designated myb1-3, that was expressed exclusively in the roots. Screening of 14,100 Arabidopsis T-DNA tagged lines identified a T-DNA insertion 500 bp upstream of the putative myb1-3 translation start site. However, analysis of this potential myb1-3 knockout line revealed that myb1-3 expression was not affected by the T-DNA insertion. To examine the cell-specific pattern of myb1-3 expression, tansgenic Arabidopsis lines containing the myb1-3 promoter driving GUS expression were generated. Staining was localized around the stele cylinder of the root, started behind the root apex, and was stronger in the young root tissues. The phenotypes of Arabidopsis lines ectopically expressing myb1-3, under the control of the CaMv 35S promoter, are currently being investigated.

8-43

Dissecting cell shape regulation with microtubule organization and cellulose synthesis mutants
Keiko Sugimoto, Nori G. Hasenbein, **Richard E. Williamson**, Geoffrey O. Wasteneys
Plant Cell Biology Group, Research School of Biological Sciences,The Australian National University
Canberra, ACT 2601, Australia

To better understand the functional relationship between cortical microtubule orientation, cellulose alignment and directional cell expansion, we carried out genetic and structural analyses with two Arabidopsis mutants, mor1 and rsw1. Both mutants are temperature-sensitive. At 29°C, cortical microtubules in mor1 shorten and randomize and cellulose synthesis in rsw1 is significantly suppressed. In both mutants, radial swelling of organs occurs after the primary phenotype (microtubule disorganization or cellulose synthesis reduction) is established. In double mutants, the radial swelling measured in root tips was additive, suggesting either (1) double mutants have more severe phenotypes because the microfibrils are not only randomized but also reduced in number or (2) that microtubule disorganization and suppression of cellulose synthesis both alter expansion properties but do so independently. To evaluate these two possibilities, we examined microtubule and microfibril orientation patterns and measured cell expansion in the roots of both mutants at the restrictive temperature. Unexpectedly, field emission scanning elecron microscopy revealed that in mor1, microfibrils were still aligned in parallel arrays, well after isodiametric expansion had replaced elongation. In rsw1 cells, on the other hand, microtubules remained transverse even though microfibrils appeared to be randomized. The additive phenotype of mor1-rsw1 double mutants is therefore consistent with mor1 and rsw1 perturbing independent mechanisms.

8-44

ANY1 - a gene that enables growth anisotropy

Angela T. Whittington, Nori G. Hasenbein, Madeleine C. Rashbrooke, Tobias I. Baskin and Geoffrey O. Wasteneys
Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, Canberra ACT 2601

Anisotropic cell expansion is an intrinsic feature of plant development, and is largely responsible for organ shape and, especially, elongation. From an EMS-mutagenized Arabidopsis thaliana Columbia population, we identified the any1 mutant, whose growth anisotropy is constitutively impaired. Any1 cells expand isodiametrically resulting in short, broad petioles, hypocotyls, roots and stems. Compared to wild type, root elongation rates are reduced by 50% although rates of cell production do not differ. In leaves, pavement cells remain polyhedral in shape rather than interdigitating and trichomes are conspicuously meringue-shaped. Closer examination by scanning electron microscopy revealed that many trichomes burst during the early stages of growth, indicating inherent weaknesses in cell walls. Trichome rupture is consistent with a partial glabrosity of leaves. Cortical microtubules are apparently undisturbed in any1.

In any1 mutants, seed set is low, and the reduced fertility may be attributed at least in part to reduced stamen filament length. We are using both complementation analyses and a co-dominant ecotype specific PCR-based marker (CAPS) approach to map the any1 locus. The results suggest any1 is a novel gene. ANY1 appears to be epistatic in F2 segregations with several diverse mutants. Progeny tests, however, indicate that double homozygotes are not produced, suggesting any1 is incompatible with at least some other mutations.

8-45

Immunolocalization of ASK1 and ASK2 proteins

Ming Yang (1), Peter Schorr (2), William Crosby (2) & Hong Ma (1)
(1)Department of Biology, Penn State University, University Park, PA 16802, USA, (2) Plant Biotechnology Institute, NRC Canada, Saskatoon, SK S7N 0W9, Canada

The yeast and human SKP1 proteins are important regulators of the mitotic cell cycle. It is an essential component of SCF complexes that specifically target proteins for ubiquitin-mediated proteolysis. SKP1 may also mediate protein phosphorylation in the yeast kinetochore. In Arabidopsis, a number of SKP1-like genes have been uncovered and named Arabidopsis SKP1-LIKE1 (ASK). In addition, mutants at one of the ASK loci, ASK1, have also been isolated. Phenotypic analysis of the ask1-1 mutant has indicated that ASK1 is required for male homologous chromosome separation and has a role in the overall growth of the plants. To gain further understanding of meiotic and/or mitotic functions of ASK proteins, antibodies against ASK1 and ASK2 were generated. Using these antibodies, intracellular localization of these two proteins is being examined in wild-type and ask1-1 microspore mother cells (MMCs) and root tip cells. Preliminary results indicated that ASK1 is present as discreet particles and away from chromosomes during male meiosis. This result suggests that ASK1 indirectly regulate homologue separation, possibly by degrading or modifying a protein that could inhibit homologue separation. It was also found that ASK1 is present in both nucleus and cytoplasm of meiotic and mitotic premetaphase cells. While localization of ASK2 in MMCs is under investigation, ASK2 appeared to be primarily present in cytoplasm of root tip cells, suggesting functional differentiation between ASK1 and ASK2. Furthermore, different levels and patterns of ASK1 and ASK2 immunostaining in different cells are being evaluated to determine if amounts and locations of these proteins are regulated in a cell cycle and/or cell type dependent manner.

9-1

The gain of function epi-mutant fwa causes late flowering

Wim J.J. Soppe (1), Steven E. Jacobsen (2), Carlos Alonso-Blanco (1), Tetsuji Kakutani (3), Anton J.M. Peeters(1) and Maarten Koornneef (1).
(1) Lab. of Genetics, Wageningen Univ.,Dreijenlaan 2, 6703 HA Wageningen, The Netherlands; (2) Dept. of MCD Biology, UCLA, Los Angeles, CA 90095, USA; (3) Dept. of Molecular Genetics, NIAR, Tsukuba, Ibaraki 305, Japan.

The transition to flowering is of crucial importance in the life cycle of a plant. In Arabidopsis many mutants have been obtained that influence the timing of flowering. The combination of physiological, genetical and molecular approaches, using these mutants, has led to a model of floral induction that consists of a photoperiod promotion pathway, a vernalisation promotion pathway and an autonomous promotion pathway. The FWA gene, belonging to the photoperiod promotion pathway, has recently been cloned and encodes a putative transcription factor. Plants which are transformed with a cosmid, containing the fwa mutant gene, show dominant late flowering. However, this late flowering phenotype of the transformants is not stable and disappears after a few generations. Expression studies by northern blot and RTPCR revealed that the FWA gene is not expressed in wild-type plants but only in the mutant fwa plant. Furthermore, analysis of the promoter of FWA indicated that it contains two repeats which are highly methylated in wild-type plants but hypomethylated in the mutant. This leads to the hypothesis that due to this hypermethylation, the FWA gene is not expressed in wild type-plants. In contrast, in the fwa mutant the repeats are hypomethylated, allowing the gene to be expressed and thus causing a late flowering phenotype. Therefore, the FWA gene represses the transition to flowering.

9-2

Vernalization in Arabidopsis - A Molecular Genetic Approach

T Gendall, P Dijkwel, U Johanson, Y Levy, C Lister, G Simpson, P Sivadon, K Torney and C Dean
Department of Molecular Genetics, John Innes Centre, Norwich, UK, NR4 7UH

Many plants survive the winter by growing vegetatively and time their flowering to coincide with the favourable conditions of spring. This control is achieved through the integration of both environmental cues and endogenous signals. We are interested in the acceleration of flowering that occurs after exposure to a long period of low temperature, or vernalization, and are studying the genetic and molecular interactions that regulate the vernalization requirement and response in Arabidopsis. The FRIGIDA (FRI) gene is a strong repressor of flowering, but this repression can be overcome by vernalization. FRI is the major determinant of flowering time in naturally occurring Arabidopsis ecotypes. We have map-based cloned FRI, and identified two distinct mutations in FRI found only in rapid cycling ecotypes. Interestingly, this allelic variation correlates well with the geographical distribution of Arabidopsis ecotypes. We have also isolated the FCA gene that strongly promotes flowering. fca mutants are late flowering, but this phenotype can be reversed by vernalization. FCA is an RNA-binding protein whose expression is regulated at multiple levels. Furthermore, we have identified a number of mutants that are defective in their vernalization response, or vrn mutants. We have map-based cloned one of these genes, VRN2, and shown that it encodes a putative transcription factor, suggesting that VRN2 may play a role in the regulation of genes involved in the vernalization response. Together, an analysis of FRI, FCA and VRN2 will allow us to begin to understand how plants perceive and respond to the cold conditions of winter, and how this signal is integrated into a signaling network that regulates flowering time.

9-3

FLF/FLC is a repressor of flowering in Arabidopsis

Candice C. Sheldon, E. Jean Finnegan, W. James Peacock, and Elizabeth S. Dennis
CSIRO Plant Industry, Canberra, ACT 2601.

We have identified a MADS-box gene, FLOWERING LOCUS F (FLF), which encodes a dominant repressor of flowering (1). FLF has recently been shown to be the same as FLOWERING LOCUS C (FLC) (2), a locus identified genetically as one of the major determinants of flowering time in naturally occurring ecotypes of Arabidopsis. In a range of flf/flc mutants and ecotypes, increasing FLF/FLC transcript level correlates with increasing lateness to flower, pointing to the FLF/FLC gene being an important controller of the transition to flowering. FLF/FLC mRNA expression is down-regulated by vernalization, an important environmental stimulus for flowering. This change in gene expression is mitotically stable, but is reset in the next generation. FLF/FLC expression is also decreased in plants with a reduced level of genomic DNA methylation, consistent with our previous suggestion that vernalization acts to induce flowering through changes in gene activity caused by a reduction in DNA methylation (3). FLF/FLC mRNA expression is regulated by genes in both the autonomous and vernalization-dependent pathways suggesting that FLF/FLC acts to integrate these two flowering pathways. In contrast, the photoperiod-responsive pathway appears to promote flowering independently of FLF/FLC. The late-flowering flf-1 mutant requires a greater amount of exogenous gibberellin (GA) to promote flowering than wild-type. This suggests that the FLF/FLC gene product may block the promotion of flowering by GA, although this appears not to be through the up-regulation of the known negative regulators of GA action: RGA, GAI and SPY.

(1) Sheldon et al (1999) Plant Cell 11, 445-458.
(2) Scott and Michaels (1999) Plant Cell 11, 949-956.
(3) Burn et al (1993) Proc Natl Acad Sci USA 90, 287-291.

9-4

Characterising GIGANTEA: A circadian-regulated gene which regulates photoperiodic flowering in ARABIDOPSIS.

Sarah Fowler1, Kim Richardson3, Karen Lee2, Hitoshi Onouchi2, Alon Samach2, Bret Morris3, George Coupland2 and Jo Putterill1.
1Plant Sciences Group, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand. 2John Innes Research Institute, Norwich Research Park, Colney, Norwich NRU 7UH, United Kingdom. 3Hort+Research, Mount Albert Research Centre, Private Bag 92169, Auckland, New Zealand.

In genetic models of flowering in Arabidopsis, genes that regulate flowering in response to photoperiod fall into a single genetic pathway called the long day pathway. This pathway was first named for mutations such as gigantea (gi) and constans (co), that delay flowering in long days, but have little or no effect in short days. The long day pathway also contains genes that are involved in daylength perception and signalling. These are usually placed upstream of genes such as CO which do not appear to regulate circadian activity. The GI gene is positioned upstream of CO because over expression of CO complemented the late-flowering phenotype of gi mutants. Recently, we isolated the GI gene via T-DNA insertional mutagenesis. GI is circadian regulated and encodes a novel putative membrane protein. The results of experiments investigating the interactions between GI and other genes in the long-day pathway will be presented. The results suggest that GI has an important role in regulating the expression of genes in the long day pathway.

9-5

The flowering-time gene FT acts downstream of CONSTANS and antagonistically with its homolog, TERMINAL FLOWER1

Yasushi Kobayashi, Hidetaka Kaya, Masaki Iwabuchi and Takashi Araki
Department of Botany, Division of Biological Science, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

Floral transition is an important decision during the post-embryonic development of flowering plants. More than eighty loci are known to regulate this process and models for genetic regulation of floral transition in which these loci are placed in several interacting pathways have been proposed (Levy and Dean, 1998). We have focused our analysis on the flowering-time gene FT and cloned the gene by T-DNA tagging approach. Expression of FT was regulated by photoperiods, and preceded to floral commitment. Ectopic over-expression of FT caused precocious flowering (abstract #155, last year). The fact that FT is regulated by photoperiod suggested the involvement of CONSTANS (CO). We examined FT expression in co background and found a delay in FT up-regulation in co-1. Precocious flowering phenotype of 35S::FT plants was affected by neither co-1 mutation nor short days. Furthermore, FT was quickly up-regulated after induction of CO activity in 35S::CO:GR plants (provided by G. Coupland). These results suggested that FT is in part downstream of CO. However, eventual up-regulation of FT in the absence of CO or in short days suggests CO- and photoperiod-independent pathway(s). FT and TFL1 belong to a small gene family in Arabidopsis. Apparently opposite phenotypes of loss-of-function mutants and ectopic over-expression transgenic plants of these two genes suggest antagonism and importance of the balance between them. We confirmed this by combining loss-of-function mutations and ectopic over-expression transgenes of FT and TFL1. In an attempt to identify other genes working with or downstream of FT, we screened for suppressor mutations for ft-1 (G171E) and 4 candidates for extragenic suppressor (suppressor of ft, sft) were isolated. We are currently testing whether these mutations can suppress other alleles and deletion of the entire locus. Also, by crossing 35S::FT transgenic plants and various late-flowering mutants, we are trying to identify possible downstream genes.

9-6

Switching on Flowers

Doris Wagner (1) Robert W.M. Sablowski (2) and Elliot M. Meyerowitz (1)
(1) California Institute of Technology,Division of Biology 156-29, Pasadena CA 91125, USA(2) Molecular Genetics Dept., John Innes Centre, Norwich NR4 7UH, UK

In Arabidopsis LEAFY (LFY) and APETALA1 (AP1) are pivotal for the transition to the reproductive phase , where instead of leaves the shoot apical meristem produces flowers. It is unclear how LFY and AP1 interact in this process. Using steroid-inducible activation of LFY, we demonstrate that AP1 is an immediate-early target of transcriptional activation by LFY. Early AP1 induction is independent of protein synthesis and occurs in the tissues and at the developmental stage where floral fate is assumed. In addition, this system revealed distinct LFY activities at different stages of reproductive development. Early, transient activation of LFY in a null mutant background converts inflorescences to flowers but does not result in class B homeotic gene activation. Competency to turn on these floral homeotic genes in response to LFY is acquired only later in development. Class B homeotic gene expression thus provides a molecular marker for different phases during reproductive development. Next, we analyzed the effect of prolonged versus transient LFY activation in the presence and absence of AP1 function. The data provide insight into the interdependence of LFY and other components of the developmental framework within which it acts.

9-7

Photoreceptors and flowering of Arabidopsis: photophysiological studies using mutants and transgenic lines

David Bagnall, Rod W. King and Cheryl Blundell.
CSIRO Division of Plant Industry,Canberra 2601

Perception of daylength in Arabidopsis involves several photoreceptors, including various phytochromes, blue light receptors and chlorophyll. The differing roles of these pigments were assessed utilising mutants and overexpression lines in specific photophysiological treatments. The light stable phytochromes B and D perceive end-of-day (EOD) and night break treatments. Promotion of flowering by brief EOD far-red (FR) light is nullified by a subsequent brief red light exposure. A PHYB-overexpressing line was late. Conversely, flowering was early and EOD effects reduced in mutants lacking phytochromes B and D or chromophore (hy1 or hy2). The similarity of PHYB and PHYD responses indicates genetic redundancy. When exposed to high irradiances (HIR), phyB mutants responded similarly to WT. FR night breaks implemented as a skeleton LD did not indicate that PHYB was important for time measurement. By contrast phyA mutants showed normal EOD flowering, but were somewhat late in LD. Thus, PHYA may modulate LD response. These mutant studies also exposed a third photoresponse where reduced photosynthetic input gave large delays in flowering. For both a phyA mutant and a blue light receptor mutant, hy4-101, there were substantial delays in flowering of soil grown, photosynthetically-dependent plants compared with those grown on agar supplemented with sucrose. This non-photoperiodic, photosynthetic promotion of flowering will contribute significantly to variation in Arabidopsis response both in laboratory and field.

9-8

Towards Understanding the Role of FPF1 in Arabidopsis floral induction.

John Chandler, Siegbert Melzer & Klaus Apel
Institut für Pflanzenwissenschaften, ETH, Universitätsstrasse 2, 8092 Zürich, Switzerland.

The gene FPF1 (Flowering Promoting Factor 1) is expressed predominantly in the peripheral zone of the apical meristem immediately following photoperiodic induction of flowering in Arabidopsis, and is also expressed later in floral meristems. Constitutive expression of the gene under control of the CaMV 35S promoter causes early flowering under long and short day conditions. The gene encodes a 12.6 kD protein which has no homology to any previously identified protein of known function. However, much evidence, such as treatments with GA and paclobutrazol, a gibberellin biosynthesis inhibitor, suggests that FPF1 is involved in GA signalling pathways and alters responsiveness to GAs in apical meristems. To further characterise which transcripts are regulated by FPF1, and therefore implicate FPF1 function in specific pathways, a differential display approach was used. Gene expression was compared in apices of plants constitutively expressing the FPF1 gene, either before induction to flower, or following a four day induction with long days, with gene expression in Ler wild type either before or after floral induction. A differentially expressed partial cDNA transcript has been isolated, which shows no homology to genes of known function and which is down-regulated in FPF1 over-expressing plants and also by GA3. In order to more directly define alterations in gibberellin biosynthesis and metabolism in apices and leaves of FPF1 over-expressing plants, compared with wild type plants, a quantitative RT-PCR approach has been used, to analyse expression of GA-regulated transcripts, such as GA4, GA5, GASA1 and LFY.

9-9

The role of DNA methylation in the vernalization response of Arabidopsis thaliana

R. K. Genger 1,2, E. J. Finnegan 1, K. A. Kovac 1,2, C.C. Sheldon 1, W. J. Peacock 1 and E. S Dennis 1
(1) CSIRO, Division of Plant Industry, Canberra, ACT 2601 (2) Division of Biochemistry and Molecular Biology, Australian National University, Canberra, ACT 0200

We have transformed Arabidopsis, ecotype C24, with an antisense construct of METI, a gene encoding a DNA methyltransferase. Antisense plants with reduced DNA methylation flowered early compared with untransformed controls, but flowered even earlier following a vernalization treatment (1). Both vernalization and METI antisense-induced demethylation cause a reduction in the level of transcripts of FLF, a dominant repressor of flowering (2). Vernalization of early flowering METI antisense plants further decreased the level of FLF mRNA, indicating that the additive effect of vernalization and demethylation could be attributed to greater repression of FLF. The vernalization signal is not transmitted to progeny and later flowering in the progeny was associated with a restoration in the level of FLF mRNA. Vernalization responsive late flowering mutants transformed with a METI antisense flower early; early flowering was associated with lower levels of FLF transcripts. These observations, together with the properties of vernalization, suggest that changes in DNA methylation mediate the vernalization response. This hypothesis is supported by our observation that prolonged cold treatment reduced DNA methylation.

1. Finnegan, EJ et al., (1996) Proc. Natl. Acad. Sci. USA 95: 5824-5829.
2. Sheldon CC et al., (1999) Plant Cell 11: 445-458.

9-10

9-11

Isolation of Early-Flowering Mutants by Activation Tagging Mutagenesis
Ilha Lee, Eun-Sook Park, Ho-Rim Lee
Department of Biology Seoul National University San 56-1 Sillim-Dong Kwanak-Gu Seoul, Korea 151-742

Floral inducion, a phase change from vegetative to reproductive development, is regulated by environmental factor and internal factor. The major environmental factors affecting on flowering are photoperiod and vernalization(cold treatment for flowering). Photoperiod induction pathway is relatively well studied at molecular level but the molecular mechanism of vernalization pathway is largely unknown. To investigate the molecualr mechanism of vernalization pathway, we adopted activation tagging mutagenesis strategy. The vector which has four times 35S enhancer with basta resistance marker was introduced randomly into Arabidopsis chromosome through Agrobacterium. The inserted 35S enhancer would cause the overexpression of nearby gene and give gain-of-function mutant phenotype. To reveal the mutants that activate vernalization pathway, we used FRI Arabidopsis line for activation tagging mutagenesis because it shows strong dependence on vernalization for flowering. Using this strategy, we isolated one dominant early-flowering mutant and two recessive early-flowering mutants. The genetics and physiology of these early-flowering mutants will be presented.

9-12

Isolation and characterisation of the FCA and CO flowering-time genes from pea and other plants.
Richard Macknight (1), Tonya Frew (2), Caroline Dean (3) Jan Grant (2) and Gail Timmerman-Vaughan (2)
(1)Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand (2) New Zealand Institute forCrop and Food Research Ltd., Private Bag 4704, Christchurch, New Zealand (3) Molecular Genetics Department,John Innes Centre, Colney lane, Norwich NR4 7UH, UK.

Over the last 30 years the site of action and physiological role of a number of pea flowering genes have been identified (Reid et al., 1996. Seminars in Cell and Dev. Biol. 7, 455). To determine if any of these well-characterised (but not cloned) pea flowering-time genes are orthologues of the Arabidopsis FCA or CO flowering-time genes, we have isolated the two genes from pea. The genes have been mapped in pea using recombinant-inbred mapping populations and RFLP markers. The pea FCA and CO genes mapped to linkage groups, IV and II respectively. No flowering time genes that map to these loci have been identified previously. To demonstrate that FCA and CO are involved in controlling the flowering-time of pea, transgenic pea plants that overexpress or express antisense versions of the two genes are being produced.

The Brassica napus FCA orthologue has been isolated. Brassica napus FCA complemented the late flowering phenotype of an Arabidopsis fca mutant. Arabidopsis FCA transcripts are alternatively processed at introns 3 and 13 (Macknight et al., 1997. Cell 89, 737-745). Similar alternatively processed FCA transcripts were detected from both Brassica napus and pea. FCA and CO homologues have also been isolated from other plants and a comparison of these genes will be presented.

9-13

Isolation and characterization of uns mutants showing unusual sugar responses

Masa-aki Ohto (1), Yasuko Furukawa (1), Etsuko Aoki (1), Shingo Hayashi (1&2) and Kenzo Nakamura (1&2)
(1)Division of Developmental Biology, National Institute for Basic Biology, Okazaki, 444-8585 (2) Graduate School of Bioagricultural Sciences, Nagoya University, Naogya 464-0814

Effects of the mutations on flowering such as co, fca, fha, fd, gi and tfl1 , but neither ft nor fwa, were minimized by 1% sucrose, when they were cultured in vitro on horizontal plates with or without sucrose. Although the flowering phenotype of wild type plants on 0% and 1% sucrose medium didn't differ, increase of the sucrose concentration from 1% to 5% cause the delay of flowering time in WT plants and all flowering mutants tested. Such delay by high levels of sucrose was, in part, responsible for the delayed activation of LFY. These results suggest that sugar might control the floral transition in Arabidopsis, both positively and negatively (Ohto et al., 9th Arabidopsis meeting, Madison, 1998). To understand the role of sugar-signalling during the floral transition, we have established 8,000 enhancer-tagged mutant lines and isolated mutants with anomalies in flowering time which also show the altered patterns of the sugar-regulated gene expression. Those mutants were named as uns after unusual sugar response. Among them, uns2, uns3 and uns4 flowered earlier than WT plants on soil under LD. Other morphological features of the mutants from WT plants and from each other could recognize them. Sugar-inducible increases of amylase activity and anthocyanin accumulation in leaf-cuttings were lower in both uns3 and uns4, or higher in uns2, than those of WT plants. Traits of the mutants are inherited dominantly in uns4 and uns3, and recessively in uns2. The mutated genes could be tagged with T-DNA. Isolation and sequencing of T-DNA flanking region of uns2 revealed the map position on the chromosome 4.

9-14

Suppressor mutants of fca-1.

Bonita Smart and Caroline Dean
Department of Molecular Genetics, John Innes Centre Norwich UK

The transition to flowering in Arabidopsis is controlled by multiple pathways. One of these, the autonomous promotion pathway functions independently of photoperiod and vernalization. One of the genes in this pathway, FCA, forms part of a post-transcriptional cascade. In order to identify downstream targets of FCA and interacting pathways, we have undertaken a suppressor mutagenesis using the strong fca allele, fca-1. Ten suppressor mutations, termed acf for the reversal of fca, have been isolated which suppress, to varying degrees, the late flowering of fca-1. Analysis of the acf mutations has taken a variety of approaches. Tests for allelism revealed that the mutants constitute nine complementation groups. Four (or 3) of the mutations appear to be semi-dominant while the remaining six (or 5) are recessive. Several of the acf fca lines show additional phenotypes in both long and short day conditions.Isolation of the acf mutations away from the fca-1 mutation has been achieved for the majority of the mutants. The flowering time of the single acf mutants has been compared to that of the doubles to try and establish if they function in the same or in interacting pathways. The single mutants have been crossed to other late flowering mutant to establish if they suppress the late flowering conferred by other mutations. Four of the ACF loci map to chromosome 5. The mapping of two other ACF loci is still underway.

9-15

Altered Expression levels of ATHB16 in Transgenic Arabidopsis Leads to Abnormal Leaf Expansion and an Altered Flowering Time Response

Yan Wang and Peter Engström
Department of Physiology Botany, Uppsala UniversityVillavägen 6, 752 36 Uppsala Sweden

ATHB, Arabidopsis thaliana homeobox genes encode homeodomain-leucine zipper transcription factors originally isolated by sequence similarity to Drosophila homeobox genes. The homeobox encodes the homeodomain which is a 60 to 62 amino acid domain consisting of three a-helices. About 20 ATHB genes have been isolated and they are highly conserved within the homeodomain region. Here we present the analysis and charactrisation of one member of this gene family, ATHB16, which encodes a protein with a molecular weight of 33,300. It maps to the bottom of chromosome 4. Altered expression levels of ATHB16 in transgenic Arabidopsis leads to differences in leaf size and in flowing time as compared to wild type. (A) Rosette leaf expansion Overexpression of ATHB16 caused a reduced leaf expansion and internode elongation, resulting mainly from a reduction in expansion and more condensed organisation of the mesophyll cells. Oppositely, down-regulation of ATHB16 activity in transgenic Arabidopsis caused over-sized rosette leaves, as a result of an increase in leaf cell size, but not from increasing of cell numbers. The leaf length-to-width ratio is not altered in the transgenic plants. We propose that the ATHB16 gene product acts to repress cell expansion during plant development. (B) Flowering time Under long-day condition, overexpression of ATHB16 delayed the transition from the vegetative to the reproductive phase. Reduced expression level of the gene had a reverse effect; plants expressing an antisense ATHB16 flowered earlier than wild type. Under short-day condition, the transgenic plants showed the reverse response; overexpression of ATHB16 causing a premature flowering and the antisense gene resulting in delayed flowering. We conclude from these results that ATHB16 may function as a negative regulator of the flowering time response to photoperiod in Arabidopsis.

9-16

Isolation of an Arabidopsis gene whose expression correlates with delayed flowering.

Dale Wilson and Alan Neale,
Department of Biological Sciences, Monash University, Melbourne Australia

The facultative long day plant Arabidopsis shows variation in flowering times both within, and between, natural populations. This variation is due to genetic differences in conjunction with the influence of several environmental factors such as photoperiod and temperature. The late-flowering ecotype Pitztal, flowers in approximately 90 days under standard growth conditions. Early-flowering ecotypes such as Columbia, flower after 20 to 30 days in similar conditions. We have previously created several early-flowering Pitztal mutant lines which now flower at similar times to these early-flowering ecotypes. The cDNA subtraction technique was utilised in an attempt to isolate differentially expressed sequences between our mutant lines and wild-type Pitztal. Approximately 40 such sequences were isolated, cloned, and characterised by sequencing and hybridisation experiments. One of these clones, highly enriched in the subtracted cDNA sample, shows strong expression in wild-type Pitztal plants and has little if any detectable expression in any of the mutant lines. An examination of expression profiles of this gene in early-flowering ecotypes showed expression of this gene is not detectable in L. erecta and only very weak expression is found in Columbia. Further characterisation of this clone is currently being conducted to examine the role of the gene in flowering.

9-17

Characterization of suppressors of tfl1-2 mutants

Hongjun Xiang, Thomas P. Jack
Department of Biological Science,Dartmouth College,Hanover, New Hampshire 03755, USA

terminal flower 1 (tfl1) mutants are early flowering and they develop terminal flowers on the shoot apex and single flowers in the axils of cauline leaves. The fact that TFL1 RNA is expressed in the subapical region of the shoot meristem but has an effect on the development of the cells of the shoot apex suggests that TFL1 functions non-cell-autonomously and that TFL1 function may involve cell-cell signaling. At present, little is known about the role of TFL1 in these processes. To identify TFL1 interacting genes, we performed a suppressor screen in a tfl1-2 mutant background. tfl1-2 is a phenotypically strong allele; when grown under long days, tfl1-2 plants flower early, produce terminal flowers, and do not produce secondary shoots. We have isolated suppressors of tfl1-2 that delay the formation of the terminal flowers and increase the number of secondary shoots. Some of these suppressors exhibit a phenotype only in a tfl1-2 mutant background. Other suppressors, by contrast, are late flowering or develop aerial rosettes in a Tfl1+ background. We will present a phenotypic analysis of these suppressors as well as progress on mapping.

10-1

FRUITFULL and SHATTERPROOF MADS-box genes Interact during Fruit Development in Arabidopsis.

Cristina Ferrándiz , Sarah J. Liljegren, Gary S. Ditta, Sherry A. Kempin, and **Martin F. Yanofsky**
Department of Biology, University of California at San Diego, La Jolla, CA 92093-0116, USA

Arabidopsis fruits are derived from the gynoecium which consists of and apical stigma, a short style, and a basal ovary. The two valves of the mature fruit are separated along their entire length by the replum, and a zone of dehiscence differentiates late in fruit development along the valve/replulm boundary. The dehiscence zone undergoes cell separation as the fruit matures, allowing the valves to detach from the replum and seed dispersal. The FRUITFULL (FUL) MADS-box gene is required for valve cell identity since valve cells fail to grow and differentiate in ful mutants. Constitutive expression of FUL (35S::FUL) leads to a conversion of replum into valve cell identity, indicating that FUL is sufficient to specify a valve cell fate in this context. In addition, because the dehiscence zone normally forms at the valve/replum boundary, it fails to form in 35S::FUL plants leading to an indehiscent gain-of-function phenotype. Interestingly, an indehiscent phenotype also occurs in shatterproof1 shatterproof2 (shp1 shp2) double mutants indicating that the normal function of SHP1 and SHP2 is to promote pod shatter. As shp1 and shp2 single mutants are indistinguishable from wild-type plants, these two genes are functionally redundant for specification of the dehiscence zone. We have continued to investigate the interactions between these genes, their roles in regulating lignification, and their regulation of downstream target genes. Our latest results, together with a model of their roles during fruit development will be presented.

10-2

Building an ovule: genetic control of patterning, polarity, identity, and directional expansion.

C. S. Gasser (1), S. C. Baker (1), J. Broadhvest (1), B. A. Hauser (1), R. K. Kuzoff (1), R. J. Meister (1), K.Schneitz (2) D. J. Skinner (1), and J. M. Villanueva (1).
(1)Section of Molecular and Cellular Biology, University ofCalifornia, 1 Shields Ave., Davis, CA, USA. and (2) Institut fuer Pflanzenbiologie, Universitaet Zuerich, Zuerich, Switzerland.

As for other aspects of plant morphogenesis, formation of Arabidopsis ovules requires early establishment of a developmental plan (pattern) along the proximal/distal and abaxial/adaxial axes. Following this pattern, regions of an ovule primordium take on specific developmental fates and undergo morphogenic changes due to differential cell expansion and cell division. We, and others, have identified mutants in at least seventeen different genetic loci regulating ovule development. Effects of these mutations indicate that several genes regulate the fundamental processes of morphogenesis outlined above. HUELLENLOS (HLL), AINTEGUMENTA (ANT), and SHORT INTEGUMENTS 2 (SIN2), have apparently independent roles in several aspects of ovule development, but also share partially redundant activity in proximal-distal patterning. INNER NO OUTER (INO) encodes a putative transcription factor which has an essential role in abaxial-adaxial polarity and subsequent asymmetric development of the outer integument. TSO1 is essential for proper orientation of cell division planes and directional cell expansion. Through these observations, and double-mutant analysis, we have assembled a model for genetic regulation of Arabidopsis ovule development. In addition, some of these mutants mimic specific evolutionary events in ovule phylogeny and our results may help elucidate the natural history of this structure. Supported by grants from NSF and USDA/NRICGP.

10-3

Distinct mechanisms promote polarity establishment in the carpels of Arabidopsis thaliana.
Yuval Eshed, Stuart F. Baum and John L. Bowman
Section of Plant Biology, UC Davis

Above ground lateral organs of plants display abaxial-adaxial asymmetry. Members of the Arabidopsis YABBY gene family specify abaxial cell fate in lateral organs. Mutations in CRC, a carpel specific member of this family, display ectopic formation of adaxial carpel specific tissues – placenta and ovules - only when the functions of other genes, such as KANADI or GYMNOS, are perturbed as well. Mutations in these genes alone do not result in loss of polar differentiation, and therefore, they act redundantly with CRC to establish polarity. Since GYMNOS encodes a uniformly expressed putative homologue of the chromatin remodeling protein, Mi-2, we argue that the unique genetic interactions do not reflect a molecular redundancy, but rather, that the genes act in distinct mechanisms to ensure proper differentiation in the abaxial regions of the carpels.

10-4

Non-cell-autonomous function of the floral homeotic gene PISTILLATA and APETALA3 is exerted by their downstream molecule.
Koji Goto, Homma Takashi, and Kazuhito Gauda.
Inst. for Chemical Research, Kyoto University.Uji, 611-0011, Japan.

Arabidopsis floral homeotic genes PISTILLATA (PI) and APETALA3 (AP3) are expressed in the second and the third whorls spanning three layers, L1, L2 and L3. Previous studies suggest that PI and AP3 work as non-cell-autonomous manner among these layers. To investigate the pathway of non-cell-autonomous organ identity signaling, we introduced PI and AP3 genes to express only in the L1 layer (L1::PI and L1::AP3). These transgene partially rescued the mutation of pi and ap3, namely immature petal and stamen formation were observed. In the stamen, formation of pollen which are originated from L2 cells was observed. To examine whether PI and AP3 proteins themselves moved across layers, we observed localization of these proteins of L1::PI and L1::AP3 flowers. Unexpectedly, PI and AP3 proteins were localized only in the cells of the L1 layer. Since PI and AP3 expression induces their own expression, namely they are regulated by autoregulation, we examined expression of PI and AP3 in the L2, L3 layers of L1::PI and L1::AP3 plants using in situ RNA hybridization, but no ectopic signal was detected. Taken together, these results suggest that organ identity signaling produced by PI and AP3 is transmitted from L1 layer to L2, L3 layers by means of downstream molecule of PI and AP3.

10-5

GYNOECIUM DEVELOPMENT IN ARABIDOPSIS

Stuart F. Baum, Yuval Eshed, Joanna Putterill*, John Alvarez§ and **John L. Bowman**
Section of Plant Biology,University of California Davis, Davis, California 95616, USA, *School of Biological Sciences, University of Auckland, Private Bag 92019 Auckland, New Zealand, §Department of Biological Sciences, Monash University, Clayton, Melbourne, Victoria 3168, Australia

One of the key defining features of angiosperms is the carpel, whose primary function is to house the ovules. The overall structure of the carpel reflects its primary functions: to enclose and protect the developing ovules, to mediate pollination, and to develop into a fruit to be used as a vector for seed dispersal. As in nearly all other angiosperm species, the gynoecium occupies the central position in the Arabidopsis flower, with the formation of the gynoecial primordium occurring after the formation of the organ primordia of the other floral organs. The superior gynoecium of Arabidopsis is typical of the Brassicaceae; it is composed of two congenitally fused carpels creating a single ovary, which is topped with a postgenitally fused short solid style and stigmatic papillae. Below the ovary is a short internode, referred to as the gynophore that connects the gynoecium to the floral receptacle. A postgenitally fused septum divides the ovary into two locules, with parietal placentae developing at the adaxial margins of fusion of the two carpels. Thus, the Arabidopsis gynoecium provides a system to examine the specification of tissue types within an organ, as well as congenital and postgenital fusion events. A morphological and anatomical developmental series is presented which is used as a basis to which various carpel mutants are compared.

10-6

SHORT INTEGUMENTS 2, a regulator of ovule integument cell division in Arabidopsis, also participates in ovule primordia growth and floral organ formation.

Jean Broadhvest, Shawn C. Baker and Charles S. Gasser.
Section of Molecular and Cellular Biology, Division of Biological Sciences, University of California, Davis, CA 95616, USA.

A mature Arabidopsis ovule is amphitropous and composed of four structural domains: the funiculus (supporting stalk), two integuments (inner and outer) and the nucellus which contains the embryo sac. As for morphogenesis of other plant organs, development of the ovule is insured by the tight regulation and coordination of genes necessary for the appropriate establishment of a primordium, patterning, cell division, and elongation. In the Arabidopsis ovule, SIN2 is involved in the regulation of cell division in both inner and outer integuments as shown by the sin2 single mutant phenotype. Double mutant lines and in situ hybridization data were generated to understand more about the genetic interactions between SIN2 and others genes regulating ovule development. Interestingly, synergistic phenotypes have revealed new roles for SIN2 in other ovule structural domains. SIN2 shows a positive interaction with HUELLENLOS suggesting a role for SIN2 in the regulation of ovule primordia growth. Genetic interaction with a weak mutant allele of TSO1 suggests that SIN2 might also have a role in proper growth of all floral organs. Models of genetic interactions and progress on the molecular isolation of SIN2 will also be presented. Supported by the NSF and the USDA/NRICGP.

10-7

Dissecting the floral homeotic AGAMOUS pathway
Xuemei Chen and Yulan Cheng
Waksman Institute Rutgers University Piscataway, NJ 08854 USA

The floral homeotic MADS domain protein AGAMOUS (AG) performs at least three functions during Arabidopsis flower development. It specifies the identities of stamens and carpels, controls floral determinacy, and negatively regulates the expression of another floral homeotic gene, APETALA1. AG had been the only gene known to perform these functions until the discovery of HUA1 and HUA2 from an enhancer screen using ag-4, a weak ag allele. Despite the involvement of the HUA genes in every aspect of AG's functions, the hua1 hua2 double mutant shows a very weak floral phenotype, i.e., slightly petaloid stamens and bulged carpels. The lack of an ag-like phenotype and the fact that numerous mutageneses in non-sensitized backgrounds only yielded ag alleles suggest that some genes in the AG pathway may have overlapping functions. If this is true, an enhancer screen in the hua1 hua2 background should allow us to identify more genes in the AG pathway. Indeed, we have recovered many enhancer lines from an EMS mutagenesis in the double mutant background. The phenotypes of the enhancer lines vary in severity from slightly more severe than hua1 hua2 to almost ag-like. The phenotypes of the enhancer lines will be presented. HUA2 was cloned with a map-based strategy. It encodes a protein with limited similarity to two newly isolated human transcriptional coactivators that interact with sequence-specific transcription factors and the general transcriptional machinery. This and the finding that AG and HUA2 appear to function at the same hierarchical level raised the possibility that HUA2 may be a cofactor of AG. We will present evidence that AG and HUA2 can physically interact in vitro.

10-8

Isolation and characterization of a nectary-specific Ntr gene of Arabidopsis
Hak Soo Seo, Jong Tae Song, Yeon Jong Koo, Jong Seob Lee (1) and **Yang Do Choi**
Department of Agricultural Chemistry and Department of Molecular Biology (1), Seoul National University, Suwon 441-744, Korea

A genomic clone for Ntr gene was isolated from Arabidopsis by using floral nectary specific gene, Ntr1, of Brassica campestris L., ssp. pekinensis as a probe. The clone encodes a protein of 381 amino acid residues in four exons and three introns. Southern blot analysis revealed the presence of a single copy of the Ntr gene in the Arabidopsis genome. Northern blot analysis showed that its transcript was approximately 1.5 knt in length and expressed mostly in nectaries of developing flower. To examine the expression pattern of the gene, about 2.25 kbp upstream promoter fragment was translationally fused to the GUS reporter gene and then transformed into Arabidopsis. It was expressed predominantly in floral nectaries but also in other flower organs at much lower level.

10-9

FRUITFULL defines valve identity gene in Arabidopsis carpels.
Cristina Ferrándiz, Shusei Sato, Sarah J. Liljegren and Martin F. Yanofsky
Department of Biology University of California at San Diego La Jolla, CA 92093-0116 USA

The FRUITFULL (FUL) gene is required for normal silique development in Arabidopsis. All the tisues within the ovary walls are affected by the ful mutation: The valves fail to differentiate and expand and frequently break out before maturation of the seeds, the style displays abnormal morphologies, and the replum adopts a zig-zag arrangement (Gu et al,1998, Development 125:1509-1517). By contrast, 35S::FUL lines display a phenotype in which the ovary walls are largely converted into valve tissue. As a result, the dehiscence zone, that in WT differentiates between the valve and the replum to ensure pod shatter, completely fails to develop. This indehiscent phenotype resembles the shatterproof1 shaterproof2 (shp1 shp2) double mutants (Liljegren et al, this meeting). We are currently investigating the possible interactions between FUL and SHP1/SHP2 at the genetic and molecular levels. Our results suggest that FUL is formally transcriptional repressor of SHP1/SHP2. To further explore the function of FUL, we have generated transgenic plants that have a constitutively activated version of FUL (FUL:VP16) under the control of different regulatory sequences. The results of these studies and a current model for the role of FUL will be presented.

10-10

Patterns of expression of the carpel development gene SPATULA
Marcus G. B. Heisler, Yasmin M. Bylstra and David R. Smyth
Department of Biological Sciences, Monash University, Clayton, Vic. 3168, Australia

Mutant studies of the SPATULA gene of Arabidopsis indicate that it acts to promote the growth of carpel margins and development of transmitting tract tissues derived from them. SPT encodes a transcription factor of the basic helix-loop-helix family. Its expression in the developing gynoecium is confined to those regions that are disrupted in spatula mutants. When the gynoecium arises at stage 6, SPT expression is strongest in those regions where carpels are congenitally fused. Later, expression occurs in the growing septum and at the apex where stigmata and transmitting tract tissues develop. Thus SPT is a regulatory gene that apparently acts autonomously to control the development of specific sub-regions of the gynoecium. ETTIN (ARF3) is another gene involved in carpel development. ettin mutant gynoecia display a reduction in valve tissue, the appearance of a gynophore and the over-proliferation of stigmatic and septal tissues (Sessions and Zambryski 1995). Significantly, spt is almost completely epistatic to ettin with regard to gynoecium development, indicating that ETTIN may confine SPT activity to subregions of the wild-type gynoecium (Alvarez and Smyth 1998). Confirming this proposal, SPT expression expands to almost completely encompass the gynoecium by stage 7 in ettin mutants, and later it becomes concentrated in those regions that develop into ectopic outgrowths. SPT transcripts were also detected within subregions of other meristematic tissues, including the apical meristem, young floral buds, and developing petals, stames and ovules. These organs are apparently unaffected in spt mutants, suggesting that SPT plays a redundant role in their development.

10-11

PI/AP3 heterodimer interacts with different MADS proteins in each whorls and modulates their DNA binding specificity; a molecular basis of the ABC model.

Takashi Homma and Koji Goto
Inst. for Chemical Research, Kyoto Univ., Uji, 611-0011, Japan

Arabidopsis floral homeotic gene, PI and AP3 encode MADS domain proteins which have DNA binding (MADS) and coiled-coil domain. PI and AP3 make hetero-dimer to act as B function of floral organ identity. PI/AP3 complex, however, lacks activator domain to fulfill the role of transcription factor. Therefore, we screened for co-activator protein which interacts with PI/AP3 complex using yeast two-hybrid system. AP1 and AGL9 were cloned when both PI and AP3 were used as a bait. AP1 and AGL9 are also members of MADS domain protein, but distinct from PI and AP3, can act as transcriptional activators. AP1, which provides A function, is expressed in the first and second whorls and AGL9 is expressed in the second, third, and fourth whorls. In yeast, AGL9 as well as AP1 interacts with AG, which provides C function. However, AP1 expression is transcriptionally repressed by AG in the Arabidopsis flower. Taken together, these results demonstrate that PI/AP3/AP1/AGL9 complex determines petal identity in the second whorl, and PI/AP3/AGL9/AG determines stamen identity in the third whorl. MADS domain proteins have very similar DNA binding specificities in vitro but specify the development of different floral organ in vivo. AP3::GUS was expressed in the leaves of triple transgenic 35S::AP1, 35S::PI and 35S::AP3 plants, whereas GUS activity was eliminated in the 35S::AP1 plants. These results suggest that PI/AP3 complex modulates the DNA binding specificity of AP1. Thus, the ability of different MADS genes to specify distinct organ identity function may depend on the whorl specific combinations of MADS domain proteins that modulate DNA binding specificity.

10-12

The putative PETAL LOSS gene of Arabidopsis encodes a protein with two trihelix DNA binding motifs

Paul Howles, Megan E. Griffith, Kristen Dorian, Georgia Stamaratis and David R. Smyth
Department of Biological Sciences, Monash University, Clayton, Vic. 3168, Australia

Mutants of the PETAL LOSS gene are impaired in the initiation and orientation of petals. The first formed flowers may have several petals but later flowers frequently have none. The orientation of those petals that do arise is frequently disrupted. When ptl is combined with B function mutants, petals are almost always reversed, suggesting that the functions of PTL and B class genes act redundantly to specify the orientation of second whorl organs within the flower.

We have mapped the PTL gene to the top of chromosome 5, just above TERMINAL FLOWER1. Using map-based cloning, we have identified a BAC that contains the PTL gene. A candidate gene within this BAC demonstrates base changes in two different ptl mutant alleles, one conferring a stop codon, the other a frame shift. The candidate gene is a member of a family of transcription factors found so far only in plants. Family members contain one or two trihelix DNA binding domains that recognise and bind to GT elements. The putative PTL gene contains two of these domains. If complementation studies confirm that the candidate gene is PTL, it will be the first member of this family whose function is indicated by an associated mutant phenotype.

10-13

CUP-SHAPED COTYLEDON genes are involved in development of gynoecum and ovule in Arabidopsis thaliana

Tetsuya Ishida (1), Mitsuhiro Aida (2), Shinobu Takada (1) and Masao Tasaka (2).
(1)Department of Botany, Division of Biological Science, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan; (2)Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0101, Japan.

Flowers are reproductive organs in higher plants. Gametogenesis occurs in stamens and gynoecia and embryogenesis occurs in gynoecia. A flower of Arabidopsis has six independent stamens and two carpels which fused to make a gynoecium. The gynoecium consists of a stigma, a style and an ovary which has two locules containing ovules. Two locules are separated by a septum, which are formed postgenitaly. Ovules are formed on the base of septum. Plants with mutations at two loci, CUC1 (CUP-SHAPED COTYLEDON1) and CUC2, show fused cotyledons, fused sepals and fused stamens. In addition, there were defects in formation of ovaries and ovules. In cuc1 cuc2 double mutant, the septum was not formed and the number of ovule decreased. In most of ovules, nucelli were not covered with integuments. CUC2 was expressed at the tips of septal primordia and the boundary between nucellus and chalaza of developing ovules in wild type. These expression patterns were partially but not completely consistent with the phenotypes of mutant gynoecium and ovule. This suggests that CUC2 may act cell non autonomously in some tissues.

10-14

Functional analysis of DAD1 which is required for jasmonate production and anther dehiscence

Sumie Ishiguro and Kiyotaka Okada
Department of Botany, Graduate School of Science, Kyoto University, Kyoto 606-8502, JAPAN

Anthers of most flowering plants dehisce and release their pollen grains at the time when the flowers are just opening. This regulated dehiscence helps effective pollination especially in self-pollinating plants such as Arabidopsis thaliana. We have identified a mutant, dad1 (defective in anther dehiscence 1), whose anthers fail to dehisce even after flowers have opened. The pollen grains contained in the undehisced anthers have developed to the tricellular stage, but cannot germinate even when they are attached to the stigma. Because it was pointed out that the jasmonate has a key role for the anther dehiscence, we examined the effect of jasmonate upon the dad1 mutant. After we applied the solution of methyl jasmonate to the young flower buds of dad1 mutants, anthers actually dehisced at the flower anthesis and released fully functional pollen grains. Similar successful self-fertilization was observed when we applied the linolenic acid, a precursor of jasmonate. We isolated the DAD1 gene by T-DNA tagging and showed that this gene encodes a putative lipase. The expression of DAD1 promoter-GUS fusion gene is appeared one-day before anthesis and restricted in the filaments of stamens. These finding suggest that the DAD1 lipase catalyze the release of free linolenic acid that is then converted to jasmonate, which induces the anther dehiscence. Characterization of DAD1 protein is in progress.

10-15

Ectopic expression of AINTEGUMENTA increases the size of floral organs
Beth A. Krizek
Department of Biological Sciences, University of South Carolina, Columbia, SC 29208 USA

AINTEGUMENTA (ANT) has been shown previously to be involved in floral organ initiation and growth in Arabidopsis. ant flowers have fewer and smaller floral organs and possess ovules which lack integuments and a functional embryo sac. Here I show that inflorescence meristems and young floral meristems of ant plants are smaller than those in wild type. Failure to initiate the full number of organ primordia in ant flowers may result from insufficient numbers of meristematic cells. Ectopic expression of ANT under the control of the constitutive 35S promoter results in the development of larger floral organs. The number and shape of these organs is not altered and the size of vegetative organs is normal. Microscopic and molecular analyses indicate that the increased size of 35S::ANT sepals is due to increased cell division, while the increased sizes of 35S::ANT petals, stamens, and carpels are primarily due to increased cell expansion. In addition, 35S::ANT ovules often exhibit increased growth of the nucellus and the funiculus. These results suggest that ANT can control organ growth in flowers by regulating not only cell division but also cell expansion.

10-16

The birch genes bpmads1, bpmads5 and bpmads6
Juha Lemmetyinen, Annakaisa Elo, Mika Lännenpää, Maarit Hölttä, Katri Kärkkäinen and Tuomas Sopane
University of Joensuu, Department of Biology, P.O.Box 111, 80101 Joensuu, Finland

One aim of our work is to study the genetic regulation of flower development in silver birch (Betula pendula Roth), which has unisexual and structurally simple flowers, very different from those of Arabidopsis and Antirrhinum. The knowledge will be used to develop non-flowering sterile birches. So far, we have isolated cDNA clones for 7 MADS-box genes (BpMADS1-7) from birch. Among these BpMADS1 is similar to AGL9, BpMADS5 to AGL8 and BpMADS6 to AGAMOUS. According to RNA gel blot analysis these genes are expressed in both male and female inflorescence, but not in any vegetative tissues. Possible functions of these genes are being studied using ectopic expression of the cDNAs in tobacco, Arabidopsis and birch. Ectopic expression of BpMADS1, BpMADS6 and especially BpMADS5 caused early flowering in some transgenic tobacco lines. Ectopic expression of BpMADS6 had also effects on sepals and petals. Some tobacco plants expressing BpMADS5 flowered when about 5 cm high, and produced similar progeny. BpMADS5 also caused our early-flowering test clone (BPM2) to flower, when 7 cm high, whereas normally this clone flowers when 30-70 cm high. In Arabidopsis 35S::BPMADS1 caused changes in flower structure leading to flowers without sepals and petals, to partial conversion of sepals to carpels or to clusters of flowers. 35S::BPMADS6 caused extremely early flowering phenotype similar to that in emf mutants. The promoter of BpMADS5 was isolated, and ligated to uidA reporter gene (GUS). In tobacco GUS gene activity was present in receptacle, stamens and carpels .

10-17

Regulation of valve margin development and fruit dehiscence by the SHATTERPROOF MADS-box genes

Sarah J. Liljegren (1), Sherry Kempin (1), Cristina Ferrándiz (1), Gary S. Ditta (1), Yuval Eshed (2), Beth Savidge (1), John L. Bowman (2), and Martin F. Yanofsky (1)
(1) Department of Biology, University of California at San Diego (2) Section of Plant Biology, University of California at Davis

Pod shatter, or fruit dehiscence, occurs late in the development of dry, dehiscent fruits to ensure seed dispersal. Despite its agronomic importance, little is known about the regulation of this complex process. We have previously identified loss-of-function alleles of two MADS-box genes, SHATTERPROOF1 (SHP1) and SHATTERPROOF2 (SHP2), which are both expressed along the valve margin of Arabidopsis fruits. Although shp1 and shp2 single mutants are indistinguishable from wild-type plants, shp1 shp2 double mutants display a striking phenotype, as the mature fruits fail to dehisce. Differentiation of the dehiscence zone and lignification of adjacent cells, which has been proposed to promote pod shatter, fail to occur in shp1 shp2 fruits. Interestingly, transgenic plants that constitutively express SHP1 and SHP2 produce fruits which show ectopic valve lignification and other phenotypes consistent with a transformation of the valve to a valve margin identity. As many similarities are apparent between 35S::SHP1 35S::SHP2 and fruitfull (ful) fruits (Gu et al., Development 125:1509-1517, 1998), as well as between shp1 shp2 and 35S::FUL fruits (C. Ferrándiz and M. Yanofsky, unpublished results), we have been exploring the antagonistic interactions between the SHP1/2 and FUL MADS-box genes. In addition, the availability of several valve margin molecular markers has allowed further investigations of the cascade of gene activity that leads to formation of the valve margin. Our current model of interactions involved in valve margin and dehiscence zone development will be presented.

10-18

Functional Characterisation of an Arabidopsis (1-3)-b-Glucan Synthase Homologue

Nicholas A. Paech (1&2), Geoffrey B. Fincher (1) and Anna M. Koltunow (2).
(1) Department of Plant Science, Waite Institute, University of Adelaide, Glen Osmond SA 5064, Australia.
(2) CSIRO Division of Plant Industry, Horticulture Unit, P.O. Box 350, Glen Osmond SA 5064, Australia.

In plants, accumulation of the (1-3)-b-glucan, callose, is associated with several developmental processes. These include transient deposition in the cell plate during cell division, accumulation around sporocytes prior to meiosis, around degenerating megaspores following meiosis and around embryogenic initials prior to parthenogenesis. Callose is also deposited in response to pathogenesis and wounding. The precise role of callose in many of these processes is yet to be determined, but the knowledge of the regulation of callose deposition would be greatly enhanced by cloning the gene(s) responsible for its synthesis. During a screen for genes expressed during early ovule development in the apomictic plant Hieracium, a cDNA clone was isolated that had homology with a (1-3)-b-glucan synthase from yeast. Northern analysis in Hieracium indicated that the mRNA is present in comparable levels in all tissues examined. Database searches identified a highly homologous region in an Arabidopsis bacterial artificial chromosome. A full-length cDNA encoded by this (1-3)-b-glucan synthase-like region was isolated from Arabidopsis using reverse transcription and the polymerase chain reaction (RT-PCR). To characterise the function of this Arabidopsis (1-3)-b-glucan synthase homologue, two distinct approaches have been taken. The first was to clone the sequence into a yeast expression vector and test whether the Arabidopsis sequence could complement the yeast fks1 mutant phenotype. The fks1 mutant has an insertion mutation in FKS1, a gene encoding yeast (1-3)-b-glucan synthase. The second approach was to clone the Arabidopsis homologue into an over-expression vector, transform into Arabidopsis and screen for phenotypic alterations in plant development.

10-19

Transformation and Expression of PPF-1 and GDA-1 Genes into Arabidopsis

Yanhui Su, Yunjian Xu and Zhu Yuxian
College of Life Sciences, Peking University, Beijing, 100871, P.R.China

Despite genetic propensity for longevity with the plant kingdom, senescence usually occurs at a precise develpmental stage after reproduction in many species. However, we found pea(Posum sativum L.)lines G2(Sn, Hr)produced flowers and fruit under either long day(LD) or short day(SD) conditions, but senesced only under LD. Morever, endogenous gibberellin(GA) levels were inversely correlated with photoperiod and senescence. Using cDNA representional difference analysis (cDNA RDA), we isolated two SD specific genes named PPF-1 and GDA-1. PPF-1 shares a substantial homology only at the deduced amino-acid level with a subtillis gene SP3J which is required for maintaining vegetative growth. Northern blots showed that this gene was only expressed under SD after floral initiation. The expression of GDA-1 was found to be SD specific, but independent of flower initiation time. Under SD conditions, when G2 pea displays an unlimited vegetative growth, PPF-1 and GDA-1 expression were sustained at a relatively high level. Treatment of LD-growen G2 pea with GA3 were able to stimulate PPF-1 and GDA-1 expression unless it was applied at a very later growth stage. To further elucidate the functions of PPF-1 and GDA-1 during plant growth and development, we cloned both genes into plant transformation inter mediate vector pMN10098 and transferred them into Arabidopsis thaliana by floral dip method. Overexpression or anti-sense expression of these two genes in the transformants may change the development phenotype of Arabidopsis, such as flowering time. Molecular analysis of the difference between transformants and wild type Arabidopsis may give usnew sight about their roles during the development and senescenceof plants.

10-20

Formation of the pollen wall in Arabidopsis revealed using mutagenesis and ultrstructural analysis

Philip E. Taylor, Jeong-Keun Choi and Ian A. Staff
School of Botany, University of Melbourne, Parkville, VIC 3052. Australia

Sporopollenin is synthesised and incorporated into the exine of the pollen wall. Its exceptional stability to non-oxidative degradation allows for the preservation of pollen grain morphology over millions of years. Mutants affecting pollen wall formation were produced and isolated in an attempt to define these morphogenetic processes using high resolution light and electro microscopy. Firstly, seeds were mutagenised in ethylmethane sulphonate and plants grown to maturity. After self-pollination, seeds were collected from 600 of the most vigorous plants and 20 seeds from each plant were sown. About 10,000 plants of the M2 generation were secreened for pollen viability with Alexander's stain. After self-pollination, seed set was estimated from silique length. About 150 putative male-sterile plants were found. Mutant lines were sustained by crossing with wild-type. Based on detailed phenotypic analysis, the stage of action of seven non-allelic male-sterile mutants with aberrations in microspore wall formation was determined. Three previously characterised ms mutants (from A. Chaudhury, CSIRO, Canberra) were included for further analysis. The characterisation of pollen wall mutants has enabled a clearer understanding of wall formation in wild-type pollen. A model detailing the ultrastructural changes in pollen wall formation is presented for Arabidopsis and comparisons made within the Brassicaceae.

10-21

Conservation of the Arabidosis thaliana actin gene ACT1 reproductive tissue specific expression pattern.

A. V. Vitale and R. B. Meagher:
The University of Georgia, Athens, GA 30602 USA

Actin genes throughout the plant kingdom exist as multi-gene families. Based on expression patterns and sequence analysis eight expressed Arabidopsis thaliana actins comprise two ancient classes and can be further divided into five subclasses. ACT1 and ACT3, the result of a duplication event 30-60 MYA, comprise one of the five ancient subclasses and are both expressed at high levels in mature pollen, embryo sac, and organ primordia. Non-coding regions of ACT1 and ACT3 share little homology with the exception of 55-bp of sequence within the 5' flanking region. Because these genes have identical expression patterns it is postulated that this conserved sequence may direct gene expression. To address this, cis-element dissections are being examined in transgenic A. thaliana lines. Other areas of the ACT1 5' flanking region are also being dissected for a role in the ACT1 expression pattern. All constructs are being examined quantitatively as well as qualitatively. Examination of the ACT1 promoter region has also been undertaken in transgenic lines of Nicotiana tabacum. Because its expression is similar to that seen in A. thaliana it can be suggested that differential regulation of actin expression is important and could be one of the reasons for the maintenance of a large gene family in the small genome of Arabidopsis. Conservation of ACT1 expression is also under investigation in the more distantly related Oryza sativa.

10-22

Analysis of fertilization-independent fruit development in the Arabidopsis fwf1 mutant

A. Vivian-Smith (1&2), A.M. Chaudhury (3), A.M. Koltunow (1)
(1) CSIRO Plant industry, Horticulture Unit, P.O. Box 350, Glen Osmond 5064, (2) Department of Plant Science, University of Adelaide, Urrbrae, 5064, (3) CSIRO Plant Industry, Black Mountain Laboratories, GPO Box 1600, Canberra, ACT 2601.

To gain a broader understanding of how seedless fruit develop, a recessive mutant that forms siliques independent of fertilization (fwf1) was isolated from a mutagenized male sterile population. fwf1 mutants show that silique development can be uncoupled from fertilization and seed development. Characterization of fwf1 may identify factors that normally link these processes. Emasculated fwf1 pistils develop 7-9 mm siliques similar to pollinated L.er. Reciprocal cross pollination of L.er and fwf1 showed that reduced seed set, in proximal positions of the fwf1 silique, was in part due to a maternal defect. To address whether the integrity of specific carpel tissues is required for parthenocarpic development and to assess if hormone perception is involved in generating the fwf1 phenotype, we crossed fwf1 with a mutant blocked in carpel development (frt1-3), a series of ovule development mutants (fis2, ant and ats) and several hormone perception mutants. We observed that ga1-3 and frt1-3 phenotypes are epistatic to fwf1. Demonstrated independence of fwf1 with gai, a mutant that blocks GA3 induced parthenocarpy, indicates fwf1 has a role independent of GA perception. When ats, a unitegmic ovule mutant is combined with fwf1, enhanced silique elongation was observed following emasculation. As ats lacks two integumentary cell layers, the enhancement of silique development in fwf1 suggests that these maternal cell layers provide inhibitory signals that control the efficiency of fwf1 siliques to develop in the absence of fertilization. The fwf1 mutation was mapped and we are using several approaches to clone fwf1.

10-23

Plant growth regulator induced fruit development in Arabidopsis

Adam Vivian-Smith (1&2), Anna M. Koltunow (1)
(1) CSIRO Plant industry, Horticulture Unit, P.O. Box 350, Glen Osmond 5064, Adelaide, (2) Department of Plant Science, University of Adelaide, Urrbrae, Adelaide 5064

Development of carpels into fruit is usually linked to fertilization and seed development in Arabidopsis. Previous research has shown that the number of fertilized ovules and endogenous GAs are critical factors in initiating fruit development. Ovules remain receptive to fertilization for a short time and ovary growth is terminated after this period if fertilization is absent. Parthenocarpic (seedless) growth occurs when growth regulator treatments are applied to unfertilized pistils or when natural genetic variants arise. To clarify roles of hormones during fruit development we applied BA, GA3, NAA and IAA to emasculated pistils of various Arabidopsis ecotypes and determined the period of pistil receptivity, compared to pollination. Pistil response varied with the level of growth regulator and ecotype tested. Landsberg was most responsive to GA3 and NAA (10 µmol pistil-1). GA3 induced development comparable to fertilized siliques, while NAA induced greater cellular expansion of the exocarp and mesocarp. Arabidopsis mutants were used to examine potential dependencies on GA biosynthesis (ga1-3, ga4-1 and ga5-1) and perception (spy-4 and gai) during parthenocarpic silique development. gai mutants, are blocked in GA perception and produce siliques only in response to pollination and NAA. Structural development in both pollinated gai and ga5-1 was also similar to auxin induced parthenocarpy. Collectively our genetic analysis reflects redundancy in hormonal control required for the initiation of silique development, and indicates that mechanisms other than GA mediated perception exist to trigger silique development in Arabidopsis.

10-24

FIL protein interacts with ETTIN protein in Yeast Two-Hybrid system

Keiro Watanabe, Shinichiro Sawa, and Kiyotaka Okada
Department of Botany, Graduate School of Science, Kyoto University Sakyo-ku, Kyoto, 606-8502, Japan.

We isolated a floral meristem identity gene FILAMENTOUS FLOWER (FIL), and found it encodes a zinc finger and a HMG-related domains. The expression of FIL gene is localized in ventral regions of cotyledons, leaves and floral organs. The adaxial epidermal cells of rosette and cauline leaves of 35S::FIL plants partially changed into the abaxial epidermal cells. FIL gene determines the dorsal identity of cotyledons. leaves and floral organs.(Sawa et al. Genes & Development, 13, 1079~1088, 1999) In order to examine proteins interacting with the FIL protein, we screened floral cDNA library by two-hybrid system using FIL as a bait, and obtained four candidate genes. One of them is ETTIN gene, which is known to control regional identity of stamens and gynoecium, and to encode putative DNA binding domains similar to the transcription factors ARF1 and IAA24. (Sessions et al. Development, 124, 4481~4491,1997) It is possible that FIL and ETTIN proteins work cooperatively in the development of stamens and carpels, because the expression pattern of both genes is overlapped.

10-25

A novel mutation that alters megaspore cell fate in Arabidopsis

Wei-Cai Yang, Jian Xu, De Ye, Venkatesan Sundaresan
Laboratory of Plant Molecular Genetics, The Institute of Molecular Agrobiology, 1 Research Link, Singapore 117604

After meiosis in the ovule, four megaspores are generated, of which only the chalazal megaspore will develop into an embryo sac, and the remaining three non-functional megaspores undergo programmed cell death. In a screen of Ds insertions carrying a Kan-R marker, a line SGT1084 was identified which showed a KanR:KanS ratio of 1:3, instead of the expected 3:1. In crosses to wild type, the KanR:KanS ratio is 1:7 with SGT1084 as female, and 1:4 with SGT1084 as male, indicating that the mutation affects both male and female gametes, but is more severe in the female. The basis for the reduced male transmission is as yet unknown. About 37% of the ovules in the heterozygous plants were aborted. Detailed analysis of the aborted ovules revealed that the normal single embryo sac was replaced by either four large cells, or by an embryo sac towards the micropylar end and an additional 3 smaller cells towards the chalazal end. Preliminary investigations suggest that in these ovules, the three non-chalazal megaspores do not undergo programmed cell death, but survive and can acquire the ability to form embryo sacs. The mutant line contains a single Ds element. DNA flanking the Ds insertion has been used to isolate genomic clones, and further molecular characterization is in progress.

10-26

The SPOROCYTELESS gene is essential for the formation of microsporocytes and megasporocytes in Arabidopsis

De Ye, Wei-Cai Yang, Jian Xu, Venkatesan Sundaresan*
Institute of Molecular Agrobiology, The National University of Singapore, 1 Research Link, Singapore 117604. *e-mail: director@ima.org.sg; FAX 65-872-7007

The formation of haploid spores marks the initiation of the gametophytic phase of the life cycle of all vascular plants ranging from ferns to angiosperms. In angiosperms, this process is initiated by the differentiation of a subset of floral cells into sporocytes, which then undergo meiotic divisions to form microspores and megaspores. There is currently little information available regarding the genes and proteins that regulate this key step in plant reproduction. We report here the identification of a mutation, SPOROCYTELESS (SPL), which blocks sporocyte formation in Arabidopsis. Analysis of the SPL mutation suggests that development of the anther walls and the tapetum and microsporocyte formation are tightly coupled, and nucellar development is dependent upon megasporocyte formation. Molecular cloning of the SPL gene shows that it encodes a nuclear protein with similarity to transcription factors, and that it is expressed in the microsporocytes and megasporocytes. These data suggest that the SPL gene product may regulate development of male and female meiocytes in Arabidopsis.

10-27

LEUNIG is required for normal congenital and postgenital fusions of gynoecium in *Arabidopsis*

Changbin Chen[1] and Hai Huang[2]
1 Department of Biology, East China Normal University, Shanghai 200062, China
2 Shanghai Institute of Plant Physiology, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China

The *Arabidopsis* gene *LEUNIG* was previously found to regulate floral organ identity. In this work we describe gynoecial phenotypes of newly isolated strong *leunig* alleles, *leunig-101, leunig-102* and *leunig-103*. Gynoecia of these strong *leunig* mutants are united only at the basal part, leaving 4 unfused parts at the apex. Among them two medial ones are styles capped with stigmas, and two lateral ones are protrusions from valves. The epidermal cells at the very distal portion of protruded valves mimic those on wild-type

styles, and those valves occasionally also have stigma-like tissues, indicating the evolutionary relation between gynoecium valve and carpel. The gynoecium with unfused apex in *leunig* arises as a unit from a basal meristematic zone, suggesting that *LEUNIG* is required for normal congenital gynoecium fusion. The epidermal cells on growing inner surfaces of *leunig* gynoecium failed to fuse after they contact each other, indicating that *LEUNIG* is essential for the proper postgenital fusion. We have also analyzed *clavata1-4 leunig-101, clavata2-1 lug-101, fruitfull-1 leunig101* and *pinoid-1 leunig-101* double mutants. *clavata1-4 leunig-101* and *clavata2-1 leunig-101* exhibited additive phenotypes of single mutants, suggesting that *LEUNIG* and *CLAVATA* genes function in different pathways. In contrast, normal *FRUITFULL* and *PINOID* gene functions are required for the expression of *leunig* phenotypes. The results of gynoecial phenotypic comparison between *leunig* and other plants at different evolutionary levels imply that the *LEUNIG* gene may play an important role in evolution of flowering plants.

11-1

Genomic Imprinting During Seed Development in Arabidopsis.

J-Ph. Vielle-Calzada (1), Ch. Spillane (2), J. Thomas (1), M. Hoeppner (1), and U. Grossniklaus (1)(2)
(1) Cold Spring Harbor Laboratory, 1 Bungtown Rd., Cold Spring Harbor NY 11724, USA (2) Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel, Switzerland.

Viable seed formation depends on the coordinated development of the embryo, the endosperm and the maternal seed coat. It has recently been shown that these interactions require maternal gene activity in the haploid (gametophytic) as well as in the diploid (sporophytic) tissues of the developing ovule. The Arabidopsis thaliana gene MEDEA (MEA) regulates cell proliferation by exerting a gametophytic maternal control during seed development. Seeds derived from embryo sacs carrying a mutant mea allele abort after delayed morphogenesis, with excessive cell proliferation in the embryo and reduced free nuclear divisions in the endosperm . Thus, it appears that mutant embryos show increased cell proliferation at the expense of the endosperm. We have cloned the MEA locus by transposon tagging and show that it encodes a SET domain protein with homology to members of the Polycomb and trithorax group. In animals, proteins of these families maintain active or repressed states of gene expression during development by modulating higher order chromatin structure. Using a combination of genetic and molecular studies, we have now shown that mea affects an imprinted gene transcribed exclusively from maternally inherited alleles after fertilization. Strikingly, the paternally inherited mea allele appears to be transcriptionally silent in both the embryo and the endosperm. The regulation and function of MEA is consistent with the parental conflict theory for the evolution of genomic imprinting.

11-2

Initiation of Endosperm Development in Arabidopsis

Ramin Yadegari, Tetsu Kinoshita, Anat Katz*, Linda Margossian, Michael J. Hannon, Daphna Michaeli, Nir Ohad*, and **Robert L. Fischer**.
Department of Plant & Microbial Biology, University of California, Berkeley, California 94720-3102. U.S.A. *Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel.

A fundamental problem in biology is to understand how fertilization initiates reproductive development. Higher plant reproduction is unique because two fertilization events in the ovule are required for sexual reproduction. One sperm fuses with the egg to form an embryo. A second sperm fuses with the adjacent central cell nucleus that replicates to form an endosperm, which is the support tissue required for embryo and/or seedling development. To investigate mechanisms that initiate reproductive development, mutations, termed fie (fertilization-independent endosperm) and mea (medea) have been isolated in Arabidopsis. fie and mea mutations affect the central cell, allowing for replication of the central cell nucleus and endosperm development without fertilization. The FIE and MEA genes encode WD motif- and SET domain-containing polycomb proteins. In Drosophila and mammals, polycomb proteins repress homeotic gene expression. These results suggest that the FIE and MEA polycomb proteins function to suppress a critical aspect of early plant reproduction, namely endosperm development, until fertilization occurs.

11-3

Fertilization independent seed development: a pathway to understanding apomixis

A.M. Chaudhury, Ming Luo, Pierre Bilodeau, Aneta Ivanova, Liz Dennis, and W.J. Peacock
Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry GPO BOX 1600, ACT 2601, Australia

We have identified and cloned genes that control different steps of seed development without fertilization (1, 2, 3). We cloned FIS1 and FIS2; FIS1 is allelic to MEDEA, a polycomb group protein with homology with enhancer of zeste gene from drosophila. FIS2 is a novel protein with zinc binding domain suggesting that it is a transcription factor. Another gene FIE has also been cloned and encodes a WD40 type protein (4). The phenotypes of these mutants and the properties of the products they code for indicates that a polycomb group protein complex negatively regulates some aspects of seed development in the absence pollination. The FIS mutants show some of the phenotypes of apomictic plants, e.g., autonomous endosperm development. Thus orthologs of the FIS genes may have similar functions in apomictic and are likely to be important in mediating apomixis. FIS genes may be regulated by a putative apomictic regulatory gene MOF (Mother of FIS). In order to isolate MOF genes that act earlier than FIS we are attempting to identify genes that regulate the expression of FIS. Experiments and approaches will be discussed that relate the role of the FIS genes to apomictic seed development.

1. Peacock et al. (1995) in Induced Mutations and Molecular Techniques for Crop Improvement. (IAEA, Vienna, Austria), pp. 117-125. 2. Chaudhury et al. (1997) Proc. Natl. Acad Sci. USA 93, 5319-5324. 3. Luo et al. (1999) Proc. Natl. Acad Sci. USA 96, 296-301. 4. Ohad et al. (1999) Plant Cell 11, 407-416.

11-4

The Arabidopsis Somatic Embryogenesis Receptor-like Kinase (AtSERK) gene.

Sacco de Vries, Carin Zwartjes, Ed Schmidt, Ingrid Rienties, Khalid Shah, Dorus Gadella and Valérie Hecht.
Laboratory of Molecular Biology, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.

The AtSERK gene, encoding an LRR type transmembrane receptor kinase is expressed in cells competent for embryogenesis in culture. The AtSERK gene is present as a single copy in the Arabidopsis genome. RT-PCR, in situ hybridization and promoter-reporter analysis reveal that the AtSERK gene is first expressed before fertilization in a polar fashion in developing ovule primordia after meiosis I, in the entire female gametophyte and nucellus and persists in the egg cell and the synergids up to fertilization. After fertilization AtSERK expression continues in all cells of the embryo including the suspensor up to the 8-celled stage after which expression disappears. This expression pattern points to the presence of a signal transduction chain of unknown function during female gametogenesis, early zygotic embryogenesis and during formation of competent cells in culture. Overexpression of AtSERK under the control of the CaMV 35S promoter results in an increased competence for somatic embryogenesis in several independent transformants, suggesting that the entire SERK mediated signal transduction chain can be activated under culture conditions. We are currently investigating the expression pattern of several genes encoding proteins that were identified as SERK interactors in yeast 2 hybrid screens and the mechanism of receptor dimerization by using Fluorescence Lifetime Imaging Microscopy on SERK-GFP fusion proteins. This system will also be employed to verify the interaction between SERK and interacting proteins in vivo. The long term aim of our research is to chart the entire signal transduction chain mediated by the SERK gene.

11-5

Identification and analysis of genes controlling late embryogenesis and embryo dormancy in plants
Lars O. Baumbusch (1), Glenn A. Galau (2), D. Wayne Hughes (2) and Kjetill S. Jakobsen (1)
(1) Division of General Genetics, University of Oslo, P.O. Box 1031 Blindern, N-0315 Oslo, Norway, (2) Department of Botany, University of Georgia, Athens, Georgia 30602, USA.

Seed dormancy is the inability to immediately germinate, despite otherwise favourable conditions, until specific environmental stimuli break this inhibition. Briefly, embryogenesis can be divided into the cotyledon stage, the maturation stage and the postabscission stage which prepares the embryo for desiccation and quiescence, and in some species, for dormancy (Galau et al. 1991). Primary dormancy breaking in Arabidopsis requires both light and imbibitition at 5 C (cold stratification). In order to identify genes exclusively controlling the postabscission and the dormancy programs in Arabidopsis an extensive screening was done for phenotypes which germinate rapidly as dry seeds without dormancy-breaking cold stratification. Both EMS mutagenized and T-DNA tagged seeds were utilised and 11 mutant lines have been more closely examined. Mapping the different lines using published and new PCR-based CAPS (Cleaved Amplified Polymorphic Sequences) and SSLP (Simple Sequence Length Polymorphism) is underway and the mutated loci have been found at multiple sites on chromosomes 1, 2, 3 and 5. Attempts are being made to clone these genes and analyse their effects on expression patterns of marker genes. mRNA and protein levels will be examined by using tools developed from cloned genes. The effects of the mutations on stress reactions are being studied.
Ref.: Galau GA, Jakobsen KS and Hughes DW (1991) Physiol Plant 81: 280-288.

11-6

A comparison of cytokinesis mechanisms during endosperm development and embryogenesis
Mikael Blom Soerensen(1), Ulrike Mayer(2), Gerd Jürgens(2), Wolfgang Lukowitz(3), Jim Haseloff(4), Christian Dumas (1)and **Frédéric Berger(1)**
(1) RDP UMR5667,ENS LYON 46 allée d'Italie 69364 Lyon cedex 07 France (2) ZMBP, Universität Tübingen Lehrstuhl fuer Entwicklungsgenetik Auf der Morgenstelle 1 72076 Tuebingen

Early endosperm development in Arabidopsis is characterised by nuclear division in absence of cytokinesis. Later,nuclei located at the periphery of the endosperm become enclosed into cell walls. This cellularisation process is initiated around the embryo and progresses toward the opposite (chalazal) pole. Further cell division occurs and multiple cell layers are established. We propose a division of the cellular endosperm development into distinct developmental stages which will be usefull to characterise mutant phenotypes. To obtain an insight into the genetics of cytokinesis in endosperm we have studied endosperm cellularisation in 11 mutants originally characterised for defects in embryo cytokinesis (Torres-Ruiz & Jürgens,1994; Assaad et al.,1996; Lukowitz et al.,1996; Mayer et al.,1999). Endosperm cellularisation is affected in some but not all of these mutants. We have characterised as well a mutant line where endosperm cellularisation is prevented whereas embryo cytokinesis is normal. In conclusion we propose that cytokinesis mechanisms are mostly shared by the endosperm and the embryo and that there are few endosperm-specific and embryo-specific pathways. Ref: Assaad et al.,1996 Mol. Gen. Genet. 253:267-277 Lukowitz et al.,1996 Cell 84:61-71 Mayer et al.,1999 European J. Cell Biol. 78:100-108 Olsen et al., 1995 BioEssay 17: 803-812 Torres-Ruiz & Jürgens 1994 Development 120: 2967-2978

11-7

Polycomb-Group mediated gene control of seed development in Arabidopsis thaliana; an interactive approach

Pierre Bilodeau, Ming Luo, Liz Dennis, Jim Peacock, Abed Chaudhury
Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry, GPO Box 1600, ACT 2601, Canberra, Australia

Polycomb-Group proteins are responsible for maintaining proper patterns of expression of homeotic and other genes in Drosophila. Recently, our group and others have isolated three different genes from Arabidopsis thaliana involved in the repression of seed development without fertilisation. This apparent role for these genes and the fact that two (FIS1, FIE) share significant sequence homology with known Polycomb-Group genes from other species suggest that the Polycomb-Group dependent gene silencing mechanisms may be broadly conserved in eukaryotes. FIS1 encodes a protein with homology to the Polycomb-group protein Enhancer of Zeste (Ez) from Drosophila and is allelic to the recently described Arabidopsis gene MEDEA (Grossniklaus et al. (1998) Science 280: 446-50). FIS2 encodes a protein with a C2H2 zinc finger at the N-terminus, several putative nuclear localisation signals and a central region containing 2 distinct polypeptide sequences (A and B repeats) repeated twelve and seven times respectively but arranged with no apparent order (Luo et al. (1999) PNAS 96: 296-301). FIE, a likely allele of fis3, has been shown to encode a WD-40 protein related to the Polycomb-Group protein Extra Sex Comb (Esc) from Drosophila (Ohad et al. (1999) Plant Cell 11:407-15). Ez and Esc type of proteins have been shown to interact directly and/or to coimmunoprecipitate from embryo extract from different eukaryote species including fly, human, and mouse. The phenotype associated with mutation of each of these is similar, suggesting that they are involved in similar developmental processes. We now possess the appropriate molecular tools to study the interactions between the Polycomb-Group proteins from Arabidopsis and their role in fertilisation and plant embryogenesis.

11-8

Overexpression of BNM3, an embryo-expressed AP2 domain protein from Brassica napus, induces ectopic embryo formation in Arabidopsis.

Kim Boutilier (1), Remko Offringa (2), Therese Ouellet (3), Henk Kieft (4), Jiro Hattori (3), Andre van Lammeren (4), Brian Miki (3), Michiel van Lookeren Campagne (1)
(1) CPRO-DLO, Wageningen, the Netherlands; (2) Leiden University, Leiden, the Netherlands

Subtractive screening of Brassica napus microspore embryo cultures was used to isolate genes whose expression is newly induced or up-regulated during the initiation of embryogenesis in vitro. One of these genes, BNM3, encodes a new member of the AP2 domain family of transcription factors. RNA gel blot analysis and mRNA in situ hybridization indicate that BNM3 expression is preferentially localized to the developing embryo. Ectopic over-expression of BNM3 in Arabidopsis results in the formation of ectopic embryos on the petioles, shoot apical meristem and cotyledon/leaf margins of seedlings. Arabidopsis lines over-expressing the BNM3 gene also exhibit elements of cytokinin over-production, suggesting a link between cytokinin signalling and the initiation of embryogenesis.

11-9

SGR2 (SHOOT GRAVITROPISM 2) is related with Arabidopsis embryogenesis.
Takehide Kato (1), Hidehiro Fukaki (2) and Masao Tasaka (3)
(1) Dept. of Bot., Div. Bio. Sci., Graduate School of Sci., Kyoto Univ. (2) New York University (3) Graduate School of Bio. Sci., Nara Institute of Sci. and Tech

In higher plants, shoot shows negative gravitropism. To study the molecular mechanisms involved in shoot gravitropism, we have isolated sgr (shoot gravitropism) mutants in Arabidopsis thaliana showing little or no gravitropic response in shoot. In this paper, we focused on sgr2. Ten sgr2 alleles have been isolated. The strong alleles showed little gravitropic response both in hypocotyl and inflorescence stem but normal in root. sgr2 is a recessive mutant but the results of genetic analysis often deviated from Mendelian. A little number of shrinked or irregular shaped seeds were found in sgr2 siliques. Some sgr2 seeds didn’ft germinate. Most of sgr2 seedlings were normal (two cotyledons, one hypocotyl and one root). However, some of the seedlings had one or three cotyledons and seedlings with double sets of cotyledons on a hypocotyl or with two sets of hypocotyls and roots were also observed in a very low frequency. Some sgr2 embryos had large holes, irregular division pattern and abnormal shapes. Almost all sgr2 zygotes had a large hole at the apical region and the first division plate was shifted down to the basal position. The proportion of abnormal embryos decreased with the development of the embryo, in contrast, the variety of abnormalities increased. SGR2 was mapped to the south of UFO in chromosome 1. We further identified a gene in which all sgr2 mutations were mapped. The putative SGR2 gene encodes a novel protein of the phospholipase family.

11-10

Characterization of Ds-insertions within two cell cycle genes of Arabidopsis.
Hong-Sien Kwee, De Ye, Wei-Cai Yang, Serguei Parinov, Mayalagu Sevugan, Venkatesan Sundaresan.
Institute of Molecular Agrobiology, 1 Research Link, National University of Singapore, Singapore 117604.
Email: director@ima.org.sg

Progression through the cell cycle is driven by the orderly action of several protein kinase complexes that are regulated at different molecular levels. Proteolysis plays an important role in the regulation of several phases of the cell cycle. Two essential roles of a large protein complex called the anaphase promoting complex (APC) are, in sequence of order, degradation of Ôglue proteinsÕ that hold the sister chromatids together at the metaphase/anaphase transition and degradation of mitotic cyclins in early anaphase. The APC is composed of the 34 amino acid tetratrico-peptide repeat (TPR) proteins Cdc16p/Cdc23p/Cdc27p and other proteins. We have an Arabidopsis loss of function mutant of a putative plant CDC16 gene with high homology to the human and yeast CDC16/Cut9, obtained through a Ds-insertion. The protein has 540 amino acids and contains ten TPR motifs that are also present in all CDC16/Cut9 proteins from yeast and human. Cytological observation shows that embryosac development is arrested after the first mitosis at the two-nucleus stage. Arrested embryosacs are then terminated and the seeds abort. The transmission of the mutation through the female (23%) is more severely affected than through the male (68%). Another Ds-insertion we have identified is within the CyclinD3 gene which has been previously described (Soni, et al., 1995, Riou-Khamlichi, et al., 1999). Preliminary results shows that embryos are arrested at the pre-heart stage in about 30-40% of the seeds. Further characterization of this mutant is in progress.

11-11

FIS genes, their expression and role in embryo development

Ming Luo*, Jenny Guerin=, Pierre Bilodeau*, Anna Koltunow=, Liz Dennis*, Jim Peacock*, and Abed Chaudhury*
*Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry, GPO Box 1600, ACT 2601, Australia; =CSIRO Division of Plant Industry, PO Box 350, Glen Osmond, SA 5064, Asutralia

FIS genes control several steps of seed development without pollination. In fis1 , fis 2 and fis3 mutants endosperm, and to a lesser extent embryo development, occurs without pollination indicating that the wild type FIS loci are negative regulators of seed development in the absence of pollination. Embryo development in these mutants is arrested following pollination giving rise to mainly non viable seedlings. In order to dissect the genetic basis of this arrest we have identified modifier gene(s) that allow the seed development to go further. The phenotypes of modified and unmodified fis mutants will be reported to shed new light on the role of endosperm in seed development. The results will be discussed in the light of a model of the action of the FIS1 FIS2 and FIS3 genes in mediating seed development in Arabidopsis. We have cloned the FIS1 and FIS2 genes and prepared promoter-GUS fusions. The expression of these promoter-GUS fusions was studied in reproductive tissues. The expression pattern of these genes and their presumed role in seed development will be discussed.

11-12

Analysis of the pollen coat and its functions in Arabidopsis thaliana

Jacob A. Mayfield and Daphne Preuss
The University of Chicago, 1103 E. 57th Street, EBC 304, Chicago, IL 60637 USA

Pollination on a dry stigma demands at least two distinct functions of the molecules involved. The dry environment requires movement of water from the stigma to the desiccated pollen grain in a regulated manner, while the need to distinguish acceptable pollen from that of other species has driven the evolution of a specific cell-cell recognition system. The molecules involved in these processes remain ill-defined; however, sterile mutations that lack a pollen coat implicate this lipid-rich extracellular structure in both functions. The pollen coat in Arabidopsis thaliana contains a small number of protein. A series of biochemical purifications coupled with protein microsequencing revealed the identity of several pollen coat proteins. Pollen from a plant with a loss-of-function mutation in one of the corresponding genes compromises hydration without otherwise affecting the pollen coat. To further dissect both the hydration phenomena and cell recognition system, generation of mutations in other pollen coat proteins is under way.

11-13

Molecular genetic approach to study the role of auxin and cytokinin during early embryogenesis in Arabidopsis thaliana

Remko Offringa (1), Dolf Weijers (1), Jim Haseloff (2), Erwin van Rijn (1) and Paul Hooykaas(1)
(1) Institute of Molecular Plant Sciences, Leiden University, The Netherlands (2) Department of Plant Sciences, University of Cambridge, United Kingdom

During early embryogenesis the body plan of dicot plants is laid down in a distinct pattern of cell layers and organs. Although the exact role of the plant hormones auxin and cytokinin in this pattern formation is still unclear, increasing evidence is emerging that auxin and its polar transport are important for the formation of bilateral symmetry during the transition from globular to heart stage. Here we present a molecular genetic approach with which the effects of cell-type specific expression of genes coding for enzymes that modify the local endogenous levels of phytohormones on the early developing arabidopsis embryo are studied. For this purpose several promoters that show cell type specific expression in the arabidopsis embryo, such as the protoderm specific LTP1 promoter (provided by S. de Vries), a promoter that provides expression in dividing cells and the STM promoter were fused to the non-plant mGAL4-VP16 transactivator encoding gene. The effector genes were placed under control of the UAS promoter, which is specifically activated by the GAL4-VP16 protein. To be able to monitor the expression driven by a specific promoter, a reporter gene encoding a gfp-gusA fusion protein (Quaedvlieg et al.,1998, PMB 37, 715-727) was fused to the UAS promoter and placed on the same construct. Separate transgenic arabidopsis lines were generated with the resulting activator and effector constructs. The selection and analysis of the different activator and effector lines will be presented. Crossings between activator and effector lines and microscopic analysis of the resulting embryos is in progress. Preliminary results indicate that the transactivation system can be used for the cell type specific expression of effector genes.

11-14

'Monogamy model' for interaction between gametophytes in pollen tube guidance of Arabidopsis and related plants

Kentaro Shimizu, Sumie Ishiguro and Kiyotaka Okada
Department of Botany, Graduate School of Science, Kyoto UniversityKitashirakawa-oiwake, Sakyo, Kyoto 606-8502 Japan

Multicellular haploid gameotphytes contribute to the sexual reproduction of seed plants. Male gametophyte (pollen tube, PT) is guided to a female gametophyte (FG) through diploid sporophytic cells of a pistil. Intercellular recognition systems between a PT and diploid cells have been extensively studied. In contrast, very little is known about the interactions between a PT and an FG, though it is necessary for the PT guidance. Remarkable feature of the PT guidance is that only one PT elongates to one FG ('prevention of polyspermy'), but it is not known whether the prevention is achieved by diploid cells or by the FG or the repulsion between PTs, or by the combination of them. In order to elucidate the guidance and prevention mechanisms, we observed the paths of PTs in wild type Arabidopsis and in maa mutants in which the development of FGs were delayed. PTs directed to mutant FGs, but they lost the way just before entering the micropyle, and elongated to random directions. Mutant FGs attracted two PTs at higher frequency than wild type FGs did. To explain the interaction between female and male gametophyte, we propose 'monogamy model' in which FG emit two-step attractive signals, and prevent polyspermy. The prevention of polyspermy by FGs could increase the inclusive fitness by helping the fertilization of other FGs. In addition, repulsion between PTs might also prevent polyspermy. Monogamy model was shown to be consistent with reproductive isolotion observed in interspecific crosses in Brassicaceae.

11-15

The Reproductive Process in Arabidopsis

Dagmar Teichmann, Ellen Wismann and Heinz Saedler
Max Planck Institut für Züchtungsforschung, Carl-von-Linné Weg 10, D-50829 Köln, Germany

We are interested in the identification and isolation of genes that control sexual reproduction in our model plant Arabidopsis thaliana. We therefore screened a transposon mutagenized Arabidopsis thaliana population consisting of 3000 plants which carry approximately 15000 insertions of the transposable element En1 from maize. En1 is highly active in the heterologous host Arabidopsis, therefore the majority of the mutants found will likely be caused by an insertion of the element. The prescreen was aimed at the isolation of mutants which showed reduced seed set or no seed set at all. Mutants with gross organ defects were excluded. Pollen production and release was tested by staining and light microscopy. Male meiosis was tested by fluorescence microscopy. Furthermore, mutations were identified as male or female specific by reciprocal crosses to wildtype plants. We are now concentrating on 6 mutants, that are impaired in the male side of reproduction. In some of these we observe aberrant anther dehiscence. Current work includes the isolation of En flanking sequences, in order to isolate sequences responsible for the mutations.

11-16

Origin and development of somatic embryos in Arabidopsis thaliana pt-1 cultures monitored with the SERK::GUS reporter gene system.

Iris R. von Recklinghausen1, Valérie F.G. Hecht2, Jan H.N. Schel1, Sacco C. De Vries2, André A.M. van Lammeren1
1 Laboratory of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands 2 Laboratory of Molecular Biology, Wageningen Agricultural University, The Netherlands

Somatic embryogenesis in vitro was studied using liquid cultures of the Arabidopsis primordia timing (pt) mutant, which is characterized by an enhanced embryogenic capacity (Mordhorst et al., Genetics, 1998). The Somatic Embryogenesis Receptor-like Kinase (SERK) gene is a suitable molecular marker for early embryogenesis in carrot (Schmidt et al., Development, 1997) as well as in Arabidopsis zygotic embryogenesis (see DeVries et al., this meeting). In order to monitor cells competent to form embryos and to follow the early stages of somatic embryogenesis, plants containing the ?-glucuronidase (GUS) reporter gene under the control of the At-SERK promotor in the pt mutant background were made (Hecht & DeVries, unpublished). SERK::GUS expression patterns were monitored from the onset of the cultures up to the establishment of a stable embryogenic callus line. At the onset of the culture, the SERK gene was expressed strongly and rather uniformly, but during later stages of callus development its expression became more spatially restricted. Furthermore, the SERK::GUS staining pattern in seedlings derived from somatic embryos was compared with that of normal seedlings. In addition, the embryogenic SERK::GUS expression was compared with non-embryogenic callus, which gave only little or no staining and varied in pattern. The similarity of the SERK::GUS staining pattern with in situ hybridizations, using the AtSERK probe on BMM sections of mature callus, confirms the specificity of the signal.

11-17

A Rop GTPase-Dependent Signaling Pathway Controls Pollen Tube Growth in Arabidopsis
Hai Li (1), Yakang Lin (1), Rachel M. Heath (1), Michael X. Zhu (2), and **Zhenbiao Yang (**1).
(1) Plant Biotechnology Center, (2) Neurobiotechnology Center, The Ohio State University, Columbus, OH 43210, USA.

Pollen tubes undergo a tortuous journey within the pistil to deliver sperms to the ovule. In cultures, pollen tubes elongate through polar tip growth. The molecular basis behind the directional pollen tube growth is poorly understood. We have shown that a signaling pathway mediated by the pollen-specific Rop GTPase Rop1At plays a critical role in the control of pollen tube growth in Arabidopsis. Pollen-specific overexpression of constitutively active rop1at mutants caused bulbous cells instead of normal cylindrical pollen tubes. Overexpression of the Rop1At wild type gene induced similar but less severe phenotypes associated with the ectopic localization of Rop1At to the plasma membrane of pollen tubes. In contrast, dominant negative rop1at mutant or antisense rop1at RNA inhibited tube growth at 0.5 mM extracellular calcium, but the growth inhibition was reversed by higher extracellular calcium. Injection of anti-Rop antibodies disrupted the tip-focused intracellular calcium gradient known to be crucial for tip growth. These results define a Rop GTPase-dependent pathway that leads to the formation of the tip-focused calcium gradient and couples the spatial and temporal control of pollen tube growth. All constructs that disturb in vitro pollen tube growth also caused reduced male fertility, suggesting that either tip growth or the Rop1At-dependent pathway is involved in guided growth of pollen tubes within the pistil.

11-18

An Arabidopsis mutant defective in late embryogenesis and postgerminative development
Kazutoshi Yamagishi (1&4), Shigeo Yoshida (1) , Robert L. Fischer (2), Robert B. Goldberg (3) and John J. Harada (4).
(1) Plant Functions Laboratory, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198, Japan, (2) Department of Plant and Microbial Biology, University of California Berkeley, California 94720-3102, USA, (3) Department of Biology, University of California Los Angeles, California 90024-1606, USA (4) Section of Plant Biology, University of California Davis, California 95616, USA

To investigate genes involved in embryogenesis, we identified embryo defective mutants from T-DNA mutagenized Arabidopsis. A mutant designated line24 arrested at a late stage of embryo development and had characteristics of leafy cotyledon mutants, i.e., accumulation of anthocyanin, desiccation intolerance, and trichomes on cotyledons. Further analysis showed that the mutant also has defects in seedling stage, i.e., seedling lethality and short hypocotyl in the dark, which are partially similar to fusca mutant. This finding suggests that the mutation may also affect postgerminative processes. Double mutant analysis showed that phenotypes of line24 double mutants with lec1, lec2, and fus1 (cop1) seemed to be synergistic. Therefore, the line24 gene could work with LEAFY COTYLEDON and FUSCA genes. We isolated the line24 gene and confirmed that this gene complements the line24 mutation. Transcripts of the line24 gene accumulated ubiquitously in embryos and vegetative organs. Interestingly, three transcripts that are inferred to be variations in splicing have been identified from wild type plants. The predicted polypeptide of these transcripts contained several WD-40 motifs. Transgene experiments are in progress to investigate which transcripts complement the line24 mutation.

12-1

Regulation of cell identity in the shoot meristem

Thomas Laux
ZMBP-Center of Plant Molecular Biology, Auf der Morgenstelle 1, D-72076 Tuebingen, Germany

Postembryonic shoot development in higher plants is marked by repetitive organ formation via a self-perpetuating stem cell system, the shoot meristem. The destiny of each stem cells daughter cell is determined by its position within the shoot meristem: cells in the center remain pluripotent stem cells, whereas cells at the periphery form organs and undergo differentiation. Genetic and molecular analysis suggest that specification of stem cell fate requires signaling from underlying cells (Moussian et al. 1998, Mayer et al. 1998). These cells are established early in embryogenesis, before the actual stem cells and by a series of asymmetric divisions become confined to the prospective shoot meristem. Recent observations suggest that mechanistic principles of stem cell regulation are shared not only between shoot and root meristems, but also between animal stem cell system and plant meristems. Moussian et al. 1998, EMBO 17, 1799-1809 Mayer et al., 1998 Cell 95, 805-815

12-2

CLV3 acts via CLV1 and CLV2 to repress WUSCHEL

Ulrike Brand (1), Jennifer C. Fletcher (2), Martin Hobe (1), Nicole Beinert (1), Elliot M. Meyerowitz (2) and **Rüdiger Simon** (1)
(1) Institut für Entwicklungsbiologie, Universität zu Köln, Gyrhofstr. 17, 50923 Köln, Germany
(2) California Institute of Technology, 1200 East California Blvd., Pasadena, CA 91125 USA

In higher plants, organogenesis occurs continuously from self-renewing apical meristems. Arabidopsis thaliana plants with loss-of-function mutations in the CLAVATA (CLV1-3) genes have enlarged meristems and generate extra floral organs. Genetic analysis indicates that CLV1, which encodes a receptor kinase, acts with CLV3 to control the balance between meristem cell proliferation and differentiation. CLV3 encodes a small, predicted extracellular protein. CLV3 acts nonautonomously in meristems, and is expressed at the meristem surface overlying the CLV1 domain. These proteins may act as a ligand/receptor pair in a signal transduction pathway that coordinates growth between adjacent meristematic regions by restricting the number of cells in the central zone and/or promoting the exit of cells into the peripheral zone where organs are initiated. We have now tested the effects of ectopic expression of CLV3 controlled by a constitutive promoter. Transgenic plants fail to maintain a functional meristem, however, they do initiate leaves in an irregular pattern, resembling wuschel mutants. Occasionally, inflorescences are formed that produce aerial leaf rosettes, and sometimes flowers that lack organs in the inner whorls. These phenotypes depend on CLV1 and CLV2 activity, as CLV3 expression has no effect in CLV1 or CLV2 mutants. We propose that CLV3 activates a CLV1/CLV2 receptor complex, leading to repression of wuschel in the meristem, which in the CLV3-overexpressing lines results in the loss of cells with central zone identity.

12-3

Radial pattern formation

Philip N. Benfey, Yrjo Helariutta, Joanna Wysocka-Diller, Jun Lim, Jocelyn E. Malamy, Hidehiro Fukaki; Biology Department, 1009 Main Building, New York University, New York, N.Y. 10003

Formation of a radial pattern of cell layers occurs during both root and shoot development. Analysis of mutations in the SCARECROW (SCR) and SHORT-ROOT (SHR) genes in Arabidopsis indicates that they are key regulators of radial patterning in both root and shoot. Both genes have been cloned and their expression patterns are consistent with their role in radial patterning. Genetic and molecular analyses indicate that SHR is upstream of SCR in a developmental pathway. Expression and sector analysis indicate that SHR acts in a non cell-autonomous manner. The SCR orthologue from maize, mzSCR has been used with other markers to analyze radial patterning during root regeneration. The expression patterns of these genes during regeneration of the root apex after QC excision support the hypothesis that cell-cell signaling plays a central role in the regeneration process.

12-4

Pattern and polarity in Arabidopsis root development

Dimitris Beis, Sabrina Sabatini, Harald Wolkenfelt and Ben Scheres.
Dpt. of Molecular Cell Biology, Utrecht University, The Netherlands

Plant organ development is initiated during embryogenesis and proceeds post-embryonically from distally positioned groups of dividing cells, the meristems. The Arabidopsis root displays a simple and regular organisation of tissues, its ontogeny during and after embryogenesis is described at the cellular level, strict lineage facilitates analysis, but yet it exhibits astonishing developmental flexibility in relation to positional information. We have investigated the role of the phytohormone auxin in cell fate specification. Auxins, with indole-3-acetic acid as the major active form, have diverse roles in growth and development that have hitherto been difficult to disentangle. We have utilised an auxin-response promoter element to visualise auxin distribution in vivo. We show that auxin is asymmetrically distributed in the root with a peak concentration in columella stem cells. Mutants in auxin transport and response suggest that this distribution is required for patterning. Re-distribution of the auxin concentration peak by laser ablation and by polar auxin transport inhibition correlates with changes in multiple cell fates and cell- and organ polarity. Thus, auxin and its transport machinery play major roles in organising pattern and polarity.

12-5

FASCIATA1 and FASCIATA2 Genes Regulate Apical Meristem Organization and Function

Takashi Araki, Hidetaka Kaya, Ken-ichiro Taoka, Yasushi Kobayashi, Tetsuo Meshi, and Masaki Iwabuchi,
Department of Botany Division of Biological Science, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

The apical meristems of higher plants give rise to all the parts of plant body during post-embryonic development. We take genetic and molecular approaches to elucidate the control mechanisms of structure and function of apical meristems. Two fasciata mutants (fas1, fas2) were originally described as mutants which affect the shoot apical meristem and result in aberrant phyllotaxy and stem fasciation (Leyser and Furner, 1992). Our previous analysis showed that both mutants also affect the root apical meristem suggesting that FAS genes are regulators of apical meristem function in general. By T-DNA tagging and candidate gene approach, we have cloned FAS1 and FAS2. Deduced FAS proteins have significant homology with two subunits of human Chromatin Assembly Factor-I (hCAF-I) (Kaya et al., abstract #461, last year). To have a better understanding of the roles of FAS genes, we examined mutant phenotype in apical meristems in detail. In shoot apical meristem, failure to maintain proper size and shape, irregular cellular arrangement and organization, and failure of organ initiation at all or at the right position were observed in fas mutants. These suggest defects in both central zone and peripheral zone. In root apical meristem, regular stereotyped arrangement of initial cells and files of cells derived from them, especially columella root cap cells, was perturbed in fas mutants. Furthermore, mis-expression of a differentiation marker (starch granules) was observed in columella initials and, in some cases, it was difficult to unequivocally identify central cells. These findings suggest the roles of the FAS genes in regulation of proper organization of root apical meristem which may be at least in part mediated via their function in central cells. Expression analysis, mainly by promoter-GUS fusions, supported these observations. Also, expression associated with cell cycle or cell proliferation was analyzed using suspension cell cultures of Arabidopsis T87 and tobacco BY2.

12-6

TORNADO (TRN) genes are required for the specification of cell type in the epidermis and lateral root cap of ARABIDOPSIS

Cnops G. (1&2), Wang X. (1), Linstead P. (2) Van Montagu M. (1) Van Lijsebettens M. (1) and Dolan L. (2).
(1) Labo Voor Genetica/VIB, Ledeganckstraat 35, 9000 Gent, Belgium (2) Cell Biology, JIC, Colney Lane, NR5 7UH Norwich, UK

tornado (trn) mutations are recessive and fall into two complementation groups, designated trn1 and trn2. All mutations are severe dwarfs with twisted organs. The phenotypes of trn1-1, trn1-3 and trn2-1 roots were characterised. Root twisting starts three days after germination and coincides with retardation in longitudinal growth. Meristem shape is altered in both trn1 and trn2 roots. Defects are especially observed in the protoderm (epidermal/ lateral root cap) cell files. In some trn roots, an irregular division is observed in the initial of the protoderm. In the meristematic region of trn roots long thin cells are observed in the epidermal cell files. These cells die in the elongation zone resulting in the formation of gaps that run along the length of the root. Evidence will be presented that these long thin cells are mis-specified into lateral root cap cells. TRN gene function is not only required for correct specification of epidermis and lateral root cap but also for correct sub-specification of the epidermis into hair and non-hair cells. In wild-type roots, hair files are separated by one or two non-hair files. Analysis of epidermal cell morphology and marker gene expression in trn1 roots revealed that subsets of cells in both files acquired an incorrect fate. Double mutant analysis indicates that TRN1 is required at an early stage of epidermal sub-specification. To unravel the function of TRN1 in epidermis/lateral root cap specification on a molecular level, chromosome walking towards TRN1 was initiated. TRN1 maps to the bottom half of chromosome 5 (Cnops et al., 1996).

12-7

Positional cloning of ACAULIS5, a gene required for internode elongation in Arabidopsis thaliana.
Yoshie Hanzawa, Taku Takahashi and Yoshibumi Komeda
Division of Biological Sciences, Graduate School of Science, Hokkaido University

The acaulis5 (acl5) mutation causes a severe reduction in the cell length of stem internodes with little or no effect on the growth of other organs (Hanzawa et al., 1997, Plant J., 12: 863). The ACL5 gene has been cloned by a map-based strategy. Using 4000 F2 progenies, the ACL5 locus was fine mapped to a 54kb region on chromosome5 which was covered with 5 overlapping phage clones. Sequence analysis revealed that a candidate gene in this region carries a single base-pair substitution in the predicted ORF. Genomic fragment containing the gene completely restored the mutant phenotype to the wild type. The ACL5 gene encodes a novel protein with highest similarity to spermidine synthase (SPDS). SPDS is known to be a key enzyme in polyamine biosynthesis from putrescine to spermidine and spermine. TLC analysis indicated that the recombinant ACL5 protein produced in E. coli has spermine synthase activity. This is the first isolation of a spermine synthase gene from plants and the direct demonstration of the involvement in plant development of polyamines. Possible functions of this gene in internode elongation will be discussed.

12-8

Characterization of the function of knotted1-like genes using Arabidopsis
Angela Hay, Naomi Ori and Sarah Hake.
Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.

A collection of Enhancer Trap (ET) transposon insertion lines has been generated in our lab and screened for expression patterns in the shoot apex. From 800 ET lines we have identified twenty five that will be useful as markers for events in shoot apical meristem (SAM) development, and possible target genes for KNAT1, a knotted1-like homeobox gene which is expressed in the SAM of Arabidopsis. In order to determine whether any genes defined by these ETs act downstream of KNAT1, we have crossed putative ET lines to 35S:KNAT1 plants and screened for changes in the ET expression patterns. Inducible kn1 or KNAT1 constructs can then be used to investigate whether or not these genes are direct targets of KNAT1. We are also using this inducible system to characterize the function of kn1-like genes in Arabidopsis. The inducible expression of kn1, a gene known to function in the SAM in maize, confers a striking phenotype in Arabidopsis. The entire leaves become highly lobed in response to pulses of kn1, similar to the phenotype conferred by overexpression of KNAT1. This inducible fusion of kn1 and the gluticorticoid receptor (GR), allows dissection of the overexpression phenotype of kn1 and may indicate aspects of kn1 function and mechanisms of shape change in leaves. We have begun to address specific questions regarding the "window of competence" within which leaf primordia respond to kn1 and the involvement of hormones in mediating this response.

12-9

terminal flower2, an Arabidopsis thaliana mutant disrupted in meristem identity and transition to reproductive phase

Katarina Landberg and Annika Sundås Larsson
Dept. of Physiological Botany, Uppsala University, Villav. 6, S-752 36 Uppsala, Sweden

A mutation in the terminal flower2 (tfl2) locus causes the Arabidopsis inflorescence meristem to produce floral organs leading to a determinate inflorescence. The tfl2 mutant phenotype is highly pleiotropic, in addition to termination tfl2 mutants show dwarfing, early flowering and a reduction in photoperiod sensitivity. The phenotype suggests that the TFL2 gene has a dual role in the meristem, in the timing of the transition from vegetative to reproductive growth as well as in the maintenance of identity.

The gene function has so far been investigated by double mutant analysis involving the meristem identity mutants terminal flower1 (tfl1), leafy (lfy) and apetala1 (ap1), indicating an interaction with AP1 but not with LFY or TFL1. To investigate a possible interaction between TFL2 and AP1 the tfl2 mutant has been combined with 35S::AP1 plants. Further, double mutant analysis combining tfl2 with early flowering3, another gene showing photoperiod insensitivity and that has been suggested to be part of a pathway regulating the meristem identity genes, are also in progress and will be presented.

Two tfl2 alleles have been isolated in the Columbia background, tfl2-1 is fast neutron induced and tfl2-2 was found in an EMS mutagenized population. Both alleles show the same phenotype. The TFL2 gene maps to the upper arm of chromosome 5, 1.3 cM south of the SSLP marker nga106. Cloning of the gene is carried out by chromosome walking and it has been positioned to three Tamu BAC clones covered by the CIC YAC 9H7. A 10 kb deletion, which completely abolishes one ORF, has been found in the tfl2-2 allele. Sequencing of the other tfl2 allele as well as complementation experiments are in progress.

12-10

Characterization of a novel zinc finger protein gene of Arabidopsis

Jong Seob Lee (1), Sook Kim (1), Dong-Keun Lee and Yang Do Choi (2).
(1) Department of Molecular Biology and Research Center for Cell Differentiation, Seoul National University, Seoul 151-742, Korea, (2) Department of Agricultural Chemistry, Seoul National University, Suwon 441-744, Korea.

We have previously characterized cDNA and genomic clones encoding a putative zinc finger protein (BcZFP1) of Brassica campestris. BcZFP1 contains two zinc finger motifs of the Cys2/His2 type. Ectopic expression of the BcZFP1 cDNA under the control of the CaMV 35S promoter altered leaf morphology of transgenic tobacco plants. Furthermore, the expression terminated the shoot apical meristem perpetuity possibly through incorporation of cells in the central zone of the shoot apical meristem into differentiating an ectopic leaf primordium. Expression of the BcZFP1-GUS gene was confined to groups of cells located underneath the outer three cell layers in the peripheral zone and was concurrent with swelling of the leaf founder cells leading to the development of a leaf primordium. These results suggest that the zinc finger protein gene may play an important role for leaf initiation or development, probably by determining the cell fate in the shoot apical meristem. To have a better understanding of the function of the zinc finger protein gene in leaf development, we isolated an Arabidopsis zinc finger protein gene which is highly homologous to the BcZFP1 gene. Screening of T-DNA insertion lines of Arabidopsis led to isolation of an heterozygous mutant in which the T-DNA insertion is located 420 bp upstream of the start codon of the AtZFP1 gene. Self-pollination of the heterozygous plant did not generate mature homozygous mutant plants, suggesting its essential role in embryo and seedling development. The heterozygous mutant seedlings exhibited an abnormal pattern of leaf initiation, generating a first solitary leaf. Furthermore, they showed alteration in the pattern of leaf initiation. We also expressed sense and antisense constructs in Arabidopsis. The phenotypes of transgenic plants are under investigation.

12-11

A mutation at the TOPLESS locus causes shoot to root transformations during embryogenesis
Jeff Long (1), Scott Woody (2) and M. Kathryn Barton (2)
(1) Program in Cell and Molecular Biology (2) Department of Genetics, UW Madison, 445 Henry Mall, Madison, Wi 53705, USA

A single, temperature sensitive mutation at the TOPLESS (TPL) locus disrupts the apical/basal polarity of the embryo and transforms the cotyledons, SAM and hypocotyl into a second root. This "apical" root produces root hairs, has a root cap, and displays a gravitropic response similar to the true root upon germination. We have performed in situ hybridizations on tpl embryos with genes specific to the SAM, the cotyledons, the hypocotyl , and the root to determine when this transformation takes place. We find that markers for the SAM (SHOOT MERISTEMLESS and UNUSUAL FLORAL ORGANS) are absent in tpl embryos while the expression of a cotyledon marker (AINTEGUMENTA) is reduced. The KNAT1 gene marks the developing hypocotyl in wild-type, but is absent from tpl embryos. SCARECROW, which is expressed in the endodermal layer of the root and hypocotyl in wild-type, is expressed more apically in tpl embryos than in the wild type. A RAM specific enhancer trap, LENNY, is expressed at both the apical and basal poles of tpl embryos. Temperature shift experiments of developing tpl mutant embryos shows that the fate of apical structures becomes fixed after the transition stage of embryogenesis.

12-12

Interactions between BEL1 and KNAT homeodomain proteins during Arabidopsis development.
Mark S. Pidkowich (1), Mohammed Bellaoui (1), Alon Samach (1), Susanned Kohalmi (2), Kumuda Kushalappa (1), William L. Crosby (2), George W. Haughn (1).
(1) Botany Department University of British Columbia, Vancouver, BC, Canada, V6T 1Z4, (2) Gene Expression Group, Plant Biotechnology Insitute National Research Council, Saskatoon, SK, Canada, S7N 0W9.

BEL1, required for proper ovule development, is a homeodomain transcription factor. The function of an individual transcription factor can depend on the formation of heterodimeric complexes with other proteins. Indeed, the deduced amino acid sequence of BEL1 contains a possible amphipathic alpha helix that could mediate such protein-protein interactions. We have used the yeast two-hybrid system to identify Arabidopsis proteins that interact with BEL1 as a means of further exploring BEL1 function. Our results indicate that BEL1 can form complexes with three members of the KNAT homeodomain family; STM, KNAT1, and KNAT5. STM, KNAT1, and KNAT5 are not obviously expressed in the developing ovule in a spatial/temporal manner that overlaps with BEL1 expression, which indicates that interactions between these KNAT proteins and BEL1 are not necessary for proper ovule formation. However, the BEL1 gene is transcribed in additional tissues outside of the ovule where it may function in combination with KNAT proteins. We have been using situ hybridization to analyze the expression of BEL1, STM, KNAT1, and KNAT5 throughout the developing plant in order to evaluate the likelihood of each possible interaction, and what processes such interactions might control.

12-13

Gene interactions in the shoot meristem: The CLAVATA and AGAMOUS genes regulate WUSCHEL expression.

Heiko Schoof, Achim Haecker and Thomas Laux
Zentrum für Molekularbiologie der Pflanzen, Universität Tübingen, Germany

The WUSCHEL gene is required for stem cell maintenance in shoot and floral meristems of Arabidopsis (1). Based on genetic data, a model of gene interactions in the shoot meristem was proposed wherein SHOOTMERISTEMLESS and the CLAVATA pathway act competitively to regulate WUS activity. Mutations in the WUS gene are epistatic to clv mutations, suggesting that the CLV pathway negatively regulates WUS. Here we show that the CLV pathway represses WUS at the transcription level. In clavata1, 2 and 3 mutants the WUS expression domain is extended one meristem cell layer up relative to wildtype, indicating that the CLV pathway is necessary to supress WUS expression in this layer. wus mutants are completely epistatic to mutations in the AGAMOUS gene with respect to floral meristem indeterminacy (2). In ag floral meristems, WUS expression is maintained after the developmental stage where it is switched off in wildtype. This correlates with indeterminacy of the meristem and initiation of further floral whorls. Thus, WUS is a central target for regulators of organ initiation.

(1) K. F. X. Mayer, H. Schoof, A. Haecker, M. Lenhard, G. Jürgens and T. Laux (1998), Cell 95, 805-815
(2) T. Laux, K. F. X. Mayer, J. Berger and G. Jürgens (1996), Development 122, 87-96

12-14

Molecular genetic studies of AtNAC1, a novel NAC box gene of Arabidopsis thaliana.

Shinobu TAKADA(1), Tetsuya ISHIDA(1), and Masao TASAKA (2)
(1)Dept. Bot., Div. Biol. Sci., Graduate School of Sci., Kyoto Univ., (2)Graduate School of Biol. Sci., NAIST

We identified two Arabidopsis genes, CUC1 (CUP-SHAPED COTYLEDON1) and CUC2, which were involved in organ separations and shoot apical meristem formations. As CUC1 and CUC2 had redundant functions, it was expected that CUC1 had similar structures to CUC2. CUC2 contained a NAC box in the 5' half region. So, it was expected that CUC1 also had a NAC box. We mapped the CUC1 locus in detail and found three NAC box containing genes (AtNAC1, AtNAC2, AtNAC3) near the CUC1 locus and cloned all of them. Unexpectedly, it was suggested that none of these was CUC1, as cuc1 had no mutated sequences in their loci and these were expressed normally in cuc1 mutants. The NAC domain and the parts of C-terminal half of AtNAC1 were highly homologous to those of CUC2. By in situ hybridization analyses, we found that AtNAC1 and CUC2 showed similar expression patterns during whorl plant life. These suggest that the function of AtNAC1 is similar to that of CUC2. Now we are analysing the transgenic plants in which AtNAC1 genes are expressed in the sense or antisense directions under the control of the CaMV 35S promorter.

12-15

Analysis of the receptor-like kinase gene expressed in the meristems.
M. Takemura, H. Kanamoto, M. Hasegawa, A. Yokota and T. Kohchi
Graduate School of Biological Sciences,Nara Institute of Science and Technology, 8916-5, Takayama, Ikoma, Nara, 630-0101, Japan

We have isolated two receptor-like kinase genes from an equalized cDNA library derived from inflorescence meristems of Arabidopsis by differential screening. These have been named as Inflorescence Meristem receptor-like Kinase genes (IMKs), IMK2 and IMK3. We determined their cDNA and genomic DNA sequences as reported in last meeting, showing that the deduced amino acid sequences contained an extracellular receptor domain consisted of a leucine-rich repeat, a transmembrane domain and a cytoplasmic serine/threonine kinase domain. In this study, we analyzed the expression of the IMK3 gene using the transformants carrying the IMK3 Promoter::GUS transgene. As a result, GUS activity was detected not only in the inflorescence meristem but also in the vegetative meristem as well as in the root meristem. Results suggested a possibility that the IMK3 gene is playing a role in shoot and root meristem functions. We also studied the intercellular localization of both IMK2 and IMK3 gene products by using their fusion constructs with GFP. Transient assay using Onion and Arabidopsis indicated that both fusion proteins were localized in the membrane. In addition, by using various constructs with deletion series of the IMK3, we found that the N-terminal which included the receptor domain and the transmembrane domain was required for their localization into the membrane. These results support the hypothesis that these receptor-like kinases act for the signal transduction across the membrane. To reveal their function in Arabidopsis, we are trying to identify the T-DNA insertion mutants.

12-16

Isolation and characterization of supershoot, a novel Cytochrome P450 involved in shoot branching pattern and vascular development.
Titima Tantikanjana, Megan Griffith, Parvesh H. Mahtani, Venkatesan Sundaresan
Institute of Molecular Agrobiology, 1 Research Link, The National University of Singapore, Singapore 117604

We identified 6 independent Ds insertion lines, generated by a target transposon tagging system, that segregate for a mutant with an abnormal shoot branching pattern. The mutants display a massive proliferation of shoots resulting in several hundred inflorescences per plant. The mutation also causes other pleiotropic effects that can be observed after 3 weeks growth. The abnormalities include reduced internode elongation, defects in vascular patterning in the late adult leaves, reduced number of floral organs, defects in the floral development and very low seed set. Senescence of detached leaves from this mutant was also delayed. Molecular analysis of 3 mutants revealed that each carried a Ds element inserted into the same gene, designated SUPERSHOOT1 (SPS1), but in different positions. The SPS1 cDNA sequence is predicted to encode a protein of 538 amino acid with sequence homology to cytochrome P450. GUS expression from the reporter gene carried by the gene trap Ds element was observed in the shoot apical meristem, at the base of adaxial of the cauline leaves, vascular tissue of the aerial parts, and at the receptacle of the flower and silique. Expression pattern of SPS1 as exhibited by the GUS reporter correlates well with the morphological abnormalities.

12-17

Down regulation of CDK activities caused progression of root cell differentiation
Masaaki Umeda, Chikage Umeda-Hara and Hirofumi Uchimiya.
Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan.

It has been proposed that pattern formation in the root meristem is controlled by a balance between signals inhibiting and promoting cell differentiation. However, little is known how cell division is involved in cell fate decisions because of limited biological tools for manipulating cell division. Here we overexpressed a cell cycle-related gene in Arabidopsis and observed suppression of cell division and progression of cell differentiation in the root meristem. CDK-activating kinase (CAK) is a cell cycle regulator which phosphorylates and activates CDKs, thus CAK is an essential factor for full activation of cell division. We have recently identified an Arabidopsis cDNA named cak1At which encoded a novel type of CAK (ref.1). Here we made transgenic plants expressing sense and antisense cak1At under the glucocorticoid-inducible promoter. In both plants, root growth was inhibited by treatment with dexamethasone (DEX), and the cyclin::GUS chimeratic protein disappeared in the root meristem, suggesting that cell division was stopped by overexpression of the transgene. Surprisingly, sustained differentiation of the vascular tissue, columellar and cortical cells was observed even in the initial cells surrounding the central cells, which was never observed in the control root. These results may suggest that cell division activity is essentially required for the maintenance of initial cells whose differentiation is believed to be inhibited by unknown signals derived from the central cells (ref.2).

Ref.1. Umeda, M., Bhalerao, R.P., Schell, J., Uchimiya, H. and Koncz, C. (1998) Proc. Natl. Acad, Sci., USA, 95: 5021-5026. Ref.2. van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P. and Scheres, B. (1997) Nature, 390: 287-289.

12-18

Isolation and characterization of vertebrate-type CDK-activating kinase
Masatoshi Yamaguchi, Masaaki Umeda, Satoko Matsubayashi and Hirofumi Uchimiya
Inst. Mol. Cell. Biosci., Univ. Tokyo

Cyclin-dependent protein kinases (CDKs) play central roles in the regulation of cell cycle. The kinase activity is regulated by phosphorylation/dephosphorylation of the conversed amino acid residues as well as binding to cyclins. CDK-activating kinase (CAK) phosphorylates at the threonine residue within the T-loop of CDK to activate its phosphorylation activity. Recently we identified Arabidopsis CAK, named Cak1At, as a suppressor of temperature-sensitive cak mutant of budding yeast (ref.1). However, it had low similarity to animal and yeast CAKs and was classified as a distinct CAK in the phylogenetic tree. We revealed that Cak1At phosphorylated only human CDK2 but not the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II which is a substrate of vertebrate CAKs. On the other hand, a rice CAK homolog, termed R2, is structurally similar to vertebrate CAKs and had CDK- and CTD-kinase activities (ref.2). Here we report the isolation of an Arabidopsis cDNA encoding a vertebrate-type CAK. This CAK (Cak2At) showed the highest similarity to rice R2 with 85% identity at the amino acid. Based on the data of expression analysis, the function of two distinct CAKs will be discussed.
Ref.1. Umeda,M., Bhalerao,R.P., Schell,J., Uchimiya,H. and Koncz,C. (1998) Proc. Natl. Acad. Sci. USA 95, 5021-5026 Ref.2. Yamaguchi,M., Umeda,M. and Uchimiya,H. (1998) Plant J. 16, 613-619

12-19

The meristem cell division control gene WIGGUM is ERA1 which encodes a farnesyltransferase beta subunit

Eva C. Ziegelhoffer, Leonard J. Medrano, Elliot M. Meyerowitz
Division of Biology 156-29 California Institute of Technology Pasadena, CA 91125

Control of cellular proliferation in plant meristems is important for maintaining the correct number and position of developing organs. One of the genes identified in the control of floral and apical meristem size and floral organ number in Arabidopsis is WIGGUM (WIG). In wig mutants, one of the most striking phenotypes is an increase in floral organ number, particularly in the outer whorls which is consistent with an increase in cell number across the width of young floral meristems. wig mutants display additional phenotypes including reduced and delayed germination, delayed flowering, longer life span, reduced internode elongation, occasional fasciation, and modest phyllotaxy defects. To better understand WIG's role in development, we cloned the WIG gene by positional cloning. All three wig alleles have substitutions in the coding region of ERA1 (enhanced response to ABA), a gene identified as a farnesyltransferase beta subunit. Protein farnesylation is a posttranslational modification in which a 15-carbon farnesyl lipid is covalently attached via a thioether linkage to a cysteine residue near the C-terminus of target proteins (including components involved in signal transduction and cell division control). To determine if farnesyltransferase in Arabidopsis is regulated at the level of WIG/ERA1 mRNA expression, we performed in situ hybridization on various tissues including embryos, seedlings, and inflorescences; WIG mRNA is expressed at low to moderate levels in most tissue types (in comparison to other known meristem control genes) and was unchanged in several mutant backgrounds including clv1, clv3, stm, and wus. Conversely, CLV1, CLV3, and STM expression is not altered in wig mutants.

12-20

Lateral root initiation in Arabidopsis thaliana

Pedro J. Casero (1) , Ilda Casimiro (1), Gloria Durán (1), Alan Marchan (2), Malcolm Bennett (2)
(1) Dpto. Ciencias Morfológicas y Biología Celular y Animal. Faculty of Sciences, University of Extremadura. 06071 Badajoz. Spain. (2) School of Biological Sciences. University Park. University of Nottingham. Nottingham NG7 2RD. UK

Lateral roots initiate from a few pericycle cells, founder cells, located in from of the xylem. These founder cells are about 100 microns in length and contain central nuclei. The lateral root initiation occurs when two adjacent founder pericycle cells, located in the same column in front of the xylem, undergo asymmetrical transverse divisions giving rise to a pair of short pericycle cell derivatives flanked by two longer ones. This means that prior to the first transverse division, both nuclei move from the centre towards the cell end nearest the other founder cell. Then each of them, divide transversally producing two cell derivatives unequal in size. Later, this also occurs in the neighbouring pericycle columns. Therefore, several pairs of these short cells appear at the same transverse level. The longer cell derivatives successively divide transversely and asymmetrically producing new short cells close to those previously formed. The short cells expand radially and undergo periclinal divisions.
This project was supported by the EC. Framework IV (BIO4CT960487), Spanish Ministry of Education and Science (UE98.0007) and Consejería de Educación y Juventud, Junta de Extremadura (BRV97A051, PRI97C165)

==========

AUTHOR INDEX

==========